UNIVERSIDADE FEDERAL DO PARÁ
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM
BIOLOGIA DE AGENTES INFECCIOSOS E PARASITÁRIOS

ASPECTOS MULTIFATORIAIS DA INFECÇÃO DO VÍRUS DA IMUNODEFICIÊNCIA HUMANA (HIV) E DO VÍRUS EPSTEIN BARR (EBV) RELATIVOS A EPIDEMIOLOGIA, IMUNOLOGIA E IMUNOGENÉTICA DOS LOCI *A*, *B* E *DRB1* DO GENE *HLA de classe* I E II

LEONN MENDES SOARES PEREIRA

Belém-PA

2022

LEONN MENDES SOARES PEREIRA

ASPECTOS MULTIFATORIAIS DA INFECÇÃO DO VÍRUS DA IMUNODEFICIÊNCIA HUMANA (HIV) E DO VÍRUS EPSTEIN BARR (EBV) RELATIVOS A EPIDEMIOLOGIA, IMUNOLOGIA E IMUNOGENÉTICA DOS LOCI A, B E DRB1 DO GENE HLA de classe I E II

Tese apresentada ao Programa de Pós-Graduação em Biologia de Agentes Infecciosos e Parasitários do Instituto de Ciências Biológicas da Universidade Federal do Pará como requisito parcial na obtenção do grau de Doutor em Biologia de Agentes Infecciosos e Parasitários.

Orientador: Prof. Dr. Antonio Carlos Rosário Vallinoto

Belém-PA

2022

LEONN MENDES SOARES PEREIRA

ASPECTOS MULTIFATORIAIS DA INFECÇÃO DO VÍRUS DA IMUNODEFICIÊNCIA HUMANA (HIV) E DO VÍRUS EPSTEIN BARR (EBV) RELATIVOS A EPIDEMIOLOGIA, IMUNOLOGIA E IMUNOGENÉTICA DOS LOCI *A, B* E *DRB1* DO GENE *HLA de classe* I E II

Tese apresentada ao Programa de Pós-Graduação em Biologia de Agentes Infecciosos e Parasitários do Instituto de Ciências Biológicas da Universidade Federal do Pará como requisito parcial na obtenção do grau de Doutor em Biologia de Agentes Infecciosos e Parasitários.

Orientador:

Profº. Drº. Antonio Carlos Rosário Vallinoto
Laboratório de Virologia, ICB-UFPA

Avaliadores:

Profº. Drº. Francisco Alves Acácio
Instituto de Ciências Biológicas (ICB/UFPA)

Profº. Drº. Eduardo José Melo dos Santos
Instituto de Ciências Biológicas (ICB/UFPA)

Profº. Drº. Igor Brasil Costa
Seção de Virologia (SAVIR/IEC)

Profº. Drº. Carlos Alberto Marques de Carvalho
Departamento de Patologia (UEPA)

Profª. Drª. Maria Alice Freitas Queiroz (suplente)
Instituto de Ciências Biológicas (ICB/UFPA)

Belém-PA

2022

“A sabedoria oferece proteção, como o faz o dinheiro, mas a vantagem do conhecimento é esta: a sabedoria preserva a vida de quem a possui.”

Eclesiastes 7: 12

Aos egrégios entes, cujas saudades deixaram.

Aos bravos remanescentes de uma guerra insólita.

AGRADECIMENTOS

Agradeço à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pela bolsa de incentivo ao doutoramento, tal como, aos recursos provenientes do Ministério da Saúde para o fomento do projeto de pesquisa.

Agradeço aos doutores Igor Costa e Felipe Freitas do Instituto Evandro Chagas (IEC) pela confiança e parceria firmada com o Laboratório de Virologia do Instituto de Ciências Biológicas (ICB-UFPA), com a qual, foi possível o desenvolvimento dessa tese.

Agradeço ao meu orientador Dr Antonio Vallinoto pela credibilidade, respeito e integridade profissional que tem demonstrado ao logo dessa qualificação acadêmica.

Em nome das doutoras Patricia Jeanne Mattos e Erika Vanessa Joge agradeço a toda a equipe do laboratório de Imunogenética da Fundação de Hemoterapia e Hematologia do Pará (HEMOPA) pelo precioso auxílio nas práticas do doutorado.

Em nome dos ilustres colegas Eliane França, Iran Costa e Igor Tenório Lima agradeço a toda a equipe dos Laboratórios do vírus Epstein-Barr e de Retrovírus da seção de Virologia do IEC pelo desenvolvimento conjunto das práticas laboratoriais.

Agradeço a todos os membros docentes e discentes do Laboratório de Virologia por essa árdua jornada de formação desde a iniciação científica até o doutoramento.

Agradeço aos valorosos pacientes pela confiança e prestígio em nossa ciência. É visando o bem-estar social do brasileiro que realizamos nossos projetos e contribuições na saúde pública.

Em nome de Gercelinda Soares, Mário Fernando Pereira, Olinda Soares (em memória), Izaura Pereira (em memória) e Ruthelene Menezes (em memória) sou grato a toda a minha família por sua dedicação, amor fraternal e escolhas que me permitiram ter boas escolhas. Nosso amor é incondicional! Também agradeço a minha amada noiva Alessandra Rafaela Bandeira por seu fundamental companheirismo ao longo dessa jornada.

“Como é bom render graças ao Senhor e cantar louvores ao teu nome, ó Altíssimo; anunciar de manhã o teu amor leal e de noite a tua fidelidade” (Salmos 92:1-2). Sou grato a Ti oh Deus, pois Sois a fonte de amor, conhecimento e misericórdia que tenho me nutrido todo o tempo.

SUMÁRIO

1. INTRODUÇÃO 1
1.1 O VÍRUS DA IMUNODEFICIÊNCIA HUMANA (HIV) 1
1.1.1 Aspectos históricos e origem do HIV 1
1.1.2 Classificação e características biológicas 2
1.1.3 Replicação do HIV 5
1.1.4 Transmissão, suscetibilidade e história natural da infecção 7
1.1.5 Epidemiologia da infecção 9
1.1.6 Diversidade e epidemiologia molecular 13
1.2 ASPECTOS DA COINFECÇÃO HIV/EBV 17
1.3 O VÍRUS ESPTEIN-BARR (EBV) 18
1.3.1 Aspectos históricos e origem do EBV 18
1.3.2 Classificação e características biológicas 20
1.3.3 Replicação do EBV 23
1.3.4 Transmissão e patogênese da infecção 27
1.3.5 Epidemiologia da infecção 30
1.3.6 Diversidade genética e epidemiologia molecular 31
1.4 ASPECTOS GERAIS DA RESPOSTA CELULAR ADAPTATIVA NAS INFECÇÕES POR HIV E EBV 33
1.5 O ANTÍGENO LEUCOCITÁRIO HUMANO 35-36
1.5.1 Características gerais 35-36
1.5.2 Aspectos genéticos e nomenclatura 38
1.5.3 Processamento e apresentação de antígenos 40
1.5.4 Diversidade genética dos lócus *A, B* e *DRB1* 45
1.5.5 Implicações do sistema HLA nas infecções pelo HIV e EBV 48
1.5.5.1 Alelos dos lócus *A, B* e *DRB1* associada ao risco e agravamento da infecção pelo HIV 48
1.5.5.2 Alelos dos lócus *A, B* e *DRB1* associados à proteção e controle da progressão da infecção pelo HIV 51
1.5.5.3 Alelos dos lócus *A, B* e *DRB1* associados ao risco e agravamento da infecção pelo EBV 53
1.5.5.4 Alelos dos lócus *A, B* e *DRB1* associados à proteção e controle da progressão da infecção por EBV 54

1.6 JUSTIFICATIVA 55
1.7 OBJETIVOS 55
1.7.1 OBJETIVO GERAL 55
1.7.2 OBJETIVOS ESPECÍFICOS 56
2. APRESENTAÇÃO DOS ARTIGOS 56
2.1 PRIMEIRO MANUSCRITO 57
2.2 SEGUNDO MANUSCRITO 76
2.3 TERCEIRO MANUSCRITO 101
2.4 QUARTO MANUSCRITO 117
3. CONSIDERAÇÕES FINAIS 180
4. REFERÊNCIAS BIBLIOGRÁFICAS 184
ANEXO 1 222
APÊNDICE 1 241
APÊNDICE 2 243

LISTA DE FIGURAS E TABELAS

Figura 1 - Estrutura do HIV 4

Figura 2 – O genoma do HIV 4

Figura 3 – Replicação do HIV 6

Figura 4 – História natural da infecção pelo HIV, sem o uso de terapia 9

Figura 5 – Prevalência global do HIV em 2020 12

Figura 6 - Taxa de detecção de AIDS (por 100.000 hab.) e percentual de declínio ou incremento, segundo UF de residência, por ano de diagnóstico no Brasil, 2010 a 2020 13

Figura 7 - Prevalência mundial dos subtipos HIV-1 do grupo M 15

Figura 8 - Distribuição de grupos HIV-2 na África Ocidental 16

Figura 9 – Modelo geral de partícula viral dos herpsevírus 21

Figura 10 - Estrutura genômica do EBV 21

Figura 11 – O ciclo de vida do EBV 27

Figura 12 – Mapa da região correspondente ao MHC, situada no cromossomo 6 37

Figura 13 – Estrutura das moléculas de HLA de classe 1 e 2 37

Figura 14 - Nomenclatura do HLA segundo as definições do Comitê de Nomenclatura dos da Organização Mundial da Saúde 40

Figura 15 – Vias de processamento e apresentação de antígenos por moléculas de HLA. 44

(Errata) Figure 1 (primerio manuscrito) 64

Figure 1 (quarto manuscrito) 141-142

Figure 2 (quarto manuscrito) 143-144

Figure 3 (quarto manuscrito) 144

Figure 4 (quarto manuscrito) 145

Figure 5 (quarto manuscrito) 146

Figure 6 (quarto manuscrito) 147

Figure 7 (quarto manuscrito) 148

Figure 8 (quarto manuscrito) 149

Figure 9 (quarto manuscrito) 150

Table 1 (quarto manuscrito) 152

Table 2 (quarto manuscrito) 159

Supplementary data 1 (quarto manuscrito) 161

Supplementary data 2 (quarto manuscrito) 165

LISTA DE ABREVIATURAS, SIGLAS E SÍMBOLOS

AIDS - Síndrome da Imunodeficiência Adquirida;

β2m - Beta-2 Microglobulina;

CASA DIA - Centro de Atenção à Saúde nas Doenças Infecciosas Adquiridas;

CDC - Centro de Controle de Doenças;

CEP - Comitê de Ética em Pesquisa com Seres Humanos;

CLIP - Cadeia Peptídica Invariante Associada à Classe II;

CNVs - Polimorfismo de Número Variável de Cópias;

CRF - Forma Recombinante Circulante;

CR2 - Receptor de Complemento tipo 2;

CyPA - Ciclofilina A;

DNA - Ácido Desoxirribonucleico;

DNA (+) - DNA de Polaridade Positiva;

DNAc (-) - DNA Complementar de Polaridade Negativa;

DNAsss - *Single-Strand Strong Stop* DNA;

IARC - *International Agency for Research on Cancer*;

IEC - Instituto Evandro Chagas;

Ig - Imunoglobulina;

IN - Integrase;

InDel - Polimorfismos do tipo Inserção ou Deleção;

IRAP - Aminopeptidase Responsiva à Insulina Endossômica;

IR1 - repetições internas;

IR2 e IR4 - Regiões Conservadas Ricas em Guanina e Citosina com 125 pb e 102 pb;

ITAM - Seqüência de Ativação Imuno-Receptor baseada em Tirosina;

ITIM - Sequência de Inibição de Imuno-Receptor baseada em Tirosina;

KIR - Receptor Semelhantes a Imunoglobulinas de Células *natural killer*;

LABVIR-ICB-UFPA - Laboratório de Virologia do Instituto de Ciências Biológicas na Universidade Federal do Pará;

LAV - Vírus Associado a Linfoadenopatia;

PTLD - Desordem Linfoproliferativa Pós-transplante;

RNA - Ácido ribonucleico;

RSV - Vírus do Sarcoma de Rous;

SAIDS - Síndrome da Imunodeficiência de Símios;

SAPE - estreptoavidina-ficoeritrina;

SCP - *Small Capsomere-interacting Protein*;

SCR - Repetições de Consenso Curtas;

SIVs - Vírus da Imunodeficiência Símia;

SIV_{CPZ} - SIV de Chimpanzés da África Central;

SIV_{GO} - SIV de Gorilas;

SIV_{SMM} - SIV de Macacos Mangabeis;

SNPs - *single nucleotide polymorphisms*;

TAP - Transportador Associado ao Processamento de Antígenos;

TCLE - Termo de Consentimento Livre e Esclarecido;

TPA - 12-O-tetradecanoilforbol-13-acetato;

dNTP - Desoxinucleotídeos Trifosfatos;

DSTs - Doenças Sexualmente Transmissíveis;

DVMO - Doadores Voluntários de Medula Óssea;

EBNA-1 - Antígeno Nuclear do tipo 1;

EBV - Vírus Epstein Barr;

EBER - Moléculas de RNA não Codificantes e não Poliadenilados de EBV;

ERAP - Peptidases Associadas ao Retículo Endoplasmático;

GILT - Tiol-redutase Lisossômica induzido por Interferon-γ;

HEMOPA - Fundação de Hemoterapia e Hematologia do Estado do Pará;

HHV4 - Gamaherpesvírus humano 4;

HIV 1 e 2 - Vírus da Imunodeficiência Humana do tipo 1 e 2;

HLA - Antígeno Leucocitário Humano;

HSH - Homens que fazem sexo com homens;

HTLV - Vírus Linfotrópico de Células T Humanas;

LB - Linfoma de Burkitt;

Ii - Cadeia Invariante;

LMP-1 - Proteína Membranar de Latência do tipo 1;

LTR - Longas Sequencias de Repetição;

MCP - *Major Capsid Protein*;

MHC-I e -II - Complexo de Histocompatibilidade de classe I e II;

NK - Células *natural killer*;

ORF - Quadro de Leitura Aberta;

pAPCs - Células Apresentadoras de Antígenos Profissionais Específicas;

PBMCs - Células Mononucleares do Sangue Periférico;

P. carinii - *Pneumocystis carinii;*

PCR-SSO - Polymerase Chain Reaction - Sequence Specific Oligonucleotide;

PICs - Complexos de Pré-integração;

PLC - Complexo de Carga de Peptídeos;

PR - Protease;

TR - Transcriptase Reversa;

IN -integrase;

Tr - Repetições Diretas nas Terminações 3’ e 5’;

URF - Formas Recombinantes Únicas;

Us e **Ul** - Domínios de Sequencias Únicas Curtas e Longas;

ZEBRA - *BamHI Z Epstein-Barr virus replication activator*;

ZREs - *Zta response elements*.

RESUMO

Ainda há uma carência de estudos multifatoriais relacionado as infecções por HIV e EBV na região amazônica brasileira. O objetivo do presente estudo foi avaliar os aspectos epidemiológicos, perfis imunológicos e a associação genética dos lócua *A, B* e *DRB1* entre jovens adultos do estado do Pará infectados pelos agentes. O total de 642 amostras coletadas entre os anos de 2018 a 2019 foram triadas com imunoensaios para a detecção do antígeno p24 e anticorpos IgG anti-p24-HIV, tal como, IgM e IgG anti-VCA-EBV; a carga viral do HIV e do EBV foram quantificadas por PCR em tempo real usando matrizes de amplificação comercial; a contagem de linfócitos T e a dosagem de citocinas foram efetuadas por citometria de fluxo; a genotipagem do EBV foi feita por PCR convencional usando primers descritos na literatura; a genotipagem do HLA de classe I e II foi realizada por PCR-SSO; dados clínicos e epidemiológicos foram obtidos de questionários próprios do projeto e relatórios médicos; dados de dispensação de antirretrovirais e monitoramento dos pacientes com HIV foram obtidos do banco de dados SICLON e SISCEL, respectivamente; as análises estatísticas foram desenvolvidas com o auxílio dos programas Bioestat 5.3, GENEPOP e GraphPad Prism 8.4.3. O uso de drogas ilícitas, a baixa escolaridade e renda familiar, o não uso de preservativos, a orientação homoafetiva, não se relecionar com um único parceiro sexual e o histórico de IST's foram indicadores associados a infecção por HIV e coinfecção HIV/EBV; o histórico de coinfecções foi associado a infecção primária por EBV. O genótipo EBV-2 foi associado a baixos níveis de carga viral do HIV-1, enquanto a carga viral do EBV-1 foi positivamente correlacionada com a carga viral do HIV-1; a apresentação tardia e a polissintomatologia foram fatores associados a manutenção imunológica; o perfil imunológico foi mais ativo na infecção primária por EBV e mais equilibrado na infecção passada, alguns achados imunológicos foram atribuídos a prevalente coinfecção por HIV nesses grupos. Os alelos HLA-B*13, B*35 e B*39 do HLA de classe I foram associados a falha na resposta terapêutica em pacientes HIV^{+}. Os alelos HLA-DRB1*03, DRB1*09 e DRB1*16 foram associados a manutenção imunológica em diferentes fases da infecção por EBV.

ABSTRACT

There is still a lack of multifactorial studies related to HIV and EBV infections in the Brazilian Amazon region. The objective of the present study was to evaluate the epidemiological aspects, immunological profiles and the genetic association of locua A, B and DRB1 among young adults in the state of Pará infected by the agents. A total of 642 samples collected between 2018 and 2019 were screened with immunoassays for the detection of p24 antigen and anti-p24-HIV IgG antibodies, such as anti-VCA-EBV IgM and IgG; HIV and EBV viral load were quantified by real-time PCR using commercial amplification arrays; T lymphocyte count and cytokine dosage were performed by flow cytometry; EBV genotyping was performed by conventional PCR using primers described in the literature; HLA class I and II genotyping was performed by PCR-SSO; clinical and epidemiological data were obtained from the project's own questionnaires and medical reports; data on antiretroviral dispensing and monitoring of HIV patients were obtained from the SICLON and SISCEL databases, respectively; Statistical analyzes were performed with the help of Bioestat 5.3, GENEPOP and GraphPad Prism 8.4.3 programs. The use of illicit drugs, low schooling and family income, not using condoms, homosexual orientation, not having a single sexual partner and having a history of STIs were indicators associated with HIV infection and HIV/EBV coinfection; history of co-infections was associated with primary EBV infection. EBV-2 genotype was associated with low levels of HIV-1 viral load, while EBV-1 viral load was positively correlated with HIV-1 viral load; late presentation and polysymptomatology were factors associated with immunological maintenance; the immunological profile was more active in the primary EBV infection and more balanced in the past infection, some immunological findings were attributed to the prevalent HIV coinfection in these groups. The HLA-B*13, B*35 and B*39 alleles of HLA class I were associated with failure to respond to therapy in HIV^+ patients. The HLA-DRB1*03, DRB1*09 and DRB1*16 alleles of HLA class II were associated with immune maintenance at different stages of EBV infection.

1. INTRODUÇÃO

1.1 O VÍRUS DA IMUNODEFICIÊNCIA HUMANA (HIV)

1.1.1 Aspectos históricos e origem do HIV

Em meados de 1981, relatórios médicos norte-americanos atentavam para a singular epidemia de casos de pneumonia por *Pneumocystis carinii* (*P. carinii*) entre homens, jovens, homossexuais, previamente saudáveis (CDC, 1981). A saúde dos infectados se alterava ao passo do avançar da doença. Alguns chegavam a desenvolver lesões tipicamente associadas ao sarcoma de Kaposi e muitos evoluíram ao óbito enquanto havia a tentativa de tratar as infecções oportunistas (MMWR, 1981).

A partir de 1982, baseado nas principais evidências epidemiológicas, a "nova doença" fora sugerida como uma "disfunção imunológica relacionada a uma exposição comum", o que concentrava as questões inerentes a sua transmissão ao estilo de vida dos que se expuseram a fluidos corporais (sangue e sêmen). Como não havia precedentes, os bancos de sangue foram diretamente afetados e permaneceram inseguros. Muitos hemofílicos foram infectados como resultado de transfusões de sangue contaminado (CDC, 1981; MMWR, 1982).

A identificação do agente etiológico da nova epidemia era um clamor científico, político e social da época. As hipóteses iniciais de que poderia se tratar de um agente viral procederam de observação da similaridade das vias de transmissão e do quadro de imunodeficiência com outras doenças de etiologia viral. Em 1983, o primeiro sinal neste sentido foi proporcionado pela descoberta de um novo retrovírus humano por Luc Montagnier e colaboradores do Instituto Pasteur, França, em amostras de linfadenopatia aguda, que ficou conhecido como vírus associado a linfoadenopatia (LAV), que apresentava características biológicas e de patogenicidade diferentes do vírus linfotrópico de células T (HTLV) (Barré-Sinoussi et al., 1983). Em contrapartida, a equipe de Gallo do Instituto Nacional do Câncer, EUA, em estudo simultâneo batizava o agente como vírus linfotrópico de células T humanas do tipo III (HTLV-III), alegando algumas características estruturais semelhantes com as cepas isoladas do HTLV, além do tropismo por linfócitos T $CD4^+$ (Poiesz et al., 1980; Popovic et al., 1984).

Em 1985 os testes laboratoriais foram desenvolvidos para detectar anticorpos anti-LAV/HTLV-III, fato que impulsionou o diagnóstico e definição da síndrome da imunodeficiência adquirida (AIDS). Segundo o Centro de Controle de Doenças (CDC), a definição expandida dos casos de AIDS especificava o teste positivo para o patógeno e a presença de quadros oportunistas associados (candidíase oral ou pulmonar, linfoma não-Hodgkin, sarcoma de Kaposi, dentre outras) (CDC, 1985). Em revisões posteriores do grupo,

foi incluído a relevância da redução no número dos linfócitos T $CD4^+$ como marcador da imunossupressão na definição clínica da AIDS, em decorrência de estudos que haviam mostrado a contagem abaixo do esperado dessas células nos pacientes infectados (Fernandez-Cruz et al., 1990).

Apenas em 1986, o Comitê Internacional de Taxonomia Viral (ICTV), presidido na época por Harold Varmus, deliberou pela identificação do patógeno como vírus da imunodeficiência humana (HIV) (Coffin et al., 1986). Ainda em 1986, foi observado em pacientes africanos de Senegal, com suspeita de AIDS, testes com perfis inconclusivos quanto a reatividade para os imunodiagnósticos em vigor. A caraterização mais detalhada do soro dos pacientes mostrou que haviam cepas virais antigenicamente distintas às de referência previamente descritas. Tratava-se de uma nova espécie comparada à descrita primordialmente, que foi então denominada HIV-2, (Clavel et al., 1986).

Em primatas não-humanos, mantidos em cativeiro, foi observada a ocorrência de um quadro sindrômico semelhante aos aspectos da AIDS em humanos, porém, sem a presença do HIV-1, recém descrito, nas amostras coletadas. A síndrome da imunodeficiência de símios (SAIDS), assim denominada, era causada por retrovírus similares ao HIV (em termos de tropismo, morfologia, efeitos citopáticos e genoma), coletivamente designados vírus da imunodeficiência símia (SIVs). Essas relações forneceram as primeiras evidências de que os grupos virais emergiram de eventos de transmissões zoonóticas entre as espécies de primatas humanos e não-humanos (Chakrabarti et al., 1987; Sharp et al., 1994).

Estudos filogenéticos subsequentes confirmaram a origem do HIV a partir dos SIVs. O HIV-1 emergiu do SIV de chimpanzés da África Central (SIV_{CPZ}), mais especificamente a cepa SIV_{CPZ}*Ptt*, com a qual também há uma relação monofilética com o vírus de gorilas (SIV_{GOR}) (Keele et al., 2006). No entanto, a espécie HIV-2 teve sua origem proposta a partir da linhagem de vírus infectantes de macacos mangabeis (*Cercocebus atys*) (SIV_{SMM}) da África Ocidental (Gao et al., 1992).

1.1.2 Classificação e características biológicas

O vírus da imunodeficiência humana do tipo 1 (*Human immunodeficiency virus 1* – HIV-1) e do tipo 2 (*Human immunodeficiency virus 2* – HIV-2) são espécies pertencentes a ordem *Ortovirales*, família *Retroviridae*, subfamília *Orthoretrovirinae* e gênero *Lentivirus* (ICTV, 2019).

A partícula viral pós brotamento possui o nucleocapsídeo em formato de anel eletro denso, cuja a morfogênese se baseia em um arranjo simples e contínuo da poliproteína Gag em uma rede hexamérica que cresce em curvatura inerente e incorpora estocasticamente novas moléculas proteicas. A infectividade viral só é adquirida mediante o processo de maturação da partícula, que se procede pela ativação da protease viral (PR) e subsequente processamento do produto do gene *gag* e estabilização do dímero de RNA genômico (Briggs et al., 2009; Sakuragi, 2011) (figura 1).

A partícula viral madura possui um diâmetro que varia de 100 a 120 nm e um envelope glicoproteico de camada dupla adquirido da célula hospedeira, sob a qual estão dispostas espículas virais triméricas das glicoproteínas gp 120 (glicoproteína de superfície) ancorados ao envelope pelos trímeros da gp 41 (glicoproteína transmembranar), além de moléculas do complexo de histocompatibilidade de classe I e II (MHC-I e MHC-II) e ciclofilina A (CyPA) do hospedeiro. Abaixo do envelope está a matriz viral, composta por subunidades da proteína p17. Envolto a matriz, está o capsídeo de formato cônico (dependendo do plano de corte) composto pela proteína p24 em arranjos de subunidades hexaméricas de 9,6 nm. A estrutura do capsídeo guarda o material genético viral (2 fitas simples de RNA de polaridade positiva), que se encontram ligadas entre si pelo terminal 5' e associadas ao nucleocapsídeo composto pela proteína p7; no capsídeo também estão as proteínas transcriptase reversa (TR), integrase (IN), PR, vif, vpr, nef, p6 e componentes da maquinaria celular hospedeira (RNA^{Lis} e desoxinucleotídeos trifosfatos (dNTP)) (Briggs et al., 2003; Costa et al., 2015; German Advisory Committee Blood, 2016) (figura 1).

O genoma do HIV tem o comprimento aproximado de 9,7 Kb, flanqueado nas extremidades por longas sequencias de repetição (LTR – *Long Terminal Repeats*), e nove sequencias de leitura em aberto que expressam proteínas distintas. Três genes são estruturais (*gag, pol* e *env*), dois regulatórios (*tat* e *rev*) e quatro acessórios (*nef, vif, vpr* e *vpu* ou *vpx*). Partindo do terminal 5', o gene *Gag* codifica as proteínas da matriz viral (p17), capsídeo (p24) e nucleocapsídeo (p7); segue-se com a leitura do gene *pol* que codifica as proteínas PR, TR e IN; o gene *Env* é adjacente e codifica o percussor das glicoproteínas gp120 e gp41; os genes regulatórios *tat* e *rev* são importantes no início da replicação viral e os genes acessórios, além de contribuírem neste processo, também participam na manutenção da patogenicidade do agente (Sauter et al., 2012; Lazar et al., 2016) (figura 2).

A espécie HIV-2 apresenta o gene *vpx* ao invés de *vpu*, o que está parcialmente relacionado a redução da patogenicidade no vírus (Motomura et al., 2008; Vincenzie Poli, 2013).

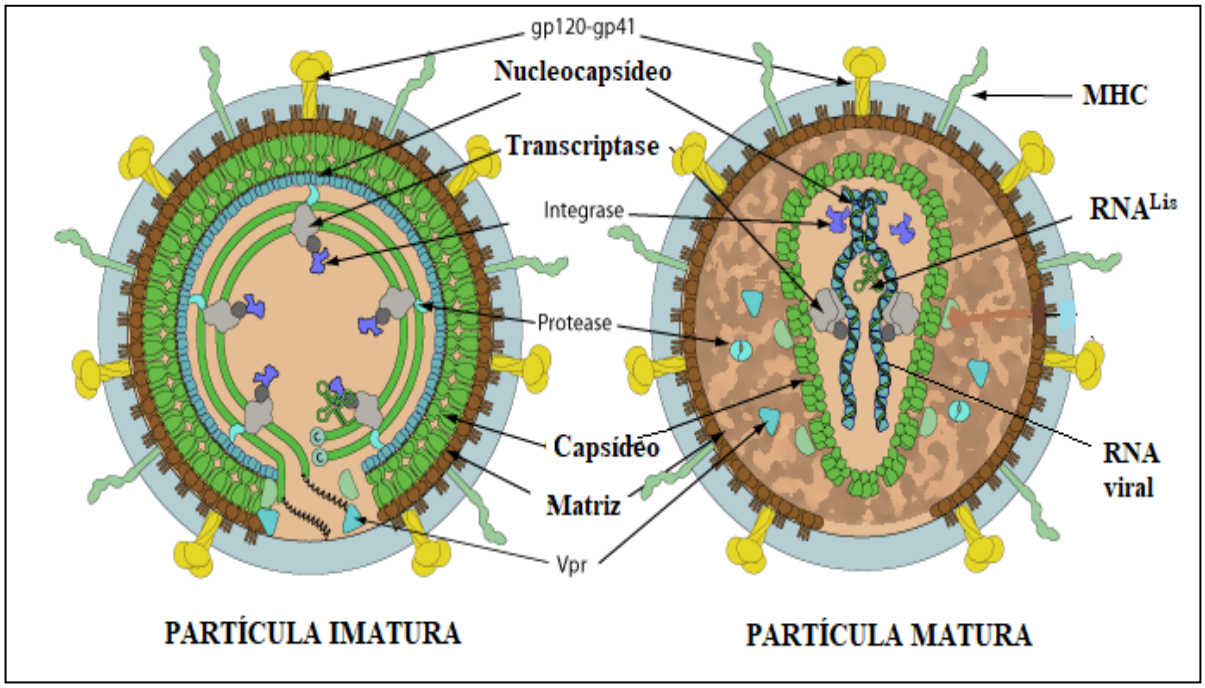


Figura 1- Representação estrutura do HIV: A partícula imatura possui o nucleocapsídeo em formato de anel eletro denso. A infectividade viral só é adquirida mediante o processo de maturação da partícula, que se procede pela ativação da protease viral e subsequente processamento do gene Gag. Adaptado de Viral Zone, 2015 (https://viralzone.expasy.org/7?outline=all_by_species).

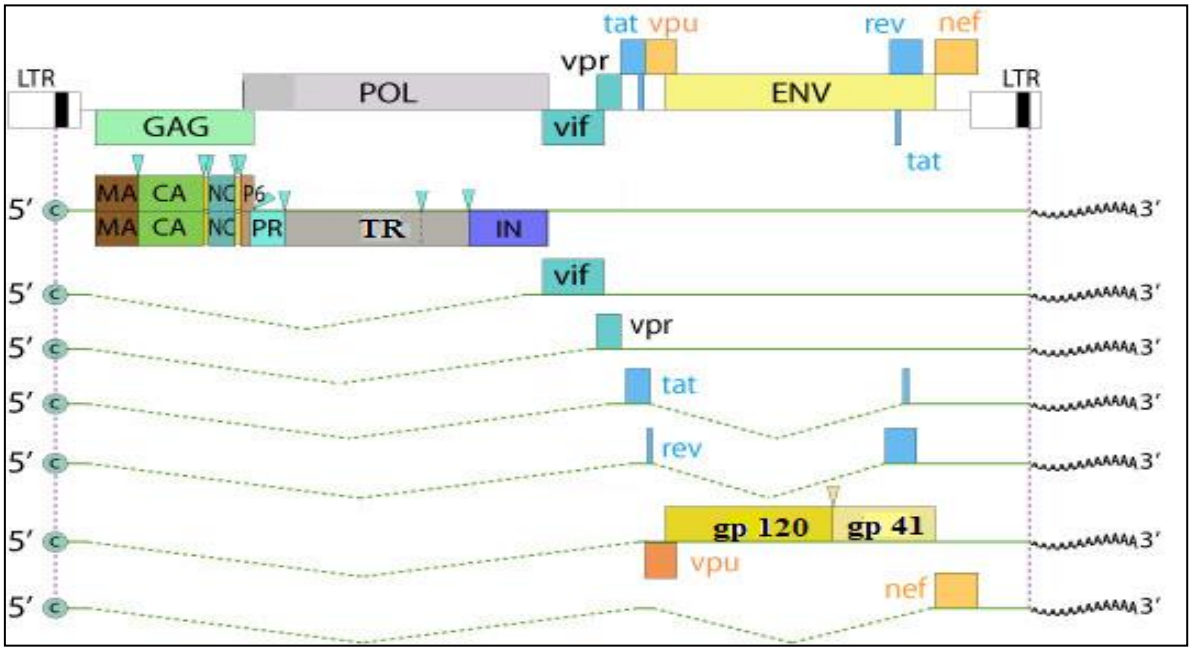


Figura 2 – O genoma do HIV-1: O material genético do vírus compõe 2 fitas simples de RNA de polaridade positiva, flanqueado nas extremidades por sequencias LTR. Um Cap 5' e uma cauda poli-a estão inseridos no início e final da sequência, respectivamente. (MA – matriz; CA – capsídeo; NC – nucleocapsídeo; PR – protease; TR- transcriptase reversa; IN – integrasse). Adaptado de Viral Zone, 2015 (https://viralzone.expasy.org/7?outline=all_by_species).

1.1.3 Replicação do HIV

A adsorção viral ocorre pela interação inicial da glicoproteína gp 120 com moléculas CD4, permitindo a exposição de sítios de ligação para os receptores de quimiocinas CCR5 e CXCR4 presentes na superfície da célula alvo. Essa interação inicia mudanças conformacionais que expõem o peptídeo de fusão da glicoproteína gp 41 em formato de pré-grampo, o que realiza a fusão da partícula viral à membrana da célula hospedeira. O capsídeo viral no citoplasma sofre desestruturação da matriz e, subsequente, exposição do RNA genômico viral, molde da transcrição reversa (Buzon et al., 2010; Chen et al., 2016)

A transcrição reversa é iniciada pela interação da TR com a molécula de RNA^{Lis} ligada a extremidade 5' do RNA genômico, sintetizando um fragmento de aproximadamente 186 bases de DNA complementar de polaridade negativa [DNAc (-)], denominado de DNAsss (*single-strand strong stop* DNA). Este fragmento de DNA é reposicionado pela TR na posição 3' do RNA genômico, onde é reiniciada e finalizada a síntese do DNAc. Concomitante ao processo, ocorre a degradação do RNA genômico pela propriedade de RNAse da TR, exceto numa região rica em purina que se adequa como "*primer*" da fita positiva de DNA [DNA (+)]. A polimerização da cadeia positiva sintetiza o sítio de iniciação PBS (*primer binding site*) na extremidade 3', no qual, o fragmento de DNA (+) hibridiza com a região correspondente no DNAc (-) onde se reinicia a síntese completa da sequência positiva (Kaushik et al., 2001; Piekna-Przybylska e Bambara, 2011; Chen et al., 2016).

A dupla fita de DNA permanece associada às proteínas virais TR, Vpr, p17 e p7, além de fatores associados a cromatina, como a proteína LEDGF/P75 (Ciuffi et al., 2005), formando assim o complexo PIC. A carioteca nuclear é transposta por PIC através de nucleoporinas presentes nos poros nucleares. Dentro do núcleo, a enzima IN catalisa o processo de integração do DNA viral por meio de dois eventos de clivagem molecular (transesterificação) e consecutiva transferência ao genoma celular (Hazuda et al., 1994; Delelis et al., 2008).

As sequências LTRs do DNA integrado contêm sinais promotores e potenciadores da transcrição gênica que recrutam fatores e coativadores transcricionais aos sítios correspondentes (Shah et al., 2014). A RNA polimerase II do hospedeiro catalisa o processo de transcrição, sob o auxílio da enzima viral Tat que prologa o processo por hiperfosforilação enzimática. O RNA mensageiro completo serve de molde para a tradução da poliproteína Gag, porém, apenas com processos de *splicing* do RNA, obtém-se as demais proteínas virais (Schiralli Lester e Henderson, 2012).

Os constituintes glicoproteicos, gp120 e gp41, resultam da clivagem do percussor gp160 e são transportados para a membrana plasmática (McCune et al., 1998). Os percussores Gag e Gag-Pol passam por modificações pós-traducionais de adição de ácido mirístico e outros componentes que auxiliam no transporte das poliproteínas à membrana plasmática e ligação em regiões conhecidas como balsas lipídicas (Campbell et al., 2001). Duas moléculas de RNA mensageiro completo ligam-se entre si pela extremidade 5' formando um dímero que constituirá o material genético da partícula em montagem (Moore e Hu, 2009). O acúmulo de elementos virais leva a deformação da membrana e início do brotamento viral; sítios ativos em Gag (p6, YPLASL e Bro) interagem com enzimas do complexo ESCRT e Alix que catalisam o processo de brotamento da partícula imatura (Peel et al., 2011). A partícula viral liberada adquiri atributos infecciosos, ao passo que a protease cliva as poliproteínas Gag e Pol ocorrendo a estabilização do RNA genômico (Moore e Hu, 2009) (figura 3).

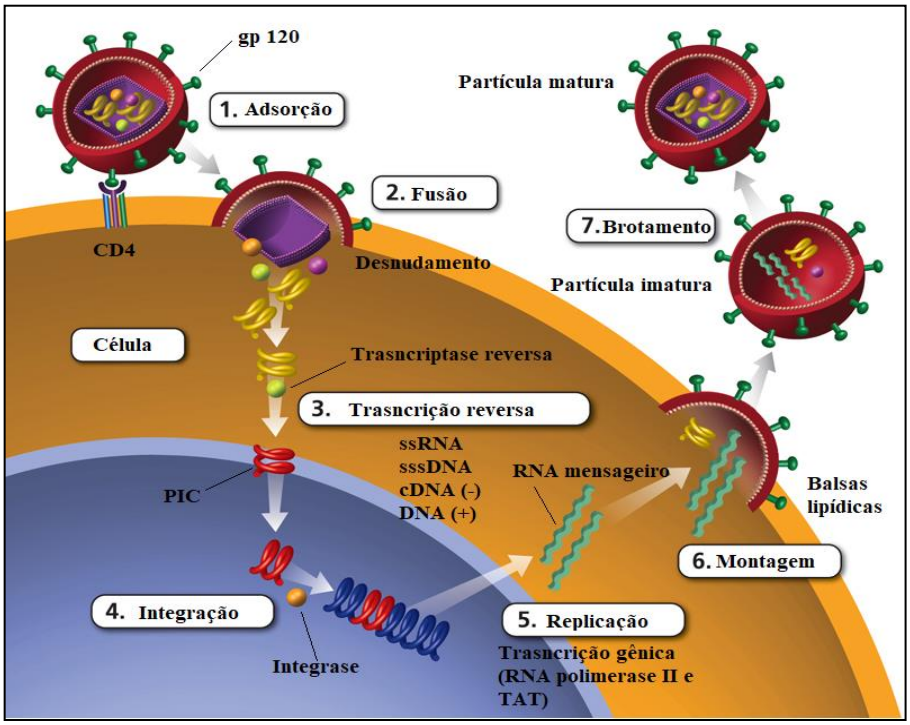


Figura 3 – Replicação do HIV: A infecção da célula hospedeira inicia com os processos de adsorção e fusão da partícula, por intermédio das proteínas gp120 e gp41 virais, tal como CD4 e receptores de quimiocinas do hospedeiro. Há um desnudamento do capsídeo e subsequente exposição do material genético viral; a transcriptase orquestra a transcrição reversa em uma molécula de DNA (+). A integração do complexo PIC ao genoma do hospedeiro ocorre através da enzima integrase. Mediada pela RNA polimerase do hospedeiro e pela proteína Tat, ocorre a expressão do genoma viral. Proteínas são traduzidas e acumuladas em regiões especificas da membrana plasmática; o acúmulo de elementos virais leva a deformação da membrana e início do brotamento viral. As partículas imaturas passam por um processo de maturação para adquirir a infecciosidade. Adaptado de AIDSinfo (https://aidsinfo.nih.gov/understanding-hiv-aids/glossary/1648/replication).

1.1.4 Transmissão, suscetibilidade e história natural da infecção

A transmissão do HIV ocorre mediante a exposição ao vírus na superfície da mucosa ou por inoculação percutânea, que ocorre através do contato sexual, parenteral, perinatal ou amamentação. Destas, a exposição do trato genital feminino, por sêmen ou sangue, é apontada como de maior risco à infecção, contribuindo com cerca de 12,6 milhões de novos casos em escala mundial. Por outro lado, o trato genital masculino exposto a secreções cervicovaginal e retal, ou sangue, contribui com cerca de 10,2 milhões de novos casos (Hladik e McElrath, 2008).

Logo após a infecção, inicia-se a fase aguda ou primária da doença, em que a carga viral é detectável em altas concentrações e há uma brusca redução na quantidade de linfócitos T $CD4^+$, podendo o indivíduo apresentar doenças (análogos a gripe, mononucleose infecciosa, meningite asséptica) ou sintomas clínicos (febre, mal-estar, mialgia, linfadenopatia, erupção cutânea, faringite e diarreia). Nesta fase ocorre intensa resposta celular que resulta na diminuição da carga viral e recuperação parcial dos linfócitos T $CD4^+$. A soroconversão de fase aguda dura em torno de 3 meses, com raros casos chegando aos 6 meses (Braun et al., 2015; Melhuish e Lewthwaite, 2018) (figura 4).

A suscetibilidade celular ao HIV está associada a expressão da molécula CD4 e dos receptores de quimiocina CCR5 e CXCR4. Neste sentido, as células mononucleares do sangue periférico (PBMCs), que incluem linfócitos, monócitos e células dendríticas, são importantes sítios para a infecção (Al-Jabri, 2003). Os linfócitos T $CD4^+$ são as principais células-alvo e estão diretamente relacionados a manutenção da carga viral quando ativadas, cujos efeitos citopáticos são marcantes na história natural da infecção (Wainberg, et al., 1987). Macrófagos teciduais produzem partículas virais em menor taxa que os linfócitos T $CD4^+$; a infecção em si não diminui a circulação de macrófagos ou monócitos, porém, pode resultar na atenuação da resposta celular por alteração da expressão de citocinas e moléculas coestimuladoras (Koppensteiner et al., 2012). Populações de células dendríticas mielóides e plasmocitóides são tanto suscetíveis a infecção pelo HIV-1 como podem transinfectar as células T $CD4^+$ devido ao íntimo contato estabelecido na apresentação de antígenos (Loré et al., 2005), sendo considerada a primeira linhagem celular a se infectar e disseminar o HIV-1 entre os linfócitos dos nódulos linfáticos (Spira et al., 1996).

Com o desenvolvimento de anticorpos específicos e a estabilidade da carga viral, inicia-se a fase assintomática ou de persistência crônica. Na maioria dos casos a redução da contagem de T $CD4^+$ ocorre lentamente ao longo de 6 a 8 anos; em uma parcela dos infectados essa redução ocorre nos primeiros 6 a 12 meses. Embora este período de latência seja assintomático,

alguns indivíduos podem desenvolver linfadenopatia generalizada e persistente, acompanhada por sudorese, mialgia, mal-estar e cansaço (Melhuish e Lewthwaite, 2018) (figura 4).

A fase de AIDS, propriamente dita, é uma consequência da progressão da infecção e disfunção imunológica, tornando o indivíduo suscetível a doenças oportunistas, nefropatias, doenças autoimunes, alterações no sistema nervoso e câncer. Com uma contagem de linfócitos T $CD4^+$ entre 200 a 350/mm^3 o indivíduo pode apresentar candidíase, purpura, varicela-zoster e infecções bacterianas por *Mycobacterium tuberculosis* e *Streptococcus pneumoniae*; na ausência de uma doença indicadora, o diagnóstico de AIDS pode ser avaliado com base na sintomatologia do paciente (anemia, trombocitopenia, linfadenopatia, etc.). Quando a contagem de linfócitos diminui para <200 células/mm^3 o paciente torna-se passível a pneumonia por *Pneumocytis carinii*, toxoplasmose cerebral, citomegalovírus associado a colite, molusco contagioso, psoríase, etc.; nesta fase a síndrome nefrótica e insuficiência renal progressiva está associada a mortalidade significativa, principalmente em pacientes afrodescendentes. Estando a contagem de linfócitos <100 células/mm^3 o paciente corre o risco de infecções bacterianas e fúngicas disseminadas, citomegalovírus latentes podem reativar-se gerando esofagite e colite, as alterações neurológicas tornam-se comuns (leucocefelopatia multifocal progressiva, linfoma primário do sistema nervoso central e encefalopatia); as variações de câncer mais comuns incluem sarcoma de Kaposi, linfoma não-Hodgkin de células B e outros localizados no ânus, cabeça e pescoço (Hanna et al., 2007; Damtie et al., 2013) (figura 4).

Um grande obstáculo na erradicação da infecção está na capacidade do HIV permanecer em latência nas células infectadas e assim obter persistência, mesmo com o uso da terapia antirretroviral. A latência viral é estabelecida nos linfócitos T $CD4^+$ logo no início da infecção, com cerca de 10^6 células infectadas por paciente; os linfócitos T $CD4^+$ de memória são os reservatórios ideais a longo prazo, visto que mantêm apenas atividades celulares básicas de sobrevivência que não oferecem um suporte para a replicação viral e não induzem resposta imunológica (Chun et al., 1997; Gondois-Rey et al., 2001). Células da linhagem monócito-macrófago abrigam o HIV latente mesmo em terapia prolongada (Lambotte et al., 2000), devido a provável resistência celular aos fármacos inibidores de protease viral (Kim et al., 1998). Outros reservatórios incluem células do sistema nervoso central, mastócitos e células *natural killer* (Alexaki et al., 2008).

No entanto, na progressão para AIDS subgrupos de infectados exibem padrões diferentes de evolução da doença. Os ditos não progressores de longo tempo permanecem assintomáticos, sem terapia antirretroviral, com elevada contagem de linfócitos T $CD4^+$, mas com permanente detecção viral, no período de 7 a 10 anos de infecção (Madec et al., 2009;

Ballana et al., 2012). Dentre os não progressores, os controladores de elite são capazes de suprimir a replicação viral na ausência de terapia por mais de 12 meses e com pelo menos três coletas indetectáveis para o vírus. Os controladores virêmicos, por outro lado, apresentam carga viral entre 1.000 e 2.000 cópias/mm^3, nestes há uma diminuição da contagem de linfócitos T CD4$^+$ quando comparado aos controladores de elite (Okulicz et al., 2009). Alguns fatores intrínsecos ao hospedeiro foram apontados como elementos que contribuem com a estabilidade da infecção nos controladores, dentre eles: a regulação positiva da quinase celular p21; alelos do HLA; o polimorfismo de deleção Δ32 de CCR5 e a suscetibilidade a coinfecção pelo GB vírus C (Sabin e Lundgren, 2013).

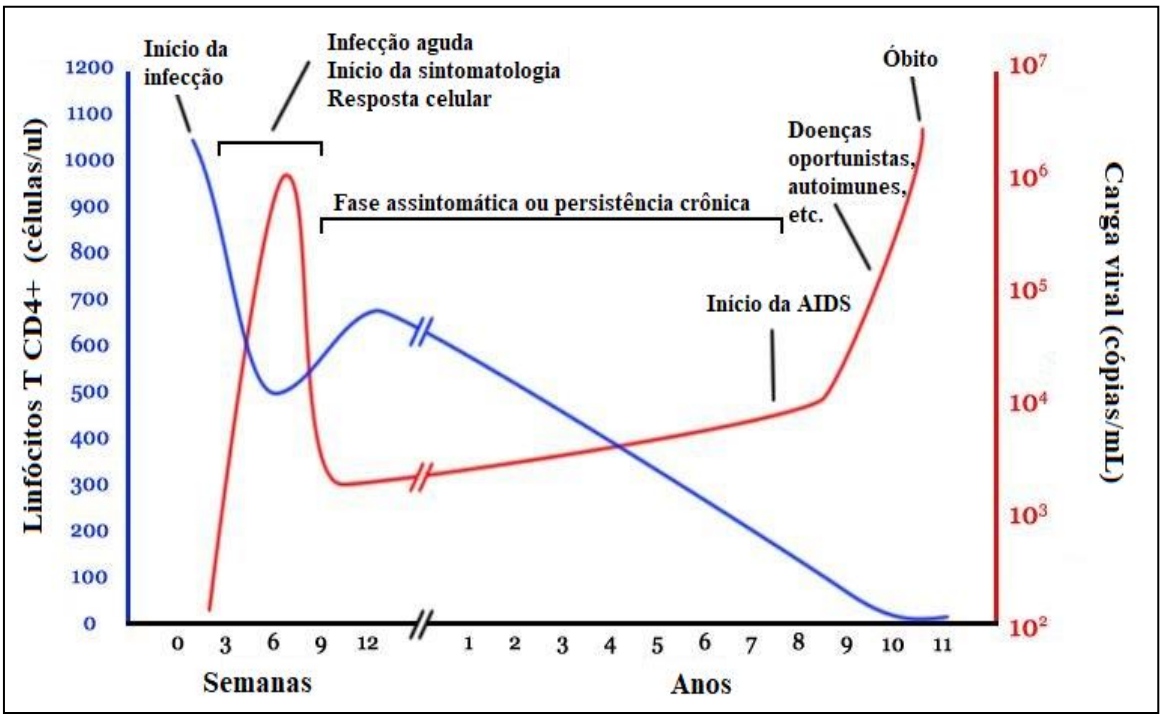


Figura 4 – História natural da infecção pelo HIV, sem o uso de terapia: As múltiplas fases da progressão da infecção envolvem a dinâmica da relação linfócitos T CD4$^+$ e carga viral plasmática. A sintomatologia clínica também varia ao longo do tempo, sendo as doenças oportunistas, nefropatias, autoimunes, no sistema nervoso e cânceres indicadores da severidade sindrômica. Adaptado de Popoola e Awodele, 2016.

1.1.5 Epidemiologia da infecção

A epidemia do HIV vem mudando desde os primeiros casos relatados em meados dos anos de 1980, passando por altas estimativas de 3,7 milhões de novas infecções observadas nos anos 1990, ao declínio do número de novos casos ao longo dos anos 2000. A expansão da cobertura antirretroviral aumentou a sobrevida das pessoas vivendo com HIV e reduziu a taxa

de morte relacionada a AIDS em países subdesenvolvidos entre os anos de 1995 a 2012 (Fettig et al., 2014). No entanto, atualmente as discussões sobre a epidemiologia do HIV surgem com vigor devido a nova pandemia do coronavírus; é provável que as pessoas vivendo com o HIV (PVHIV) sejam especialmente vulneráveis aos efeitos diretos e indiretos da pandemia. Ainda se discute quais os efeitos da coinfecção para os PVHIV e quais os impactos das medidas de controle nos resultados de saúde desse grupo (Lesko & Bengtson, 2021).

No final de 2020, cerca de 37.7 milhões de pessoas viviam com o HIV no mundo, cerca de 0.7% dos adultos. O continente africano continuava a ser gravemente afetado, com cerca de 1 a cada 25 adultos infectados (2/3 da população mundial infectada), porém, o declínio global de mortes tem tido progressos na África Subsaariana, Oriental e Meridional, reflexo da rápida expansão do tratamento na região (UNAIDS, 2018; WHO, 2020) (figura 5).

Estimativas apontam para uma redução no pico de novas infecções, sem distinção de idade, de 3,4 milhões em 1996 para 1,8 milhões em 2017. Novamente a África Subsaariana teve reduções mais acentuadas, com cerca de 30% de declínio, principalmente nas regiões leste e sul; na América Latina a tendência permaneceu estável (declínio de 1%); porém, no Oriente Médio, Norte da África, Europa Oriental e Ásia Central, o número anual de novas infecções dobrou em menos de 20 anos. Nas mulheres há uma porcentagem desproporcional de novos casos entre indivíduos com 15 anos ou mais, apenas na África, elas representavam cerca de 59% dos novos casos em 2017 (UNAIDS, 2018; UNAIDS, 2019).

Em 2020, profissionais do sexo, homens que fazem sexo com homens, usuárias de drogas e pessoas trans com seus parceiros sexuais somaram cerca de 65% das infecções por HIV em todo o mundo. Com cerca de 93% das novas infecções fora da África subsaariana e 39% das novas infecções na África subsaariana (UNAIDS, 2021).

No Brasil, a epidemia do HIV é classificada como estável em nível nacional, com prevalência geral de 0,6%. No entanto, a mesma varia geograficamente, com níveis mais altos no sul e sudeste do país (UNAIDS, 2017). Entre os anos de 2007 e 2021, foram notificados 381.793 casos de HIV e, em 2020, foram diagnosticados 32.701 novos casos. Os casos de AIDS em 2020 chegaram a 29.917 notificações com uma taxa de detecção de 14,1/100 mil habitantes, totalizando, no período de 1980 a junho de 2021, 1.045.355 casos detectados no país. Desde o ano de 2012, observa-se uma diminuição na taxa de detecção de aids no Brasil, que passou de 22,0/100 mil habitantes (2012) para 14,1/100 mil habitantes em 2020, configurando um decréscimo de 35,7%. (Brasil, 2021).

Cinco estados brasileiros apresentaram aumento nas taxas de detecção, foram eles: Sergipe (23,1%), Alagoas (18,5%), Rio Grande do Norte (9,7%), Amapá (1,7%) e Paraíba

(1,5%). Em 2020 os estados do Amazonas, Rio Grande do Sul e Roraima apresentaram as maiores taxas, com 28,7, 21,8 e 21,2 casos por 100 mil habitantes, respectivamente. O Acre foi o estado com a menor taxa, com 5,6 casos/100 mil habitantes. Entre as capitais, apenas Rio Branco e Brasília tiveram taxas inferiores à nacional: 9,7 e 12,4 casos/100 mil habitantes, respectivamente (figura 6).

Estudos realizados na região Amazônica brasileira mostraram um significativo aumento no número absoluto de casos entre os anos de 2001 e 2012, cerca de 10,34 novos casos em 2001 para 21,8 em 2012; a taxa de incidência em homens dobrou em comparação às mulheres ao longo do tempo; e nas populações indígenas permaneceu baixa e estável em cerca de 0,11%, embora o isolamento geográfico das populações tenha dificultado o controle dos dados (Oliveira et al., 2015). Em uma análise retrospectiva de bancos de sangue no mesmo período, a prevalência de soropositivos foi maior em homens juvenis (16 a 20 anos) e candidatos a doação voluntária pela primeira vez (Viga-Yurtsever et al., 2015). Entre os anos de 2010 e 2015 a prevalência do HIV foi elevada também entre candidatos a doação de sangue atendidos em unidades que adotam o voto de auto exclusão (Souza et al., 2019).

Em outros estudos permanece a prevalência do número de casos em homens (Miranda et al., 2017), porém, alerta-se para o progresso na taxa de mulheres infectadas, principalmente gestantes e de baixa escolaridade (Machado et al., 2017). A frequência no número de casos de usuários de drogas não injetáveis (Gomes et al., 2017; Machado et al., 2017) desperta a vigilância epidemiológica para a região amazônica, pois, embora não exponha a transmissão parenteral, pode promover outras variáveis de risco, principalmente sexuais, como demostrado em outras regiões brasileiras (Miranda et al., 2014).

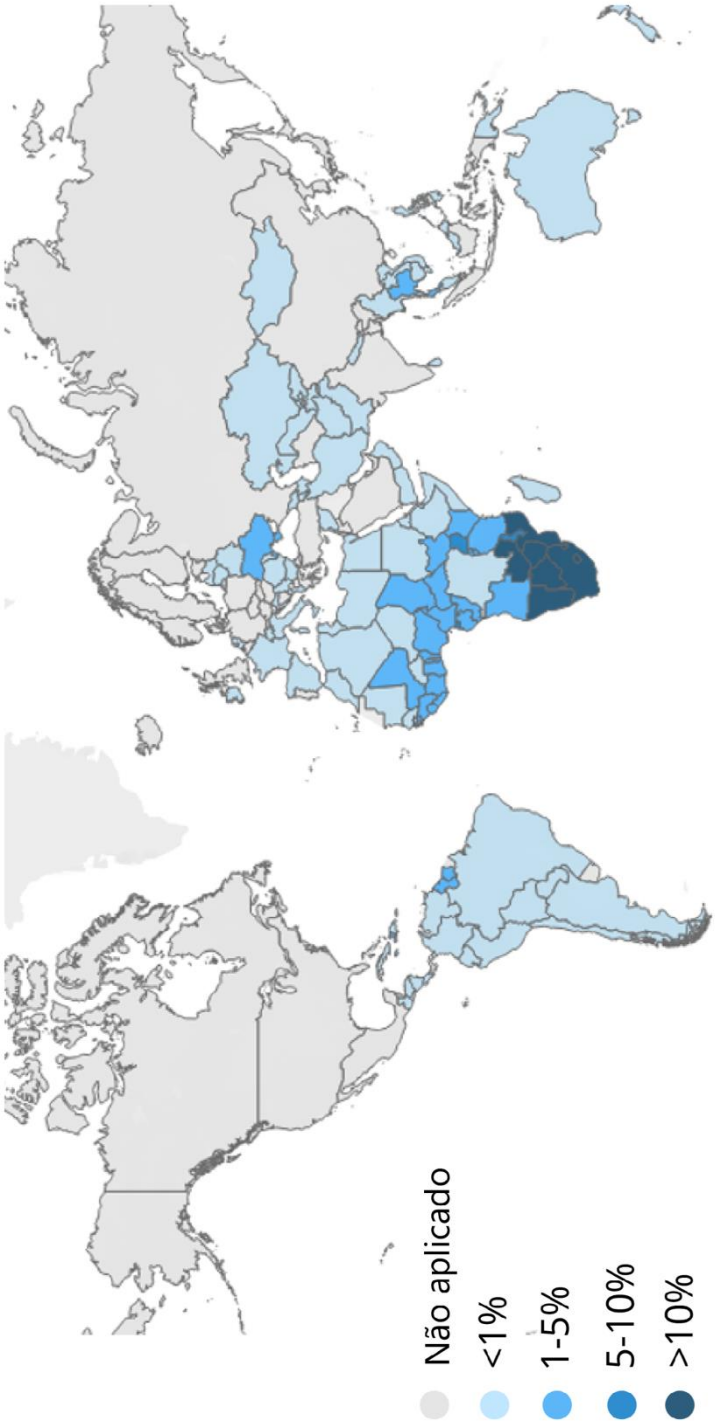


Figura 5 - Prevalência global do HIV em 2020. Adaptado de Kaiser Family Fundaction (https://www.kff.org/global-health-policy/fact-sheet/the-global-hivaids-epidemic/).

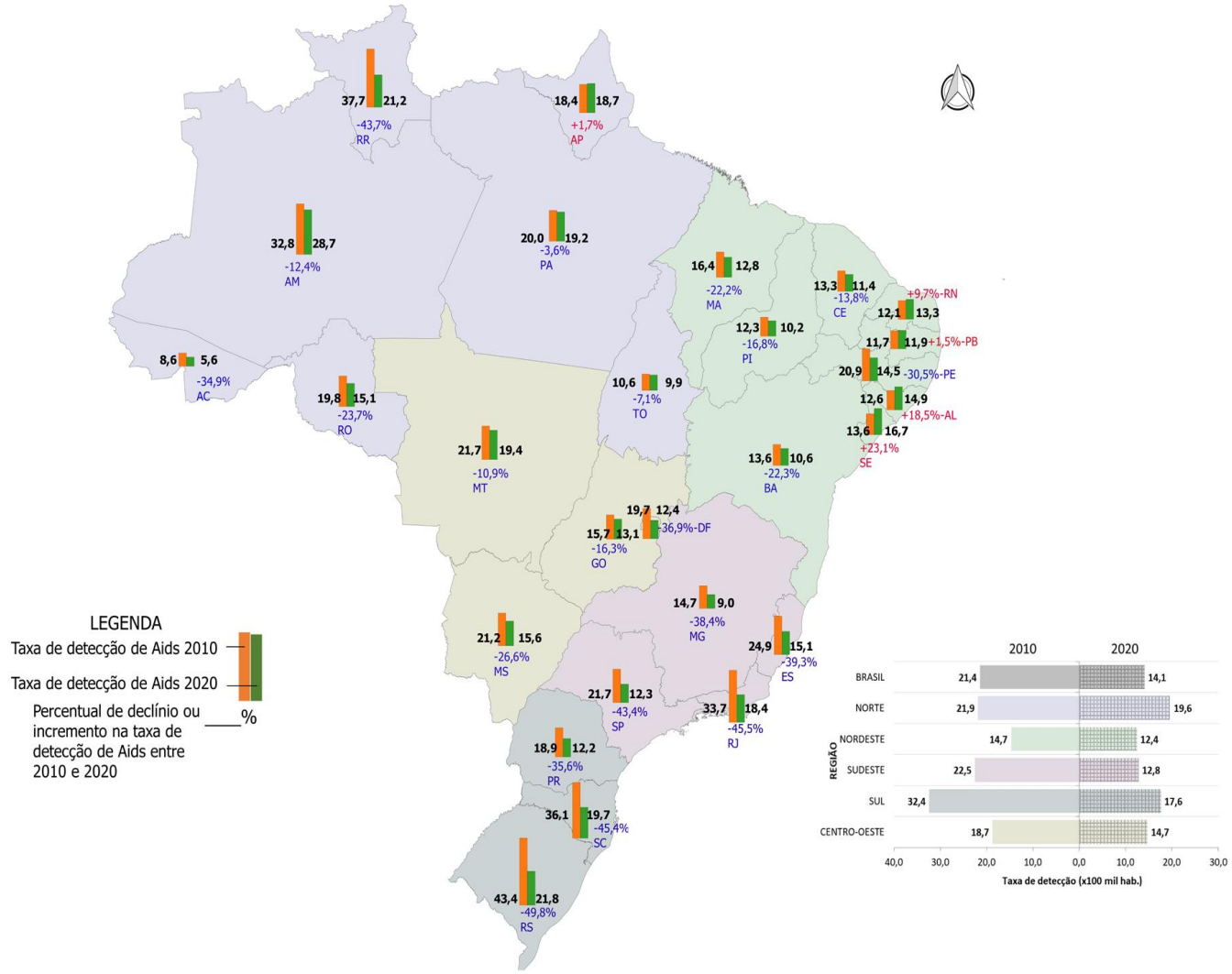


Figura 6 - Taxa de detecção de AIDS (por 100.000 hab.) e percentual de declínio ou incremento, segundo UF de residência, por ano de diagnóstico no Brasil, 2010 a 2020.
Adaptado de Brasil, 2021

1.1.6 Diversidade e epidemiologia molecular

A diversidade genética do HIV se deve a fatores intrínsecos relacionados a inatividade de revisão da transcriptase reversa (Perelson et al., 1996), e a capacidade de recombinação de diferentes cepas virais quando coinfectam uma mesma célula hospedeira (Blackard et al., 2002). A emergência da resistência antirretroviral também reflete na diversidade do HIV, vista a aquisição de cepas resistentes tanto em infecções recentes (resistência primária), quanto após um período de tratamento (resistência secundaria, ou adquirida) (Taylor et al., 2008).

O HIV-1 é subdivido nos grupos M (*major*), O (*outlier*), N (*non-M, non-O*) e P (Costa et al., 2015). O grupo M é o mais prevalente em escala mundial. Divide-se nos subtipos A (1-4), B, C, D, F (1-2), G, H, J e K, caracterizados por similar distância genética entre si e, em alguns casos, relações geográficas e epidemiológicas. Formas recombinantes agrupam mosaicos genéticos intersubtipos que podem estar distribuídos em pelo menos três indivíduos sem ligação epidemiológica direta (forma recombinante circulante – CRF), ou isolada apenas em um hospedeiro (forma recombinante única – URF) (Hemelaar et al., 2006). São conhecidas mais de 40 CRFs, sendo os subtipos E e I, que outrora foram ditos "puros", reclassificados nas formas CRF01_AE e CRF06_cpx, respectivamente (Gao et al., 2001). Recentemente, o subtipo L foi proposto como uma nova variante do grupo M a partir de amostras coletadas na República Democrática do Congo (Yamaguchi *et al*., 2020).

O subtipo C predomina na África subsaariana, países do Leste da África e Índia. O subtipo A prevalece na África Central, no Irã e na Europa Ocidental, sendo a variante A1 a mais prevalente dentre as demais. O subtipo B é o mais disseminado, predominando nos EUA, na Europa Ocidental, no Japão e na Austrália. Estudos recentes destacam o aumento da prevalência de formas não-B na Europa Ocidental e Central; do subtipo B na Líbia; e a diminuição da prevalência do subtipo B na transmissão sexual entre chineses. As formas recombinantes CRF01_AE e CRF14_BG têm impacto nas epidemias do sudeste asiático e continuam em expansão na Europa Meridional, respectivamente. Há uma emergência das formas BF na América do Sul e da forma CRF35_AD entre usuários de drogas do Afeganistão e do Irã (Delgado et al., 2002; Thomson et al., 2004; Kijak et al., 2007; Sanders-Buell et al., 2007; Santos e Soares, 2010; Beloukas et al., 2016; Yuan et al., 2016; Daw et al., 2017) (figura 7).

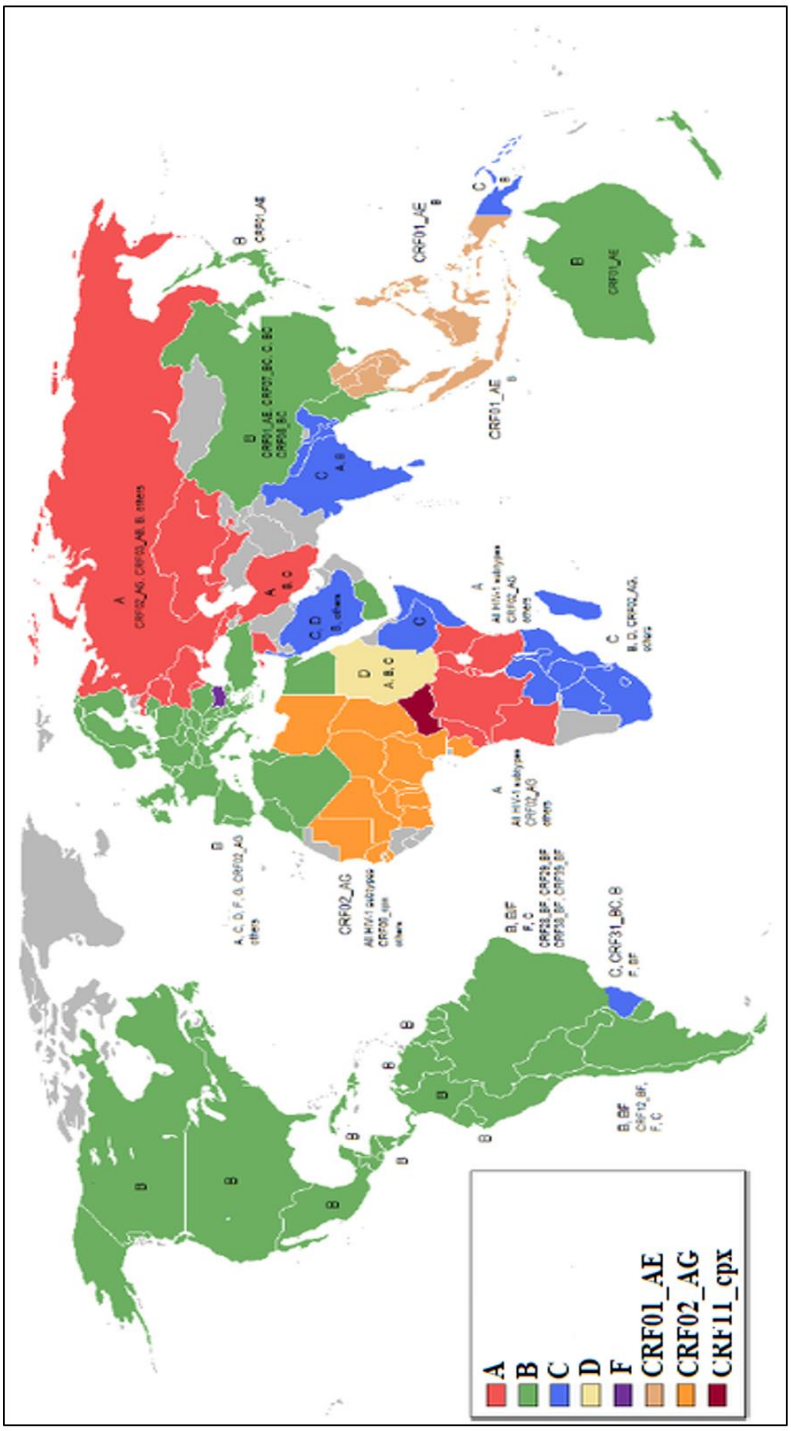


Figura 7- Prevalência mundial dos subtipos HIV-1 do grupo M: O subtipo B predomina nas Américas e o subtipo A na Eurásia. O continente africano é um mosaico de subtipos e formas recombinantes. Adaptado de Santos e Soares, 2010.

O grupo O está distribuído na África Ocidental e Central. Os grupos N e P permanecem raros, com a circulação restrita a Camarões na África (Brennan et al., 2008; Plantier et al., 2009).

No Brasil, o subtipo B e as formas recombinantes F1 e BF1 prevalecem na maioria das regiões do país (Guimarães et al., 2015). Dados recentes mostram a cocirculação das variantes

B, F1, B’ (brasileira) e BF1; o aumento na detecção do subtipo C; a detecção ocasional do subtipo D na região Sudeste; estando as variantes D e BF1 diretamente associadas a progressão à AIDS (Leite et al., 2017). No Centro-oeste, o subtipo B permanece com a maior prevalência, seguido da forma recombinante CRF60_BC, e o subtipo D representando cerca de 1% da epidemiologia regional (Tanaka et al., 2019). Destaca-se o aumento da prevalência do subtipo C e das formas recombinantes CB na região Sul do Brasil (Librelotto et al., 2015) e de formas não-B no Nordeste (Delatorre et al., 2017). Na região amazônica brasileira, houve um impulso epidemiológico orquestrado pelos subtipos B e F (Vicente et al., 2000), algo que foi observado em comunidades tradicionais da região (Machado et al., 2006). A hipótese de uma rota epidemiológica Sudeste – Norte foi ratificada pela identificação do subtipo D e de formas recombinantes recentes (CRF02_AG) no Pará (Machado et al., 2009).

Nove linhagens distintas do HIV-2 foram identificadas (A-I). Dentre estes, os subtipos A e B são frequentes, os demais foram observados em um único paciente, e o subtipo F ainda se subdividi nas cepas F1 e F2, encontradas em dois pacientes distintos (Ayouba et al., 2013). Diferente da explosiva diversidade do HIV-1, apenas duas formas recombinantes foram identificadas para o HIV-2: a variante circulante CF01-AB e a variante única 7312A do grupo B. A baixa virulência e transmissibilidade do HIV-2 são fatores sugeridos para o diminuto número de eventos de recombinação neste vírus (Visseaux et al., 2016) (figura 8).

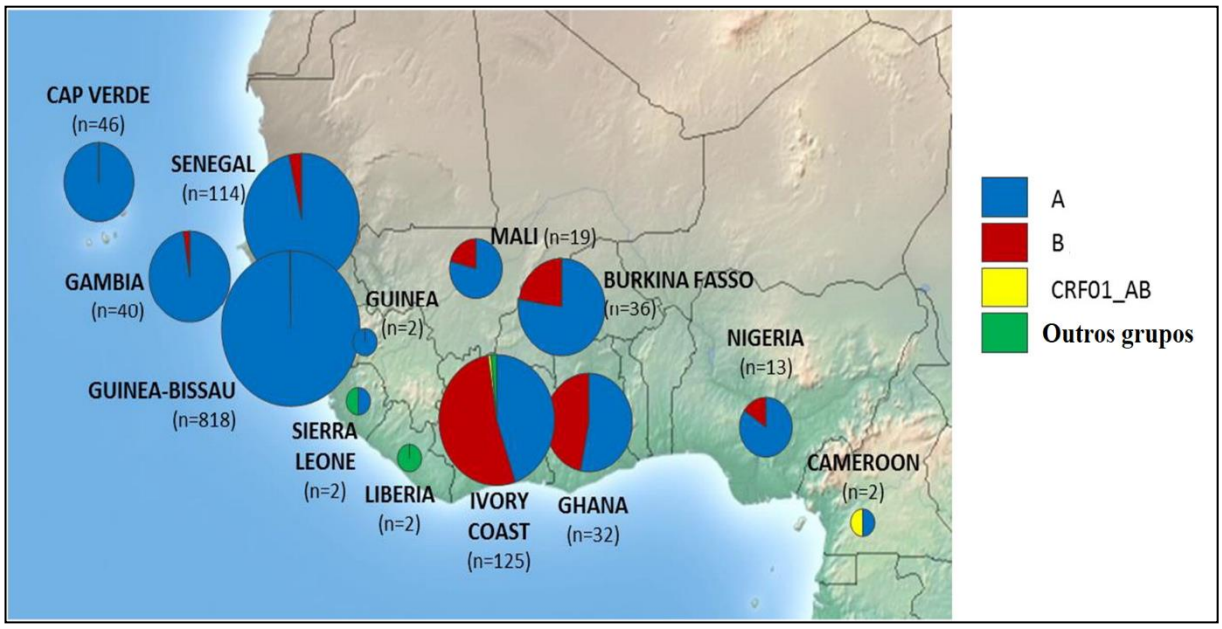


Figura 8 - Distribuição de grupos HIV-2 na África Ocidental. Adaptado de Visseaux et al., 2016.

1.2 ASPECTOS DA COINFECÇÃO HIV/EBV

O espectro de doenças associadas à infecção pelo HIV se deve a progressivos eventos de disfunção e ativação imunológica, como a destruição dos linfócitos T CD4+ e a ativação de células mononucleares causadoras de dano tecidual (Lucas & Nelson, 2015). Tal fato torna-se crítico quando a infecção evolui para o quadro de AIDS, no qual a severa imunossupressão no hospedeiro o deixa a mercê de sintomas e sinais clínicos referentes a neoplasias malignas (Friis et al., 2012). Nesse contexto, a coinfecção pelo EBV é apontada como um dos fatores que contribui com a morbidade dos pacientes infectados com HIV, por ser um precursor de neoplasias linfáticas, como o linfoma de Burkitt (Guarner et al., 1991; Santilli et al., 2010), linfoma de efusão primária (Lacoste et al., 2000), linfoma difuso de grandes células B (Chao et al., 2012), dentre outros.

Estudos mostram o isolamento do DNA do EBV na mucosa oral (Grando et al., 2005) e no tecido sanguíneo de crianças portadoras do HIV (Slyker et al.,2013; Petrara et al., 2014), neste sentido, supõem-se que a alta incidência da infecção pelo EBV durante os cinco primeiros anos de vida dos pacientes, observada em coortes africanas, sugere que a transmissão materna seja uma via importante na manutenção da coinfecção (Slyker et al.,2013). Sendo a translocação microbiana e o estado de ativação imunológica persistente, induzidos pelo HIV, fatores que podem influenciar à replicação do EBV e a expansão de células B infectadas (Petrara et al., 2014).

Em estudos com adultos, possíveis diferenças na infecção pelos subtipos 1 e 2 do EBV são observadas entre portadores e não portadores do HIV. Pacientes imunocompetentes usualmente apresentam infecção por apenas um genótipo viral, enquanto ambos os genótipos podem coexistir em pacientes imunocomprometidos (van Baarle et al., 1999; 2000).

As alterações no perfil imunológico do hospedeiro, induzidas pelo HIV, podem favorecer a expressão de genes do EBV correlacionados a suscetibilidade a lesões, como as tipicamente observadas na leucoplasia pilosa na cavidade oral (Santos et al., 2014), tal como a suscetibilidade a outros agentes infecciosos, como a tríplice infecção de HIV, EBV e papiloma vírus (HPV), na qual a presença do EBV foi associada a prevalência do HPV na mucosa anal desses pacientes (Gianella et al., 2016).

Porém, o tratamento com a terapia antirretroviral foi associado a diminuição da viremia tanto do HIV, quanto do EBV, e ao aumento dos níveis de linfócitos TCD8+ circulantes nos

pacientes (Giron et al., 2013). Em pacientes coinfectados e moderadamente imunossuprimidos, a terapia levou ao declínio da carga do EBV, enquanto que os indivíduos com resposta não ideal, não restauraram o controle do EBV. Para os autores, a supressão duradoura da replicação do HIV é um pré-requisito para restabelecer o controle imunológico do EBV (Friis et al., 2010).

Paralelamente, resultados promissores forma obtidos no tratamento de pacientes com linfoma não-Hodgkin coinfectados com HIV; o regime quimioterápico EPOCH alcançou a remissão em 22 dos 24 pacientes com sobrevida livre de progressão de 23 meses, todos com fatores prognósticos favoráveis (Harrington, 2005).

1.3 O VÍRUS ESPTEIN-BARR (EBV)

1.3.1 Aspectos históricos e origem do EBV

Em meados de 1957, emergia na África Subsaariana casos de uma síndrome de linfoma, caracterizados pela presença de tumores de crescimento rápido na mandíbula, abdômen, osso, coluna vertebral e, raramente, nas glândulas salivares, que ficou conhecida como Linfoma de Burkitt (LB) (Burkitt e O'Conor, 1961). Os pesquisadores Denis Burkitt e Gregory O'Conor, não apenas descreveram a doença, como notaram que a sua distribuição formava um "cinturão epidemiológico" ao longo de regiões tropicais quentes e úmidas da África Central, como também observado na epidemiologia da malária. Não obstante, havia semelhanças na apresentação clínica do LB com a leucemia linfocítica bovina, uma doença cuja a etiologia era viral (O'Conor, 1961). Dessa forma, os indícios apontavam para uma provável origem viral do LB e vetorização por artrópodes infectados (Burkitt, 1969).

Em 1961, Burkitt realizava uma palestra em Londres sobre seus achados quando conheceu o patologista Anthony Epstein, quem lhe convencera a enviar amostras dos tumores extraídos para análises laboratoriais. Epstein tinha experiências com o vírus do sarcoma de Rous (RSV) (causador de tumores em galináceos), além de ter desenvolvido estudos em microscopia eletrônica, o que lhe tornou receptivo a hipótese de oncongenia viral proposta por Burkitt (Epstein, 1999). Apenas em 1963, a partir de uma linhagem celular viável de LB, a equipe de Epstein conseguiu observar partículas virais nas células cultivadas; os vírus tinham características morfológicas claramente compartilhadas entre os herpesvírus humanos, porém, sem induzir uma infecção lítica, foi denominado como vírus Epstein-Barr (EBV) (Epstein et al., 1964).

Pesquisas subsequentes idealizadas por Werner e Gertrude Henle foram necessárias à conclusão da hipótese do novo herpesvírus estar associado aos casos de linfoma. A equipe dos pesquisadores demonstrou a potencial capacidade de transformação induzida pelo EBV em co-cultivo de células B infectadas com células B saudáveis (Henle et al., 1967), e o isolamento do material genético viral em biópsias de LB (ZurHausen et al., 1970). Um estudo epidemiológico publicado em 1978 foi categórico ao mostrar que crianças africanas que tiveram contato prévio e prolongado com o EBV desenvolveram LB (Thé et al., 1978).

Um novo avanço sucedeu casualmente, também na equipe dos Henles, quando um colaborador desenvolveu um quadro febril intenso, seguido de outros sintomas, típico de mononucleose infecciosa, cujos testes sorológicos positivaram para o EBV após o estabelecimento da doença. A correlação entre o EBV e a mononucleose foi confirmada em um estudo sorológico com amostras de pacientes coletadas antes e após a doença (Henle et al., 1968).

No cenário científico da época tinham-se provas suficientes que comprovavam o EBV como um oncovírus humano capaz de causar doenças clinicamente distintas. O reconhecimento da via de transmissão se deu por estudos que isolaram o vírus em amostras de saliva de pacientes com mononucleose e que observaram a replicação viral em células epiteliais orais, sugerindo a transmissão oral como a principal rota de infecção do EBV (Niederman et al., 1976; Greenspan et al., 1985). Esses achados refutaram a hipótese inicial de Burkitt (Burkitt, 1969), porém, as relações instigadas entre a distribuição do LB e da malária proporcionaram associações conseguintes entre as doenças (Young et al., 2016).

O gatilho para o isolamento de herpesvírus similares ao EBV em primatas não humanos se deu pela observação de padrões bioquímicos e sorológicos equivalentes com as amostras de pacientes soropositivos para o EBV (Landon et al., 1968; Bocker et al., 1980). Análises moleculares posteriores revelaram a presença de microRNAs conservados entre as cepas virais que datavam mais de 13 milhões de anos de divergência evolutiva, provavelmente devido à importância dessas moléculas no processo de replicação do grupo (Cai et al., 2006). Tratava-se de uma linhagem de linfocriptovírus de primatas não humanos ortólogos ao EBV e que possuíam estruturas genômicas colineares e proteínas homólogas significativas em diferentes estágios da infecção, porém, com singularidades na fisiopatologia e história natural da infecção entre os diferentes hospedeiros (Cho et al., 2001; Mühe e Wang, 2015).

O estudo filogenético da família *Herpesviridae* interpreta o desenvolvimento das linhagens virais como sendo um evento sincrônico de coespeciação com os diferentes hospedeiros mamíferos, que para o EBV, considera-se estreitamente relacionado à coevolução

vírus-primatas (McGeoch et al., 2006). A transmissão horizontal, geralmente por manuseio de carne contaminada, é pensada como rota de transmissão interespecífica entre os hominídeos, provavelmente ocorrido em uma era remota do desenvolvimento evolutivo dos grandes símios (Ehlers et al., 2010).

1.3.2 Classificação e características biológicas

O vírus Epstein-Barr, também conhecido como Gamaherpesvírus humano 4 (*Human gammaherpesvirus 4* – HHV4), pertence a ordem *Herpesvirales*, família *Herpesviridae*, subfamília *Gammaherpesvirinae* e gênero *Lymphocryptovirus* (ICTV, 2019).

A partícula viral é formada por um capsídeo icosaédrico composto por 162 capsômeros, envolto por um envelope lipoprotéico externo, sobre o qual, estão dispostas as espiculas glicoproteicas de superfície: gp350, gH, gB, gp42, gM, gp78, gN, gp150 e gL; preenche o espaço entre o capsídeo e o envelope um tegumento viral de proteínas amorfas (Santos e Silva, 2015; Smatti et al., 2018) (figura 9). A estrutura tridimensional do capsídeo dos herpesvírus é similar entre si devido ao número de triangulações determinadas pela disposição das proteínas MCP (*Major Capsid Protein*), a presença da proteína SCP (*Small Capsomere-interacting Protein*) nos hexômeros, e a presença de complexos heterotriméricos nas junções a cada três capsômeros (Germi et al., 2012).

O genoma do EBV é formado por uma molécula de DNA linear de fita dupla com cerca de 172 kpb que codifica mais de 85 genes. Domínios de sequências repetidas dividem o genoma viral de modo a favorecer a codificação gênica, são elas: repetições diretas nas terminações 3’ e 5’ (Tr); repetições internas (IR1); domínios de sequencias únicas curtas (U_S) e longas (U_L); e regiões conservadas ricas em guanina e citosina (G/C), com 125 pb (IR2) e 102 pb (IR4), localizadas no quadro de leitura aberta (ORF) correspondente a origem da replicação viral (Arvin et al., 2007; Santos e Silva, 2015). A nomenclatura das ORFs do EBV foi estabelecida segundo um mapa de fragmentos de restrição BamHI, onde os fragmentos encontrados foram ordenados decrescentemente de A à Z com base em seus tamanhos, ou com base na aplicação dos mesmo na biossíntese viral (Bouvard et al., 2009) (figura 10).

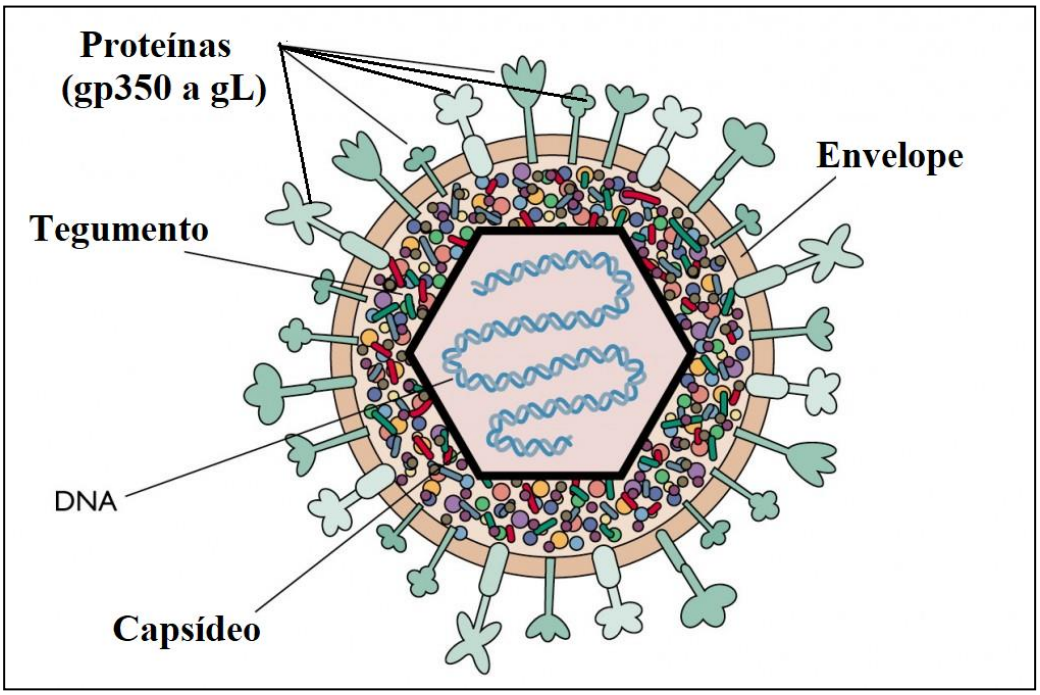


Figura 9 – Modelo geral de partícula viral dos herpsevírus. Adaptado de Smith, 2014 (https://ultimate-herpes-protocol.weebly.com/blog/archives/10-2014).

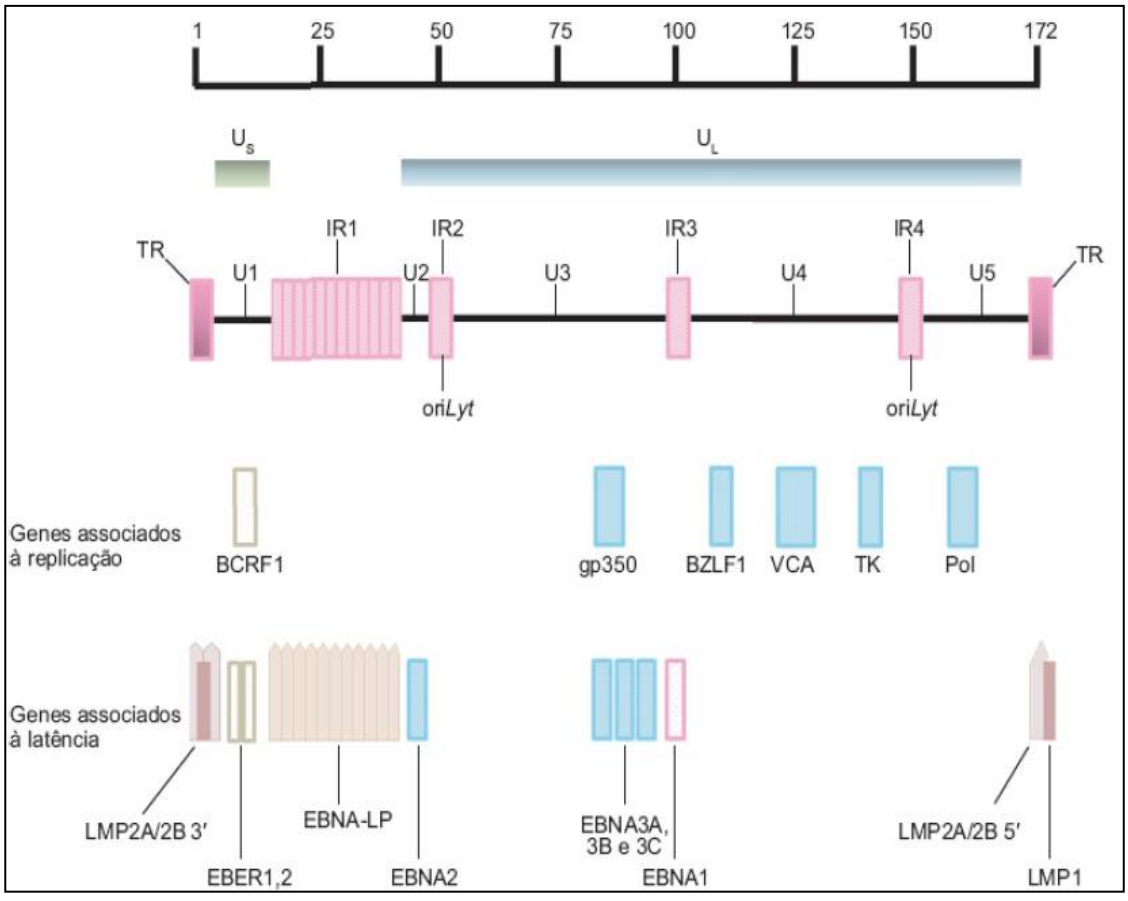


Figura 10 - Estrutura genômica do EBV. O genoma consiste em uma molécula de DNA de fita dupla de aproximadamente 172 kpb com sequências únicas, curta (US) e longa (UL), organizadas em séries de domínios únicos (U1 a U5); repetições internas (IR1 a IR4) e regiões terminais (TR). Adaptado de Santos e Silva, 2015.

As proteínas do EBV estão, em sua maioria, relacionadas a manutenção da replicação viral e montagem da partícula. Não obstante, o genoma também consiste de genes latentes que não são traduzidos durante a fase lítica, tal como genes de RNA associados à latência expressos neste período (Yates et al., 1984).

O antígeno nuclear do tipo 1 (EBNA-1) é expresso em ambas fases de infecção, sendo essencial à persistência nas células do hospedeiro por exercer os papeis de transtivador gênico e replicação episomal do genoma viral (Yates et al., 1985; Canaan et al., 2009). As proteínas EBNA-2 e EBNA-LP cooperativamente participam da transcrição gênica viral e transformação celular (Portal et al., 2011). Membros da família EBNA-3, que incluem EBNA-3A, EBNA-3B e EBNA-3C, possuem uma estrutura gênica similar e participam como coativadores de EBNA-2 na regulação da expressão de genes de latência (Robertson et al., 1996).

A proteína membranar de latência do tipo 1 (LMP-1) altera a sinalização das células B por mimetizar o receptor CD40 e ativa as vias NF-kβ, quinase JNK e p38 na transformação oncogênica (Yang et al., 2004). A LMP-2A pode bloquear a sinalização do receptor de células B por vias dependentes de ERK/MAPK, tal como, favorece o desenvolvimento de linfomas em ensaios *in vitro* e *in vivo* (Mancao e Hammerschimidt, 2007; Fukuda e Kawaguchi, 2014).

Pequenas moléculas de RNA não codificantes e não poliadenilados de EBV (EBER-1 e EBER-2) são transcritos pela RNA polimerase do tipo III na latência viral e atuam como indutores tumorigênicos, competem resistência a apoptose por inibição da cinase PKR e modulam positivamente a expressão das citocinas IL-10, IL-6 e IL-9 (Kang e Kieff, 2015). Durante a latência também são expressos micro RNAs oriundos dos fragmentos BamHI, BART e BARF1 do EBV, que dentre a amplitude de funções estão: transformação e crescimento progressivo da célula; evasão da resposta imunológica (Cai et al., 2006; Seto et al., 2010).

Os gamaherpesvírus possuem componentes moleculares homólogos as proteínas da família BCL-2, responsável pela regulação da apoptose celular. A proteína BHRF1 inibe subconjuntos de proteínas pró-apoptóticas do hospedeiro, contribuindo na infecciosidade viral e linfomagênesis. De outro modo, a proteína BALF1 regula a atividade antiapoptótica de BHRF1 em diferentes linhagens celulares, similar ao *feedback* negativo observado entre outros membros da família BCL-2 (Bellows et al., 2002; Kvansakul et al., 2010).

A mudança do estado de latência viral para uma fase replicativa (lítica) é orquestrada pelo fator de transcrição inicial imediato do EBV (ZEBRA), também conhecido como BZLF1, uma proteína de 245 aminoácidos, pertencente à família dos fatores de transcrição bZIP, cuja

função é ativar promotores de gene líticos virais por interação em regiões genômicas específicas (ZREs) e regular negativamente a expressão de genes ligados a latência ou resposta imune celular (Kenney et al., 1989; Petosa et al., 2006). A proteína BRLF1 em semelhança atua como ativador transcricional imediato de genes líticos iniciais do EBV; independente de qual fator imediato seja primeiramente ativado, um requer a expressão do outro, e ambos são necessários para a ativação completa dos genes precoces (Zalani et al., 1996; Adamson et al., 2000).

A evasão imunológica similarmente é condicionada a proteína BNLF2a, que, por bloquear a formação heterodimérica de moléculas transportadoras de antígenos (TAP) ao longo do processamento destes, interfere diretamente na via de apresentação de antígenos dependente de MHC-I (Wycisk et al., 2011). A proteína BCRF1 atua tanto como homologa a citocina IL-10, e desta forma atenua a resposta imunológica do hospedeiro (Niiroet al., 1992), quanto como transativador de genes tardios da replicação viral (Gruffat et al., 2012).

Dois genótipos do EBV são conhecidos, EBV-1 e EBV-2, cujas diferenças genéticas são observadas na sequência nucleotídica dos genes EBNA-2 e membros da família EBNA-3 (Sample et al., 1990).

1.3.3 Replicação do EBV

O clássico tropismo do EBV pelos linfócitos B se deve a interação predominante e de alta afinidade das glicoproteínas de envelope gp350/220 com o receptor de complemento tipo 2 (CR2 ou CD21) na superfície celular. Repetições de consenso curtas (SCR) em CR2 auxiliam na aproximação do vírus à membrana celular e desencadeia a endocitose viral. Sinalizações subsequentes tem consequências nos eventos a jusante (Tanner et al., 1987; Martin et al., 1991) (figura 11).

A via de CR2 é menos eficaz na adsorção viral em linhagens epiteliais, devido à baixa expressão do receptor e de moléculas coestimuladoras (CD19 e CD35) *in vivo* (Fingeroth et al., 1999). Nessas células, foi proposto outros mecanismos de entrada do vírus, independente de CR2: Na mucosa, o complexo antígeno-anticorpo formado entre a imunoglobulina A (IgA) e gp350/220, pode facilitar a fixação do vírus sobre a superfície basolateral da célula epitelial via receptor polimérico de IgA (Sixbey e Yao, 1992). A unidade proteica composta pelas glicoproteínas covalentemente ligadas gH e gL podem interagir com um receptor ainda não identificado na membrana das células epiteliais, convencionalmente definido como gHgLR (Molesworth et al., 2000). A proteína de superfície codificada pelo gene BMRF2 pode interagir

com integrinas da membrana celular (dentre elas: β1, α5, α3 e αv), principalmente na infecção através da superfície basolateral (Tugizov et al., 2003; Xiao et al., 2007) (figura 11).

Nas células B, o processo de fusão à membrana celular é desencadeado pela glicoproteína gp42 e auxiliada pelo complexo gHgL e proteína gB. A gp42 se liga às moléculas de MHC-II, seguida pela internalização e liberação do nucleocapsídeo no citoplasma, mediante a formação de bolsas hidrofóbicas que acomodam proteínas virais e celulares úteis no processo de entrada do vírus na célula (Mullen et al., 2002). Nas células epiteliais, a fusão ocorre em pH neutro, por fusão direta sem endocitose; sugere-se que o complexo gHgL participe diretamente da fusão em células epiteliais, porém, ainda permanece incerto o papel de gB nesse processo (Borza et al., 2004) (figura 11).

Ainda não estão compreendidos os fenômenos pós-fusão referentes ao desnudamento do nucleocapsídeo e o transporte do genoma viral ao núcleo. Teorias propõem que as pesquisas devem ser voltadas para entender as diferentes rotas de adsorção e grau de permissividade das linhagens celulares suscetíveis (Hutt-Fletcher, 2007; Chesnokova e Hutt-Fletcher, 2014) (figura 11).

Na fase de latência viral, o genoma permanece no núcleo celular em estado episssomal extracromossômico. Os episssomas circulares replicam-se uma vez a cada ciclo celular, de forma bidirecional, por meio da DNA polimerase do hospedeiro, geralmente iniciando a partir de uma região genômica conhecida como *ori*P. Estes episssomas são segregados de maneira uniforme para as células filhas, de tal forma que seja mantido um número de cópias constante na infecção latente (Ott et al., 2011; Frappier, 2012). Um elemento replicador de 65 pb, localizado próximo a origem *ori*P, (elemento DS) é fundamental na replicação do DNA viral quando presente a proteína EBNA1 (Bashaw e Yates, 2001). Uma origem alternativa para a replicação episssomal foi determinada em culturas de células Raji transfectadas com plasmídeos transformados com EBV; trata-se da região "Raji*ori*", acerca de 25 kpb de *ori*P, cuja a síntese de novas moléculas de DNA, embora eficiente, não sobrepuja a replicação mediada por *ori*P e o elemento DS (Wang e Sugden, 2008; Hammerschmidt e Sugden, 2013) (figura 11).

Durante a latência, ocorre a expressão dos genes dos antígenos nucleares (EBNA-1, 2, 3A, 3B, 3C e LP), das proteínas latentes de membrana (LMP-1, LMP-2A e LMP-2B), das moléculas de RNA não-poliadeniladas (EBER-1 e 2) e dos transcritos com múltiplos eventos de *splicing* (BamHI, BART e BARF1) (Ambinder & Mann, 1994; Okano, 2000; zurHausen, 2000). Estas atuam na transativação gênica (Shimakage & Sasagawa, 2001), na transformação celular (Ohga et al., 2002) e resistência à apoptose (Takada &Nanbo, 2001) (figura 11).

É provável que a diferenciação de células B após a estimulação do receptor de células B, ou por estímulos *in vitro* com éster de forbol, 12-O-tetradecanoilforbol-13-acetato (TPA), butirato de sódio e ionóforos, possam induzir a fase lítica do EBV, por ativação da transcrição dos genes BZLF1 e BRLF1, a partir do genoma viral latente (Falk e Ernberg, 1999; Laichalk e Thorley-Lawson, 2005). A citocina TGF-β também pode induzir a infecção lítica em linhagens de linfoma de Burkitt *in vitro*, e poderiam potencialmente reativar o EBV *in vivo* (Adler et al., 2002). Os promotores que conduzem a transcrição de BZLF1 e BRLF1 são inativos em células B na forma latente da infecção, porém, podem ser ativados pelos estímulos líticos citados (Kenney, 2007).

Na fase lítica do EBV, a replicação do DNA se inicia a partir de uma origem gênica diferente, denominada *ori*Lyt, e requer um conjunto de proteínas de replicação conservadas dos herpesvírus e distintas das observadas no ciclo latente, tal como a DNA polimerase viral (Yates, 1996). Os genes deste ciclo expressam proteínas categorizadas em imediatas, iniciais e tardias. Os genes imediatos BZLF1 (ZEBRA) e BRLF1 codificam proteínas com função de ativador transcricional que iniciam uma cascata de eventos determinantes na expressão dos antígenos precoces (com as funções de metabolismo, replicação e bloqueio do processamento de antígenos), seguida pela expressão dos genes tardios [proteínas estruturais de montagem da partícula; e genes relacionados a evasão da resposta imunológica (BCRF1)] (Feederle et al., 2000; Okano, 2000; Gulley, 2001; Santos e Silva, 2015) (figura 11).

A replicação do EBV na fase lítica resulta na amplificação dos genomas virais na forma de concatâmeros longos que são subsequentemente clivados em unidades lineares não metiladas e empacotados nos capsídeos em formação (Gibson & Roizman, 1971; Bloss & Sugden, 1994). Baseado no modelo do herpesvírus simples tipo 1 (HSV-1), a primeira unidade estrutural fechada é denominada procapsídeo, uma estrutura esférica composta pela proteína principal (BCLF1), tríplex proteicos de BORF1 e BDLF1, a proteína "*scaffold*" de direcionamento (BDRF1), a protease viral (BVRF2) e a menor proteína do capsídeo (BFRF3). A maturação do procapsídeo ocorre pela clivagem da proteína BDRF1 pela protease, ao longo do empacotamento do DNA, e o término da montagem pela proteína BFRF3 (Gao et al., 1994; Newcomb et al., 1996, Henson et al., 2009) (figura 11).

O capsídeo formado adquire um envelope primário ao brotar através das membranas nucleares internas do espaço perinuclear. A fusão do envelope primário à membrana nuclear externa transporta a partícula viral para o citoplasma, onde o espaço entre o capsídeo e o envelope é preenchido por uma camada de proteínas tegumentares. Essas proteínas promovem os estágios iniciais da replicação dos herpesvírus, incluindo a transativação dos genes virais

iniciais. O capsídeo revestido por tegumento é envolvido novamente por uma camada lipoproteica à medida que brota nas organelas citoplasmáticas; para o EBV, esse envelope secundário é obtido nas cisternas *cis* do complexo de Golgi. Vesículas contendo partículas maduras são então transportadas para a membrana plasmática, onde se fundem e liberam o vírus no espaço extracelular (Wisner e Johnson, 2004; Sugimoto et al., 2008; Nanbo et al., 2018) (figura 11).

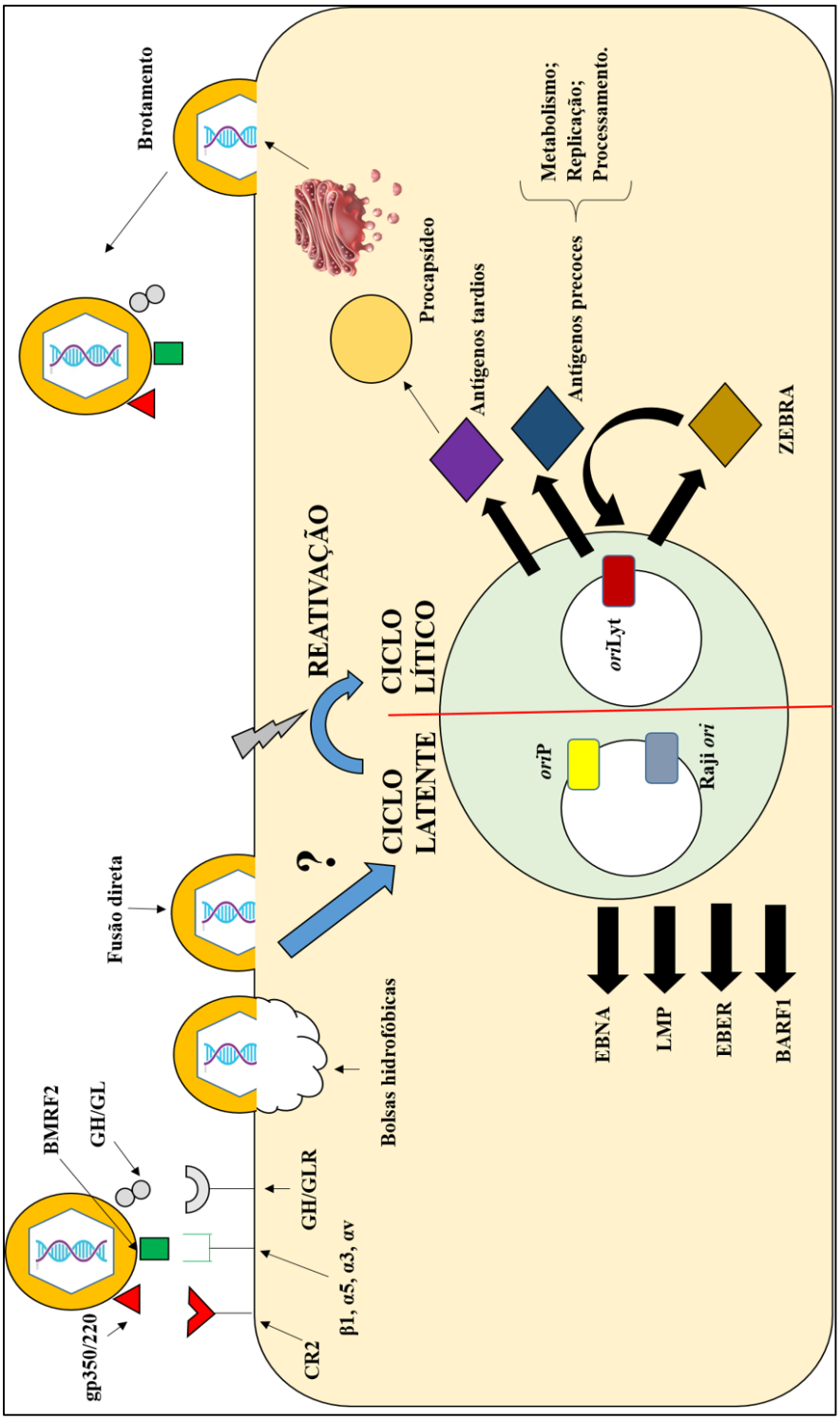


Figura 11 – O ciclo de vida do EBV: A adsorção viral ocorre pela interação das glicoproteínas gp350/220, gh/gl e BMRF2 com receptores e integrinas da superfície celular. A fusão pode ocorrer de forma direta ou através da formação de bolsas hidrofóbicas, dependendo da linhagem infectada. Ainda não está claro o mecanismo de descapsidação e transporte do material genético ao núcleo celular. Duas fases distintas do ciclo de vida foram identificadas: a fase latente resulta na manutenção do episoma viral através da expressão de proteínas latentes que atuam na transativação gênica, transformação celular e resistência a apoptose; na fase lítica ocorre a expressão de genes de proteínas estruturais relacionadas a montagem da partícula viral. Produção própria.

1.3.4 Transmissão e patogênese da infecção

A transmissão do EBV ocorre, frequentemente, através do contato com a secreção orofaríngea contaminada. Nos adolescentes e adultos, o beijo é apontado como principal via de transmissão, enquanto que o compartilhamento de objetos contaminados com saliva é a

principal rota entre crianças menores (Hjalgrim et al., 2007). Entre adultos soronegativos para EBV, o contato próximo com casos soropositivos em um espaço restrito e compartilhado raramente leva a infecção (Storrie e Sphar, 1976), no entanto, em crianças com hábitos semelhantes, a infecção é regular (Crawford et al, 2002).

A transmissão transplacentária e por amamentação são modos de transmissão não significativos e apenas observados em raras circunstâncias (Fleisher e Bologonese, 1984; Kusuhara et al, 1997). A transfusão sanguínea e por transplante de órgãos são particularmente preocupantes devido à associação da infecção por EBV como um fator de risco para a desordem linfoproliferativa pós-transplante (PTLD) (Aguilar et al, 1999; Scheenstra et al, 2004).

O EBV foi detectado em secreções cervicais de adolescentes e mulheres adultas, e no sêmen e tecido peniano (Enbom et al, 2001; Israele et al, 1991; Kapranos et al, 2003); porém, ainda se discute a sua transmissão sexual. As evidências biológicas encontradas em indivíduos sexualmente ativos, especialmente, quando portadores de infecções sexualmente transmissíveis (ISTs), são indícios justificáveis da transmissão sexual; entretanto, permanecem sem prioridades nos argumentos clínicos das ISTs (Pagano et al., 2007).

Na transmissão oral, a hipótese inicial sugeria que o vírus infectava inicialmente as células epiteliais da orofaringe, da nasofaringe e das glândulas salivares, onde frequentemente a replicação ocorreria (Allday & Crawford, 1988). No entanto, estudos posteriores comprovaram que, na realidade, os linfócitos B da orofaringe representam o sítio primário da infecção (Niedobitek et al., 1997). Logo após a transmissão, o vírus infecta o tecido linfóide do anel de Waldeyer no epitélio da orofaringe, seguido pela infecção dos linfócitos B nas criptas das tonsilas (Hutt-Fletcher, 2007). Posteriormente, o vírus se propaga pela corrente sanguínea, chegando a acometer 10% ou mais dos linfócitos circulantes. A proliferação das células infectadas resulta no aumento do tecido linfoide em diferentes regiões somáticas e a elevação dos títulos de imunoglobulina M (IgM) heterófilas. O EBV pode também determinar a diferenciação de linfócitos B em plasmócitos e promover grande produção de anticorpos (gamopatia policlonal). Em conjunto, essa sequência de eventos leva à imortalização dos linfócitos B, que então se proliferam vigorosamente e mantêm o DNA viral latente no núcleo (Figueiredo, 2009; Schooley, 2009).

Um modelo amplamente aceito de persistência do EBV é conhecido como modelo do centro germinativo, o qual propõe que o vírus emprega a expressão sequencial de programas de transcrição de latência que impulsionam as células B *naïves* infectadas a se tornarem células proliferativas que entram no compartimento de células B de memória, onde ocasionalmente se dividem como parte da homeostase. Desta forma, o vírus infecta um *pool* de células quiescentes

de vida longa, nas quais ele pode persistir. Eventualmente, um subconjunto de células B de memória infectadas pode reativar a fase lítica do vírus, em associação com sinais de diferenciação terminal. Este processo resulta na biossíntese de novos vírus que podem ser liberados na saliva para propagação infecciosa ou infectar novas células B *naïves*, completando assim o ciclo (Kraus et al., 2001; Thorley-Lawson, 2001; Thorley-Lawson et al., 2013).

Embora a infecção pelo EBV prevaleça em crianças, nessas a maioria dos casos são assintomáticos e silenciosos. Porém, em adolescentes e adultos a infecção pode resultar na manifestação da mononucleose infecciosa (Abbott et al., 2017). Algumas características são indicativas do motivo pela qual as manifestações ocorrerem com maior frequência em adolescentes e em adultos, como: (i) a existência de maior carga viral na infecção relacionada à atividade sexual, quando comparada à aquisição pelo contato exclusivamente pela saliva, o que poderia induzir uma miríade de respostas de linfócitos T $CD8^+$, os quais têm participação no desenvolvimento das manifestações clínicas, através da liberação de citocinas (Crawford et al., 2002); (ii) a existência prévia de imunidade à infecções por outros agentes virais (imunidade heteróloga), que poderia justificar os achados clínicos mais específicos em indivíduos com maior idade (Selin et al., 2006) e (iii) o incremento da atividade dos linfócitos T $CD8^+$ que induziria uma diminuição do quantitativo de células *natural killer* (NK), supostamente relacionada com a redução da gravidade dos sintomas (Andrews et al., 2008; Williams et al., 2005).

A mononucleose infecciosa é caracterizada por expressiva variação clínica, associada principalmente a idade, ao *status* imunológico e à presença de comorbidades no hospedeiro. A apresentação clínica clássica consiste na tríade de febre alta, faringite e linfadenomegalia; geralmente é observado um quadro de fadiga, mal-estar e mialgia antecedente ao início da doença (Son e Shin, 2011; Oliveira et al., 2012). As manifestações podem ser agudas, como úlceras genitais e dacriocistite aguda; ou tardias, como autoimunidade e aterosclerose. Alguns podem ser relativamente benignos, como fadiga, alergias e paralisia do nervo facial, contudo, outros podem ser fatais, como ruptura esplênica e insuficiência hepática. As demais complicações incluem miocardite, disfunção renal, hepatite e colecistite acalculosa e várias entidades hematológicas, neurológicas e respiratórias (Bolis et al., 2016).

A infecção primária é combatida por uma forte resposta celular específica, levando o vírus a estabelecer a persistência característica da infecção latente. Nessa fase, a perda do controle proliferativo, associado a disfunções imunológicas, são apontados como mecanismos tumorigênicos da infecção pelo EBV, o que o ratifica, desde a sua descoberta, como um organismo parasitário potencialmente carcinógeno (Hsu e Glaser, 2000; Knecht et al., 2001).

De acordo com a *International Agency for Research on Cancer* - IARC, o EBV é classificado como um carcinógeno do grupo I (IARC, 2019).

O EBV está associado tanto a neoplasias de origem linfoide como a neoplasias sólidas (Thorley-Lawson et al., 2013). Estudos mostram que o EBV está associado, além do clássico linfoma de Burkitt, a linfomas do tipo Hodgkin (Deacon et al., 1993), ao carcinoma de nasofaringe (Fan & Gulley, 2001; Raab-Traub, 2002), carcinoma gástrico (ZurHausen et al., 2000), neoplasias da musculatura lisa (Lee et al., 1995; McClain et al., 1995), carcinoma intracervical (Landers et al., 1993), dentre outros. A diversidade de padrões de expressão de genes latentes observados denota a complexidade da infecção pelo EBV (figura 13), sendo possível que o vírus esteja associado a neoplasias em outros sítios, porém ainda não tenha sido detectado pelas técnicas empregadas (Lima & Rabenhorst, 2006).

A extensão da associação ao EBV varia com cada neoplasia e metodologia empregada nos estudos. Em alguns casos apenas 1/4 a 1/3 dos pacientes apresentam positividade para a infecção. Este fato sugere que o EBV não é absolutamente necessário para o estabelecimento e manutenção de uma neoplasia. Embora o vírus possa imortalizar células B *in vitro*, seu papel *in vivo* na tumorigênese ainda não foi totalmente esclarecido. Além disso, seu papel na tumorigênese das células T ou NK não é claro. As características genéticas do hospedeiro podem estar relacionadas com a patogênese do câncer, embora não se possa descartar a possibilidade de que cepas específicas de EBV sejam prevalentes em certas áreas endêmicas (Kimura et al., 2013).

1.3.5 Epidemiologia da infecção

Nos países em desenvolvimento, a infecção primária ocorre logo na primeira infância, e a soroconversão é vista frequentemente entre 3 e 4 anos de idade. Em populações heterogêneas, a soroprevalência chega a 97% em indivíduos saudáveis, principalmente na idade adulta, semelhante à escala global (Hjalgrim et al., 2007; Smatti et al., 2017).

Nos países desenvolvidos a infecção é comum na adolescência, variando de 0 a 70% na infância e chegando a mais de 90% na idade adulta (Hjalgrim et al., 2007; Trottier et al., 2012). De fato, a prevalência de anticorpo anti-EBV diminuiu em indivíduos norte-americanos com idade entre 6 a 19 anos entre 2003 a 2010 (Balfour et al., 2013). Uma bimodalidade epidemiológica, com picos em crianças abaixo de 5 anos e novamente após os 10 anos de idade, foi observada também em países desenvolvidos. Discute-se nesses estudos se a transmissão oral

entre parceiros sexuais na adolescência e início da vida adulta, ou entre pais e filhos possa influenciar nas taxas descritas (Edwards e Woodroof, 1979; Lai et al., 1975).

Não há diferenças significativas na soroprevalência em razão do sexo em crianças até o início da adolescência, quando se passa a observa a maior prevalência nas mulheres (Crawford et al., 2002). Padrões socioeconômicos similarmente podem influenciar no número de casos, cujas condições precárias têm sido associadas à infecção precoce por EBV, enquanto a infecção tardia é típica em populações de alto nível socioeconômico (Henle et al., 1969).

No Brasil, essa tendência é observada na progressiva prevalência sorológica com o aumento da idade, atingindo um pico na segunda década de vida e sem viés sexual. Há um alerta para a soroprevalência em crianças e adolescentes de baixo nível socioeconômico (Figueira-Silva e Pereira, 2004), tal como, para a predominância da infecção em indivíduos com suspeita de doenças no sistema nervoso e coinfectados pelo HIV (Bastos et al., 2014).

Aproximadamente 143.000 mortes em 2010 foram atribuídas a malignidades associadas ao EBV. Porém, este índice pode estar subestimado, uma vez que algumas das neoplasias associadas ao vírus têm uma baixa prevalência em estudos epidemiológicos (Khan e Hashim, 2014). Apenas dentre os casos de linfoma, 95% dos linfomas de Burkitt, 90% de linfomas não-Hodgkin e mais de 90% dos distúrbios linfoproliferativos em imunocomprometidos estão associados à infecção pelo EBV (Magrath, 1991; Thompson e Kurzrock, 2004).

No Brasil, embora um estudo de caso-controle não tenha considerado a infecção pelo EBV um fator de risco para a doença de Hodgking (Kirchhoff et al., 1980), estudos subsequentes associaram o vírus a 87% dos casos entre os anos de 1954 a 1979, na mesma região geográfica. Destaca-se, porém, que em 54 anos houve uma diminuição na prevalência da infecção para cerca de 46%, o que se especula ser uma mudança nos padrões clínico-patológicos do linfoma nessa região em decorrência de transições socioeconômicas experimentadas (Campos et al., 2018). Outros achados mostram a presença da infecção em 60% dos casos de linfoma de Burkitt (Hassan et al., 2008) e de microRNAs do vírus como potenciais biomarcadores do linfoma difuso de células B em idosos (Andrade et al., 2014).

1.3.6 Diversidade genética e epidemiologia molecular

O genótipo 1 (EBV-1) tem alta prevalente entre indivíduos saudáveis caucasianos (74%) e asiáticos (85%). Os achados populacionais em caucasóides mostraram que 19% dos avaliados estava infectado com o tipo 2 (EBV-2) e 7% coinfectados com ambos os tipos. Nos asiáticos, cerca de 4% estava infectado com EBV-2 e 11% com ambos os tipos. Na África Oriental, a alta

prevalência do EBV-2 sugere a predominância dessa cepa na região (Young et al., 1987; Chang et al., 2009).

Há uma notável proporção epidemiológica na prevalência dos tipos de EBV, tal como, a coinfecção entre as duas cepas. Nos imunocomprometidos a coinfecção é mais comumente detectada, sugerindo que esta, provavelmente, seja adquirida durante o estado de depleção imunológica ou que a imunidade pode diferencialmente controlar a reativação e persistência dos subtipos de EBV em um indivíduo (Apolloni e Sculley, 1994; Yao et al., 1996). Relatos de recombinações intertipos para os genes da família EBNA também foram constatados em diferentes regiões geográficas estudadas (Palser et al., 2015).

Sabe-se que as inferências filogenéticas baseadas nas variações do gene EBNA justificam a classificação dos tipos do EBV, porém, novas propostas incluem a agregação de outras sequências em múltiplos genes virais como forma de contribuir com a resolução da classificação, posto que, estudos vêm atentando para a não correlação das variantes dos genes EBNA com sequências de outras regiões genômicas (Fassone et al., 2002; Klemenc et al., 2006).

Nesse sentido, grande parte das investigações recentes se fundamenta no estudo da proteína LMP-1, devido ao alto grau de polimorfismos quando comparado aos demais genes. As variações da proteína classificam o EBV em sete grupos principais, cuja nomenclatura reflete na sua origem geográfica ou a localização de descrição: Alasca (Ala); China (Ch1), (Ch2) e CAO (isolada em pacientes com carcinoma de nasofaringe); Mediterrâneo (Med +) e (Med−); Carolina do Norte (NC); e Tailândia (SEA1) e (SEA2) (Alfieri et al., 1996; Tzellos e Farrell, 2012; Smatti et al., 2018).

A coinfecção de múltiplas variantes de LMP-1 foi encontrada em indivíduos com mononucleose, com Linfoma de Hodgkin e pacientes coinfectados com o HIV. Há um destaque para a variante CAO que, também, foi isolada em 55,6% dos poloneses, 62,5% dos taiwaneses e 28% dos argentinos avaliados, sendo todos os indivíduos saudáveis. Em uma população multiétnica de indivíduos saudáveis, a prevalência dessa variante chega a 30,6% (Hu et al., 1991; Correa et al., 2004; Tzellos e Farrell, 2012; Polz et al., 2014; Smatti et al., 2017).

Em pacientes com mononucleose a diversidade de LMP1é menor durante a infecção aguda e vai aumentando ao longo do curso, particularmente no tecido epitelial, o que sugere a possível seleção de variantes para transmissão ou para amplificação pós-transmissão. A intensa variabilidade é compatível à noção de que a variância de LMP1 é introduzida durante a replicação do genoma viral na produção de partículas na orofaringe (Renzette et al., 2014).

As variantes do gene BZLF-1 (ZEBRA) foram definidas segundo a repetição de 29 pares de base localizada no primeiro intron do gene (entre 102654 e 102529 pb). A prevalência da variante dentre os pacientes com carcinoma de nasofaringe varia de 18,7% a 81,3% em chineses e 6,5% a 93,5% em africanos e europeus. Nos saudáveis, a proporção de portadores é maior, com cerca de 21,2% a 81,8% e 20% a 80,0%, respectivamente às regiões geográficas (Chen et al., 1996; Grunewald et al., 1998).

Diversidade genética também é observada na sequência do promotor ZP do gene BZLF-1. Dentre as variantes identificadas (Zp-P, Zp-V3, Zp-V4 e Zp-PV), a ocorrência de Zp-P foi observada, frequentemente, em indivíduos saudáveis das Américas e Europa. Zp-V3 é mais comum em pacientes com câncer de diferentes regiões geográficas. Para os autores, estudos sistemáticos são necessários antes que se possam inferir conclusões firmes em relação a associações das variantes em Zp com os aspectos geográficas e neoplasias relacionadas (Gutierrez et al., 2002; Martini et al., 2007).

Variações polimórficas não sinônimas foram encontradas nos genes da glicoproteína gp350/220, BRRF2, BPLF1 e EBNA-1 de linhagens linfobaltóides em crianças quenianas. Sequências conservadas foram mais evidentes em genes líticos, exceto pelas variações no gene EBNA-1 de EBV-2. Diferentes pressões evolutivas nas duas classes de genes é uma possível explicação para o achado (Simbiri et al., 2015).

1.4 ASPECTOS GERAIS DA RESPOSTA CELULAR ADAPTATIVA NAS INFECÇÕES POR HIV E EBV

A imunidade adaptativa contra antígenos virais se inicia com a ativação das células apresentadoras de antígenos (especialmente células dendríticas) mediante a exposição. O perfil de moléculas co-estimuladoras e a expressão de antígenos leucocitários humanos (HLA) sobre a superfície dessas células são fatores necessários para o estímulo de linfócitos T CD4+ e CD8+, as principais células efetoras da resposta adaptativa celular (Janeway, 2001).

Na infecção pelo HIV, é notória a imunodominância dos linfócitos T CD8+ no contexto de resposta celular, em virtude da própria depleção sobre os linfócitos T CD4+ induzida pelo vírus (Ramduth et al., 2005). No entanto, embora haja um desgaste dessa população celular, as mesmas não estão totalmente ausentes, além do que, anormalidades no desenvolvimento da resposta citotóxica é consistente com a redução de estímulos de células T CD4+, o que destaca a interação das linhagens na manutenção da resposta anti-HIV, como demostrado em modelos experimentais (Janssen et al., 2003). Efetivamente, a literatura fornece dados precisos que

destacam importância das células T CD4+ no priming, manutenção de memória e maturação funcional dos linfócitos TCD8+, mesmo na infecção crônica (McMichael &Rowland-Jones, 2012).

Na fase aguda, diferentes populações de células T CD4+ são massivamente reduzidas, particularmente no tecido linfóide intestinal (GALT). Porém, um remanescente de células CD4+ HIV-específicas e não infectadas escapam, apesar da alta viremia, e sustentam a tentativa de reboot imunológico que, entretanto, rapidamente é perdida à medida que a infecção progride (Rosenberg et al., 2000; Douek et al., 2009). A progressão lenta da doença, quando na ausência de tratamento, está associada a respostas mais vigorosas de células T CD4+. Em controladores, o perfil de expressão gênica das células T CD4+ é semelhante ao das pessoas não infectadas (Vigneault et al., 2011).

Na infecção aguda pelo HIV, as células NK representam a população citolítica dominante, antes do desenvolvimento de respostas imunitárias adaptativas induzidas por linfócitos T CD8+. Com a cronificação da doença, há uma proeminente atividade das células CD8+, porém, ambas as linhagens celulares cooperam para conter a replicação viral (Alter et al., 2007). Ressalva-se que, nos estágios iniciais, podem haver mais fenômenos virológicos a nível de tecidos do que no sangue propriamente dito, visto que a resposta de linfócitos T CD8+ nos linfonodos ocorre previamente ao sangue periférico (Altfeld et al. 2002). Contudo, há relatos que associam a resposta das células TCD8+ de fase aguda com o declínio na viremia inicial, fato que sugere uma atividade, no mínimo, parcialmente efetiva, predominantemente direcionada contra epítopos de Env e Nef (Turnbull et al., 2009).

A resposta de células T CD8+ específicas permanecem detectáveis durante todo o curso da doença, sendo mais amplas em indivíduos com infecção progressiva, quando comparado aos controladores (Pereyra et al., 2008). Na fase crônica, a especificidade da resposta à Gag parece estar associada à menor carga viral (Kiepiela et al., 2007). Todavia, a capacidade de afetar a função das células T CD4+ e células apresentadoras de antígenos leva a diminuição do pool circulante de células T CD8+ efetoras e de memória, resultando em falha no controle imunológico (Gulzar & Copeland, 2004).

Semelhante a imunopatogênese do HIV, as células NK também desempenham um papel na proteção contra a infecção primaria do EBV ou na patogênese da mononucleose infecciosa. Dentre os principais mecanismos de ação, está o controle da transformação de células B infectadas, muito útil na manutenção da tumorigênese induzida pelo vírus. Apesar disso, a imunidade adaptativa, por sua vez, é inequivocamente necessária para a robusta resposta anti-EBV, mesmo na infecção primária (Tangye et al., 2017).

A contagem de células T CD8+ no sangue periférico aumenta em até 10 vezes nesta fase quando comparado a indivíduos assintomáticos. Sendo a especificidade da resposta mais voltada a antígenos de fase lítica, compreendendo cerca de 50% do pool dessa linhagem celular em circulação (Taylor et al., 2015). Simultaneamente, ocorre a elevação dos níveis séricos de citocinas pró-inflamatórias e imunorreguladoras (IFN-γ, TNF, IL-6, IL-10 e TGF-β) (Panikkar et al., 2015).

Um modesto aumento na proporção de linfócitos T CD8+ EBV-específicos é observado em pacientes assintomáticos (em torno de 15% do total de células T CD8+ em circulação), contraindo-se entre 2–5% de células T CD8+ de memória após o controle virológico (Hislop et al., 2007).

As células T CD4+ EBV-específicas compreendem até 1% do pool da linhagem circulantes em pacientes com mononucleose. A resposta dessas células é direcionada para um repertório mais amplo de antígenos virais, principalmente para epítopos de fase latente (Taylor et al., 2015). Essa observação sugere que, embora numericamente pequenas, as células T CD4+ podem agir no controle da infecção por EBV. O que é consistente com os resultados de terapia celular adotiva em pacientes com doença linfoproliferativa pós-transplante associada ao EBV (Haque et al., 2007). Em um estudo de dinâmica viral em crianças EBV+, a porcentagem de células T CD4+ de memória central foi significativamente maior na infecção crônica quando comparado à aguda (Xing et al., 2013).

Em suma, durante as infecções virais, há uma subsequente ativação de células citotóxicas e células T CD4+ específicas para o vírus. Neste contexto, o HLA desempenha um papel essencial no estímulo da resposta celular frente aos desafios patogênicos (Wit et al., 2016). Após sua descoberta, os loci de HLA têm se destacado como os principais candidatos para suscetibilidade a doenças infecciosas. Na verdade, a hipótese original associa a variabilidade de HLA à força seletiva induzida pela infecção, particularmente infecções virais (Doherty e Zinkernagel, 1975). A suscetibilidade surge através da interação complexa de fatores genéticos ambientais e do hospedeiro, e muito dos esforços recentes têm sido na identificação de variantes funcionais de regiões gênicas e seus efeitos na condição patológica. (Blackwell et al., 2009).

1.5 O ANTÍGENO LEUCOCITÁRIO HUMANO

1.5.1 Características gerais

O antígeno leucocitário humano (*Human Leucocyte Antigen* - HLA) compreende um grupo de glicoproteínas de membrana que atuam como receptores especializados para peptídeos antigênicos, sendo oriundos a partir de genes específicos localizados no braço curto do cromossomo 6, denominados de complexo principal de histocompatibilidade (*Major Histocompatibility Complex* - MHC) (Abbas & Lichtman, 2005; Shiina et al., 2009).

O MHC é dividido de acordo com a estrutura e a função dos produtos gênicos em três regiões: classe I (HLA-A, -B, -C, -E, -F e -G), classe II (HLA-DR, -DQ, -DM e –DP) e classe III (Horton et al., 2008). As regiões de classe I e II codificam dois grupos de proteínas estruturalmente distintas; e a região de classe III, com 75 loci distribuídos dentro de 0,9 Mb de DNA, encontra-se localizada entre as regiões de classe I e II e contém um grupo de genes cujos produtos estão envolvidos nas respostas inflamatórias, tais como as proteínas do sistema complemento, citocinas, proteínas do choque térmico, entre outras. (Williams, 2001; Shiina et al., 2004, Shiina et al., 2009) (figura 12).

As moléculas HLA de classe I são formadas por duas cadeias polipeptídicas: uma cadeia α (pesada), codificada pelo MHC de classe I, com peso molecular de 44kD, e uma cadeia β_2 codificada no cromossomo 15, com peso molecular de 12 kD. Essas porções interagem entre si por ligações não covalentes. O HLA de classe I é composto por um domínio extracelular aminoterminal (no qual se ligam peptídeos), um domínio extracelular semelhante à imunoglobulina, um domínio transmembrânico e um domínio citoplasmático. A cadeia α ainda se divide em α1, α2 e α3, estando o sítio de ligação com peptídeos entre as cadeias α1 e α2 (Marsh et al., 2000; Horton et al, 2004; Abbas & Lichtman, 2005) (figura 13).

As moléculas do HLA de Classe II são formadas por duas cadeias polipeptídicas ligadas não covalentemente entre si, sendo a cadeia α (32 a 34 kD) um pouco maior do que a cadeia β (29 a 32 kD). Nas moléculas da classe II, ambas as cadeias polipeptídicas contêm grupos oligossacarídeos, aminoterminais extracelulares e carboxiterminais intracelulares, e mais de dois terços da cada cadeia estão localizados no espaço extracelular. As duas cadeias da molécula HLA de classe II são codificadas por dois diferentes genes MHC. As porções extracelulares das cadeias α e β são subdivididas em dois segmentos denominados α1 e α2, β1 e β2. A região de ligação dos peptídeos na molécula HLA de classe II é formada pela interação das duas cadeias, mais especificamente entre os segmentos α1 e β1 (Marsh et al, 2000; Horton et al., 2004; Magalhães et al, 2004) (figura 13).

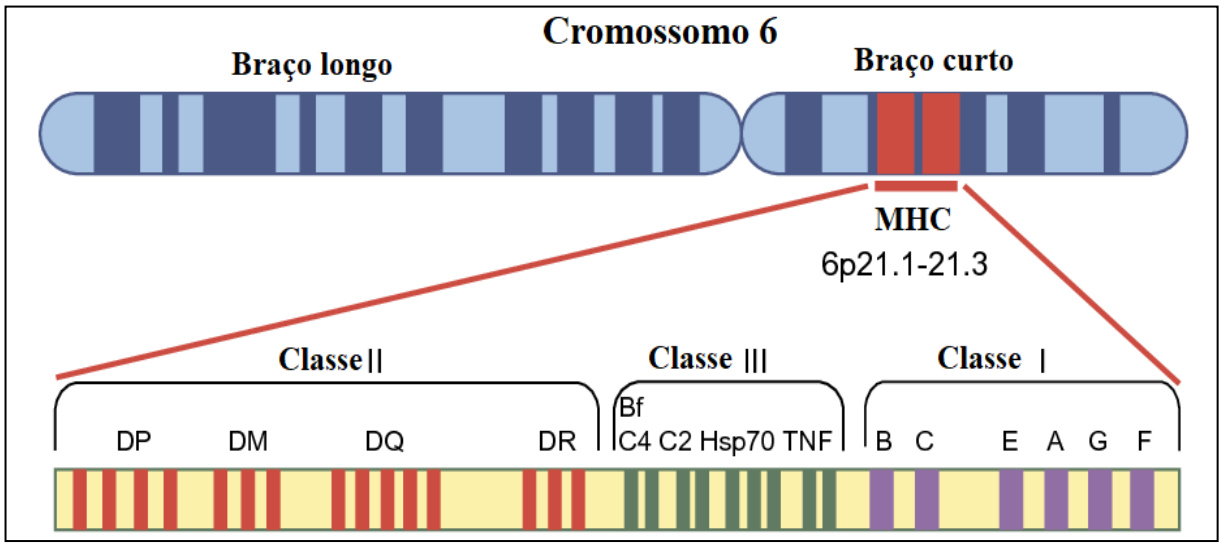


Figura 12 – Mapa da região correspondente ao MHC, situada no cromossomo 6. Adaptado de Regnell e Lernmark, 2017.

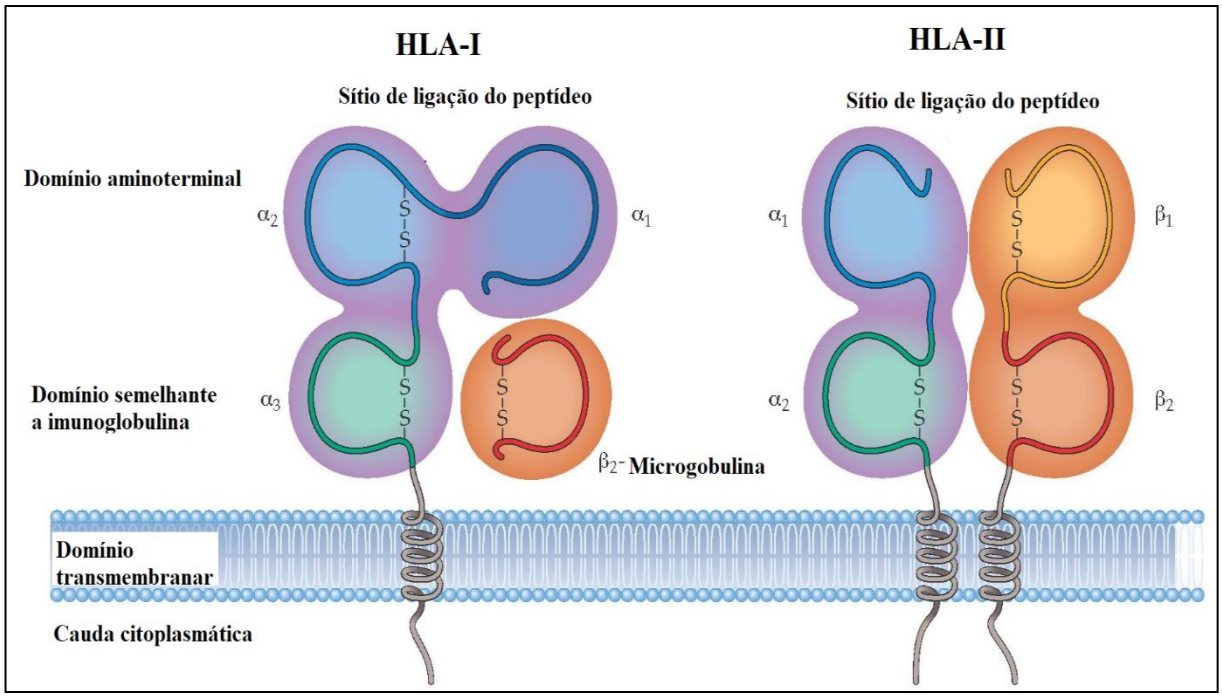


Figura 13 – Estrutura das moléculas de HLA de classe 1 e 2. Adaptado de https://www.onlinebiologynotes.com/major-histocompatibility-complex-mhc-structure-types-and-functions/

A apresentação de peptídeos antigênicos mediada por HLA tem papéis fundamentais em numerosas funções imunológicas, como a seleção clonal, indução de tolerância, imunidade mediada por células T e participa da produção de citocinas e anticorpos por estímulo à resposta inflamatória. Logo, há um extenso interesse no papel do HLA principalmente no contexto de rejeição de transplantes e suscetibilidade a doenças (Trowsdale & Knight, 2013).

Porém, embora a apresentação de antígenos tenha inicialmente definido o HLA como iniciador e mantenedor da imunidade adquirida, tornou-se evidente que essa molécula poderia também ser reconhecida por receptor semelhantes a imunoglobulinas de células natural killer (KIR). Isto criou um papel adicional para as moléculas HLA clássicas na condução da resposta imune inata, no qual a indução de KIR mediada por HLA proporciona sinais positivos ou negativos às NK através, respectivamente, da sequência de ativação de imuno-receptor baseada em tirosina (ITAM) ou da sequência de inibição de imuno-receptor baseada em tirosina (ITIM). Embora HLA e KIR estejam localizados em cromossomos diferentes e, portanto, são herdados independentemente, ambos são altamente polimórficos, e há grande potencial para determinadas combinações de alelos desses fatores para resultar em interações benéficas ou deletérias (KelLey & Trowsdale, 2005).

1.5.2 Aspectos genéticos e nomenclatura

Os genes *HLA* tem como característica principal o seu extremo polimorfismo. É o conjunto de genes que apresenta o maior grau de polimorfismo entre todos os loci expressos do genoma humano. Teoricamente, é possível que cada indivíduo apresente um conjunto diferente de alelos, embora a probabilidade de irmãos compartilharem o mesmo haplótipo seja de 25%. Os alelos de HLA têm segregação mendeliana, com o predomínio de codominância alélica; além disso, há um claro desequilíbrio de ligação gênica, que denota que certos alelos ocorrem junto com uma frequência maior do que a esperada por acaso. Variações nas combinações esperadas de alelos na população, comparado à formação aleatória de haplótipos, pode estar relacionada ao desequilíbrio de ligação (Buhler et al, 2012; Cardozo et al., 2014).

Polimorfismos de um único nucleotídeo (*single nucleotide polymorphisms* - SNPs) são os tipos mais comuns de variações observadas no MHC. Polimorfismos do tipo inserção ou deleção (InDel), número variável de cópias (CNVs) e conversão gênica são outros mecanismos de geração de diversidade genética nesses loci gênicos (Stewart et al., 2004; Chen et al., 2009; Street et al., 2011; Trowsdale & Knight, 2013).

A diversidade nucleotídica sinônima em genes humanos não *HLA* é cerca de 0,03% a 0,11% (Li & Sadler, 1991). Por outro lado, a diversidade nucleotídica sinônima é cerca de 4,0% para classe I e de 8,0% para o locus *DRB1* (Satta 1992; Takahata, 1993). Autores concluíram que essa diferença não pode ser atribuída à alta taxa de mutação nos loci *HLA*, porque a taxa de substituição nucleotídica sinônima estimada para *DRB1* é baixa quanto à observada para outros genes (Klein et al., 1986; Satta, 1992).

Sugere-se que ao longo da evolução dos vertebrados repetidos eventos de duplicação e conversão gênica foram a força motriz originaria da diversidade do sistema *HLA* (Kasahara et al., 1996; 1997). As informações disponíveis sobre a variabilidade, estrutura e função desse sistema, mostram que os padrões de variações ocorreram a partir de eventos de seleção natural, uma vez que a frequência de indivíduos homozigotos para os loci *HLA* é significativamente inferior ao esperado em condições neutras (Tiercy et al., 1992; Mack et al., 2000; Cao et al., 2004). Estudos mostram que as variações de nucleotídeos estão concentradas nos éxons que codificam a fenda de ligação ao peptídeo e o sítio de interação com o receptor da célula T, locais estes em que alterações podem promover mudanças de aminoácidos que estão em contato direto com o peptídeo ligado ao receptor de célula T. Essas características das sequências *HLA* podem indicar que o polimorfismo é o resultado de um processo de seleção natural. As regiões com maior evidência da atuação da seleção são as que interagem diretamente com os antígenos, indicando que os microrganismos patogênicos sejam fonte de seleção atuante nos genes *HLA* (Parham et al., 1988; Valdes et al., 1999).

Nessa hipótese de seleção dirigida por patógenos, as mudanças gênicas entre patógenos e hospedeiros ocorreriam paralelamente: os patógenos tenderiam a acumular mutações de escape, e as novas moléculas HLA que apresentassem de forma eficiente essas mutações seriam adaptativas e aumentariam de frequência na população de hospedeiros, recomeçando o ciclo (Borghans et al, 2004). Isto poderia explicar o grande número de alelos, as altas taxas de heterozigose e a grande persistência dos alelos nas populações ao longo de gerações.

O Comitê de Nomenclatura dos Fatores do Sistema HLA da Organização Mundial da Saúde normatiza a nomenclatura do sistema HLA durante Workshops Internacionais de Histocompatibilidade regularmente aplicados (Torres e Moraes, 2011).

Em suma, o nome de cada alelo *HLA* tem um número único correspondente até quatro conjuntos de dígitos delimitados por pontos que separam o grupo de alelos; a especificidade do mesmo; a mutação silenciosa; e as variações na região não codificante. Os dois dígitos anteriores ao primeiro delimitador descrevem os grupos de alelos, que geralmente correspondem aos antígenos determinados por métodos sorológicos de baixa resolução (por exemplo, *HLA-A, HLA-DR*). Em seguida, os alelos definidos por alta resolução recebem números sequenciais correspondentes às suas diferentes sequências, numeradas pela ordem de descobrimento. A nomenclatura do locus C incorpora a letra "w" (por exemplo, *HLA-Cw1*, *HLA-Cw2*) para diferenciá-lo do sistema do complemento. Para *HLA* classe II, após a designação do HLA e do seu locus gênico, a letra "A" ou "B" é adicionada para representar as cadeias polimórficas α e β do *HLA-DR, HLA-DQ e HLA-DP* (por exemplo, *HLA-DQA, HLA-*

DRB, HLA-DPB). Como algumas regiões possuem vários genes para as cadeias α e β, cada locus recebe um número correspondente (por exemplo, *HLA-DQB1*). Em seguida, conforme definido para *HLA classe I*, quatro a oito dígitos são adicionados após um asterisco (por exemplo, *HLA-DQB1 * 03: 01*) (Marsh et al., 2010) (figura 14).

A forma da expressão de determinados alelos é sinalizada pela introdução de sufixos adicionais. O sufixo N (*null*) indica alelos que não codificam produtos proteicos; L (*low*) proteína de baixa expressão; S (*secreted*) molécula encontrada na forma solúvel; C (*cytoplasm*) quando os produtos estão expressos no citoplasma; A (*aberrant*) quando há dúvidas sobre onde ocorre a expressão; e Q (*questionable*) quando não está definido se a mutação gera ou não modificação na expressão proteica (Bodmer et al., 1999). Em novas atualizações, ficou estabelecido que os sufixos A e C não seriam mais utilizados como forma de expressão de alelos (Marsh et al., 2010).

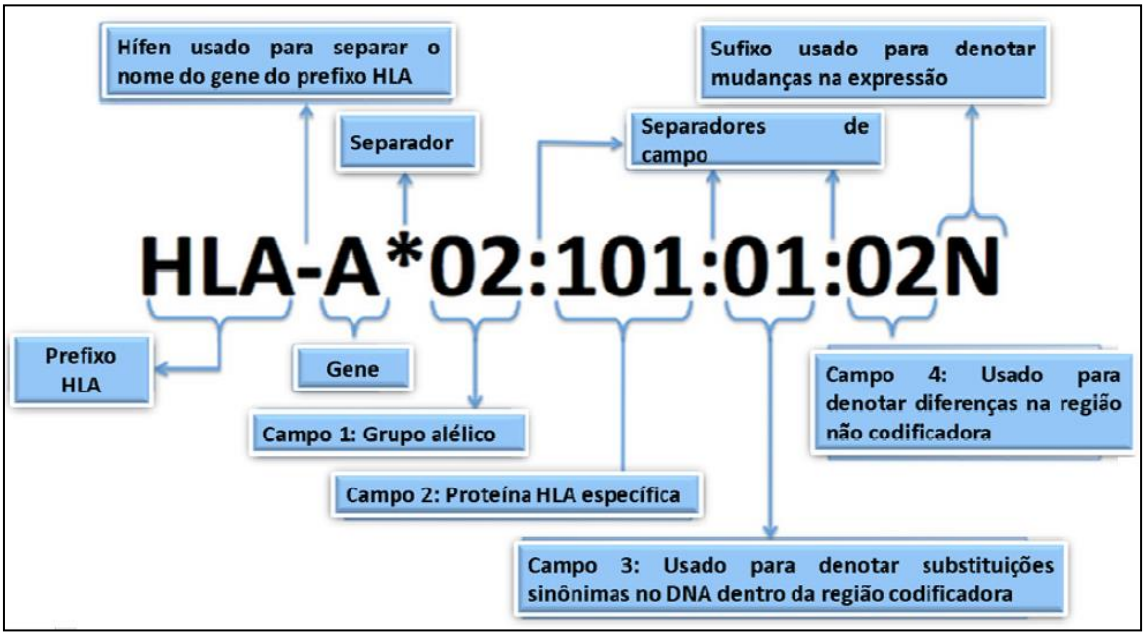


Figura 14 - Nomenclatura do HLA segundo as definições do Comitê de Nomenclatura dos da Organização Mundial da Saúde. Adaptado de (http://hla.alleles.org/alleles/index.html).

1.5.3 Processamento e apresentação de antígenos

Duas vias clássicas de processamento e apresentação de antígenos foram identificadas (figura 15 A e B): Para o HLA de classe I (via endógena), as proteínas intracelulares são degradadas em complexos especializados em proteólise enzimática denominados de proteassomos e transportadas para o retículo endoplasmático pelo transportador dependente de

adenosina trifosfato associado ao complexo proteico de processamento de antígeno (TAP), muitos destes polipeptídeos são ubiquitinados e, assim, marcados para degradação proteossômica (Neefjes et al., 2011).

No retículo, os peptídeos podem se ligar às moléculas de HLA de classe I através de complexos com a beta-2 microglobulina (β2m); a glicoproteína Tapasina tanto mantêm a estabilidade do dímero de classe I vazio no retículo endoplasmático, como liga as moléculas de HLA de classe I ao complexo TAP em associação com as moléculas chaperonas calreticulina e a tioloxidoredutase ERp57. Juntas, essas moléculas formam o complexo de carga de peptídeos (PLC) encarregado de introduzir o peptídeo e favorecer sua inserção correta na molécula HLA de classe I. Peptidases associadas ao retículo endoplasmático (ERAP1 e ERAP2) efetuam ajustes dos peptídeos até o tamanho de 8 a 10 aminoácidos, ideal para o acoplamento ao HLA de classe I. O heterodímero formado trafega ao complexo de Golgi para adição de açúcares e posteriormente para a superfície celular para o reconhecimento pelas células T CD8+ (Basu e Srivastava, 1999; Williams et al., 2002; Saric et al., 2002; Cruz-Tapias et al., 2013)

Moléculas HLA de classe I ligam peptídeos curtos e diferentes alelos HLA de classe I têm especificidades de ligação distintas, o que implica que um amplo espectro de peptídeos pode ser exibido em diferentes indivíduos. Em geral, antígenos apresentados pelo HLA de classe I são derivados de infecções intracelulares causadas por vírus, ou de proteínas sintetizadas no citosol, como proteínas maduras que completaram seu ciclo celular ou produtos ribossômicos defeituosos (Robinson et al., 2015).

Para o HLA de classe II (via exógena), os peptídeos são oriundos da proteólise de proteínas extracelulares fagocitadas, além do mais, proteínas endógenas também podem ser apresentadas nas moléculas HLA de classe II quando degradadas por autofagia. Outras formas de captura incluem a ligação ao receptor CD205, que transporta o antígeno diretamente ao endossomo tardio (MIIC), no qual é feita a montagem final; e pela transferência intercelular por exossomos, uma fonte de antígenos tumorais para ação de células T citotóxicas (Mahnke et al., 2000; Wolfers et al., 2001; Lerena et al., 2008).

As cadeias α e β do HLA de classe II interagem com uma proteína não polimórfica chamada cadeia invariante (*Ii*), esta interação estabiliza a estrutura da molécula de HLA de classe II, evitando a ligação de peptídeos no retículo endoplasmático. *Ii* é ancorado na membrana do retículo, e a porção citosólica da molécula direciona o complexo através de compartimentos endossômicos MIIC. Dentro do MIIC, o *Ii* é degradado e pode ser substituído por um peptídeo derivado da degradação do material endocitado nos endossomos ou lisossomas. As enzimas proteolíticas, tais como catepsinas, que geram péptidos de proteínas

internalizadas também atua sobre a *Ii* e degradam-na, deixando apenas um fragmento peptídico chamado de CLIP (Cadeia Peptídica Invariante Associada à Classe II), que se situa no sítio de ligação de peptídeos. A substituição de CLIP é catalisada pelo heterodímero de classe II HLA-DM, que também age como chaperona mantendo a estrutura intacta enquanto a troca se realiza. Em linfócitos B, HLA-DO influencia a eficiência de HLA-DM como aceptor de peptídeos, inibindo sua atividade em pH mais neutro. Uma vez alcançado o compartimento endolisossomal, sendo o pH 4,5, a função de HLA-DM é restaurada, abrindo o caminho para a ligação de peptídeos de origem extracelular. Um peptídeo de alta afinidade ao se ligar forma um complexo estável que é transportado até a superfície celular e apresentado a células T CD4+ (Kropshofer et al., 1996; Mosyak et al., 1998; Cruz-Tapias et al., 2013).

As proteínas HLA de classe II são codificadas por vários genes (HLA-DRA, HLA-$DRB_{1,3,4,5}$, HLA-DPA1, HLA-DPB1, HLA-DQA1, HLA-DQB1) e também mostram um nível muito alto de polimorfismo nos humanos (exceto o HLA-DRA). HLA de classe II formam heterodímeros que ligam peptídeos mais longos (12-20 aminoácidos) em um sítio de ligação de extremidade aberta. Ao contrário do HLA de classe I, as moléculas de HLA de classe II são principalmente expressas em células apresentadoras de antígenos profissionais específicas (pAPCs), tais como células dendríticas ou células B, e raramente também por células cancerosas como o melanoma (Gfeller e Bassani-Sternberg, 2018).

As células dendríticas podem apresentar antígenos extracelulares em moléculas de HLA de classe I através de um processo denominado “apresentação cruzada de antígenos” (figura 15 C). Este é um fenômeno importante na geração de respostas antivirais e tumorais, ou na indução da tolerância imunológica (Embgenbroich e Burgdorf, 2018).

Na via de apresentação citosólica, as proteínas são internalizadas em compartimentos endossômicos e posteriormente transcolados para o citosol. Ainda se discute quais mecanismos atuam neste processo, porém, admite-se que o conteúdo lisossomal não pode ser liberado no citosol de maneira desregulada. Os antígenos precisam ser desdobrados e as pontes dissulfídicas reduzidas por enzimas redutases, como a GILT (tiol-redutase lisossômica induzido por interferon-γ), antes da translocação ocorrer. Isto suporta a hipótese de que a translocação de antígenos é altamente regulada e pode envolver um complexo de poros transmembranares (Giodini e Cresswell, 2008; Singh e Cresswell, 2010). Posteriormente, as proteínas são degradadas pelo proteassomos e transportados por TAP para o retículo endoplasmático. Em seguida, os peptídeos são clivados pela ERAP1 e pela aminopeptidase responsiva à insulina endossômica (IRAP) e, finalmente, carregados em moléculas HLA de classe I (Rock et al., 2010).

A via de apresentação vacuolar é independente de TAP, visto que os antígenos internalizados são degradados diretamente em fagossomos pela protease lisossomal Catepsina S. Os peptídeos formados são então carregados em moléculas de HLA de classe I e transportados para a superfície celular (Merzougui et al., 2011). Neste contexto, as células dendríticas possuem mecanismos preventivos que impedem a rápida degradação do antígeno lisossomal e, dessa forma, favorecem a apresentação cruzada, são eles: baixa expressão de proteases lisossomais, tais como asparaginas endopeptidases e Catepsinas L, S, D e B (Delamarre et al., 2005); alcalinização ativa dos endossomos, mediada por V-ATPase e por NOX2, que regula a ativação das proteases dependentes de pH (Trombetta et al., 2003; Savina et al., 2006); por fim, estas células expressam receptores de fagocitose que visam especificamente compartimentos endossômicos de baixa degradação (Burgdorf et al., 2007).

Vias alternativas de apresentação cruzada foram observadas no transporte de antígenos pré-processados de uma célula doadora para uma célula dendríticas através de junções comunicantes (Gap). Após o transporte, os peptídeos derivados do antígeno podem entrar na via de apresentação do HLA de classe I. Curiosamente, a célula doadora não necessariamente é uma célula apresentadora de antígeno, oferecendo a possibilidade de que as células dendríticas possam obter esses peptídeos diretamente das células infectadas (Neijssen et al., 2005).

Outra via alternativa é denominada de "*cross-dressing*", na qual, a célula dendríticas adquiri uma molécula de HLA de classe I já carregada com um peptídeo derivado de antígeno transferida de uma célula doadora, provavelmente pelo contato célula-a-célula. Embora processo já tenha sido demonstrado *in vivo*, a relevância fisiológica exata do *cross-dressing* e sua contribuição em comparação com as outras vias de apresentação cruzada continua a ser elucidada (Dolan et al., 2006).

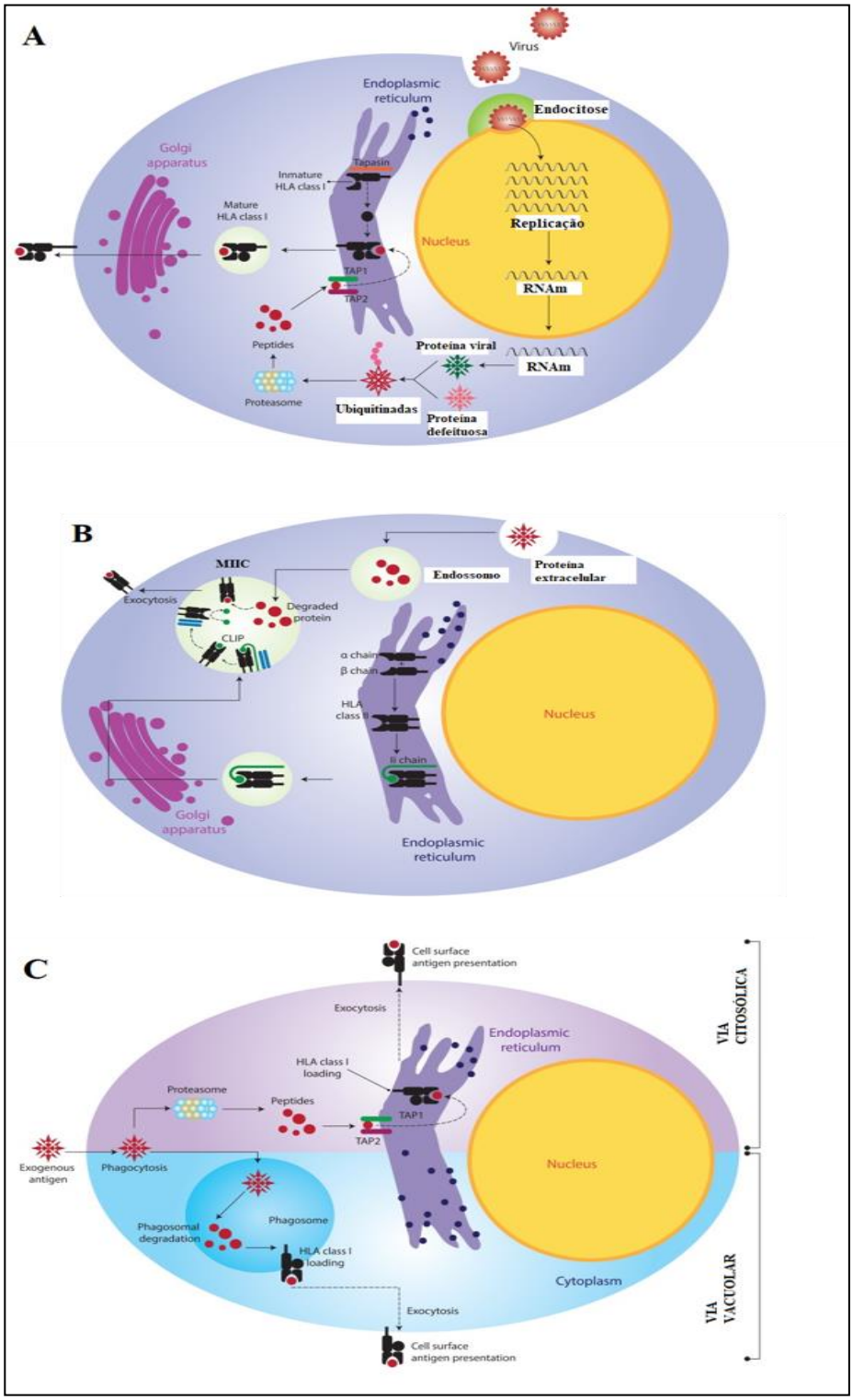


Figura 15 – Vias de processamento e apresentação de antígenos por moléculas de HLA. (**A**) Na via endógena, proteínas são degradadas pelo proteassomo em pequenos peptídeos que são transportados por TAP para o retículo endoplasmático. HLA de classe I é sintetizado nos ribossomos e transcolado também para o retículo, onde é montado e ligado ao peptídeo. (**B**) Na via exógena, proteínas extracelulares são fagocitadas e degradadas nos endossomos. Paralelamente, no retículo endoplasmático as cadeias α e β do HLA de classe II e as moléculas *Ii* são montadas em heterotrímeros. Em vesículas, *Ii* é degradado em CLIP e ocupa o sítio de ligação ao antígeno até a substituição por este. (**C**) Na apresentação cruzada, após a fagocitose, os antígenos podem ser processados pelo proteassomo, seguindo a via clássica de apresentação

por HLA de classe I (citosólica) ou reimportadas no fagossomos e ser apresentado em moléculas de HLA de classe I ali presentes (vacuolar). Adaptado de Cruz-Tapias et al., 2013.

1.5.4 Diversidade genética dos lócus *A, B* e *DRB1*

Existem singularidades na distribuição dos alelos do *HLA* em escala global, com regiões geograficamente separadas mostrando vários graus de diversidade. Essa distribuição fornece uma visão sobre a migração humana e pode ajudar a compreender a exposição a mecanismos de seleção anteriores que culminaram nos padrões observados (Tshabalala et al., 2015).

Em um estudo multipopulacional de larga escala foi observado que, de fato, há uma estrutura geográfica significativa observada nos loci do *HLA*, confirmando a relação entre a geografia e a variabilidade molecular entre as populações. Análises mais detalhadas realizadas em escala regional, entretanto, apresentam resultados diferentes, indicando que a estimativa da distância genética entre algumas populações depende muito se a variabilidade molecular de seus alelos é considerada ou não (Buhler & Sanchez-Mazas, 2011).

Em grupos americanos prevalentemente caucasoides, destacou-se, para o lócus *A*, a prevalência dos grupos *HLA-A*02*, *HLA-A*01* e *HLA-A*03*, sendo a maior diversidade observada no *HLA-A*02* e *HLA-A*24*. O lócus *B*, como esperado, foi o mais polimórfico, porém, tendo apenas dois alelos com frequências acima de 10% (*HLA-B*07:02:01* e *HLA-B*08:01:01:01*); o grupo *HLA-B*15* foi o mais diversa, seguido por *HLA-B*35*. O lócus *HLA-DRB1* foi o mais polimórfico dos loci de classe II, com 49 alelos distintos detectados; *HLA-DRB1*15:01:01:01* foi o mais frequente, seguido por *HLA-DRB1*07:01:01:01* e *HLA-DRB1*03:01:01:01* (Creary et al., 2019).

Em europeus, observou-se similaridades na prevalência de grupos de alelos do lócus *A* quando comparado aos descendentes americanos. No entanto, para os lócus *B* e *DRB1* ocorreram alterações na sequência dos alelos, sendo os alelos *HLA-B*44*, *HLA-B*35* e *HLA-B*08*, para o lócus *B*, e os alelos *HLA-DRB1*11, HLA-DRB1*03* e *HLA-DRB1*13*, para o lócus DRB1, os mais prevalentes nas populações europeias (Inotai et al., 2015).

Em asiáticos, uma amostragem chinesa revelou a prevalência dos alelos *HLA-A*11:01, HLA-A*02:07, HLA-A*24:02* e *HLA-A*02:03*, para o lócus *A*. Esses quatro alelos representaram mais de 68% do total da população. No lócus *B*, os alelos *HLA-B*46:01, HLA-B*15:02* e *HLA-B*40:01* ocorreram com maior frequência. No lócus *DRB1*, os três alelos com maior frequência foram *HLA-DRB1*15:01, HLA-DRB1*16:02* e *HLA-DRB1*12:02*. O haplótipo *HLA-A~B* mais frequente foi *HLA-A*33:03~B*58:01*; o haplótipo *HLA-A~DRB1*

mais comum foi *HLA-A*11:01~DRB1*15:01*; e no haplótipo *HLA-B~DRB1*, os mais frequentes foram *HLA-B*46:01~DRB1*14:01*. Ademais, os haplótipos *HLA-A*33:03~B*58:01, HLA-A*03:01~DR*11:04* e *HLA-B*07:05~DR*10:01* tiveram o maior desequilíbrio de ligação padronizado (Pei et al., 2018).

Um estudo anterior que avaliou a extensão das diferenças genéticas nas populações do Sul e Leste Asiáticos, mostrou uma distinta diversidade entre as subpopulações amostradas, observadas também na frequência dos haplótipos (Saw et al., 2015). Curiosamente, discute-se que a seleção natural não é uma explicação satisfatória da variação do *HLA* observada em asiáticos. Os fatores demográficos, como migrações populacionais para o sul, explicam melhor os padrões genéticos observados. A força do fluxo gênico durante as migrações subsequentes do Norte e do Sul é apontada como a razão do padrão de diversidade genética relacionado aos aspectos geográficos (Di & Sanchez-Mazas, 2014).

Em negroides sul africanos um estudo mostrou um espectro mais amplo de representação alélica, com menor dominância de alelos únicos, sendo os alelos *HLA-A*30:01, HLA-B*58:02* e *HLA-DRB1*13:01* os mais prevalentes. Foram identificados também os alelos *HLA-A*30:01:02, HLA-A*30:02:02, HLA-A*68:27, HLA-B*42:06* e *B*45:07* até então não mostrados no grupo étnico (Paximadis et al., 2012). Em algumas subpopulações sul africanas os alelos *HLA-A*11:01* e *HLA-B*40:06* parecem ter uma frequência mais considerada (Tshabalala et al., 2015). Dados atuais mostram o aumento da frequência dos alelos *HLA-A*30:02:01* e *HLA-DRB1*15:03:01* no mesmo grupo étnico (Loubser et al., 2020).

Em um estudo com cinco populações subsaarianas não houve um único alelo dos lócus A ou B predominante em qualquer uma das populações, exceto o alelo *HLA-A*30:02* em zambianos. Porém, apesar do compartilhamento de alelos, diferenças nas frequências de um número significativo de alelos foram restritas apenas a poucas populações africanas, que para os autores, pode ter decorrido de mutações nos alelos mais comuns (Cao et al., 2004).

No Brasil, a herança genética é bastante heterogênea, no entanto, a singular dinâmica de interação regional torna cada população única em termos de ancestralidade genética (Boquett et al., 2019).

Em um estudo com nativos da tribo Terena, grupo linguístico Aruak, localizados no centro sul brasileiro, para o lócus *A*, os alelos mais frequentes foram *HLA-A*02:01, HLA-A* 68:12* e *HLA-A* 31:01*; para o lócus *B*, os alelos mais frequentes *foram HLA-B* 48:03, HLA-B* 40:04* e *HLA-B*39:03*; para o lócus *DRB1*, os alelos mais frequentes foram *HLA-DRB1*16:02, HLA-DRB1*08:02* e *HLA-DRB1*14:02*. O lócus *A* foi representado por apenas oito alelos, dos quais seis eram de origem indígena pertencentes a quatro linhagens (*A*02,*

*A*24, A*68, A*31*). Em contraste, o *HLA-B* teve uma variação maior com dezessete alelos sendo de origem nativa. Dos doze alelos *DRB1*, apenas oito foram presumivelmente de origem ameríndia, porém, os alelos *HLA-DRB1*01:01, HLA-DRB1*08:04, HLA-DRB1*12:01* e *HLA-DRB1*13:02* podem ser decorrentes da miscigenação com os caucasóide e negroide (Lázaro et al., 1999).

A genotipagem do *HLA* de classe II em sete tribos distribuídas entre a Amazônia oriental, centro sul brasileiro, Paraguai e Peru mostrou que os alelos comuns do lócus *DRB1* pertenciam às linhagens *HLA-DRB1*04, HLA-DRB1*08, HLA-DRB1*09, HLA-DRB1*14* e *HLA-DRB1*16*. Os alelos pertencentes às linhagens *HLA-DRB1*01, HLA-DRB1*03, HLA-DRB1*07, HLA-DRB1*10, HLA-DRB1*11, HLA-DRB1*12, HLA-DRB1*13* e *HLA-DRB1*15* ocorrem em frequências comparativamente baixas, sendo indicados como marcadores de fluxo gênico populacional. As populações apresentavam diversidade alélica e haplotípica limitada, com perfis genéticos diferentes entre si. Os alelos mais frequentes eram comuns entre asiáticos orientais, o que apoiou a hipótese de origem asiática dos nativos americanos (Tsuneto et al., 2003).

Estudos em populações urbanas brasileiras são mais comuns entre doadores e receptores de transplantes da região sudeste: Em um estudo de análise retrospectiva de dados de prontuários de candidatos a transplante renal em São Paulo, observou-se a prevalência de indivíduos supostamente caucasoides em relação a negros. Nesta população, os alelos mais frequentes foram *HLA-A*02, HLA-B*35* e *HLA-DRB1*11*. Os haplótipos mais frequentes encontrados foram *HLA-A*01~HLA-B*08~HLA-DRB1*03* entre caucasianos e *HLA-A*29~HLA-B*15~HLA-DRB1*04* entre negros (Ravazzi-Gauch et al., 2016).

Em contraste, em um estudo realizado em doadores de medula óssea do mesmo estado, porém com um número amostral menor, foi observado que os grupos de alelos mais comuns em descendentes europeus e miscigenados africanos/europeus foram *HLA-A*02, HLA-B*35 e HLA-DRB1*13*, enquanto os grupos *HLA-A*02, HLA-B*35 e HLA-DRB1*11* foram mais comuns em descendentes africanos (Ayo et al., 2014).

Existem dados publicados de comunidades quilombolas também. Como exemplo, um estudo realizado em doze comunidades do Vale do Ribeira (São Paulo) que mostrou a prevalência dos alelos *HLA-A*02:01, HLA-A*23:01, HLA- B*45:01, HLA- B* 42:01, HLA-B*08:01, HLA-DRB1*15:03 e HLA-DRB1*07:01*. Entre os 150 haplótipos descritos, 70 foram encontrados no NMDP (Programa Nacional de Doadores de Medula Óssea - *National Marrow Donor Program*), sendo estes mais frequentes em africanos, europeus e hispânico, respectivamente (Nunes et al., 2016).

Em um banco de dados com 108 amostras de brasileiros de populações heterogêneas da região urbana o alelo *A*02:01* foi o mais frequentes e os alelos *A*24:04, A*25:01* e *A*80:01* foram os menos frequentes para o lócus *A*. O alelo *B*07:02:01* foi o mais frequente no lócus *B*. O alelo *DRB1*03:01* foi o mais frequente no lócus *DRB1* (Gonzalez-Galarza et al., 2020).

Na região norte, no banco de dados do Registro Nacional de Doadores de Medula Óssea (REDOME) do estado do Pará foi mostrado que os alelos *A*02* e *A*24* são os mais frequentes para o lócus *A*, enquanto que o alelo *A*43* foi o menos frequente. Para o lócus *B*, os alelos *B*15* e *B*35* foram os mais frequentes, enquanto que o alelo *B*59* foi o menos frequente. Para o lócus *DRB1*, os alelos *DRB1*04* e *DRB1*13* foram os mais frequentes, enquanto que o alelo *DRB1*12* foi menos frequente (Gonzalez-Galarza et al., 2020). Frequencias semelhantes foram observadas em um estudo com doadores e receptores de transplante renal realizado no mesmo estado, no qual, houve a prevalência dos grupos alélicos *HLA-A*02*, *HLA-B*35* e *HLA-DRB1*04*. Foram observados 1288 haplótipos, sendo o *HLA-A*02~HLA-B*44~DRB1*07* mais frequente e correspondente a etnia caucasoide (Barros et al., 2021).

1.5.5 Implicações do sistema *HLA* nas infecções pelo HIV e EBV

1.5.5.1 Alelos dos lócus *A, B* e *DRB1* associada ao risco e agravamento da infecção pelo HIV

O sistema *HLA* desempenha papéis em vários estágios da infecção e progressão do HIV (Kaur & Mehra, 2009). Estes incluem: a seleção do repertório de epítopos virais que serão apresentados aos linfócitos T auxiliares e citotóxicos (Chakraborty et al., 2013); a indução da citotoxicidade antiviral mediada por células NK (Parsons et al., 2012) e a seleção de mutações no vírus, modulando assim a cinética do escape viral (Chang et al., 2015). Investigações que mapearam epítopos virais mutantes associados ao gene *HLA* têm chegado à conclusão de que alelos raros podem levar a uma evolução viral adicional comparado com os alelos mais comuns, particularmente em relação aos genes *env*, *nef* e *pol* do HIV-1. Assim, o HIV evade-se da pressão imunológica em indivíduos com alelos *HLA* comuns, mantendo altos níveis de replicação e consequentemente maior carga viral. Por outro lado, quando o vírus infecta hospedeiros com alelos raros, este sofre uma pressão seletiva que acaba gerando formas variantes adicionais que possuem menor fitness viral, consequentemente diminuindo a carga viral (Rousseau et al., 2009).

Para o lócus *A*, um estudo em parceiros sorodiscordantes mostrou que a diversidade do gene *HLA* influenciou na transmissão heterossexual do HIV, no qual o alelo *HLA-A*36:01*, dentro do haplótipo *A*36~Cw*04~B*53*, foi o marcador mais desfavorável na transmissão do

HIV-1 entre parceiros índices (Dalmau et al., 2009; Tang et al., 2008). O grupo alélico *HLA-A*36* também foi associado de forma independente aos altos níveis de carga viral em africanos zambianos soroprevalentes (Tang et al., 2010). Por outro lado, um estudo realizado na República dos Camarões mostrou que o grupo alélico *HLA-A*32* foi associado ao risco de aquisição da infecção no contexto de transmissão vertical (Mekue et al., 2019).

Em vietnamitas, haplótipos contendo a variante *HLA-A*29* foram associados a mutações no HIV-1, maiores cargas virais e menores contagens de linfócitos T $CD4^+$, sugerindo que a adaptação viral e escape do controle imune mediado por *HLA* ocorreu (Chikata et al., 2018). Em uma coorte espanhola, o alelo *HLA-A*02:01* foi associado ao risco de infecção devido à baixa frequência em indivíduos soronegativos expostos ao HIV (Rallón et al., 2017).

Em uma população celular de linfócitos T $CD8^+$ cultivada a partir de amostras de japoneses HIV $^+$, o acúmulo da mutação YF9 em *Nef*, que foi selecionada por células restritas ao *HLA-A*24:02*, afetou a capacidade de supressão da replicação do HIV-1 (Murakoshi et al., 2018). De fato, já havia sido mostrado que *Nef* regula negativamente a expressão do *HLA-A* (Cohen et al., 1999), embora não tenha sido observado esse efeito sobre portadores do alelo *HLA-A*02* (Chen et al., 2012). É digno e nota destacar que em um estudo de associação genômica ampla (GWAS) em afro-americanos, sugere-se que alelos do lócus *A* que possuem resíduos de aminoácidos fora do sulco de ligação com o receptor de células T (TCR) mostram uma redução na afinidade de ligação com os linfócitos T $CD8^+$ (McLaren et al., 2012).

Para o lócus *B*, um estudo realizado com sul africanos infectados pelo HIV mostrou que um número significativamente maior de respostas de células T $CD8^+$ foi restrita ao *HLA-B* se comparado as moléculas *HLA-A.* Além deste fato, foi verificada uma maior pressão de seleção imposta ao HIV-1 por certos alelos *HLA-B* do que por alelos *HLA-A,* indicando que o principal foco de atividade viral seja o lócus *HLA-B* (Kiepiela et al., 2004). A união desses alelos na formação de haplótipos também tem servido como marcadores na patogênese da infecção pelo HIV.

Os grupos alélicos *HLA-B*35* (Scorza et al., 1988) e *B*53* (Dorrell et al., 2001) são classicamente associados com a progressão rápida da infecção. Tem sido observado que a sobrevida prolongada de caucasianos portadores do vírus foi atribuída a heterozigose completa para os loci do sistema *HLA* de classe I, e/ou pela baixa frequência de alelos associados à doença, como o *HLA-B*35* e *HLA-Cw04* (Carrington et al., 1999; O´Brien & Nelson, 2004; Poropatich & Sullivan, 2011). Em linhagens de linfócitos T $CD8^+$ restritas ao *HLA-B*35:01* foi mostrado que a resposta imunológica contra epítopos mutantes de *Nef* tendem a favorecer o escape viral (Motozono et al., 2013), embora se discuta os desfechos clínicos da progressão da

infecção em portadores de distintas variantes específicas do grupo *HLA-B*35* (Willberg et al., 2010). Para os portadores do grupo alélico *HLA-B*53* um fator adicional se refere a associação do alelo à síndrome de reação medicamentosa para os usuários do antirretroviral raltegravir, principalmente em descendentes africanos (Thomas et al., 2017).

Adicionalmente, o alelo *HLA-B*22* foi associado a progressão rápida da infecção (Gao et al., 2005). As variantes *HLA-B*07:02* e *HLA-B*08:01* são sugeridas com de risco em pacientes caucasoides infectados pelo subtipo B do HIV-1 (International HIV Controllers Study et al., 2010).

Os alelos *HLA* de classe I são ainda divididos em alótipos com base na sequência de aminoácidos presente nos resíduos 77 e 80-83 da hélice alfa 1, determinando assim os agrupamentos Bw4 (*A*09, A*23:9, A*24:9, A*24:03, A*25:10, A*32:19, B*05:102, B*05:103, B*13, B*17, B*27, B*37, B*38:16, B*44:12, B*47, B*49:21, B*51:05, B*52:05, B*53, B*57:17, B*58:17, B*59, B*63:15, B*77:15*) e Bw6 (*B*07:03, B*08, B*14, B*18, B*22, B*27:08, B*35, B*39:16, B*39:01, B*39:02, B*40, B*40:05, B*41, B*42, B*45:12, B*46, B*48, B*50:21, B*54:22, B*55:22, B*56:22, B*60:40, B*61:40, B*62:15, B*64:14, B*65:14, B*67, B*70, B*71:70, B*72:70, B*73, B*75:15, B*76:15, B*78, B*81, B*82*) que interagem com receptores de imunoglobulinas das células natural killer (KIR) (Goulder & Walker, 2012; Robinson et al., 2015).

Dentre estes, o agrupamento Bw6 pare estar mais associado a rápida progressão da infecção (Welzel et al., 2007; Jiang et al., 2013), e em maior associação quando em homozigose (Qing et al., 2006). Porém, em uma coorte australiana, a homozigose do Bw4 parece prever uma recuperação prejudicada dos linfócitos T $CD4^+$ após o início da terapia antirretroviral combinada (Rauch et al., 2008).

Para o lócus DRB1, a variante *DRB1*13:02* foi associada a cargas virais elevadas, enquanto *DRB1*12:01* apresentou valores significativamente menores e restringiu a resposta das células T $CD4^+$ p24-específicas em peruanos (Oriol-Tordera et al., 2017). Em chineses, análises de regressão logística revelaram que as variantes *DRB1*01:01* e *DRB1*15:01* foram apontados como genes de resistência da infecção pelo HIV-1 (Li et al., 2017), no entanto, há controvérsias quando confrontado com os resultados obtidos em usuários de drogas infectados, da mesma etnia, cuja variante *DRB1*15:01* de fato foi apontada como um fator de resistência à infecção, porém, a variante *DRB1*01:01* como um fator de suscetibilidade (Diao et al., 2014). Em chineses Han, haplótipos contendo os alelos *HLA-DRB1*09, HLA-DRB1*08, HLA-DRB1*15, HLA-DRB1*04* e *HLA-DRB1*12* foram super-representados em pacientes com HIV-1 (Huang et al., 2009).

A proteína Nef do HIV regula negativamente a expressão de *HLA-DR* e favorece a regulação da cadeia invariante associada à forma imatura do *HLA* de classe II durante a infecção aguda pelo HIV (Mahiti et al., 2015). No entanto, um estudo revelou que as células T $CD4^+$ de memória que expressam *HLA-DR* foram detectadas tanto em pacientes com infecção aguda quanto os crônicos em terapia prolongada. Além disso, a proporção de células HLA-DR^+ infectadas aumentou após 15 anos de terapia e expressaram níveis mais elevados de marcadores de ativação, exaustão e proliferação celulares em comparação com sua contraparte HLA-DR^- (Lee et al., 2019).

1.5.5.2 Alelos dos lócus *A, B* e *DRB1* associados à proteção e controle da progressão da infecção pelo HIV

A influência do sistema *HLA* no efeito clínico garante ajudar na investigação e entendimento da patogênese da doença, o que pode auxiliar na geração de uma vacina efetiva. A característica de heterozigose para os alelos *HLA* de classe I é considerada como uma vantagem seletiva contra a progressão para a AIDS devido a habilidade do hospedeiro em apresentar um maior repertório de antígenos virais, desenvolvendo, dessa forma, uma resposta mais ampla por linfócitos T. Isto dificulta a rápida emergência de mutantes que pudessem escapar da resposta imunológica, o que prolonga consideravelmente o período antes do desenvolvimento da síndrome (Kaur & Mehra, 2009; Zhang et al., 2011; Peterson et al., 2013).

O primeiro grande estudo populacional mostrando o papel modulador dos alelos de *HLA* de classe I na infecção pelo HIV revelou uma relação entre os perfis de *HLA* e o tempo de desenvolvimento até a AIDS, com os alelos *HLA-B*27* e *HLA-B*57* tendo um grande impacto na diminuição da velocidade de progressão da doença (Kaslow et al., 1996). Dados sequentes demostram que 28-40% dos pacientes infectados pelo HIV-1 que prolongaram em 10 ou mais anos à AIDS eram heterozigotos para todos os loci *HLA* de classe I (Carrington et al., 1999).

Similarmente, um estudo realizado em 2009 investigou o papel da transmissão materna de vírus mutantes em mulheres portadoras do alelo protetor *HLA-B*57*. Os pesquisadores encontraram que as crianças $B*57^+$ nascidos de mães $B*57^+$ podiam controlar a viremia e também possuíam partículas virais com mutações atenuadas dentro do epítopo viral TW10-Gag. Por outro lado, os $B*57^-$ nascidos de mães $B*57^+$ progrediram rapidamente à AIDS (Schneidewind et al., 2009).

Novamente nos casos de transmissão vertical, Goulder e colaboradores reportaram que crianças *HLA-* $B*27^+$ nascidas de mães que também eram *HLA-* $B*27^+$ não apresentaram bom

prognóstico clínico. Em contraste, aqueles que herdaram este alelo de seus pais mostraram-se progressores lentos ou não progressores (Goulder et al., 2001).

Outros estudos que sugerem alelos no lócus *B* como protetores incluem: o alelo *HLA-B*52* em japoneses (Chikata et al., 2017); *HLA-B*44* e *HLA-B*53* em crianças africanas (Mekue et al., 2019); *HLA-B*42* e *HLA-B*81* em adultos africanos (Ogunshola et al., 2018); e o *HLA-B*13* em uma coorte multicêntrica prevalentemente caucasoide (Carlson et al., 2012).

Dentre os estudos que relacionam os alelos do lócus *A* à proteção contra a infecção, observamos que o *HLA-A*02* mostra um efeito protetor em chineses Han (Li et al., 2017). De fato, já havia sido sugerido que a eficácia da vacina RV144 anti-HIV-1 foi maior nos portadores do alelo *HLA-A*02* (Gartland et al., 2014). Entretanto, ao alelo representou um padrão oposto em uma coorte espanhola, sendo associado a suscetibilidade a infecção (Rallón et al., 2017).

O haplótipo *HLA-A*30~B*13~C*06* foi associado a progressão mais lenta da infecção em chineses Han, provavelmente devido uma resposta citotóxica apropriada anti-*Gag* nessa população (Zhang et al., 2015). O *HLA-A*74:01* pode estar influenciando na aquisição do HIV-1 em uma coorte da África Oriental (Koehler et al., 2010). Enquanto que o alelo *HLA-A*03:01* parece estar mais frequente em mexicanos não infectados pelo HIV-1 (Valenzuela-Ponce et al., 2018).

Um controle imunológico restrito à classe II também é sugerido na infecção crônica pelo HIV, uma resposta antiviral de linfócitos TCD8$^+$ que expressam *HLA* de classe II foi mostrada ocorrer naturalmente ao longo da infecção (Ranasinghe et al., 2016). Porém, ainda há controvérsias quanto ao papel do *HLA* de classe II em eventos de pressão seletiva que levam a atenuação do HIV (Wright et al., 2012). Em um estudo de caracterização imunológica da GALT, pacientes controladores exibiram células T predominantemente expressando *HLA-DR*. Este fenótipo, previamente associado a um melhor controle da infecção, foi correlacionado com baixa carga viral e maior contagem de células T CD4. Além disso, uma correlação positiva deste fenótipo com maior expressão dos fatores de transcrição Foxp3 e RORγT sugeriu um papel chave para as células T reguladoras e Th17 na manutenção da ativação imune e integridade da mucosa intestinal (Gonzalez, et al., 2016).

Em indivíduos de descendência caucasiana europeia o alelo *HLA-DRB1*15:02* foi associado à baixa viremia e a capacidade de apresentar promiscuamente uma maior amplitude de peptídeos com menor avidez funcional quando comparado aos alelos *HLA-DRB1* ligados à alta avidez (Ranasinghe et al., 2013). O alelo *HLA-DRB1*13* parece estar associado a proteção contra o câncer cervical em pacientes sul africanas coinfectadas HIV/HPV (Chambuso et al., 2019); aos níveis reduzidos da carga viral do HIV-1, novamente, em sul africanos (Julg et al.,

2011); e a ativação celular em mulheres norte americanas coinfectadas pelo Hepacivírus C (Kovacs et al., 2020); embora o alelo tenha sido observado em menor frequência em argentinos soronegativos quando comparado aos infectados (Motta et al., 2002). O alelo *HLA-DRB1*01:01* foi associado a proteção em chineses Han (Li et al., 2017). Em pacientes sul africanos o alelo *HLA-DRB1*11:01* foi associado a proteção contra a tuberculose no contexto de coinfecção com o HIV, mesmo para indivíduos com a contagem de células T $CD4^+$ abaixo de 350 células/mm^3 (Seedat et al., 2021).

1.5.5.3 Alelos dos lócus *A, B* e *DRB1* associados ao risco e agravamento da infecção pelo EBV

No que diz respeito à infecção pelo EBV, estudos já associaram loci genéticos dentro da região HLA ao desenvolvimento de mononucleose infecciosa, sugerindo que a presença de polimorfismos na região *HLA* de classe I influencia na resposta imunológica por linfócitos T $CD8^+$ durante a infecção primária por EBV e persistência do vírus (Mcaulay et al., 2007). Em um estudo *ex vivo*, a manutenção de uma resposta anti-EBV equilibrada foi associada ao alelo *HLA-B*08* restrito à proteína precoce BZLF1 (Silins et al., 1997), porém, a resposta restrita do alelo *HLA-A*02:01* a epítopos de proteínas da fase lítica viral parece instável e associada a expressão de marcadores de morte celular (Sauce et al., 2009).

Marcadores presentes no loci *HLA* de classe I, incluindo SNPs *rs2530388* e *rs6457110*, estão associados ao risco do desenvolvimento de Linfoma de Hodgkin, relacionado à infecção pelo EBV. Os presentes SNPs apresentam forte desequilíbrio de ligação com alelos *HLA-A*, em que se evidenciou o alelo *HLA-A*01* relacionado ao aumento do risco de Linfoma de Hodgkin associado a infecção pelo EBV (Diepstra et al., 2005; Niens et al., 2007). Dessa forma, pacientes *HLA-A*01* homozigotos, apresentam 10 vezes mais chance de desenvolver Linfoma associados ao EBV, quando comparado com pacientes *HLA-A*02* homozigotos, demostrando a mais forte relação entre infecção por um vírus e genes HLA no desenvolvimento de um processo maligno (Mcaulay et al., 2007). Parte desse processo decorre da ineficaz apresentação de antígenos por células *HLA-A*01*$^+$ (Brennan & Burrows, 2008) e outros estudos sugerem que essa associação também pode ocorrer independente do histórico de infecção pelo EBV (Johnson et al., 2015).

Em uma coorte prevalentemente caucasoide inglesa, o alelo *HLA-A*26* foi associado a maior incidência de transtorno linfoproliferativo pós-transplante (DLPT) em pacientes soropositivos para o EBV (Reshef et al., 2011), no entanto, em uma população também

caucasoide, porém, com maior influência europeia ocidental, o DLPT esteve mais associado ao alelo *HLA-A*03* e independente da sorologia do EBV (Wheless et al., 2008).

Um estudo desenvolvido em Singapura mostrou que o haplótipos *HLA-A*02~B*46* está associado com aumento no risco relativo do desenvolvimento de carcinoma de nasofaringe em infectados pelo EBV. Somado a isso, o risco relativo apresentou-se maior, quando ambos os alelos ocorreram simultaneamente (Chan et al., 1983; Li et al., 2009). Em chineses foi observado que os alelos *HLA-A*02:11* e *HLA-A*11:01* foram associados a perfis clínicos indicativos da infecção pelo EBV em pacientes que receberam transplante de células tronco hematopoiéticas haploidênticas (Zhou & Lu, 2019).

Não limitado ao carcinoma, em pacientes com esclerose múltipla infectados pelo EBV há relatos de associações da carga viral aos alelos *HLA-B*07* e *HLA-DRB1*15*, enquanto nos portadores da variante *HLA-A*02* a carga viral foi menor, sem diferenças nos títulos de anticorpos anti-EBV em relação às variantes de *HLA* (Agostini et al., 2018).

A associação de polimorfismos e o status sorológico para o EBV já foi estabelecida. Uma associação significativa entre o perfil sorológico para o EBV e os SNPs *rs477515/rs2516049*, localizado no gene *HLA-DRB1*, na qual, o nível de expressão *de HLA-DRB1* foi significativamente relacionado a soropositividade direcionada a proteína EBNA-1 do EBV. No mesmo estudo, também se evidenciou que embora os genes *HLA-DRA* e *HLA-DRB9* parecem ser associados significativamente com SNPs no gene EBNA-1, seus níveis de expressão não são significativamente correlacionados com anticorpos anti-EBNA-1. No entanto, os presentes genes citados apresentam-se como fortes candidatos a susceptibilidade ao EBV (Rubicz et al.,2013).

1.5.5.4 Alelos dos lócus *A, B* e *DRB1* associados à proteção e controle da progressão da infecção por EBV

São poucos os relatos de alelos do *HLA* associados a proteção contra a infecção por EBV. Em holandeses, o alelo *HLA-A*02* foi considerado um fator protetor contra o linfoma de Hodgkin clássico exclusivamente em pacientes infectados (Huang et al., 2011); uma modesta resposta celular anti-LMP2A e EBNA3A/3B/3C foi atribuída ao alelo (Jones et al., 2016). Em outra coorte de prevalência caucasoide, foi atribuído o papel protetor do subconjunto de alotipos Bw4 contendo isoleucina na posição 80 (Bw4 80Ile) contra a infecção primária por EBV (Durovic et al., 2013).

Foi mostrado em um estudo australiano que o alelo *HLA-B*18:01* apresenta de forma específica um epítopos derivado do EBV que consegue ativar uma resposta imunológica sustentável, porém, com provável reatividade cruzada para mimetopos humanos (Rist et al., 2015). Em italianos, epítopos do gene EBNA-3 são apresentados de forma eficaz pelos alelos *HLA-B*07:02* e *HLA-B*27:05* em pacientes com espondilite anquilosante, porém, a resposta imunológica induzida é ambígua ao passo que contribui para a capacidade de proteção contra patógenos e predispõem à autoimunidade (Tedeschi et al., 2016). O alelo *HLA-B*35* também foi associado a manutenção de uma resposta imunológica efetiva contra antígenos derivados do gene EBNA-3 (Dragon et al., 2020).

1.6 JUSTIFICATIVA

Dessa forma, o conjunto de estudos publicados sugerem que a identificação de fatores epidemiológicos, perfis imunológicos e perfis genéticos dos alelos do *HLA* de classe I e II relativo as infecções por HIV e EBV auxiliam no entendimento da prevalência em grupos sociais específicos, nas diferenças na elaboração das repostas imunes e na influência direta na apresentação de epítopos e, consequentemente, na montagem de uma resposta eficaz. Tais fatos contribuem no controle das infeções por EBV e HIV, levando a diferenças individuais na transmissão, suscetibilidade e principalmente na progressão das infecções. Porém, observamos uma carência de estudos de associação desses marcadores às infecções por HIV e EBV na região amazônica brasileira; avaliar esses perfis nessa população permitirá abranger aspectos da variabilidade regional ao notório conhecimento sobre as relações parasita hospedeiro no contexto de prevalência, resposta imune e predisposição genética.

1.7 OBJETIVOS

1.7.1 Objetivo Geral

Avaliar aspectos multifatoriais relativos a epidemiologia, perfis imunológicos e a associação dos lócus *A, B* e *DRB1* dos genes *HLA* de classe I e II nas infecções por HIV e EBV, buscando identificar possíveis marcadores vinculados às infecções em uma população da região amazônica brasileira.

1.7.2 Objetivos Específicos

- Determinar a prevalência de casos de infecção e coinfecção por HIV e EBV em indivíduos da região amazônica brasileira em um dado período;
- Identificar fatores epidemiológicos e aspectos clínicos associados as infecções e coinfecções por HIV e EBV em indivíduos da região amazônica brasileira;
- Identificar perfis imunopatológicos das infecções e coinfecções por HIV e EBV com base nos dados de carga viral, concentração de linfócitos T e a dosagem de citocinas da resposta imunológica;
- Relacionar os perfis imunopatológicos das infecções e coinfecções por HIV e EBV com agravamentos clínicos e atraso na busca imediata por atendimentos específicos;
- Investigar o grau de diversidade genética dos lócus *A, B* e *DRB1* do *HLA* de classe I e II em indivíduos da região amazônica brasileira;
- Associar a frequência alélica dos lócus *A, B* e *DRB1* do *HLA* de classe I e II às infecções e coinfecções por HIV e EBV;
- Relacionar os alelos associados as infecções e coinfecções por HIV e EBV a carga viral, a concentração de linfócitos T e a perfis de citocinas da resposta imunológica;
- Relacionar os alelos associados a infecção por HIV com as variações longitudinais dos marcadores imunológicos e virais de progressão da infecção, tal como, as alterações na dispensação de esquemas terapêuticos.

2. APRESENTAÇÃO DOS ARTIGOS

Nesta tese são apresentados três artigos e uma propostas de manuscrito à publicação que tratam dos aspectos epidemiológicos, imunológicos e imunogenéticos da infecção por HIV e EBV em pacientes do estado do Pará.

No primeiro manuscrito identificamos os fatores de risco epidemiológicos relacionados a infecção primária por EBV em pacientes coinfectados por HIV; conjuntamente, estreitamos as relações desses fatores com os marcadores virológicos das infecções ativas e avaliamos se o tempo de atraso na busca por atendimentos específicos (apresentação tardia) poderiam estar relacionados aos aspectos comportamentais dos pacientes. Também avaliamos perfis clínicos de mono e coinfectados e sua relação com a apresentação tardia.

No segundo manuscrito mostramos que os genótipos do EBV foram associados a diferentes perfis imunológicos nos pacientes com infecção primária coinfectados por HIV.

Neste estudo também sugerimos qual o extrato sanguíneo mais adequado para a quantificação da carga viral na infecção primária por EBV e a relação entre os marcadores imunológicos e virais com a sintomatologia e o tempo de apresentação tardia.

No terceiro manuscrito propomos a associação dos alelos *B*13, B*35* e *B*39* do lócus *B* no *HLA* de classe I a não eficácia da resposta terapêutica no que tange a reconstituição imunológica em pacientes infectados por HIV. Além do mais, inferimos um grau de homologia entre diferentes grupos étnicos com base na similaridade das frequências alélicas observadas.

No quarto manuscrito sugerimos que os alelos *DRB1*03, DRB1*09* e *DRB1*16* do lócus *DRB1* no *HLA* de classe II foram associados à manutenção da resposta imunológica em diferentes perfis sorológicos da infecção por EBV. Também avaliamos quais aspectos clínicos e epidemiológicos estavam associados aos perfis de infecção e os relacionamos com os alelos de risco. Uma provável relação entre o perfil genético com a genotipagem do EBV foi discutida.

Os estudos foram desenvolvidos em colaboração com os laboratórios do vírus Epstein Barr e Retrovírus da seção de virologia do Instituto Evandro Chagas, tal como, estreitamos parceria com o departamento de Imunogenética da Fundação de Hemoterapia e Hematologia do estado do Pará, tendo o aluno participado ativamente de todos os processos metodológicos.

2.1 PRIMEIRO MANUSCRITO

Título: Epidemiological risk factors associated with primary infection by Epstein–Barr vírus in HIV-1-positive subjects in the Brazilian Amazon region.

Lista de autores: Leonn Mendes Soares Pereira, Eliane dos Santos França, Iran Barros Costa, Igor Tenório Lima, Amaury Bentes Cunha Freire, Francisco Lúzio de Paula Ramos, Talita Antonia Furtado Monteiro, Olinda Macedo, Rita Catarina Medeiros Sousa, Felipe Bonfim Freitas, Igor Brasil Costa & Antonio Carlos Rosário Vallinoto.

Status da publicação: Publicado.

Nome da revista: Scientific Reports.

ISSN: 2045-2322

Fator de impacto: 4.380 (Qualis A1)

DOI: | https://doi.org/10.1038/s41598-021-97707-4

www.nature.com/scientificreports

scientific reports

OPEN

Epidemiological risk factors associated with primary infection by Epstein–Barr virus in HIV-1-positive subjects in the Brazilian Amazon region

Leonn Mendes Soares Pereira[1], Eliane dos Santos França[2], Iran Barros Costa[2], Igor Tenório Lima[2], Amaury Bentes Cunha Freire[3], Francisco Lúzio de Paula Ramos[3], Talita Antonia Furtado Monteiro[2], Olinda Macedo[4], Rita Catarina Medeiros Sousa[2,3], Felipe Bonfim Freitas[4], Igor Brasil Costa[2,6,7] & Antonio Carlos Rosário Vallinoto[1,4,7✉]

To identify the prevalence and risk factors for primary Epstein–Barr virus (EBV) infection in human immunodeficiency virus (HIV)-1-positive adult treatment-naïve patients between January 2018 and December 2019 in a state of the Brazilian Amazon region. A total of 268 HIV-1 positive patients and 65 blood donors participated in the study. Epidemiological data were obtained from medical records and through a designed questionnaire. EBV infection was screened by the semiquantitative detection of anti-viral capsid antigen (VCA) EBV IgM and IgG, followed by molecular detection of the EBNA-3C gene. The plasma viral loads of HIV-1 and EBV were quantified using a commercial kit. The prevalence of primary coinfection was 7.12%. The associated risk factors were education level, family income, history of illicit drug use and sexually transmitted infections, homosexual contact and condom nonuse. Approximately 58.5% had late initiation of highly active antiretroviral therapy, which influenced the risk of HIV-EBV 1/2 multiple infection (odds ratio (OR): 4.76; 95% CI 1.51–15.04) and symptom development (p = 0.004). HIV viral load was associated with patient age (OR: 2.04; 95% CI 2.01–2.07; p = 0.026) and duration of illicit drug use (OR: 1.57; 95% CI 1.12–2.22; p = 0.0548). EBV viral load was associated with younger age (OR: 0.82; 95% CI 0.79–1.03; p = 0.0579). The replication of both viruses was associated with symptom development (HIV = OR: 2.06; 95% CI 1.22–3.50; p = 0.0073; EBV = OR: 8.81; 95% CI 1–10; p = 0.0447). The prevalence of HIV/EBV coinfection was lower than that observed in other studies, and social vulnerability and promiscuous sexual behavior were associated risk factors. A long time of HIV-1 infection, without therapy, influenced the risk of coinfection and disease progression. The viral loads of both viruses may be associated with some epidemiological aspects of the population.

The Epstein–Barr virus (EBV), also known as human gammaherpesvirus 4 (HHV4), belongs to the family *Herpesviridae*, genus *Lymphocryptovirus*[1,2]. Two EBV genotypes are classically known, EBV-1 and EBV-2, whose genetic differences lie in the sequence encoding the viral nuclear antigens (EBNA2, -3A, -3B and -3C)[3,4]. Both genotypes have a cosmopolitan distribution, although EBV-1 is more prevalent, especially in Western countries and Asia[5], while EBV-2 is more prevalent in Central African countries[6].

In developed countries, EBV infection is common in adolescence, ranging from 0 to 70% in childhood and reaching more than 90% in adulthood; in the latter age group, primary infection is clinically symptomatic[7–9]. In

[1]Laboratory of Virology, Institute of Biological Sciences, Federal University of Pará, Belém, Pará, Brazil. [2]Epstein-Barr Virus Laboratory, Virology Section, Evandro Chagas Institute, Ananindeua, Pará, Brazil. [3]Department of Epidemiology and Surveillance, Evandro Chagas Institute, Ananindeua, Pará, Brazil. [4]Laboratory of Retroviruses, Evandro Chagas Institute, Virology Section, Ananindeua, Pará, Brazil. [5]School of Medicine, Federal University of Pará, Belém, Pará, Brazil. [6]Graduate Program in Biology of Infectious and Parasitic Agents, Institute of Biological Sciences, Federal University of Pará, Belém, Pará, Brazil. [7]These authors jointly supervised this work: Igor Brasil Costa and Antonio Carlos Rosário Vallinoto. ✉email: vallinoto@ufpa.br

developing countries, primary EBV infection occurs in early childhood and is usually asymptomatic, reaching 97% seroprevalence in adulthood in heterogeneous populations, a value that is similar at the global scale[10,11]. In these countries, there is an alert for seroprevalence in children and adolescents with low socioeconomic status[12] and for the predominance of infection in individuals with suspected coinfection with human immunodeficiency virus (HIV)-1[13].

Because it is a precursor of lymphatic neoplasms, EBV is identified as one of the factors that contributes to the morbidity of patients infected with HIV-1[14–16]. Coinfection is related to progressive dysfunction events and impaired immune surveillance caused by HIV that favor persistent pathogens[17]. This factor becomes critical when the infection progresses to AIDS, in which severe immunosuppression results in clinical signs and symptoms related to malignant neoplasms[18,19].

Although most studies focus on the pathological basis of viral carcinogenesis, it is still necessary to understand aspects of primary EBV infection in the context of coinfection. In this sense, EBV coinfection has been studied in HIV-1-seropositive children, in whom EBV DNA was isolated from the oral mucosa[20] and blood tissue[21,22]. It is assumed that due to the high incidence of EBV infection during the first 5 years of life, maternal transmission is an important route in maintaining coinfection[21,23]. Additionally, microbial translocation and persistent immune activation induced by HIV are factors that may influence EBV replication and the expansion of infected B cells[22].

In adults, exposure through unprotected sex can favor HIV/EBV coinfection[24]. However, there is controversy regarding the detection of EBV in different HIV-1 infection profiles[25]. A recent study reports higher than 68% primary EBV infection in patients with HIV not on highly active antiretroviral therapy (HAART)[26], similar to the rate observed in patients on the HAART regimen[27]. These findings indicate the complexity of the relationship between viruses that can be attributed to intrinsic factors of coinfection, regardless of the therapeutic status of the patient[28].

There are few scientific studies on the epidemiology of HIV/EBV coinfection in the Brazilian Amazon and northern region of the country. Most research is focused on the carcinogenic effects of viral interactions[29,30]. To date, we have only found 1 study, by Jacome-Santos et al., that reported the overall prevalence of coinfection by strictly analyzing the presence of EBV in the oral mucosa of people living with HIV (PLHIV)[31]. Thus, the aim of the present study was to describe the prevalence of primary EBV infection in HIV-positive adult therapy-naïve and to identify the epidemiological characteristics associated with this profile.

Materials and methods

Sample characterization and ethical aspects. This was an observational, cross-sectional and analytical study conducted in mutual collaboration between the Laboratory of Virology of the Federal University of Pará (LABVIR-UFPA), the Evandro Chagas Institute (IEC) and the Hemotherapy and Hematology Foundation of Pará (HEMOPA), with the selection of participants who regularly resided in municipalities of the state of Pará, Brazil, from January 2018 to December 2019, originating from the Center for Health Care in Acquired Infectious Diseases (CASA DIA), HEMOPA and IEC.

All participants were clinically evaluated at the respective referral centers and subjected to complementary investigation via serological and molecular biology tests, based on which the participants were categorized as follows: 249 HIV-1 monoinfected patients (p24+; anti-HIV-1 IgG+); 19 HIV-1-positive patients with primary EBV infection (p24+; anti-HIV-1 IgG+; anti-viral capsid antigen (VCA)-EBV IgM+; anti-VCA-EBV IgG−; confirmation by molecular biology); and 65 uninfected individuals (volunteer blood donors, based on a screening panel of blood banks defined by ministerial ordinance[32]).

Epidemiological data, such as sex, age, place of residence, and clinical anamnesis of patients, were obtained from clinical records and an epidemiological questionnaire administered at the time of care. In compliance with resolutions 466/2012 and 347/05 of the Brazilian National Health Council, which establishes guidelines and regulatory standards for research involving human beings, the project proposal was submitted for ethical review and approved by the Human Research Ethics Committee of IEC (Protocol n. 3.121.265; CAAE n. 73927717.3.0000.0019). All participants were informed about the study objectives and signed an informed consent form. The collected biological samples were stored in a biorepository until use.

Participants included in the study were 18 years of age or older, of both sexes, carriers of HIV-1 and/or EBV, antiretroviral therapy-naïve and signed the informed consent form. Participants who did not meet the inclusion criteria or who were on antiviral therapy before sample collection were excluded.

Collection, extraction and confirmatory methods. Five milliliters of peripheral blood was collected into vacuum tubes containing EDTA as an anticoagulant. DNA was extracted from whole blood following the protocol provided with the QiaAmp DNA Mini Kit (Qiagen, Germany).

HIV-1 infection was screened by qualitative and simultaneous detection of p24 antigen and anti-HIV-1 and anti-HIV-2 IgG antibodies by enzyme immunoassay (Murex HIV Ag/Ab Combination, DiaSorin, UK); serological confirmation was performed using an Immunoblot rapid DPP HIV-1/2 kit (Bio-Manguinhos, FIOCRUZ) following the manufacturer's recommendations. The samples from CASA DIA did not require complementary tests for the diagnosis of HIV-1 because the institution has its own screening panel, to which patients enrolled in the institution are subjected.

Infection by EBV was screened by semiquantitative detection of anti-EBV IgM and IgG antibodies by enzyme immunoassay (Ridascreen EBV VCA R-Biopharm, Germany) following the manufacturer's recommendations. The identification of EBV genotypes was performed by nested PCR, targeting the EBNA-3C gene, using the primers described by Lorenzetti et al.[33] ((1st round) (F: 5′-AGATGGTGAGCCTGACGTG-3′/R: 5′-GCATCC TTCAAAACCTCAGC-3′)) and by Sample et al.[3] ((2nd round) (F: 5′-AGAAGGGGAGCGGTGTGTTGT-3′/R: 5′-GGCTGTTTTTGACGTCGGC-3′)) and following the recommended conditions: primers (10 pmol/µL);

$MgCl_2$ (50 mM); dNTP (10 mM); and Taq (5U/μL); 1st round cycles—1 cycle of 95° C/3′, 20 cycles of (94 °C/45″; 56 °C/45″; 72 °C/45″), 1 cycle of 72 °C/7′; and 2nd round cycles—1 cycle of 95° C/3′; 35 cycles of (94 °C/45″; 56 °C/45″; 72 °C/45″); 1 cycle of 72 °C/7′. The presence of a 153-bp fragment was considered positive for EBV-1, and the presence of a 246-bp fragment was considered positive for EBV-2. The positive EBV-1 and EBV-2 controls were isolated from the B95 and P3HP1 lymphoid cell lines, respectively.

Quantification of HIV-1 and EBV viral load. The HIV-1 plasma viral load was quantified by real-time PCR using an Abbott mSample Preparation System RNA extraction kit and an Abbott Real-Time HIV-1 amplification reagent kit (Abbott, Chicago, Illinois, USA) following the manufacturer's recommendations.

The EBV viral load was quantified by real-time PCR following the protocol provided with the XGEN Master EBV kit (Mobius Life Science, Pinhais, Paraná, Brazil). Plasma samples were used to quantify the viral load.

Statistical analysis. The epidemiological data were compared among the study groups using the G test. Fisher's exact test was applied exclusively in comparisons whose data were arranged in 2 × 2 contingency tables.

For significant comparisons, the degree of dependence between the epidemiological data and the study groups was calculated using simple logistic regression for 1 variable and multiple logistic regression for all variables, in which epidemiological data were included as independent variables and the presence or absence of infections as dependent variables. Due to the statistical similarity between the epidemiological profile of HIV-1 monoinfected and HIV-1/EBV coinfected patients, we assumed the presence of monoinfection or coinfection as success (1) and the absence of both as nonsuccess (0). The epidemiological factors that maintained statistical significance in the multiple regression were considered risk factors for monoinfection or coinfection.

We also calculated the dependence of the increase in viral load on the epidemiological and behavioral variables of patients. In this context, we calculated the mean viral load values and classified them, for HIV, into low (0), when between undetectable and up to 10,000 RNA copies and high (1), when greater than 10,000 copies, and, for EBV, into low (0), when between undetectable and up to 45 DNA copies, and high (1), when higher than 45 copies. The quantitative epidemiological variables were included in the function as nonbinary data, and exclusively for the length of illicit drug use, we discarded patients with noncontinuous sporadic use and grouped the remaining time of use into less than 1 year, 1 to 5 years, or greater than 5 years.

The G test was applied in other analyses of categorical data, such as in the comparison of the profile of sexually transmitted infections (STIs); behavioral changes according to the HIV-1 infection time (since diagnosis) and symptomatology. We used curve fitting to determine the frequency distribution model of cases of monoinfection and coinfection according to the collection time.

All statistical analyses were performed using the programs GraphPad Prism 3.03 (San Diego, CA, USA) and Bioestat 5.0[34].

Ethics approval and consent to participate. All methods and experimental protocols were carried out in accordance with regulations 466/2012 and 347/05 of the Brazilian National Health Council and were approved by the Human Research Ethics Committee of Instituto Evandro Chagas (Protocol n. 3.121.265; CAAE n. 73927717.3.0000.0019). All participants were informed about the study objectives and signed an informed consent form. The collected biological samples were stored in a biorepository until use.

Results

We detected primary EBV infection in only 19 of the 268 HIV+ patients evaluated (7.12%) (Table 1) and did not detect the presence of primary EBV infection in blood donor samples. The frequency of coinfection cases increased linearly throughout the study, approximately sixfold per month of collection ($p = 0.0581$; R^2: 0.476), although the increase in HIV-1 monoinfection was slight ($p = 0.1394$; R^2: 0.326) (Fig. 1A). The prevalence of coinfection cases was higher in the group of patients 18 to 28 years of age, specifically in men; the number of infected women stood out only in the group of patients 29 to 39 years of age (Fig. 1B). The EBV-1 genotype was the most prevalent among the coinfected patients (47.37%); EBV-2 and multiple infection by the 2 genotypes (EBV-1/2) occurred at the same frequency (26.32%) (Fig. 1C).

The epidemiological profiles of patients monoinfected with HIV-1 and coinfected with HIV/EBV were statistically similar; therefore, we grouped them for comparison with the noninfected group. Evaluating the variables individually, we observed that age was a risk factor for monoinfection or coinfection, being prevalent in the groups of young patients between 18 and 39 years of age ($p = 0.0078$; odds ratio (OR): 1.57; 95% CI 1.20–2.05). Only a history of cigarette use ($p = 0.0013$; OR: 2.95; 95% CI 1.64–5.30) and illicit drug use ($p < 0.0001$; OR: 24.53; 95% CI 3.34–180.14) were considered risk factors; present use was not relevant because most individuals claimed to have ceased these practices. Most aspects related to sexual practice and sexuality were associated with monoinfection or coinfection: history of STIs ($p < 0.0001$; OR: 14.05; 95% CI 4.29–45.95) and homosexual contact ($p < 0.0001$; OR: 10.87; 95% CI 4.32–27.34) had the highest probability ratios, followed by lack of a steady partner ($p < 0.0001$; OR: 4.81; 95% CI 2.25–10.25) and occasional condom use ($p < 0.0001$; OR: 1.46; 95% CI 1.06–2.01). An active sex life was associated with the noninfected group ($p = 0.0110$; OR: 0.36; 95% CI 0.18–0.72), whereas biological sex, history of sexual contact with sex workers and first sexual contact were not associated with infected patients (Table 1).

In contrast, higher education levels and higher family income were considered protective factors against monoinfection and coinfection, with the majority of noninfected individuals having completed secondary education or higher ($p < 0.0001$; OR: 0.21; 95% CI 0.13–0.32) and showing a greater representation of incomes ranging from 4 to greater than 10 times the minimum wage ($p = 0.0418$; OR: 0.48; 95% CI 0.33–0.70). Notably, current alcohol consumption was more strongly associated with noninfected patients ($p = 0.0006$; OR: 0.29; 95% CI

Groups	HIV	HIV/EBV	Not infected	Simple logistic regression			Multiple logistic regression		
Factors	n: 249	n: 19 (7.12)	n: 65	p-values	OR	IC (95%)	p-values	OR	IC (95%)
Sex									
Female	49 (19.7)	6 (31.6)	17 (26.2)	NS*					
Male	200 (80.3)	13 (68.4)	48 (73.8)						
Age									
18–28	126 (50.6)	10 (52.6)	15 (23.1)	0.0078*	1.57	1.20–2.05	NS		
29–39	66 (26.5)	7 (36.8)	30 (46.2)						
40–50	40 (16.1)	1 (05.3)	10 (15.4)						
51–61	13 (05.2)	1 (05.3)	9 (13.8)						
62–72	4 (01.2)	0	1 (01.5)						
Education									
Not literate	1 (00.4)	0	0	<0.0001*	0.21	0.13–0.32	0.0028	0.06	0.01–0.04
Literate	34 (13.7)	2 (10.5)	0						
Elementary school 1	46 (18.5)	6 (31.6)	4 (06.6)						
Elementary school 2	131 (52.6)	9 (47.4)	21 (32.3)						
High school	36 (14.5)	2 (10.5)	26 (40.0)						
University education	1 (00.4)	0	14 (21.5)						
Family income									
Unknown	9 (03.6)	1 (05.3)	1 (01.5)	0.0418*	0.48	0.33–0.70	0.0002	0.45	0.27 – 0.67
(<1) salário	41 (16.5)	8 (42.1)	9 (13.8)						
(1–3) wage	180 (72.3)	10 (52.6)	37 (56.9)						
(4–6) wages	15 (06.0)	0	12 (18.5)						
(7–10) wages	2 (00.8)	0	3 (04.6)						
(> 10) wages	3 (01.2)	0	3 (04.6)						
Cigarette use									
Historic									
No	113 (45.4)	10 (52.6)	46 (70.8)	0.0013*	2.95	1.64–5.30	NS		
Yes	136 (54.6)	9 (47.4)	19 (29.2)						
Current usage									
No	101 (74.3)	6 (66.7)	17 (89.5)	NS*					
Yes	35 (25.7)	3 (33.3)	2 (15.5)						
Alcohol consumption									
Historic									
No	31 (12.4)	2 (10.5)	9 (13.8)	NS*					
Yes	218 (87.6)	17 (89.5)	56 (86.2)						
Present use									
No	109 (50.0)	10 (58.8)	13 (23.2)	0.0006*	0.29	0.15–0.58	NS		
Yes	109 (50.0)	7 (41.2)	43 (76.8)						
Illicit drug use									
Historic									
No	177 (71.1)	14 (73.7)	63 (96.9)	<0.0001*	24.53	3.34–180.14	0.0367	13.74	1.18–160.46
Yes	72 (28.9)	5 (26.3)	2 (03.1)						
Present use									
No	70 (97.2)	5 (100.0)	2 (100.0)	NS*					
Yes	2 (02.8)	0	0						
Routes of administration									
Oral	5 (06.9)	0	0	NS*					
Inhale	65 (90.3)	5 (100.0)	2 (100.0)						
Injectables	0	0	0						
Family history of cancer									
No	147 (59.0)	12 (63.2)	34 (52.3)	NS*					
Yes	101 (41.0)	7 (36.8)	31 (47.8)						
Sexual orientation									
Heterosexual	106 (42.6)	9 (47.4)	63 (97.0)	<0.0001*	10.87	4.32–27.34	0.0008	06.80	2.23–20.75
Homosexual	107 (43.0)	7 (36.8)	1 (01.5)						
Bisexual	36 (14.5)	3 (15.9)	1 (01.5)						
Continued									

Groups	HIV	HIV/EBV	Not infected	Simple logistic regression			Multiple logistic regression		
Factors	n: 249	n: 19 (7.12)	n: 65	p-values	OR	IC (95%)	p-values	OR	IC (95%)
Active sex life									
Yes	160 (64.3)	11 (57.9)	54 (83.1)	0.0110*	0.36	0.18–0.72	NS		
No	89 (35.7)	8 (42.1)	11 (16.9)						
First sexual contact									
No relationships	0	0	1 (01.6)	NS#					
Do not remember	1 (00.4)	0	0						
< 9 years	0	0	0						
10–15 years	115 (46.2)	13 (68.4)	16 (24.6)						
16–20 years	118 (47.4)	5 (26.3)	45 (69.2)						
21–25 years	14 (05.6)	1 (05.3)	3 (04.6)						
> 25 years	1 (00.4)	0	0						
Steady partner									
No	121 (48.6)	5 (26.3)	9 (13.8)	< 0.0001*	4.81	2.25–10.25	0.0349	2.87	1.00–2.92
Yes	128 (51.4)	14 (73.7)	56 (86.2)						
Relations with sex workers									
No	191 (76.7)	16 (84.2)	55 (84.6)	NS#					
Yes	58 (23.3)	3 (15.8)	10 (15.4)						
Condom use									
Occasionally	143 (57.4)	9 (47.4)	17 (27.0)	< 0.0001	1.46	1.06–2.01	0.0034	6.23	1.83–21.19
Never	30 (12.0)	4 (21.1)	25 (39.7)						
Always	76 (30.6)	6 (31.6)	21 (33.3)						
History of STIs									
No	148 (59.4)	8 (42.1)	62 (95.4)	< 0.0001*	14.05	4.29–45.95	0.0024	13.1	2.50–69.06
Yes	101 (40.6)	11 (57.9)	3 (04.6)						

Table 1. Description of socio-epidemiological aspects and risk factors associated with HIV/EBV co-infection. NS: not significant; *Fisher's exact test; #G Test.

0.15–0.58). A family history of cancer and route of illicit drug administration were not associated with infected patients; most individuals reported no cases of cancer in the family, and for patients with a history of illicit drug use, most ingested them (Table 1).

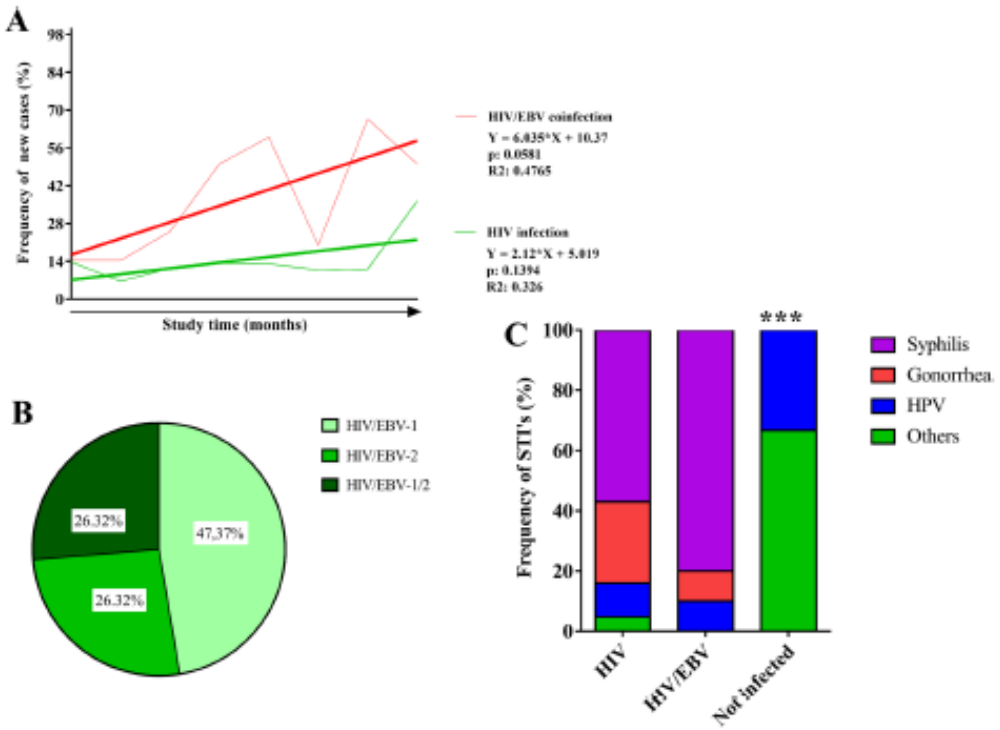
We defined the risk factors for monoinfection or coinfection based on the multiple logistic regression results. In this context, education level, family income, history of illicit drug use, history of STIs, sexual orientation, and presence of a steady partner and condom nonuse remained significant. History of illicit drug use was the strongest risk factor for monoinfection or coinfection ($p = 0.0367$; OR: 13.74; 95% CI 1.18–160.46), and high family income was the strongest protective factor ($p = 0.0002$; OR: 0.45; 95% CI 0.27–0.67) (Table 1).

We generated a risk/potential matrix of exposure to monoinfection or co-infection that compiled risk factors categorized as sexual or social; the color gradient was based on the frequency of cases screened by the intersection of social and sexual factors (Fig. 1D). The level of risk/exposure was higher in individuals who did not use condoms, used illicit drugs, had low education and low income. High-income individuals had a low level of risk/exposure only when related to non-promiscuous sexual behaviors. Heterosexual orientation was considered a low risk/exposure factor only when associated with not using illicit drugs or high education and high income.

We also investigated the time of HIV-1 infection since diagnosis in the monoinfected and coinfected groups. A considerable portion of the patients (48.42%) lived for less than 1 month with HIV-1 and sought specific care immediately after diagnosis; however, approximately 51.58% of the patients lived between 1 and more than 12 months with the virus and without the use of HAART. When asked, patients with a longer time since diagnosis reported initial skepticism of the infection. Their point of view changed with the onset of symptoms, including through advice from third parties (data not shown).

In an intergroup analysis, we observed that a time of HIV-1 infection greater than 12 months was more frequent in patients coinfected with HIV/EBV-1/2 (40%; $p = 0.0017$) and that the chance of multiple infections was approximately 5 times higher (OR: 4.76; 95% CI 1.51–15.04). Most patients in the HIV and HIV/EBV-2 groups had a recent infection diagnosis (< 1 month) (49% and 80%, respectively). A time of infection between 1 and 6 months (78%) prevailed among HIV/EBV-1 coinfected patients, but without statistical significance (Fig. 2A).

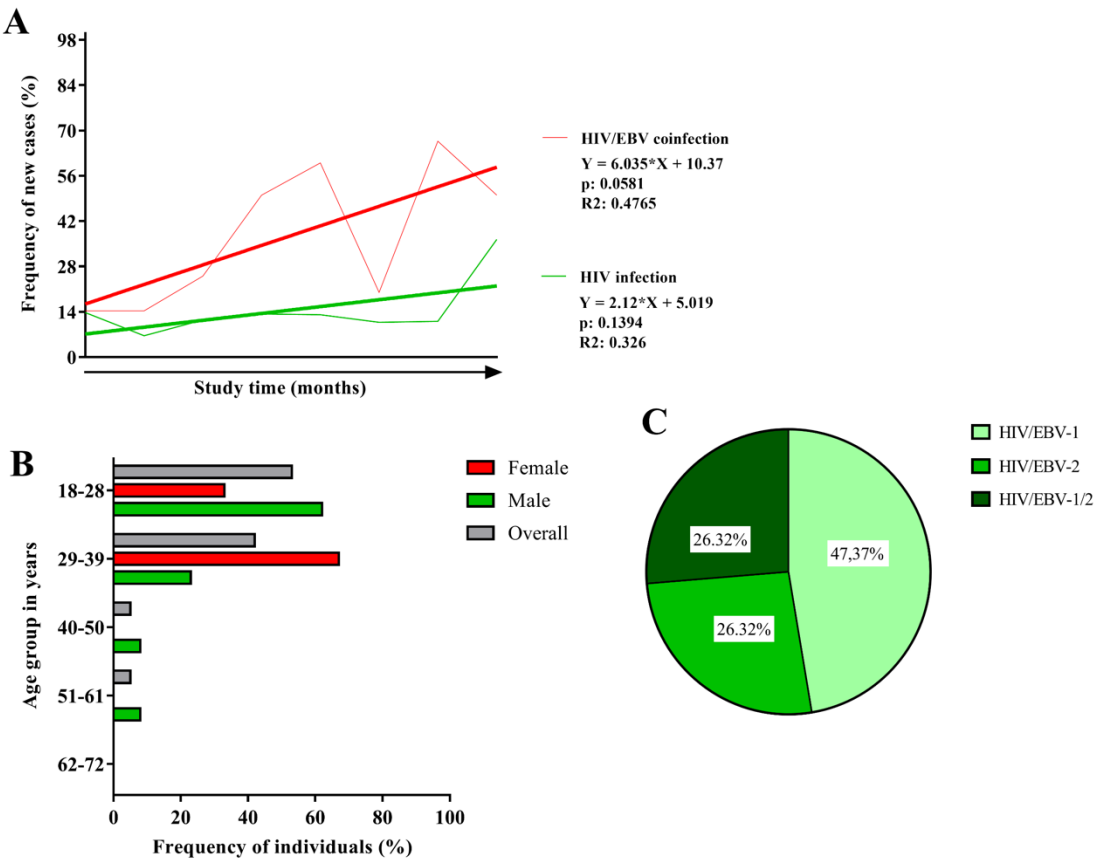
We evaluated the behavior of patients based on the time of HIV-1 infection since diagnosis (Fig. 2B). We did not observe significant differences in behavioral profiles; however, regarding sexual practice, we noted an increase in contact with sex workers for patients living longer with the infection; in contrast, the frequency of condom use after diagnosis, an active sex life, and the presence of a steady partner decreased. Regarding nonsexual behavior, alcohol consumption was a constant practice among patients, but we observed a gradual decline in the use of illicit drugs. Additionally, we observed an increase in the frequency of smokers only among patients diagnosed between 7 and 12 months prior.



D

		SOCIAL FACTORS		
SEXUAL FACTORS		Illicit drugs	Low education level	Low income
	No condom use	0.61	0.74	0.79
	Homosexual	0.13	0.47	0.51
	No sexual partner	0.15	0.40	0.44
	STI's	0.13	0.35	0.38
		Illicit drugs	Low education level	Low income
	Condom use	0.09	0.13	0.06
	Heterosexual	0.44	0.10	0.06
	Sexual partner	0.32	0.07	0.02
	No STI's	0.28	0.06	0.03
		No illicit drugs	High education level	High income
	No condom use	0.21	0.09	0.11
	Homosexual	0.22	0.32	0.35
	No sexual partner	0.33	0.38	0.40
	STI's	0.36	0.42	0.45
		No illicit drugs	High education level	High income
	Condom use	0.03	0.01	0
	Heterosexual	0.13	0.04	0.01
	Sexual partner	0.11	0.06	0.04
	No STI's	0.12	0.07	0.04

Figure 1. Frequency of cases and degree of exposure: (**A**) Frequency of new cases of HAART-free HIV-1 patients coinfected with HIV/EBV in the period between January 2018 and December 2019. (**B**) Prevalence of cases of HIV/EBV coinfection stratified based on age and sex. (**C**) Prevalence of EBV genotypes among HIV/EBV coinfected patients. (**D**) Potential risk/exposure to HIV-1 monoinfection or co-infection. The color gradient was proposed based on data on the frequency of individuals screened according to the intersection of social and sexual factors.



(Errata) Figure 1. Frequency of cases and degree of exposure: (A) Frequency of new cases of HAART-free HIV-1 patients coinfected with HIV/EBV in the period between January 2018 and December 2019. (B) Prevalence of cases of HIV/EBV coinfection stratified based on age and sex. (C) Prevalence of EBV genotypes among HIV/EBV coinfected patients. (D) Potential risk/exposure to HIV-1 monoinfection or co-infection. The color gradient was

proposed based on data on the frequency of individuals screened according to the intersection of social and sexual factors.

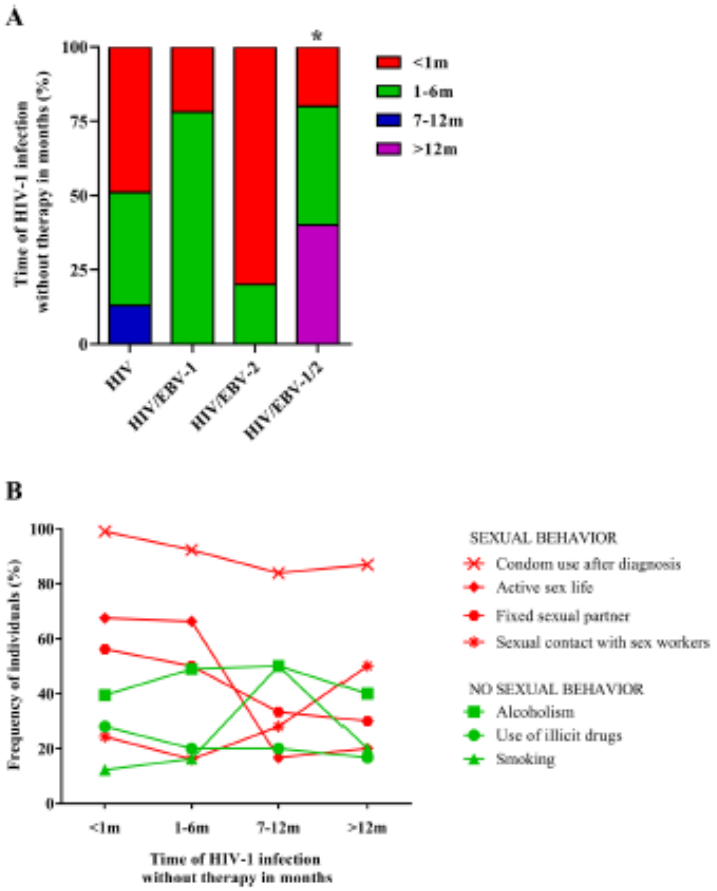


Figure 2. Late initiation of treatment for HIV-1 infection: (**A**) Frequency for initiation of HIV-1 infection treatment and monitoring, in months, after the primary diagnosis. Patients with multiple infection (HIV/EBV-1/2) initiated treatment later. *$p<0.05$. (**B**) Evaluation of behavioral profiles at different initiation times for HIV-1 infection treatment and monitoring. Condom use after diagnosis was the most frequent behavior at all treatment initiation times.

We asked the patients about the symptoms presented at the time of collection and grouped them as asymptomatic (without symptoms), oligosymptomatic (from 2 to 3 symptoms) and polysymptomatic (from 4 to 5 symptoms). Asymptomatic patients prevailed in our analyses (51.91%); only in the group of patients coinfected with HIV/EBV-1/2 were oligosymptomatic patients predominant (60.0%), but the difference was not statistically significant ($p=0.96$) (Fig. 3A). Sore throat and fever were the most frequent symptoms in all groups (77.78% and 71.72%, respectively), muscle and joint pain were not reported in the HIV/EBV-1/2 group (Fig. 3C), and no significant prevalence of symptoms was observed in the intergroup analysis ($p=0.96$).

We evaluated the profile of STIs among individuals with a history of these infections. The occurrence of syphilis and gonorrhea were the most frequent reports in the monoinfected and coinfected groups. The profile of STIs of noninfected individuals was significantly different from that of the other groups ($p<0.0001$), with other STIs (66.67%), such as herpes (33.35%) and HPV infection (33.33%), prevailing; candidiasis was an alleged condition in 33.5% of the control group (Fig. 3B).

We evaluated the frequency of the symptomatological groups based on the time since HIV-1 diagnosis (Fig. 3D). We observed a significant decrease in the frequency of asymptomatic patients with an increase in the time since diagnosis ($p=0.04$). Conversely, the rate of oligosymptomatic and polysymptomatic patients increased among patients living with HIV-1 for longer.

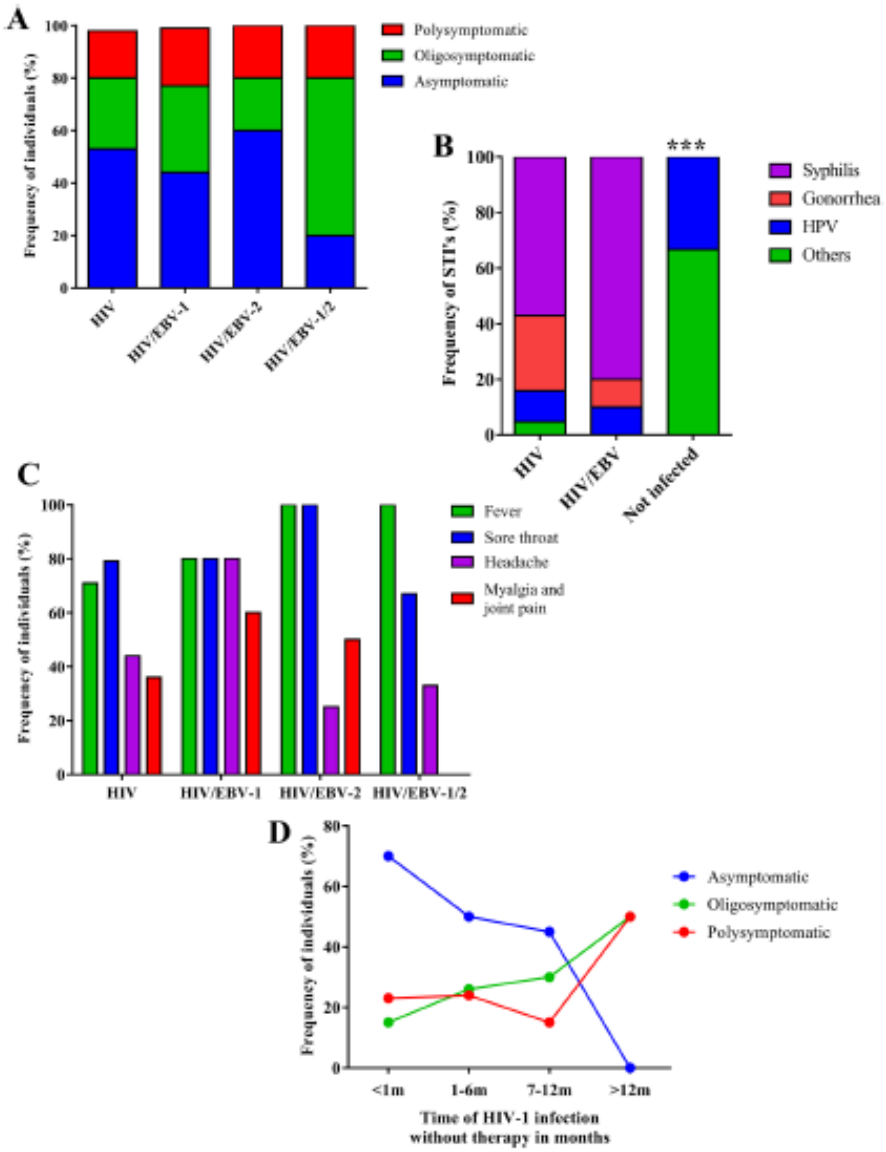


Figure 3. Symptoms: (**A**) Frequency of symptomatological categories among the studied groups. (**B**) STI diversity among individuals with a history of sexually transmitted infections in the studied groups. Blood donors had a poorly diversified history. *** ***: $p<0.001$. (**C**) Diversity of symptoms between the studied groups. Fever and sore throat were the most frequent. (**D**) Evaluation of the frequency of the symptomatological categories at different initiation times for HIV-1 infection treatment. There was an extreme reduction in the frequency of asymptomatic patients among patients for whom treatment was initiated greater than 12 months after diagnosis.

Increased HIV viral load was associated with patient age at the time of collection (OR: 2.04; 95% CI 2.01–2.07; $p = 0.026$) and with the length of illicit drug use in individuals with an illicit drug use history (OR: 1.57; 95% CI 1.12–2.22; $p = 0.0548$) (Fig. 4A). For EBV infection, no significant association was observed between viral load and patient's age, although there seems to be a tendency for older age to be associated with lower viral load values

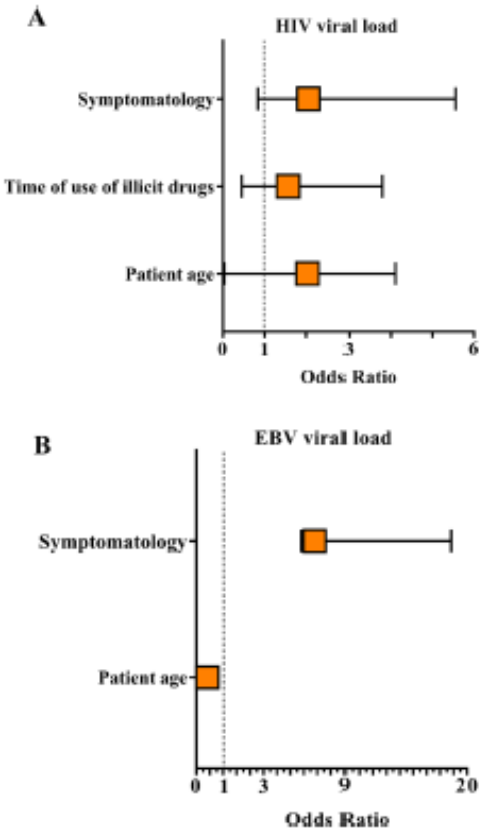


Figure 4. Epidemiological factors associated with increased viral loads: (**A**) epidemiological factors associated with increased HIV viral load. (**B**) Epidemiological factors associated with increased EBV viral load. The x-axis is the odds ratio (OR) values. Horizontal lines represent the 95% confidence interval (CI) for each OR value. Vertical dashed lines delimit an OR equal to 1; therefore, OR values greater than 1 were considered risk factors for increased viral loads. For EBV, when the age of the patients obtained an OR value less than 1, the increase in viral load was inversely proportional to the factor. Symptomatology was a factor dependent on the viral load.

(OR: 0.82; 95% CI 0.79–1.03; p = 0.0579) (Fig. 4B). The replication of both viruses was associated with patient symptoms, especially the EBV viral load, which was approximately 9 times higher in polysymptomatic patients (HIV = OR: 2.06; 95% CI 1.22–3.50; p = 0.0073; EBV = OR: 8.81; 95% CI 1–10; p = 0.0447).

Discussion

In this study, we identified a prevalence rate of approximately 7% of primary EBV infection in adult HAART-free PLHIV in a state of the Brazilian Amazon region. Our findings were similar to the epidemiological data on the general prevalence of primary infection among immunocompetent adults in emerging countries[35–37]. However, there were discrepancies when compared to data from other studies with patients immunocompromised by HIV-1; our findings were lower than the values observed even in countries with similar socioeconomic profiles[26,38,39].

Most of the differences between studies are due to the method used for sample screening, which is based on the detection of anti-VCA or anti-Epstein–Barr virus nuclear antigen (EBNA) as markers of EBV infection. The serological panel adopted in the present study was based on the detection of anti-VCA IgM and IgG antibodies, for which we assumed the profile IgM (+) IgG (−) as suspected primary infection, which was confirmed by molecular biology. Although the detection of anti-EBNA IgG antibodies is also suggested as a parameter to distinguish the infectious phases of the disease[40], methods similar to those used in the present study were indicated for the recognition of primary infection by EBV, with an estimated sensitivity above 98%, and applied in the discrimination of false positive cases[41]. We emphasize that serum anti-VCA IgM levels emerge early and

reach their serological peak within the first 5 days of disease onset, a period in which there is an increase in the clinical severity and viral load of EBV in the oral mucosa and peripheral blood[42]. Therefore, we suggest that the panel used in the present study may be a clinically relevant method in the identification of primary and recent EBV infection.

In Brazil, most studies have focused on the detection of EBV in specific organ sites of PLHIV with particular clinical profiles[31,43,44]. The methodologically closest study did not detect the same clinical profile in patients on HAART in southeastern Brazil[45]. We emphasize, however, that in the relatively time period included in the present study, the coinfection rate increased approximately sevenfold per month of collection, while the rate of new cases of PLHIV, understood as therapy-naïve based on the continuous care cascade recommended by the Brazilian Ministry of Health[46], tended to be stable, with a nonsignificant increase of twofold per month of collection.

Although the number of new HIV cases identified is stable nationally, especially in the northern region of the country[47,48], our data warn of an increase in coinfections in PLHIV, a predisposition observed in Brazil[49] and internationally[50], especially among young men.

We observed a prevalence of EBV-1 among HIV/EBV coinfected patients, confirming the epidemiology of the herpesvirus analyzed in different organ tissues of PLHIV with different clinical profiles[51,52]. Reports of EBV-2 prevalence in coinfected patients occur in specific clinical conditions after transient reactivation events of cell sites infected by different types of EBV[53].

There is controversy regarding the predominant epidemiological profile among HIV/EBV-1 coinfected patients. Reports indicate that male sex, Caucasian ethnicity and heterosexual preference are factors that influence the distribution of cases[54–56]. In the present study, male individuals predominated, as well as a singular representation of homosexual and bisexual orientation, and we did not evaluate ethnic markers, such as self-reported race. Therefore, we assume a multiethnic representation in our sample with a probable predominance of European ancestry, as already described for populations in the Brazilian Amazon[57,58]. HIV/EBV-2 coinfection combined with multiple infection by both genotypes accounted for more than half the number of coinfections, a finding that emphasizes the susceptibility to the less frequent EBV genotype or to superinfection in HIV-immunocompromised patients[59,60]. It is argued that this is due both to changes in the immunological profile[61] and in the exposure behavior of the host[62].

The epidemiological analysis revealed similarities between the monoinfected and coinfected profiles. Apparently, in the context of HIV coinfection, the presence of primary EBV infection is not associated with specific risk factors. In fact, both infections have points of convergence related mainly to unprotected sexual exposure and possible contact with contaminated blood products[63–66]. In the present study, we found a prevalence of factors that are directly and indirectly related to sexual exposure, which makes this the main route of coinfection in the studied context.

The predominant socioeconomic profile was young adult men with a low education level, low family income, and history of illicit drug use. Unsatisfactory education is a risk factor congruent with HIV infection due to the lack of aggregate knowledge about prevention mechanisms of STIs and unprotected sexual exposure[67,68]. When functional, education empowers individuals to both reflect on the extent of contact between people and improves their ability to understand and act in favor of preventive health[69,70].

It is argued that the lifestyle associated with a high education level can lead to both an increase and a decrease in the risk of HIV infection, depending on the balance of the different influences on behavior[71]. In the present study, more vulnerable socioeconomic profiles were associated with the risk of infection; in contrast, favorable profiles were prevalent among blood donors, a feature relevant to this altruistic activity[72]. This finding illustrates the sociodemographic complexity between the factors 'education' and 'income' that result in better conditions of access and social participation. That in Brazil they may be closely related to the way in which the individual is inserted in the community social organization and is motivated to act and support their relationships[73]. In contrast, low socioeconomic status contributes to less health knowledge and awareness of blood donation needs[74].

It is suggested that most of the factors that predispose an individual to HIV are associated with family or individual income because this creates environmental risks inherent to precarious access to health, the practice of "sex for survival" (sex as a means of earning income), and even the ability to deal with the consequences of the condition[75,76]. In a context of progressive poverty, HIV mortality and morbidity rates were predicted by lower incomes[77]. Analogously, there is an aggravation of social vulnerability in PLHIV of specific ethnic groups, women, and those with physical health limitations[78]. In contrast, even in already-infected key populations, a relative increase in income results in improvements in access to basic health care[79].

A history of illicit drug use was the greatest risk factor for monoinfection or coinfection in the present study, among which drugs taken orally prevailed. Recent studies have shown that an increase in the number of noninjectable drug users (NIDUs) infected by HIV trigger epidemiological surveillance in the Amazon region because although this group does not expose itself, a priori, to parenteral transmission, they may be at the mercy of other risk variables, especially sexual factors[80,81], as observed in the decrease in condom use among NIDU sex workers, even between serodiscordant partners[82]. A worrying problem is the use of polydrug use combined with unprotected sex with multiple partners[83], especially in Brazil, where although HIV prevalence rates among drug users have been declining in recent years[48], they can still vary between 10 and 25-fold higher than that estimated in the general population[84], and most users do not have basic knowledge about the severity of the infection[85].

From the point of view of sexual practice, homosexual or bisexual orientation without the presence of a steady partner and occasional condom use were predominant characteristics among monoinfected and coinfected individuals. In fact, men who have sex with men (MSM) correspond to one of the main groups particularly vulnerable to HIV (key populations) and who historically do not have adequate access to health services. Data suggest that approximately 47% of new infections worldwide were associated with key populations and their sexual partners in 2017; in that evaluation, the MSM group represented 57% of new cases in Western Europe and

Central and North America[86,87]. However, in developed countries, MSM exposed to high risks report willingness to participate in prophylaxis measures once informed of these prevention efforts[88].

In developing countries, the high proportion of seropositive MSM who are unaware of their serological status is highly worrying and represents a large gap between basic health care practices and national guidelines[89]. In Brazil, recent reports highlight the high prevalence of HIV among MSM, whose main risk factors include environments of vulnerability; stigma and discrimination; behavioral profiles; sexual practices; and lackluster policies and programs[90,91]. Notably, there is a low rate of satisfactory knowledge on HIV infection observed among Brazilian MSM[92].

As already mentioned, multiple sex partners is one of the characteristics composing the behavioral profiles of MSM[93,93], and a history of STIs is one of the consequences of this practice[94], as suggested in the present study. Recent warnings highlight the low rates of disclosure of serological status among sex partners, which is worrisome given the persistence of viral transmissibility, especially when the majority continues to practice unprotected sex[95]. It is argued that specific social networks may be endorsing this exposure behavior[96]. In the present study, we showed that unprotected sex with multiple partners, particularly for young men, are relevant attributes in the prevalence of monoinfection or coinfection. Notably, most infected individuals had their first sexual contact in preadolescence (10 to 15 years), a trend that has been observed in previous studies[97].

Regarding protected sex, a recent report points to the existence of accessibility barriers for purchasing condoms, even in developed countries, and the need for a multifaceted approach to overcome these barriers[98]. In Brazil, despite an ongoing policy of free provision of condoms in referral units[99], there is still a deficit in adherence to condom use, as suggested in the present study. We do not know whether this scenario is due to poor adherence itself, as a behavioral attitude[100], to stigmatization in accessibility[101], or to population ignorance regarding the national condom distribution policy. Future studies addressing this issue are essential as a strategic basis for preventive measures in the Brazilian Amazon region.

A history of STIs was the second factor most strongly associated with monoinfection or coinfection, with a prevalence of reported cases of syphilis and gonorrhea, respectively. Recent studies have shown that the highest risk for STIs is observed in young MSM from the most vulnerable socioeconomic groups[90,92]; among these, those diagnosed with syphilis and gonorrhea have the highest rate of HIV coinfection[102], similar to our findings. These data are particularly worrying for Brazil because the incidence rates of acquired syphilis have been increasing since 2010[103], and there is an estimated global increase in cases of gonorrhea, especially in MSM in developing countries[104]. The specific detection of these STIs can guide resource-intensive interventions, such as pre-exposure prophylaxis (PrEP), among PLHIV[102].

In summary, we propose a risk/exposure potential matrix for monoinfection or coinfection based on sexual and social risk factors. We showed that the highest risk/exposure occurs among individuals with social vulnerability and promiscuous sexual behavior, among whom condom nonusers were more susceptible to viral infections.

However, condom use had a risk more associated with low education and low family income, similar to that presented among immigrants with similar socioeconomic profiles. In this scenario of preventive failure, we assume that social factors can directly influence the quality of aggregated knowledge about proper condom handling and use and awareness of STI/AIDS[105]. Alternatively, high education is associated with affordable family income and consequent adoption of safer sexual practices and access to preventive health services[106], as suggested for individuals with high purchasing power, whose risk/exposure remained modest regardless of sexual factors.

We noted that on a monthly scale, the care and monitoring of HIV infection in a portion of patients was initiated late, with an interval between 1 and 24 months after becoming aware of the infection. However, the majority started the process quickly, approximately 1 month after the primary diagnosis.

These results are promising given persistent reports of late diagnosis among PLHIV. The greatest concern lies with the increase in the degree of morbidity and mortality inherent to the late initiation of treatment, although a trend toward a decrease in overall lethality is observed in these patients, despite maintaining a high frequency of late or advanced presentation of HIV-infected patients and the evolution of associated factors[107]. It is argued that in these cases, the immediate initiation of HAART may have favored the gradual immune recovery of patients and impacted mortality[108]. In Brazil, management guidelines advocate the immediate start of therapeutic regimens regardless of the patient's clinical or immunological status[46].

One strategy to encourage the early diagnosis of STIs is to promote testing outside the health setting[109,110]. Brazil again stands out for encouraging this practice in official and independent campaigns that aim not only to diagnosis but also to provide information regarding and encourage condom use[111,112].

It is understood that older age and heterosexual orientation are the main factors influencing late start of monitoring in PLHIV[113–116]; however, little is discussed about the risk behavior profile of this group. In the present study, we observed that the exposure behavior was similar between the different times since diagnosis, indicating that prolonged experience with HIV did not significantly alter the behavioral profile of patients who maintained promiscuous practices related to infection and inherent comorbidities. Although there was a decrease in the percentage of sexual practice in patients for whom treatments was initiated late, most patients had no steady partner and maintained sexual contact with sex workers, some of whom did not use condoms.

We also show that the risk of HIV/EBV-1/2 multiple infection was higher in patients with longer HIV-1 infection, which emphasizes our warning because coinfections are common in patients who start treatment late, have the highest associated risk rate, and are also correlated with advanced clinical disease stages[117,118]. For EBV, this becomes more specific because the prevalence of EBV-1 and of multiple infection predominate in the most immunosuppressed patients; however, it is not known which factors may be involved in this scenario[44,119].

Regarding the symptomatological aspects, asymptomatic individuals prevailed in all groups. Symptoms, when present, although still noncomplex and clinically nonspecific, were similar between both the initial phase of HIV infection and primary symptomatic EBV infection[120–123]. Particularly with EBV, these symptoms are associated

with age groups above 18 years old and the host's aggravated immune status[64], attributes that were prevalent among coinfected patients in the present study.

With the late initiation of HAART, there was a clear progression in coinfection symptoms, evidenced by the increase in cases of oligosymptomatic and polysymptomatic patients with HAART initiation greater than 12 months after the primary diagnosis. Although our results indicate that most patients sought specific medical care at the onset of the disease, we show how neglect of the condition can affect the pathological status of HIV infection, given that antiretroviral therapy initiated as soon as possible delays the progression of the disease and viral transmission[124] and has secondary benefits[125,126].

We observed that some of the epidemiological and behavioral factors were also associated with biological aspects of coinfection. Exclusively for HIV, patients with longer use of illicit drugs were more likely to have a high viral load. Specifically, we noted that most users used cocaine or marijuana, which are associated with effects on the immune system indicative of compromised resistance to HIV[127], which favors not only the risk of infection but also intensive therapeutic control[128]. We suggest that the continued use of these illicit drugs may be conducive to both the acquisition and progression of infection because the time of initiation of HAART was not associated with viral load, which points to a complex interaction between illicit drug use and virological activity, which may be as significant as exposure to therapy[129].

The advancement of age as a risk factor for increased HIV viral load was a finding different from those reported in previous studies[130–132]. However, in the present study, all patients were treatment-naïve, unlike those participating in the aforementioned studies, revealing the possible effects of immunosenescence in the face of HIV infection[133], even in a predominantly young population.

In contrast, the chances of the EBV viral load increasing were higher in younger patients, in contrast that what was observed in HIV patients. Our findings reflect those observed in other epidemiological studies, in which it is argued that the early acquisition of primary infection results in higher and more sustained levels of EBV viremia. The presence of coinfections may also impair specific immune surveillance for one or both pathogens, leading to worsening of the disease[134,135].

For both viruses, symptomatology was a factor dependent on viral load; the higher the viral load is, the greater the chances of patients presenting with a polysymptomatic condition, especially in EBV infection. For HIV, in cohorts with a high prevalence of infection, it was shown that viral load is a determining factor of disease progression[136], an observation more characteristic of the acute phase of infection[125]. However, studies indicate that viral subtype and chronic immune activation are also aspects that predict the course of progression in populations of different ethnic origins[137,138]. For EBV, the plasma viral load is directly proportional to the patient's symptoms, especially within the first 2 weeks of symptom onset; in the acute phase, viral replication is intense in peripheral blood, with the virus contained in infected B cells and a portion being released into the plasma[64,139]. In specific clinical cases, the plasma viral load, even when low, is already an effective biomarker[140,141].

Conclusions

Based on the results of the present study, we conclude that the prevalence of primary coinfection with EBV in HIV therapy-naïve patients in a state of the Brazilian Amazon region was lower than that observed in other similar regions. This finding may be associated with the methods used in the different studies, with social vulnerability and promiscuous sexual behavior being the risk factors associated with this population. We warn of the effect of the late initiation of HIV infection monitoring and the consequences of this decision on the risk of coinfections by EBV and disease progression, and we suggest that some epidemiological factors may influence the increase in viral load of both viruses.

Data availability

Data supporting the findings of this study are available from Igor Brasil Costa, but restrictions apply to the availability of these data, which were used under license for the present study and therefore are not publicly available. However, data are available from the authors upon reasonable request and with permission from Igor Brasil Costa.

Received: 10 June 2021; **Accepted:** 30 August 2021
Published online: 16 September 2021

References

1. Odumade, O. A., Hogquist, K. A. & Balfour, H. H. Jr. Progress and problems in understanding and managing primary Epstein–Barr virus infections. *Clin. Microbiol. Rev.* **24**(1), 193–209. https://doi.org/10.1128/CMR.00044-10 (2011).
2. International Committee on Taxonomy of Viruses (ICTV). https://talk.ictvonline.org/taxonomy/ (Acessado em 04 Sept 2020).
3. Sample, J. *et al.* Epstein–Barr virus types 1 and 2 differ in their EBNA-3A, EBNA-3B, and EBNA-3C genes. *J. Virol.* **64**(9), 4084–4092 (1990).
4. Cohen, J. I. Epstein–Barr virus infection. *N. Engl. J. Med.* **343**(7), 481–492. https://doi.org/10.1056/NEJM200008173430707 (2000).
5. Tzellos, S. & Farrell, P. J. Epstein–Barr virus sequence variation biology and disease. *Pathogens.* **1**(2), 156–174. https://doi.org/10.3390/pathogens1020156 (2012).
6. Ibrahim Hazem, A. H. *et al.* Epstein–Barr virus (EBV) genotypes among human immunodeficiency virus (HIV)-related B-cell lymphomas and B-cell post-transplant lymphoproliferative disorders (B-PTLD)–late-onset lymphomas, especially in the HIV setting, are associated with type-B-EBV. *Eur. J. Haematol.* **85**(3), 227–230. https://doi.org/10.1111/j.1600-0609.2010.01460.x (2010).
7. Hjalgrim, H., Friborg, J. & Melbye, M. The epidemiology of EBV and its association with malignant disease. In *Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis* (Cambridge University Press, 2007).
8. Trottier, H. *et al.* Transfusion-related Epstein–Barr virus infection among stem cell transplant recipients: A retrospective cohort study in children. *Transfusion* **52**(12), 2653–2663 (2012).

9. Fourcade, G. *et al.* Evolution of EBV seroprevalence and primary infection age in a French hospital and a city laboratory network, 2000–2016. *PLoS ONE* **12**(4), e0175574. https://doi.org/10.1371/journal.pone.0175574 (2017) (**eCollection 2017**).
10. Smatti, M. K. *et al.* Prevalence and molecular profiling of Epstein Barr virus (EBV) among healthy blood donors from different nationalities in Qatar. *PLoS ONE* **12**(12), e0189033. https://doi.org/10.1371/journal.pone.0189033 (2017).
11. Gerpe, N. M. F. *et al.* Distinctive EBV infection characteristics in children from a developing country. *Int. J. Infect. Dis.* **93**, 139–145. https://doi.org/10.1016/j.ijid.2020.01.044 (2020).
12. Figueira-Silva, C. M. & Pereira, F. E. L. Prevalence of Epstein–Barr virus antibodies in healthy children and adolescents in Vitória, State of Espírito Santo, Brazil. *Rev. Soc. Bras. Med. Trop.* **37**(5), 409–412 (2004).
13. Bastos, M. S. *et al.* Detection of Herpesvirus, Enterovirus, and Arbovirus infection in patients with suspected central nervous system viral infection in the Western Brazilian Amazon. *J. Med. Virol.* **86**(9), 1522–1527 (2014).
14. Lacoste, V. *et al.* Virological and molecular characterisation of a new B lymphoid cell line, established from an AIDS patient with primary effusion lymphoma, harbouring both KSHV/HHV8 and EBV viruses. *Leuk. Lymphoma.* **38**(3–4), 401–409. https://doi.org/10.3109/10428190009087032 (2000).
15. Chao, C. *et al.* Epstein–Barr virus infection and expression of B-cell oncogenic markers in HIV-related diffuse large B-cell Lymphoma. *Clin. Cancer Res.* **18**(17), 4702–4712 (2012).
16. Lang, F., Pei, Y., Lamplugh, Z. L. & Robertson, E. S. Molecular biology of EBV in relationship to HIV/AIDS-associated oncogenesis. *Cancer Treat. Res.* **177**, 81–103. https://doi.org/10.1007/978-3-030-03502-0_4 (2019).
17. Hernández, D. M. *et al.* Loss of T-cell multifunctionality and TCR-Vβ repertoire against Epstein–Barr virus is associated with worse prognosis and clinical parameters in HIV+ patients. *Front. Immunol.* **9**, 2291. https://doi.org/10.3389/fimmu.2018.02291 (2018).
18. Friis, A. M., Åkerlund, B., Gyllensten, K., Aleman, A. & Erniberg, I. Host-Epstein–Barr virus relationship affected by immunostimulation in HIV-infected patients representing distinct progressor profile groups. *Scand. J. Infect. Dis.* **44**(5), 388–392 (2012).
19. Riedel, D. J., Tang, L. S. & Rositch, A. F. The role of viral co-infection in HIV-associated non-AIDS related cancers. *Curr. HIV/AIDS Rep.* **12**(3), 362–372. https://doi.org/10.1007/s11904-015-0276-6 (2015).
20. Grando, L. J. *et al.* Viral coinfection in the oral cavity of HIV-infected children: Relation among HIV viral load, CD4+ T lymphocyte count and detection of EBV, CMV and HSV. *Braz. Oral Res.* **19**(3), 228–234 (2005).
21. Slyker, J. A. *et al.* Clinical and virologic manifestations of primary Epstein–Barr virus (EBV) infection in Kenyan infants born to HIV-infected women. *J. Infect. Dis.* **207**(12), 1798–1806 (2013).
22. Petrara, M. R. *et al.* Epstein–Barr virus load in children infected with human immunodeficiency virus type 1 in Uganda. *J. Infect. Dis.* **210**(3), 392–399 (2014).
23. Gumbo, H. *et al.* Congenital and postnatal CMV and EBV acquisition in HIV-infected Zimbabwean infants. *PLoS ONE* **9**(12), e114870. https://doi.org/10.1371/journal.pone.0114870 (2014).
24. Moriuchi, M. & Moriuchi, H. Increased susceptibility to HIV-1 of peripheral blood lymphocytes in acute infection with Epstein–Barr virus. *J. Med. Virol.* **71**, 343–346. https://doi.org/10.1002/jmv.10494 (2003).
25. Fellner, M. D. *et al.* Circulating Epstein–Barr virus (EBV) in HIV-infected patients and its relation with primary brain lymphoma. *Int. J. Infect. Dis.* **11**(2), 172–178. https://doi.org/10.1016/j.ijid.2006.04.001 (2007).
26. Yan, Y. *et al.* Evaluation of Epstein–Barr virus salivary shedding in HIV/AIDS patients and HAART use: A retrospective cohort study. *Virol. Sin.* **33**(3), 227–233. https://doi.org/10.1007/s12250-018-0028-z (2018).
27. Ling, P. D. *et al.* Epstein–Barr virus DNA loads in adult human immunodeficiency virus type 1-infected patients receiving highly active antiretroviral therapy. *Clin. Infect. Dis.* **37**(9), 1244–1249. https://doi.org/10.1086/378808 (2003).
28. O'Sullivan, C. E. *et al.* Epstein–Barr virus and human immunodeficiency virus serological responses and viral burdens in HIV-infected patients treated with HAART. *J. Med. Virol.* **67**(3), 320–326. https://doi.org/10.1002/jmv.10080 (2002).
29. Guimarães, A. G. D. P. *et al.* Coinfection of Epstein–Barr virus, cytomegalovirus, herpes simplex virus, human papillomavirus and anal intraepithelial neoplasia in HIV patients in Amazon, Brazil. *J. Coloproctol.* **32**(1), 18–25. https://doi.org/10.1590/S2237-93632012000100003 (2012).
30. Rebelo-Pontes, H. A. *et al.* Burkitt's lymphoma of the jaws in the Amazon region of Brazil. *Med. Oral Patol. Oral Cir. Bucal.* **19**(1), e32–e38. https://doi.org/10.4317/medoral.18936 (2014) (**Published 2014 Jan 1**).
31. Jácome-Santos, H. *et al.* Epstein–Barr virus (EBV) in periodontal sites of human immunodeficiency virus (HIV)-positive individuals in North Brazil: A cross-sectional study. *Quintessence Int.* **51**(1), 18–26. https://doi.org/10.3290/j.qi.a43616 (2020).
32. BRASIL. Ministério da Saúde. Anexo I Do Anexo IV-Portaria De Consolidação Nº 5, DE 28 DE Setembro De 2017. (Gabinete do ministro, 2017).
33. Lorenzetti, M. A. *et al.* EBNA1 sequences in Argentinean pediatric acute and latent Epstein–Barr virus infection reflect circulation of novel South American variants. *J. Med. Virol.* **82**(10), 1730–1738 (2010).
34. Ayres, M., Ayres Júnior, M., Ayres, D. L. & Santos, A. S. BioEstat 5.0: aplicações estatísticas nas áreas de ciências biológicas e médicas, CNPq, 272 (Sociedade Civil Mamirauá, 2008).
35. Moeini, M., Ziyaeyan, M., Asaei, S. & Behzadi, M. A. The incidence of Epstein–Barr virus primary infection among suspected patients referred to Namazi Hospital of Shiraz, Iran. *Jundishapur J. Microbiol.* **8**(4), e16109. https://doi.org/10.5812/jjm.8(4)2015.16109 (2015).
36. Cui, J. *et al.* Anti-Epstein–Barr virus antibodies in Beijing during 2013–2017: What we have found in the different patients. *PLoS ONE* **13**(3), e0193171. https://doi.org/10.1371/journal.pone.0193171 (2018).
37. Beader, N., Kolarić, B., Slačanac, D., Tabain, I. & Vilibić-Čavlek, T. Seroepidemiological study of Epstein–Barr virus in different population groups in Croatia. *Int. Med. Assoc. J.* **20**(2), 86–90 (2018).
38. Abdollahi, A., Shoar, S., Rasoulinejad, M. & Shetkhbahaei, S. Seroprevalence of Epstein–Barr virus among HIV positive patients moreover and its association with CD4 positive lymphocyte count. *Acta Med. Iran.* **52**(12), 916–921 (2014).
39. Okoniko, I. O., Makinde, T. S., Okoniko, B. J. & Ogbu, O. Immunological and epidemiological evaluation of EBV infections among HIV-1 infected individuals in Abakaliki, Nigeria supports the potential use of neutrophils as a marker of EBV in HIV disease progression and as useful markers of immune activation. *J. Immunoassay Immunochem.* **41**(2), 158–170. https://doi.org/10.1080/15321819.2019.1705483 (2020).
40. De Paschale, M. & Clerici, P. Serological diagnosis of Epstein–Barr virus infection: Problems and solutions. *World J. Virol.* **1**(1), 31–43. https://doi.org/10.5501/wjv.v1.i1.31 (2012).
41. Guerrero-Ramos, A., Patel, M., Kadakia, K. & Haque, T. Performance of the architect EBV antibody panel for determination of Epstein–Barr virus infection stage in immunocompetent adolescents and young adults with clinical suspicion of infectious mononucleosis. *Clin. Vaccine Immunol.* **21**(6), 817–823. https://doi.org/10.1128/CVI.00754-13 (2014).
42. Grimm, J. M. *et al.* Prospective studies of infectious mononucleosis in university students. *Clin. Transl. Immunol.* **5**(8), e94. https://doi.org/10.1038/cti.2016.48 (2016).
43. Carvalho, K. S. S. *et al.* PCR detection of multiple human herpesvirus DNA in saliva from HIV-infected individuals in Teresina, State of Piauí, Brazil. *Rev. Soc. Bras. Med. Trop.* **43**(6), 620–623. https://doi.org/10.1590/S0037-86822010000600003 (2010).
44. Santos, L., Azevedo, K., Silva, L. & Oliveira, L. Epstein–Barr virus in oral mucosa from human immunodeficiency virus positive patients. *Rev. Assoc. Med. Bras.* **60**(3), 262–269. https://doi.org/10.1590/1806-9282.60.03.016 (2014).

45. Kuschnaroff, T. M. *et al.* Prevalência da infecção pelo vírus Epstein–Barr em voluntários doadores de sangue e indivíduos com AIDS na cidade de São Paulo. *Arq Med Hosp Fac Ciênc Med Santa Casa São Paulo* **52**(1), 8–13 (2007).
46. Brasil. Ministério da Saúde. Protocolo Clínico e Diretrizes Terapêuticas para Manejo da Infecção pelo HIV em adultos (Departamento de Vigilância, Prevenção e Controle das Infecções Sexualmente Transmissíveis, do HIV/Aids e das Hepatites Virais, 2018).
47. UNAIDS Data 2017. http://www.unaids.org/sites/default/files/media_asset/20170720_Data_book_2017_en.pdf (Acessado em 28 Oct 2020).
48. Brasil. Ministério da Saúde. Boletim Epidemiológico HIV/AIDS 2019. (Departamento de Vigilância, Prevenção e Controle das Infecções Sexualmente Transmissíveis, do HIV/Aids e das Hepatites Virais, 2019).
49. Luppi, C. G. *et al.* Factors associated with HIV co-infection in cases of acquired syphilis reported in a Reference Center for Sexually Transmitted Diseases and AIDS in the municipality of São Paulo, Brazil, 2014. *Epidemiol. Serv. Saude.* **27**(1), e20171678. https://doi.org/10.5123/s1679-49742018000100008 (2018) (**English, Portuguese**).
50. Secco, A. A. *et al.* Sexually transmitted infections in persons living with HIV infection and estimated HIV transmission risk: Trends over time from the DC Cohort. *Sex Transm. Infect.* **96**(2), 89–95. https://doi.org/10.1136/sextrans-2019-054216 (2020).
51. Willoughby, C. E. *et al.* Epstein–Barr virus (types 1 and 2) in the tear film in Sjogren's syndrome and HIV infection. *J. Med. Virol.* **68**(3), 378–383. https://doi.org/10.1002/jmv.10214 (2002).
52. Grande, S. R. *et al.* Relationship between herpesviruses and periodontopathogens in patients with HIV and periodontitis. *J. Periodontol.* **82**(10), 1442–1452. https://doi.org/10.1902/jop.2011.100723 (2011).
53. Buisson, M. *et al.* Development of an Epstein–Barr virus type 2 (EBV-2)-associated hepatic B cell non-Hodgkin's lymphoma in an HIV-1-infected patient following a change in the EBV dominant type. *Leukemia* **13**(2), 298–301. https://doi.org/10.1038/sj.leu.2401268 (1999).
54. Van Baarle, D. *et al.* High prevalence of Epstein–Barr virus type 2 among homosexual men is caused by sexual transmission. *J. Infect. Dis.* **181**(6), 2045–2049 (2000).
55. Bohaina, T. F. *et al.* Polymerase chain reaction genotyping of Epstein–Barr virus in scraping samples of the tongue lateral border in HIV-1 seropositive patients. *Mem. Inst. Oswaldo Cruz.* **103**(4), 326–331. https://doi.org/10.1590/s0074-02762008000400002 (2008).
56. Giron, L. B. *et al.* Impact of Epstein–Barr virus load, virus genotype, and frequency of the 30 bp deletion in the viral BNLF-1 gene in patients harboring the human immunodeficiency virus. *J. Med. Virol.* **85**(12), 2110–2118. https://doi.org/10.1002/jmv.23722 (2013).
57. Salzano, F. M. & Sans, M. Interethnic admixture and the evolution of Latin American populations. *Genet. Mol. Biol.* **37**, 151–170 (2014).
58. da Silva, E. M. *et al.* Effect of genetic ancestry to the risk of susceptibility to gastric cancer in a mixed population of the Brazilian Amazon. *BMC Res. Notes.* https://doi.org/10.1186/s13104-017-2963-4 (2017).
59. Buisson, M. *et al.* Changes in the dominant Epstein–Barr virus type during human immunodeficiency virus infection. *J. Gen. Virol.* **75**(Pt 12), 431–437. https://doi.org/10.1099/0022-1317-75-2-431 (1994).
60. Correa, R. M. *et al.* Epstein Barr virus genotypes and LMP-1 variants in HIV-infected patients. *J. Med. Virol.* **79**(4), 401–407. https://doi.org/10.1002/jmv.20782 (2007).
61. Gianella, S., Ginocchio, C. C., Daar, E. S., Dube, M. P. & Morris, S. R. Genital Epstein Barr Virus is associated with higher prevalence and persistence of anal human papillomavirus in HIV-infected men on antiretroviral therapy. *BMC Infect. Dis.* **16**, 24 (2016).
62. Chen, M. J., Scheer, S., Nguyen, T. Q., Kohn, R. P. & Schwarcz, S. K. HIV coinfection among persons diagnosed as having sexually transmitted diseases, San Francisco, 2007 to 2014. *Sex. Transm. Dis.* **45**(8), 563–572. https://doi.org/10.1097/OLQ.0000000000000789 (2018).
63. Crawford, D. H. *et al.* A cohort study among university students: Identification of risk factors for Epstein–Barr virus seroconversion and infectious mononucleosis. *Clin. Infect. Dis.* **43**(3), 276–282. https://doi.org/10.1086/505400 (2006).
64. Dunmire, S. K., Verghese, P. S. & Balfour, H. H. Jr. Primary Epstein–Barr virus infection. *J. Clin. Virol.* **102**, 84–92. https://doi.org/10.1016/j.jcv.2018.03.001 (2018).
65. Atuhaire, L., Adetokunboh, O., Shumba, C. & Nyasulu, P. S. Effect of female sex work-targeted community-based interventions along the HIV treatment cascade in sub-Saharan Africa: A systematic review protocol. *BMJ Open* **10**(10), e039495. https://doi.org/10.1136/bmjopen-2020-039495 (2020).
66. Kumari, S. Prevalence and trends of hepatitis B virus, hepatitis C virus, human immunodeficiency virus 1, 2 and syphilis infections among blood donors in a regional transfusion center in Punjab, India: A 3 years study. *Indian J. Sex. Transm. Dis. AIDS.* **41**(1), 22–29. https://doi.org/10.4103/0253-7184.196887 (2020).
67. Szwarcwald, C. L. *et al.* Factors associated with HIV infection among female sex workers in Brazil. *Medicine* **97**(1S Suppl 1), S54–S61. https://doi.org/10.1097/MD.0000000000009013 (2018).
68. da Fonseca, A. J. *et al.* Knowledge, perception and seroprevalence of HIV/STIS among young adults in Brazilian Amazon region: A population-based study. *J. AIDS Clin. Res.* **10**, 784. https://doi.org/10.4172/2155-6113.1000784 (2019).
69. Kilian, A. H. *et al.* Reductions in risk behaviour provide the most consistent explanation for declining HIV-1 prevalence in Uganda. *AIDS* **13**(3), 391–398. https://doi.org/10.1097/00002030-199902250-00012 (1999).
70. Fylkesnes, K. *et al.* The HIV epidemic in Zambia: Socio-demographic prevalence patterns and indications of trends among childbearing women. *AIDS* **11**(3), 339–345. https://doi.org/10.1097/00002030-199703110-00012 (1997).
71. Hargreaves, J. R. & Glynn, J. R. Educational attainment and HIV-1 infection in developing countries: A systematic review. *Trop. Med. Int. Health.* **7**(6), 489–498. https://doi.org/10.1046/j.1365-3156.2002.00889.x (2002).
72. Zucoloto, M. L., Gonçalez, T., Custer, B., McFarland, W. & Martinez, E. Z. Comparison of the demographic and social profile of blood donors and nondonors in Brazil. *Health Soc. Care Community.* **27**(2), 330–336. https://doi.org/10.1111/hsc.12680 (2019).
73. Gonçalez, T. T. *et al.* Motivation and social capital among prospective blood donors in three large blood centers in Brazil. *Transfusion* **53**(6), 1291–1301. https://doi.org/10.1111/j.1537-2995.2012.03887.x (2013).
74. Zago, A., da Silveira, M. F. & Dumith, S. C. Blood donation prevalence and associated factors in Pelotas, Southern Brazil. *Rev. Saude Publica.* **44**(1), 112–120. https://doi.org/10.1590/s0034-89102010000100012 (2010) (**English, Portuguese**).
75. Farmer, P. *Women, Poverty and AIDS: Sex, Drugs, and Structural Violence* (University of California Press, 2002).
76. Mutenje, M. J., Nyakudya, I. W., Katsinde, C. & Chikuvire, T. J. Sustainable income-generating projects for HIV-affected households in Zimbabwe: Evidence from two high-density suburbs. *Afr. J. AIDS Res.* **6**(1), 9–15. https://doi.org/10.2989/16085900709490364 (2007).
77. Bachmann, M. O. & Booysen, F. L. Relationships between HIV/AIDS, income and expenditure over time in deprived South African households. *AIDS Care* **16**(7), 817–826. https://doi.org/10.1080/09540120412331290220 (2004).
78. Conover, C. J., Arno, P., Weaver, M., Ang, A. & Elinor, S. L. Income and employment of people living with combined HIV/AIDS, chronic mental illness, and substance abuse disorders. *J. Ment. Health Policy Econ.* **9**(2), 71–86 (2006).
79. da Costa, L. M. *et al.* Prevalence and risk factors for human immunodeficiency virus infection among female sex workers: Distinct offers of sexual services in a municipality of the Brazilian Amazon. *AIDS Res. Hum. Retroviruses.* **35**(9), 826–832. https://doi.org/10.1089/AID.2019.0032 (2019).

80. Gomes, S. T. M. *et al.* Immunological and virological characterization of HIV-1 viremia controllers in the North Region of Brazil. *BMC Infect. Dis.* **17**(1), 381. https://doi.org/10.1186/s12879-017-2491-9 (2017).
81. Machado, L. F. *et al.* Lower genetic variability of HIV-1 and antiretroviral drug resistance in pregnant women from the state of Pará, Brazil. *BMC Infect. Dis.* **17**(1), 270. https://doi.org/10.1186/s12879-017-2392-y (2017).
82. Avila, M. M. *et al.* High frequency of illegal drug use influences condom use among female transgender sex workers in Argentina: Impact on HIV and syphilis infections. *AIDS Behav.* **21**(7), 2059–2068. https://doi.org/10.1007/s10461-017-1766-x (2017).
83. Grabovac, I., Mellinger, M., Schalk, H., Leichsenring, B. & Dorner, T. E. Prevalence and associations of illicit drug and polydrug use in people living with HIV in Vienna. *Sci. Rep.* **8**(1), 8046. https://doi.org/10.1038/s41598-018-26413-5 (2018).
84. Guimarães, M. L. *et al.* Assessing the HIV-1 epidemic in Brazilian drug users: A molecular epidemiology approach. *PLoS ONE* **10**(11), e0141372. https://doi.org/10.1371/journal.pone.0141372 (2015).
85. Bertoni, N. *et al.* Knowledge of AIDS and HIV transmission among drug users in Rio de Janeiro. *Brazil. Harm Reduct J.* **8**, 5. https://doi.org/10.1186/1477-7517-8-5 (2011).
86. UNAIDS. Key Population. http://www.unaids.org/en/topic/key_populations (Acessado em 29 Oct 2020).
87. WHO. HIV Key Populations. http://www.euro.who.int/en/health-topics/communicable-diseases/hivaids/policy/policy-guidance-for-key-populations-most-at-risk2 (Acessado em 04 Nov 2020).
88. Connochie, D., Tingler, R. C. & Bauermeister, J. A. Young men who have sex with men's awareness, acceptability, and willingness to participate in HIV vaccine trials: Results from a nationwide online pilot study. *Vaccine.* **37**(43), 6494–6499. https://doi.org/10.1016/j.vaccine.2019.08.076 (2019).
89. Lahuerta, M. *et al.* HIV Prevalence and related risk factors in men who have sex with men in Bamako, Mali: Findings from a bio-behavioral survey using respondent-driven sampling. *AIDS Behav.* **22**(7), 2079–2088. https://doi.org/10.1007/s10461-017-1793-7 (2018).
90. Kerr, L. *et al.* HIV prevalence among men who have sex with men in Brazil: Results of the 2nd national survey using respondent-driven sampling. *Medicine* **97**(1S Suppl 1), S9–S15. https://doi.org/10.1097/MD.0000000000010573 (2018).
91. Rios, L. F., Paiva, V. & Brignol, S. Passivos, ativos and versáteis: Men who have sex with men, sexual positions and vulnerability to HIV infection in the northeast of Brazil. *Cult. Health Sex.* **21**(5), 510–525. https://doi.org/10.1080/13691058.2018.1491063 (2019).
92. Guimarães, M. D. C. *et al.* HIV/AIDS knowledge among MSM in Brazil: A challenge for public policies. *Rev Bras Epidemiol.* **22**(Suppl 1), e190005. https://doi.org/10.1590/1980-549720190005.supl.1 (2019) (**English, Portuguese**).
93. Guimarães, M. D. C. *et al.* Comparing HIV risk-related behaviors between 2 RDS national samples of MSM in Brazil, 2009 and 2016. *Medicine* **97**(1S Suppl 1), S62–S68. https://doi.org/10.1097/MD.0000000000009079 (2018).
94. Wang, Z. *et al.* Co-occurring psychosocial problems and multiple sexual partners among men who have sex with men in Shanghai, China: A syndemic approach. *J. Sex. Res.* **55**(7), 892–901. https://doi.org/10.1080/00224499.2017.1399333 (2018).
95. Mengwai, K., Madiba, S. & Modjadji, P. Low disclosure rates to sexual partners and unsafe sexual practices of youth recently diagnosed with HIV; Implications for HIV prevention interventions in South Africa. *Healthcare* **8**(3), 253. https://doi.org/10.3390/healthcare8030253 (2020).
96. Chittamuru, D., Icard, L. D., Jemmott, J. B. 3rd. & O'Leary, A. Prospective predictors of multiple sexual partners among African American men who have sex with men. *Arch Sex Behav.* **47**(7), 2081–2090. https://doi.org/10.1007/s10508-018-1207-6 (2018).
97. Heeren, G. A. *et al.* Multiple partners and condom use among students at a South African University. *J. Evid. Based Soc. Work.* **11**(5), 437–444. https://doi.org/10.1080/15433714.2012.759468 (2014).
98. McCool-Myers, M., Myo, A. & Carter, J. A. Barriers to purchasing condoms in a high HIV/STI-risk urban area. *J. Community Health.* **44**(4), 836–843. https://doi.org/10.1007/s10900-019-00670-5 (2019).
99. Brasil. Ministério da Saúde. ANEXO 05 da Norma Técnica–Incentivo HIV/Aids e outras DST – Nº 01/2002 (Portaria Nº 2314, de 20 de dezembro de 2002) (Gabinete do ministro, 2002).
100. Rutakumwa, R., Mbonye, M., Kiwanuka, T., Bagire, D. & Seeley, J. Why do men often not use condoms in their relationships with casual sexual partners in Uganda?. *Cult. Health Sex.* **17**(10), 1237–1250. https://doi.org/10.1080/13691058.2015.1053413 (2015).
101. Wilson, A. M. & Ickes, M. J. Purchasing condoms near a college campus: Environmental barriers. *Sex. Health.* **12**(1), 67–70. https://doi.org/10.1071/SH14155 (2015).
102. Katz, D. A., Dombrowski, J. C., Bell, T. R., Kerani, R. P. & Golden, M. R. HIV incidence among men who have sex with men after diagnosis with sexually transmitted infections. *Sex. Transm. Dis.* **43**(4), 249–254. https://doi.org/10.1097/OLQ.0000000000000423 (2016).
103. Brasil. Ministério da Saúde. Boletim Epidemiológico Sífilis 2020 (Departamento de Vigilância, Prevenção e Controle das Infecções Sexualmente Transmissíveis, do HIV/Aids e das Hepatites Virais, 2020).
104. Kirkcaldy, R. D., Weston, E., Segurado, A. C. & Hughes, G. Epidemiology of gonorrhoea: A global perspective. *Sex. Health.* **16**(5), 401–411. https://doi.org/10.1071/SH19061 (2019).
105. Zobolt, F. *et al.* Correlation between knowledge on transmission and prevention of HIV/STI and proficiency in condom use among male migrants from Africa and Middle East evaluated by a Condom Use Skills score using a wooden penile model. *BMC Res. Notes.* **10**(1), 216. https://doi.org/10.1186/s13104-017-2520-1 (2017).
106. Hargreaves, J. R. *et al.* Systematic review exploring time trends in the association between educational attainment and risk of HIV infection in sub-Saharan Africa. *AIDS* **22**(3), 403–414. https://doi.org/10.1097/QAD.0b013e3282f2aac3 (2008).
107. Raffetti, E. *et al.* The risk of late or advanced presentation of HIV infected patients is still high, associated factors evolve but impact on overall mortality is vanishing over calendar years: Results from the Italian MASTER Cohort. *BMC Public Health* **16**(1), 878. https://doi.org/10.1186/s12889-016-3477-z (2016).
108. Cid-Silva, P. *et al.* Late HIV diagnosis but earlier antiretroviral treatment initiation in northwest Spain: Impact of current treatment guidelines. *J. Int. Assoc. Provid. AIDS Care.* **18**, 2325958218821940. https://doi.org/10.1177/2325958218821940 (2019).
109. Goodall, L. & Leen, C. Late diagnosis of HIV: Could this be avoided?. *Scott. Med. J.* **56**(2), 84–86. https://doi.org/10.1258/smj.2011.011032 (2011).
110. Meléndez, J. *et al.* Late Presentation and missed opportunities for HIV diagnosis in Guatemala. *AIDS Behav.* **23**(4), 920–928. https://doi.org/10.1007/s10461-018-2331-y (2019).
111. UNODC. HIV e AIDS: Campanhas. Extraído de: https://www.unodc.org/lpo-brazil/pt/hiv-aids/campanhas.html (Acessado em 15 Jan 2021).
112. Brasil. Ministério da Saúde. Nova campanha contra HIV/aids estimula público jovem a realizar a testagem. Brasília: Departamento de Doenças de Condições Crônicas e Infecções Sexualmente Transmissíveis. Extraído de: http://www.aids.gov.br/pt-br/noticias/nova-campanha-contra-hivaids-estimula-publico-jovem-realizar-testagem (Acessado em 15 Jan 2021).
113. Kundro, M. A., Terwel, S. R., Tolbaro, J. J., Viloria, G. A. & Losso, M. H. Late diagnosis of HIV infection in asymptomatic patients. *Medicina* **76**(5), 273–278 (2016).
114. Gardner, A. T., Napier, R. & Brown, B. Risk factors for "late-to-test" HIV diagnosis in Riverside County, California. *Medicine* **95**(39), e5021. https://doi.org/10.1097/MD.0000000000005021 (2016).
115. Bath, R. E., Emmett, L., Verlander, N. Q. & Reacher, M. Risk factors for late HIV diagnosis in the East of England: Evidence from national surveillance data and policy implications. *Int. J. STD AIDS.* **30**(1), 37–44. https://doi.org/10.1177/0956462418793327 (2019).

116. Palacios-Baena, Z. R., Martín-Ortega, M. & Ríos-Villegas, M. J. Profile of new HIV diagnoses and risk factors associated with late diagnosis in a specialized outpatient clinic during the 2014–2018 period. *Med. Clin.* **155**(11), 482–487. https://doi.org/10.1016/j.medcli.2020.01.035 (2020).
117. Scognamiglio, P. *et al.* Unawareness of HCV serostatus among persons newly diagnosed with HIV. *J. Infect. Public Health.* **12**(5), 733–737. https://doi.org/10.1016/j.jtph.2019.01.055 (2019).
118. Ríos-Hincapié, C. Y. *et al.* Delays in HIV and TB diagnosis and treatment initiation in co-infected patients in Colombia. *Int. J. STD AIDS.* **31**(5), 410–419. https://doi.org/10.1177/0956462419881074 (2020).
119. Traore, L. *et al.* EBV and HHV-6 circulating subtypes in people living with HIV in Burkina Faso, impact on CD4 T cell count and HIV viral load. *Mediterr. J. Hematol. Infect. Dis.* **9**(1), e2017049. https://doi.org/10.4084/MJHID.2017.049 (2017).
120. Son, K. H. & Shin, M. Y. Clinical features of Epstein–Barr virus-associated infectious mononucleosis in hospitalized Korean children. *Korean J. Pediatr.* **54**(10), 409–413 (2011).
121. Oliveira, R. S. M., Benzaken, A. S., Saraceni, V. & Sabidó, M. HIV/AIDS epidemic in the State of Amazonas: Characteristics and trends from 2001 to 2012. *Rev. Soc. Bras. Med. Trop.* **48**(1), 70–78 (2015).
122. Braun, D. L. *et al.* Frequency and spectrum of unexpected clinical manifestations of primary HIV-1 infection. *Clin. Infect. Dis.* **61**(6), 1013–1021 (2015).
123. Melhuish, A. & Lewthwaite, P. Natural history of HIV and AIDS. *Medicine* **46**(6), 356–361 (2018).
124. Maartens, G., Celum, C. & Lewin, S. R. HIV infection: Epidemiology, pathogenesis, treatment, and prevention. *Lancet* **384**(9939), 258–271. https://doi.org/10.1016/S0140-6736(14)60164-1 (2014) (**Erratum in: Lancet. 2014 20;384(9948): 1098**).
125. Almeida, F. J., Kochi, C. & Sáfadi, M. A. P. Influence of the antiretroviral therapy on the growth pattern of children and adolescents living with HIV/AIDS. *J. Pediatr.* **95**(Suppl 1), 95–101. https://doi.org/10.1016/j.jped.2018.12.007 (2019).
126. Dharan, N. J. *et al.* Benefit of early versus deferred antiretroviral therapy on progression of liver fibrosis among people with HIV in the START randomized trial. *Hepatology* **69**(3), 1135–1150. https://doi.org/10.1002/hep.30296 (2019).
127. Cabral, G. A. Drugs of abuse, immune modulation, and AIDS. *J. Neuroimmune Pharmacol.* **1**(3), 280–295. https://doi.org/10.1007/s11481-006-9023-5 (2006).
128. Ladak, F. *et al.* Substance use patterns and HIV-1 RNA viral load rebound among HIV-positive illicit drug users in a Canadian setting. *Antivir. Ther.* **24**(1), 19–25. https://doi.org/10.3851/IMP3265 (2019).
129. Liang, J. *et al.* Longitudinal patterns of illicit drug use, antiretroviral therapy exposure and plasma HIV-1 RNA viral load among HIV-positive people who use illicit drugs. *AIDS* **34**(9), 1389–1396. https://doi.org/10.1097/QAD.0000000000002551 (2020).
130. European Collaborative Study. Level and pattern of HIV-1-RNA viral load over age: Differences between girls and boys?. *AIDS* **16**(1), 97–104. https://doi.org/10.1097/00002030-200201040-00012 (2002).
131. Goodkin, K. *et al.* Older age and plasma viral load in HIV-1 infection. *AIDS* **18**(Suppl 1), S87-98 (2004).
132. George, S., McGrath, N. & Ont, T. The association between a detectable HIV viral load and non-communicable diseases comorbidity in HIV positive adults on antiretroviral therapy in Western Cape, South Africa. *BMC Infect. Dis.* **19**(1), 348. https://doi.org/10.1186/s12879-019-3956-9 (2019).
133. Deeks, S. G. HIV infection, inflammation, immunosenescence, and aging. *Annu. Rev. Med.* **62**, 141–155. https://doi.org/10.1146/annurev-med-042909-093756 (2011).
134. Piriou, E. *et al.* Early age at time of primary Epstein–Barr virus infection results in poorly controlled viral infection in infants from Western Kenya: Clues to the etiology of endemic Burkitt lymphoma. *J. Infect. Dis.* **205**(6), 906–913. https://doi.org/10.1093/infdis/jir872 (2012).
135. Balfour, H. H. Jr. & Verghese, P. Primary Epstein–Barr virus infection: Impact of age at acquisition, coinfection, and viral load. *J. Infect. Dis.* **207**(12), 1787–1789. https://doi.org/10.1093/infdis/jit096 (2013).
136. Eller, M. A. *et al.* HIV type 1 disease progression to AIDS and death in a rural Ugandan cohort is primarily dependent on viral load despite variable subtype and T-cell immune activation levels. *J. Infect. Dis.* **211**(10), 1574–1584. https://doi.org/10.1093/infdis/jiu646 (2015).
137. Touloumi, G. *et al.* Impact of HIV-1 subtype on CD4 count at HIV seroconversion, rate of decline, and viral load set point in European seroconverter cohorts. *Clin. Infect. Dis.* **56**(6), 888–897. https://doi.org/10.1093/cid/cis1000 (2013).
138. McPhee, E. *et al.* Short Communication: The interaction of HIV set point viral load and subtype on disease progression. *AIDS Res. Hum. Retroviruses.* **35**(1), 49–51. https://doi.org/10.1089/AID.2018.0165 (2019).
139. Balfour, H. H. Jr. *et al.* A prospective clinical study of Epstein–Barr virus and host interactions during acute infectious mononucleosis. *J. Infect. Dis.* **192**(9), 1505–1512. https://doi.org/10.1086/491740 (2005).
140. Chan, K. C. A. *et al.* Analysis of plasma Epstein–Barr virus DNA to screen for nasopharyngeal cancer. *N. Engl. J. Med.* **377**(6), 513–522. https://doi.org/10.1056/NEJMoa1701717 (2017) (**Erratum in: N Engl J Med. 2018 Mar 8;378(10):973**).
141. Sorgato, C. C. *et al.* EBV and CMV viral load in rheumatoid arthritis and their role in associated Sjögren's syndrome. *J. Oral Pathol. Med.* **49**(7), 693–700. https://doi.org/10.1111/jop.13036 (2020).

Acknowledgements

We thank the entire technical and administrative staff of the participating institutions for helping to recruit the study patients, as well as all patients and blood donors for their trust and willingness to participate in the study.

Author contributions

T.A.F.M., O.M., R.C.M.S., F.B.F., I.B.C. and A.C.R.V. were the creators of the project. L.M.S.P., E.S.F., A.B.C.F. and F.L.P.R. were responsible for collecting the samples in the reference centers. L.M.S.P., E.S.F., I.B.C. and I.T.L. developed the project's methodological activities. L.M.S.P. applied the statistical analysis and wrote the article. I.B.C. and A.C.R.V. reviewed the article.

Funding

This study was funded by specific incentives from the Health Surveillance Department of the Brazilian Ministry of Health and Conselho Nacional de Desenvolvimento Científico e Tecnológico–CNPQ (#301869/2017-0). We thank the Coordination for the Improvement of Higher Education Personnel (CAPES) for granting a scholarship (process number: 88882.183970/2018-01).

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to A.C.R.V.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2021

2.2 SEGUNDO MANUSCRITO

Título: Epstein–Barr Virus (EBV) Genotypes Associated with the Immunopathological Profile of People Living with HIV-1: Immunological Aspects of Primary EBV Infection.

Lista de autores: Leonn Mendes Soares Pereira, Eliane dos Santos França, Iran Barros Costa, Igor Tenório Lima, Amaury Bentes Cunha Freire, Francisco Lúzio de Paula Ramos, Talita Antonia Furtado Monteiro, Olinda Macedo, Rita Catarina Medeiros Sousa, Felipe Bonfim Freitas, Igor Brasil Costa & Antonio Carlos Rosário Vallinoto.

Status da publicação: Publicado.

Nome da revista: Viruses.

ISSN: 1999-4915

Fator de impacto: 5.127 (Qualis A1)

DOI: https://doi.org/10.3390/v14020168

viruses

MDPI

Article

Epstein–Barr Virus (EBV) Genotypes Associated with the Immunopathological Profile of People Living with HIV-1: Immunological Aspects of Primary EBV Infection

Leonn Mendes Soares Pereira [1], Eliane dos Santos França [2], Iran Barros Costa [2], Igor Tenório Lima [2], Amaury Bentes Cunha Freire [3], Francisco Lúzio de Paula Ramos [3], Talita Antonia Furtado Monteiro [2], Olinda Macêdo [4], Rita Catarina Medeiros Sousa [5], Felipe Bonfim Freitas [4], Igor Brasil Costa [2,6,†] and Antonio Carlos Rosário Vallinoto [1,7,*,†]

1 Laboratory of Virology, Institute of Biological Sciences, Federal University of Pará (UFPA), Belém 66075-110, PA, Brazil; leonnpereira@hotmail.com
2 Epstein-Barr Virus Laboratory, Virology Department, Evandro Chagas Institute (IEC), Ananindeua 67030-000, PA, Brazil; elianesantos_85@hotmail.com (E.d.S.F.); irancosta@iec.gov.br (I.B.C.); igortenorio003@gmail.com (I.T.L.); talitamonteiro@iec.gov.br (T.A.F.M.); igorcosta@iec.gov.br (I.B.C.)
3 Epidemiology and Surveillance Service, Evandro Chagas Institute (IEC), Ananindeua 67030-000, PA, Brazil; amaurybentes@iec.gov.br (A.B.C.F.); franciscoramos@iec.gov.br (F.L.d.P.R.)
4 Retrovirus Laboratory, Virology Department, Evandro Chagas Institute (IEC), Ananindeua 67030-000, PA, Brazil; olindamacedo@iec.gov.br (O.M.); felipefreitas@iec.gov.br (F.B.F.)
5 School of Medicine, Federal University of Pará (UFPA), Belém 66075-110, PA, Brazil; ritaclossett@uol.com.br
6 Graduate Program in Virology, Evandro Chagas Institute (IEC), Ananindeua 67030-000, PA, Brazil
7 Graduate Program in Biology of Infectious and Parasitic Agents, Institute of Biological Sciences, Federal University of Pará (UFPA), Belém 66075-110, PA, Brazil
* Correspondence: vallinoto@ufpa.br
† Co-senior authors.

check for updates

Citation: Pereira, L.M.S.; dos Santos França, E.; Costa, I.B.; Lima, I.T.; Freire, A.B.C.; de Paula Ramos, F.L.; Monteiro, T.A.F.; Macedo, O.; Sousa, R.C.M.; Freitas, F.B.; et al. Epstein–Barr Virus (EBV) Genotypes Associated with the Immunopathological Profile of People Living with HIV-1: Immunological Aspects of Primary EBV Infection. *Viruses* **2022**, *14*, 168. https://doi.org/10.3390/v14020168

Academic Editor: Zhi-Ming Zheng

Received: 12 November 2021
Accepted: 11 January 2022
Published: 18 January 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

Abstract: Background: The aim of the present study was to evaluate the immunological profile of adult HIV-1$^+$ patients coinfected with primary Epstein–Barr virus (EBV) infection who were free of antiretroviral drugs and inhabitants of the Brazilian Amazon region. Materials and methods: Primary EBV infection was screened by the semiquantitative detection of IgM and IgG anti-VCA. Genotypes were determined by conventional PCR. EBV and HIV viral load (VL) were quantified by real-time PCR. Cytokine dosage and cell quantification were performed by cytometry. Results: Only HIV-1$^+$ individuals had primary EBV infection (7.12%). The EBV-1 genotype was the most prevalent (47.37%). The VL of HIV-1 was lower in the HIV/EBV-2 group. CD4$^+$ T lymphocytes were inversely proportional to the VL of EBV in HIV/EBV-1/2 multi-infected patients. The HIV/EBV-2 group had the lowest cytokine levels, especially IFN-γ and IL-4. Different correlations were proposed for each coinfection. The late search for specific care related to HIV infection directly affected the cytokine profile and the number of CD8$^+$ T lymphocytes. Symptoms were associated with the increase in VL of both viruses and cytokine profile. Conclusions: Different immunological profiles were associated with EBV genotypes in primary infection, with EBV-2 being more frequent in patients with low levels of HIV viral load. With late infection monitoring and consequent delay in the initiation of HAART, clinical changes and effects on the maintenance of the immune response were observed.

Keywords: HIV-1; EBV; coinfection; immunopathological profile

1. Introduction

Epstein–Barr virus (EBV) is considered one of the risk factors for morbidity in people living with HIV (PLHIV) because it is a precursor of neoplasms in this group [1–3]. Coinfection is related to progressive dysfunction events and impaired immune surveillance, which becomes critical when progressing to AIDS, in which severe immunosuppression results in pathological signs of malignant neoplasms [4–6].

In primary EBV infection, the pathological manifestations of coinfection are more studied in children, in whom HIV and persistent EBV replication results in a severe spectrum of clinical symptoms, including splenomegaly, hepatomegaly, and pneumonia, which increase the risk of hospitalization recurrence [7,8].

The discussion becomes more complex when considering that the prevalence of EBV genotypes may be associated with the immune profile of the host coinfected with HIV. EBV-1 is more prevalent in patients with fewer helper T lymphocytes and a high HIV viral load, while EBV-2 predominates in more responsive immune profiles comprising numerous helper T lymphocytes and a low HIV viral load [9–11]; However, it is not clear whether this trend is a pattern observed only in primary EBV infection.

In this context, the aim of this study was to evaluate the immunological profile of adult PLHIV who were free of antiretroviral drugs (HAART) and presented with primary coinfection with different EBV genotypes; we sought to associate immunological markers with the viral dynamics of HIV/primary EBV coinfection and the clinical aspects of the patients to reveal the possible impacts of the late attendance of the patient.

In developing countries, primary EBV infection is more frequent in childhood, especially in children of low socioeconomic status [12,13], which presents a challenge regarding the aims of this study. However, we emphasize that there are few studies on HIV/primary EBV coinfection in the Brazilian Amazon and the northern region of the country; therefore, our findings may contribute to discussions on the epidemiological scenario of HIV/primary EBV coinfection in Brazil, as well as on the complex viral interaction intrinsic to the context of coinfection.

2. Materials and Methods

2.1. Sampling

This was an observational, cross-sectional, and analytical study conducted at the Laboratory of Virology at the Federal University of Pará (LABVIR-UFPA), the Evandro Chagas Institute (IEC), the Center for Health Care in Acquired Infectious Diseases (CASA DIA, for its acronym in Portuguese), and the Hemotherapy and Hematology Foundation of the State of Pará (HEMOPA, for its acronym in Portuguese), where 20 weekly samples from participants living in the state of Pará, Brazil, were selected from January 2018 to December 2019.

Samples were collected from 333 participants who were categorized as 249 PLHIV no primary EBV infection (p24+, anti-HIV-1 IgG [+], anti-VCA-EBV IgM [−], and anti-VCA-EBV IgG [+ or −]), 19 primary EBV and HIV-1 coinfected (p24+, anti-HIV-1 IgG [+], anti-VCA-EBV IgM [+], and anti-VCA-EBV IgG [−]); confirmation by molecular biology assay), and 65 no HIV-1 infection or primary EBV infection (p24[−], anti-HIV-1 IgG [−], anti-VCA-EBV IgM [−], and anti-VCA-EBV IgG [+ or −]). All participants answered an epidemiological questionnaire administered at the time of the interview.

The project was submitted for ethical review and approved by the Human Research Ethics Committee of the IEC (Protocol: 3.121.265; CAAE: 73927717.3.0000.0019). All participants were informed about the study objectives and signed an informed consent form.

Participants included in the study were 18 years old or older, of both sexes, and HIV-1 and/or EBV carriers, were not using antiviral therapy, and signed the informed consent form.

2.2. Confirmatory Methods

Peripheral blood samples were collected from the participants. DNA was extracted from the plasma and buffy coat using a QiaAmp DNA Mini Kit (Qiagen, Düsseldorf, Nordrhein-Westfalen, Germany) following the manufacturer's recommendations.

Samples were screened for HIV-1 infection by the qualitative detection of p24 antigen and anti-HIV-1 and anti-HIV-2 IgG antibodies using an enzymatic immunoassay (Murex AG/AB Combination DiaSorin, Dartford, Kent, UK); serological confirmation was per-

formed using the Immunoblot rapid DPP HIV-1/2 kit (Bio-Manguinhos, Rio de Janeiro, RJ, Brazil) following the manufacturer's recommendations.

Infection by EBV was screened by the semiquantitative detection of anti-EBV IgM and IgG antibodies using an enzymatic immunoassay (Ridascreen EBV VCA R-Biopharm, Darmstadt, Hesse, Germany). The identification of the EBV genotypes was performed by nested PCR targeting the EBNA-3C gene using the primers described by Lorenzetti et al. [14] (1st round–(F: 5′-AGATGGTGAGCCTGACGTG-3′/R: 5′-GCATCCTTCAAAACCTCAGC-3′) and Sample et al. [15] (2nd round–F: 5′-AGAAGGGGAGCGGTGTGTTGT-3′/R: 5′-GGCTGTTTTTGACGTCGGC-3′). The reaction components and cycling conditions were as follows: 10 pmol/µL of primers, $MgCl_2$ (50 mM), dNTP (10 mM), and Taq (5 U/µL); 1st round–1 cycle of 95 °C/3′, 20 cycles of (94 °C/45″; 56 °C/45″; and 72 °C/45″), and 1 cycle of 72 °C/7′; and 2nd round–1 cycle of 95 °C/3′; 35 cycles of (94 °C/45″; 56 °C/45″; and 72 °C/45″); and 1 cycle of 72 °C/7′. The presence of a 153 bp fragment was considered positive for EBV-1, and the presence of a 246 bp fragment was considered positive for EBV-2.

2.3. Quantification of T Lymphocytes and Measurement of Cytokines

The quantification of T helper ($CD45^{high}CD3^+CD4^+CD8^-$), T cytotoxic ($CD45^{high}CD3^+CD4^-CD8^+$), double positive (DP) ($CD45^{high}CD3^+CD4^+CD8^+$), and double negative (DN) ($CD45^{high}CD3^+CD4^-CD8^-$) lymphocytes was performed by immunophenotyping and flow cytometry using BD FACSCalibur (4 colors) equipment, FACSCount™ Reagents, and TriTEST™/TruCount monitoring kits (BD Biosciences, San Jose, CA, USA). We used the BD Multiset™ Software v3.1 software (BD Biosciences, San Jose, CA, USA) already standardized for analysis of lymphocyte populations related to HIV infection.

The plasma concentrations of the cytokines IL-17A, IFN-γ, TNF, IL-10, IL-6, IL-4, and IL-2 were determined with a cytometric bead array (CBA) using BD FACSCanto™ II equipment and a BD™ CBA Human Th1/Th2/Th17 Cytokine kit (BD Biosciences, San Jose, CA, USA).

2.4. Quantification of HIV-1 and EBV Viral Loads

The quantification of the HIV-1 plasma viral load was performed by real-time PCR using an Abbott Sample Preparation System RNA extraction kit and the Abbott Real-Time HIV-1 amplification matrix (ABBOTT, Chicago, IL, USA).

The quantification of the EBV viral load was based on an estimation matrix by real-time PCR, following the protocol of the XGEN MASTER EBV kit (Mobius Life Science, Pinhais, PR, Brazil). We used buffy coat and plasma samples to quantify the viral load.

2.5. Statistical Analysis

In the sample characterization, we compared sociodemographic data, spontaneous delay in seeking specific care (late attendance) and symptomatology among groups using the G test. Fisher's exact test was used exclusively for data dispersed in 2 × 2 tables.

We applied the Kruskal–Wallis test for the analysis of variance in viral loads, number of lymphocytes and cytokine levels among the groups studied; we opted for the Student-Newman–Keuls method as a complement to compare the ranked means.

We adopted the same methodology in the assessment of immunological changes based on the time of delay in seeking specific care and symptomatology. We chose a nonparametric test after determining that the distribution of the samples did not meet the assumption of normality, as calculated by the Lilliefors test.

The Spearman coefficient was calculated in the correlation of the EBV viral loads quantified in different blood extracts with the other factors studied. We used curve fitting to determine the correlation between viral loads and the correlation between the EBV viral load and the number of lymphocytes.

Correlation matrices between all immunological and virological factors analyzed were proposed for the group of PLHIVs without primary EBV infection and for the coinfected groups based on Pearson's coefficient (r).

A dispersion diagram was inferred based on multivariate discriminant analysis of the immunological factors and viral load of the studied groups.

The statistical analyses were performed using GraphPad Prism 3.03 (San Diego, CA, USA) and BioEstat 5.0 [16].

3. Results

3.1. Sample Characterization

Of the 333 participants evaluated between January 2018 and December 2019, only 19 were positive for primary EBV infection, all of them infected with HIV-1; we obtained a prevalence of 7.09% of coinfected individuals (Table 1), with the majority living in the metropolitan mesoregion of Belém city (98.20%).

Table 1. Sample characterization of the studied groups according to sex, age, delay in seeking specific care, and reported symptoms.

Characteristics	PLHIV No Primary EBV	HIV/Primary EBV 19 (7.09)			No HIV/Primary EBV	Statistic
		HIV/EBV-1	HIV/EBV-2	HIV/EBV-1/2		
Sample number	249	9 (47.37)	5 (26.32)	5 (26.32)	65	
Sex						
Feminine	49 (19.7)	5 (56.0)	1 (20.00)	1 (20.0)	17 (26.2)	06.22 (0.23) #
Masculine	200 (80.3)	4 (44.0)	4 (80.00)	4 (80.0)	48 (73.8)	
Age (years)						
18–38	186 (74.70)	8 (88.89)	3 (60.00)	5 (100.00)	43 (66.15)	07.31 (0.82) *
39–59	59 (23.69)	1 (11.11)	2 (40.00)	0	20 (30.77)	
60–80	4 (01.61)	0	0	0	2 (03.08)	
Delay in seeking assistance (months)						
<1 m	122 (49.00)	2 (22.0)	4 (80.0)	1 (20.0)	-	27.47 (0.001) *
1–6 m	95 (38.00)	7 (78.0)	1 (20.0)	2 (40.0)	-	
7–12 m	32 (13.00)	0	0	0	-	
>12 m	0	0	0	2 (40.0)	-	
Symptomatology						
Fever	177 (71.08)	7 (77.77)	5 (100.00)	5 (100.00)	-	6.54 (0.69) *
Headache	110 (44.18)	7 (77.77)	4 (80.00)	2 (40.00)	-	
Myalgia and joint pain	90 (36.14)	5 (55.55)	1 (20.00)	0	-	
Sore throat	197 (79.12)	7 (77.77)	5 (100.00)	3 (60.0)	-	
Groups						
Asymptomatic	132 (53.01)	2 (22.22)	3 (60.00)	1 (20.00)	-	6.09 (0.41) *
Oligosymptomatic	67 (26.91)	3 (33.33)	1 (20.00)	3 (60.00)	-	
Polysymptomatic	50 (20.08)	4 (44.44)	1 (20.00)	1 (20.00)	-	
Infection history						
No	142 (57.07)	3 (42.86)	3 (60.00)	2 (40.00)	62 (95.00)	16.70 (0.67) *
Fungi	1 (00.26)	0	0	0	1 (01.67)	
Bacterial (STD)	86 (34.45)	4 (57.14)	2 (40.00)	3 (60.00)	0	
HPV	10 (4.11)	0	0	0	1 (01.67)	
Herpes	9 (3.60)	0	0	0	1 (01.67)	
Hepatitis	1 (00.51)	0	0	0	0	

#: Fisher's exact test (p-value); *: Yest G (p value).

All 65 uninfected individuals were healthy blood donors with no history of comorbidities. The 249 PLHIV no primary EBV infection and the 19 HIV/primary EBV coinfected were outpatients with a positive pre-diagnosis for HIV with or without a history of associated comorbidities (Table 1). Both uninfected and PLHIV no primary EBV infection had positive anti-VCA IgG serology, indicating previous contact with the EBV.

Among those coinfected, the EBV-1 genotype was the most prevalent (47.37%), followed by the EBV-2 genotype and multi-infection with both genotypes, both of which occurred in the same proportion in the population (26.32%) (Table 1). There was a predominance of male individuals aged 18 to 38 years in all groups, except for HIV/primary EBV-1 coinfected patients, among whom women were more prevalent (Table 1).

3.2. Evaluation of the EBV Viral Load in Different Blood Extracts and Its Correlation with HIV-1 Viral Load

We evaluated which blood extract would be the most appropriate for the quantification of EBV viral load in primary infection. We observed that the biomarker in the buffy coat was considerably higher than in the blood plasma [$p < 0.0001$; (HIV/EBV-1 plasma: median: 33.33 IQ (25–75%): 6–67.33); (HIV/EBV-1 buffy coat: median: 3668 IQ (25–75%): 1663.33–4793.33); (HIV/EBV-2 plasma: median: 16.67 IQ (25–75%): 8.67–23.33); (HIV/EBV-2 buffy coat: median: 21,322.67 IQ (25–75%): 970.67–27,473.33); (HIV/EBV-1/2 plasma: median: 14 IQ (25–75%): 10–66.67); (HIV/EBV-1/2 buffy coat: median: 3591.33 IQ (25–75%): 3562–24,485.33)] (Figure 1A). However, when calculating the correlation of the viral load with the other markers studied, we observed that the plasma viral load generated the most significant correlations (Figure 1B); therefore, plasma was chosen as the blood extract for the subsequent analyses. In Table S1, we detail the statistical data referring to the Spearman coefficient (rs) and respective probability value (p) for each proposed correlation. Notably, only the symptoms of the patients were associated with the quantified viral load in both blood extracts (rs plasma: 0.55; p: 0.015/rs buffy coat: 0.52; p: 0.022). A priori, this first analysis was focused only on the choice of the ideal sample for the quantification of the viral load; the biological significance of the associations generated was explored throughout the study.

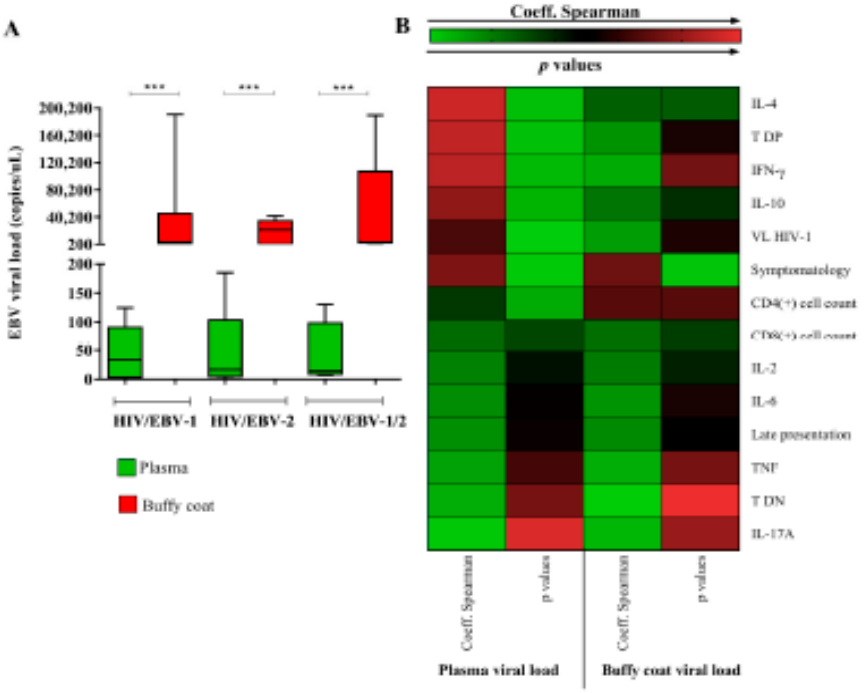


Figure 1. Analysis of the EBV viral load in different blood extracts. (**A**) Graph showing the quantification of the EBV viral load in the plasma and buffy coat of the HIV/primary EBV coinfected groups. ***: $p < 0.0001$. (**B**) Heatmap illustrating the absolute values of the Spearman coefficient and respective p value for the correlations between the EBV viral load quantified in plasma and in the buffy coat with the other factors studied. The most significant correlations were observed for plasma viral load; in the buffy coat, the viral load was associated only with the patients' symptoms. The statistical data for each correlation are detailed in Supplementary Table S1.

In an intergroup analysis, the median plasma viral load in the HIV/EBV-1 group was higher (median: 33.33 (IC 95%): 6–67.33) but not statistically significant (p: 0.962) (Figure 1 A).

The median log10 of the HIV-1 viral load was lower in the HIV-EBV-2 group when compared to the others [p: 0.0207; (HIV: med: 4.73 IQ (25–75%): 4.03–5.35); (HIV/EBV-1: med: 5.27 IQ (25–75%): 4.38–5.74); (HIV/EBV-2: med: 3.84 IQ (25–75%): 2.27–4.34); (HIV/EBV-1/2: med: 4.35 IQ (25–75%): 3.67–5.34)] (Figure 2A).

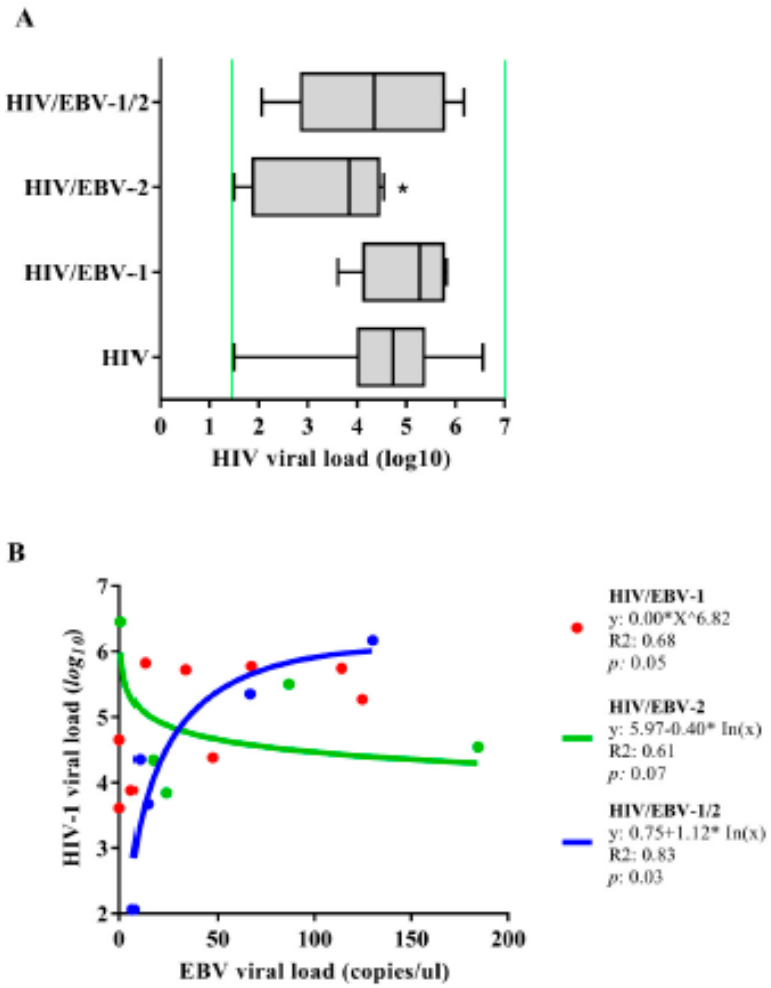


Figure 2. Quantification of viral load: (**A**) Quantification of the HIV-1 plasma viral load. Green lines indicate the limits of detection for the assay used (<log1.6 (40 copies)—>log7 (10,000,000 copies)); *: $p < 0.05$. (**B**) Regression graph for viral loads of HIV-1 and EBV.

We conducted a regression analysis of the viral loads of both viruses in the coinfected patients (Figure 2B). In the HIV/EBV-1 group, the geometric regression indicated a trend between the markers (p: 0.05; R2: 0.68), in which the EBV-1 viral load was distributed in a symmetrical curve with a peak of 125 copies/µL, followed by a decrease as the

log10 of the HIV-1 viral load increased. We found an inversely proportional relationship between the viral loads in the HIV/EBV-2 group that was best represented by a decreasing logarithmic regression (p: 0.07; R2: 0.61) in which the HIV-1 viral load slowly decreased as the EBV-2 viral load increased. In contrast, we observed the opposite for the HIV/EBV-1/2 multi-infected group; the regression between viral loads was directly proportional and represented by an increasing logarithmic function (p: 0.03; R2: 0.83). In this case, the HIV-1 viral load gradually increased as the viral loads of EBV-1 and EBV-2 increased.

3.3. Different Immunological Profiles Were Associated with the EBV Genotypes

The median number of helper T lymphocytes was higher only in the no HIV/primary EBV group when compared to others [$p < 0.0001$; (HIV: med: 317.5 IQ (25–75%): 165.25–499.25); (HIV/EBV-1: med: 374 IQ (25–75%): 120.429); (HIV/EBV-2: med: 456 IQ (25–75%): 215–534); (HIV/EBV-1/2: med: 134 IQ (25–75%): 115–217); (no HIV/primary EBV: med: 1009 IQ (25–75%): 795–1218)] (Figure 3A). The regression of the EBV viral load in relation to the $CD4^+$ T count was significant only in the HIV/EBV-1/2 group, in which we observed a logarithmic decrease in the multi-infection viral load as the lymphocyte count increased (p: 0.04; R2: 0.80). There was a trend of exponential increase in EBV viral load as a function of helper T lymphocytes in the HIV/EBV-2 group (p: 0.09; R2: 0.66). We did not observe a correlation between the EBV viral load and the $CD4^+$ T count in the HIV/EBV-1 group. The closest mathematical regression obtained was a geometric function (p: 0.62; R2: 0.037) (Figure 3B).

The median cytotoxic T lymphocyte count was lower only in the no HIV/primary EBV group when compared to others [$p < 0.0001$; (HIV: med: 1050 IQ (25–75%): 737.25–1442.25); (HIV/EBV-1: med: 1015 IQ (25–75%): 951–1959); (HIV/EBV-2: med: 1127 IQ (25–75%): 854–2819); (HIV/EBV-1/2: med: 987 IQ (25–75%): 638–1034); (no HIV/primary EBV: med: 561; IQ (25–75%): 450–745)] (Figure 3C). We did not find a correlation between the EBV viral load and the cytotoxic T lymphocyte count in the coinfected groups; there were only trends of a linear increase in the viral load as a function of cell count in the HIV/EBV-1 group (p: 0.25; R2: 0.19), an exponential decrease in the HIV/EBV-2 group (p: 0.24; R2: 0.42), and a linear decrease in the HIV/EBV-1/2 group (p: 0.56; R2: 0.12) (Figure 3D).

The number of DN T lymphocytes was higher in the HIV/EBV-2 group when compared to the others [(p: 0.046; (HIV: med: 65 IQ (25–75%): 39–101.25); (HIV/EBV-1: med: 60 IQ (25–75%): 47–95); (HIV/EBV-2: med: 116 IQ (25–75%): 91–141); (HIV/EBV-1/2: med: 31 IQ (25–75%): 18–54); (no HIV/primary EBV: med: 76; IQ (25–75%): 56–108)] (Figure 3E). We did not find a correlation between the EBV viral load and the DN count; there were only trends of a linear increase in the viral load as a function of cell count in the HIV/EBV-1 group (p: 0.16; R2: 0.26), a logarithmic increase in the HIV/EBV-2 group (p: 0.92; R2: 0.042), and a linear decrease in the HIV/EBV-1/2 group (p: 0.60; R2: 0.10) (Figure 3F).

The number of DP T lymphocytes was higher in the HIV/EBV-1 group when compared to the others [(p: 0.056; (HIV: med: 4 IQ (25–75%): 2–8); (HIV/EBV-1: med: 7; IQ (25–75%): 4–9); (HIV/EBV-2: med: 3 IQ (25–75%): 2–6); (HIV/EBV-1/2: med: 3 IQ (25–75%): 1–3); (no HIV/primary EBV: med: 10; IQ (25–75%): 5–16)] (Figure 3G). A significant regression was observed only in the HIV/EBV-1/2 group, i.e., a decreasing inversely proportional relationship between the viral load and lymphocyte count (p: 0.001; R2: 0.96); there were only nonsignificant trends of a linear increase in viral load as a function of the lymphocyte count in the HIV/EBV-2 group (p: 0.10; R2: 0.64) and a geometric trend for the variables in the HIV/EBV-1 group (p: 0.00; R2: 0.96) (Figure 3H).

In the analysis of cytokine concentrations, the median concentrations IFN-γ levels were lower in the HIV/EBV-2 group when compared to the others [(p: 0.051; (HIV: med: 11.66 IQ (25–75%): 9.80–13.48); (HIV/EBV-1: med: 10.92 IQ (25–75%): 10.14–11.94); (HIV/EBV-2: med: 3 IQ (25–75%): 2–6); (HIV/EBV-1/2: med: 13.57 IQ (25–75%): 10.24–24.02); (no HIV/primary EBV: med: 12.61; IQ (25–75%): 10.87–14.19)] (Figure 4B).

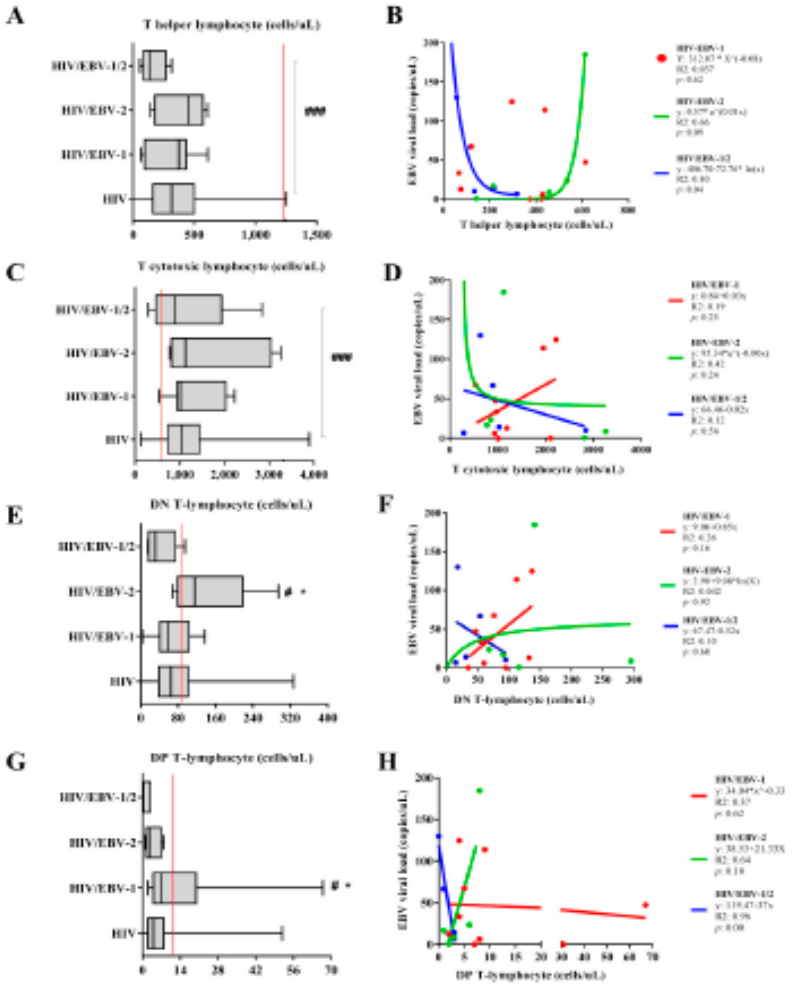


Figure 3. Cell quantification: Box–plots of the quantification of (**A**) helper, (**C**) cytotoxic, (**E**) double-negative, and (**G**) double–positive T lymphocytes in the studied groups. Red lines indicate the median cell count in no HIV/primary EBV individuals. #: high score in relation to no HIV/primary EBV individuals. *: high count among those infected. ###: $p < 0.001$; # *: $p < 0.05$. Cartesian graph of the regression between EBV viral load and the quantification of (**B**) helper, (**D**) cytotoxic, (**F**) double-negative, and (**H**) double–positive T lymphocytes in the studied groups.

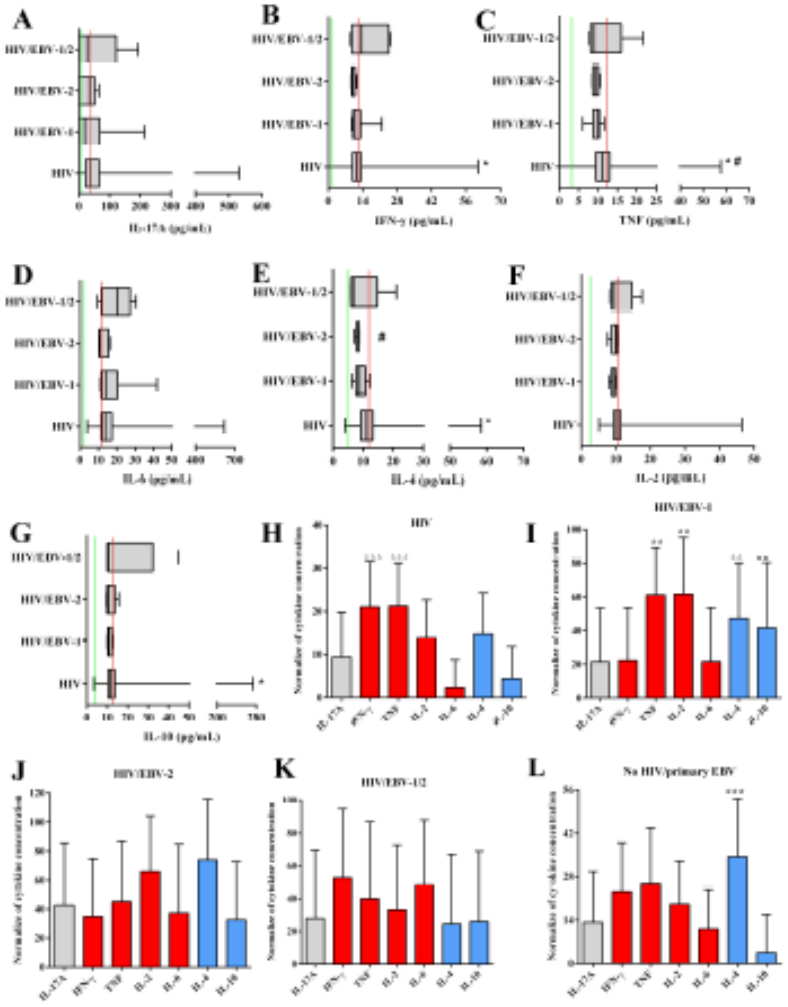


Figure 4. Cytokine levels: Box–plots of the cytokines IL-17A (**A**), IFN-γ (**B**), IL-4 (**C**), TNF (**D**), IL-6 (**E**), IL-2 (**F**), and IL-10 (**G**). Red lines indicate the median cytokine levels in the no HIV/primary EBV group. Green lines indicate the limit of detection for each cytokine defined by the assay manufacturer (IL-17A: 18.9 pg/mL; IFN-γ: 3.7 pg/mL; IL-4: 4.9 pg/mL; TNF: 3.8 pg/mL; IL-6: 2.4 pg/mL; IL-2: 2.6 pg/mL; IL-10: 4.5 pg/mL). #: different levels compared to the no HIV/primary EBV group. *: different levels among the infected groups. Graph of the comparison of immunological profiles based on the normalized concentration of cytokines evaluated in the HIV (**H**), HIV/EBV-1 (**I**), HIV/EBV-2 (**J**), HIV/EBV-1/2 (**K**), and no HIV/primary EBV (**L**) groups. Gray: TH17 cytokines; red: TH1 cytokines; blue: TH2 cytokines. *: $p < 0.05$; **: $0.005 > p > 0.001$; ***: $p < 0.001$.

The no HIV/primary EBV group had higher TNF levels than the other groups [(p: 0.002; (HIV: med: 11.16 IQ (25–75%): 9.37–13.01); (HIV/EBV-1: med: 9.77 IQ (25–75%): 9.25–10.24); (HIV/EBV-2: med: 9.52 IQ (25–75%): 8.72–9.91); (HIV/EBV-1/2: med: 8.98 IQ

(25–75%): 8.33–10.30); (no HIV/primary EBV: med: 12.065; IQ (25–75%): 10.16–14.02)] (Figure 4C).

HIV/EBV-2 coinfected individuals had the lowest IL-4 values among all groups [(p: 0.0001; (HIV: med: 11.14 IQ (25–75%): 9.24–13.27); (HIV/EBV-1: med: 8.82 IQ (25–75%): 8.52–9.79); (HIV/EBV-2: med: 8.61; IQ (25–75%): 8.52–8.62); (HIV/EBV-1/2: med: 6.79 IQ (25–75%): 6.07–8.42); (no HIV/primary EBV: med: 12.78; IQ (25–75%): 10.07–14.28)] (Figure 4E).

The evaluation of normalized cytokine data indicated the prevalence of the TH1 profile in the HIV mono-infected group, with higher levels of IFN-γ cytokines ($p < 0.0001$; med: 19.12; IQ (25–75%): 16.07–22.10) and TNF ($p < 0.0001$; med: 19.36; IQ (25–75%): 16.26–22.57) (Figure 4H), and codominance of the TH1 and TH2 profiles in the HI/EBV-1 group, with higher levels of TNF (p: 0.0035; med: 66.67; IQ (25–75%): 57.38–75.04), IL-2 (p: 0.0035; med: 57.00; IQ (25–75%): 41.06–92.75), IL-4 (p: 0.0035; med: 41.83; IQ (25–75%): 36.83–58.00) and IL-10 (p: 0.0035; med: 29.33; IQ (25–75%): 9.89–77.74) (Figure 4I). We did not observe a prevalence of immunological profiles in the HIV/EBV-2 and HIV/EBV-1/2 coinfected groups, and all cytokines had similar concentrations (Figure 4J,K). In the no HIV/primary EBV group, compared with the other cytokines, the IL-4 levels were highest ($p < 0.0001$; med: 35.86; IQ (25–75%): 21.45–45.00) (Figure 4L).

In the group PLHIV no primary EBV infection, HIV-1 viral load was positively correlated with IL-10 (p: 0.04, r: 0.12) and IL-6 (p: 0.05, r: 0.11) dosages, and negatively correlated with helper T lymphocytes ($p < 0.0001$, r: −0.38), DN T lymphocytes (p: 0.003, r: −0.19) and DP T lymphocytes ($p < 0.0001$, r: −0.30). Interestingly, helper T lymphocyte counts were positively correlated with cytotoxic T lymphocytes, with the linear graph being the best regression model among the data ($p < 0.0001$, R2: 0.2177) (Figure 5), DN T lymphocytes ($p < 0.0001$, r: 0.39), and DP T lymphocytes ($p < 0.0001$, r: 0.43), such as IL-17A dosages (p: 0.05, r: 0.12); and negatively correlated with IL-10 (p: 0.03, r: −0.14) and IL-6 (p: 0.04, r: −0.13) dosages. The cytotoxic T lymphocyte count was correlated only with the T DN ($p < 0.0001$, r: 0.37) and T DP ($p < 0.0001$, r: 0.26) lines. The strongest system correlations were observed between cytokines, with p values ranging from 0.04 to <0.0001 and r values ranging from 0.10 to 0.90; only IL-6 was only correlated with IFN-γ ($p < 0.0001$, r: 0.40) and IL-10 (p: 0.003, r: 0.24) (Figure 6A).

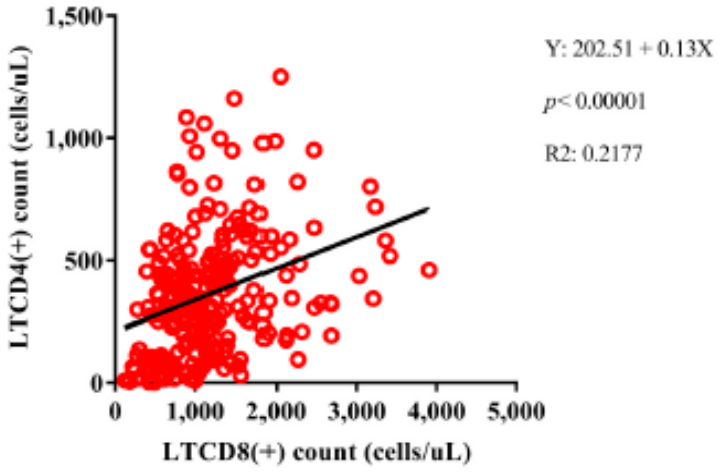


Figure 5. Positive linear regression model between helper T lymphocyte (LTCD4+) and cytotoxic T lymphocyte (LTCD8+) counts among PLHIV no primary EBV infection.

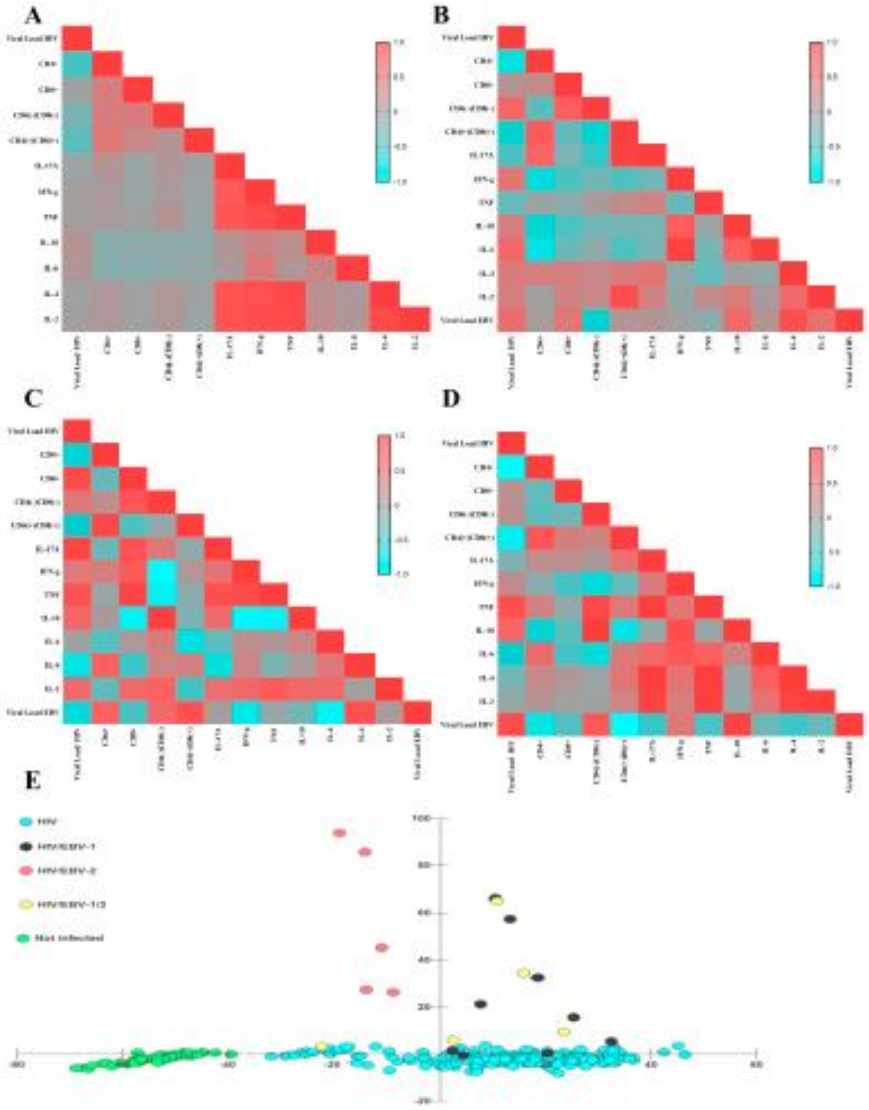


Figure 6. Matrix of correlations and clusters. (A–D) Heatmap graphs illustrating the Pearson coefficient of each analyzed correlation between immunological and virological factors for different groups: (A) PLHIV no primary EBV infection; (B) HIV/EBV-1; (C) HIV/EBV-2; (D) HIV/EBV-1/2. The color spectrum varies from blue (negative correlations) to red (positive correlations), as per the legend in the figures. (E) Discriminant dispersion diagram of the studied groups based on their immunological and viral load profiles. The HIV/EBV-2 group did not overlap with the coinfected cluster.

In the HIV/EBV-1 group, HIV viral load was positively correlated with IL-6 dosage (p: 0.04, r: 0.58) and IFN-γ (p: 0.0, r: 0.45), as was the EBV viral load (p: 0.03, r: 0.52); and negatively correlated to helper T lymphocyte counts (p: 0.04, r: −0.69) and DP T lymphocytes (p: 0.04, r: −0.52). EBV viral load was positively correlated with IL-4 dosage (p: 0.001, r: 0.82) and IL-10 (p: 0.03, r: 0.56). Helper T lymphocyte count was positively correlated with DP T lymphocytes (p: 0.03, r: 0.72), as was IL-17A dosage (p: 0.05, r: 0.61); and negatively correlated with IFN-γ dosage (p: 0.05, r: −0.63), and IL-6 (p: 0.03, r: −0.71). The cytotoxic T lymphocyte count was correlated only with the T DN lineage (p: 0.04, r: 0.67). PD T lymphocyte count was positively correlated with IL-17A dosage ($p < 0.0001$, r: 0.95) and IL-2 (p: 0.002, r: 0.84). Among cytokines, IFN-γ dosage was correlated to IL-10 (p: 0.04, r: 0.69) and IL-6 ($p < 0.0001$, r: 0.97); IL-6 dosage was also correlated with IL-10 (p: 0.05, r: 0.66) (Figure 6B).

In the HIV/EBV-2 group, HIV viral load was positively correlated with cytotoxic T lymphocyte count (p: 0.05, r: 0.87) and IL-17A dosage (p: 0.03, r: 0.92); and negatively correlated with T helper lymphocyte count (p: 0.03, r: −0.57) and EBV viral load (p: 0.02, r: −0.62). EBV viral load was positively correlated with T helper lymphocyte counts (p: 0.05, r: 0.65); and negatively correlated with IL-6 ($p < 0.0001$, r: −0.91) and IFN-γ (p: 0.005, r: −0.751) dosages. Helper T lymphocyte count was positively correlated with DN T lymphocytes (p: 0.04, r: 0.85) and negatively correlated with cytotoxic T lymphocytes (p: 0.02, r: −0.28). Cytotoxic T lymphocyte count was positively correlated with TNF assay (p: 0.03, r: 0.92) and negatively correlated with IL-10 (p: 0.04, r: −0.89). DN T lymphocyte count was positively correlated with IL-10 dosage (p: 0.01, r: 0.95) and negatively correlated with IFN-γ dosage (p: 0.003, r: −0.98) and TNF (p: 0.05, r: −0.86). Among cytokines, IL17-A was correlated with IFN-γ dosage (p: 0.005, r: 0.81); IFN-γ was correlated to TNF (p: 0.01, r: 0.87) and with IL-10 (p: 0.002, r: −0.93); TNF was also correlated with IL-10 (p: 0.03, r: −0.91) (Figure 6C).

In the HIV/EBV-1/2 group, HIV viral load was positively correlated with EBV viral load (p: 0.05, r: 0.85) and TNF dosage (p: 0.009, r: 0.79), and negatively correlated to helper T lymphocyte counts (p: 0.003, r: −0.98) and DP T lymphocytes (p: 0.05, r: −0.85). EBV viral load was positively correlated with IL-10 dosage (p: 0.05, r: 0.92) and negatively correlated with helper lymphocyte count (p: 0.02, r: −0.78), DN T lymphocytes (p: 0.05, r: −0.32) and DP T lymphocytes (p: 0.002, r: −0.98). Helper T lymphocyte count was negatively correlated with EBV viral load (p: 0.05, r: −0.77). Cytotoxic T lymphocyte count was positively correlated with DN T lymphocyte count (p: 0.02, r: 0.94). Among the cytokines, IL-17A dosage was correlated to TNF (p: 0.009, r: 0.96), IL-4 (p: 0.0007, r: 0.99), and IL-2 (p: 0.01, r: 0.95); TNF was correlated to IL-6 (p: 0.005, r: 0.98) and IL-4 (p: 0.006, r: 0.97); IL-4 was correlated to IL-2 (p: 0.02, r: 0.94) (Figure 6D).

In the dispersion diagram, the grouping of no HIV/primary EBV individuals was the most distinct among the others. Among the infected patients, we observed an overlap of the profiles for the HIV/EBV-1 and HIV/EBV-1/2 groups, which tended to be close to those mono-infected with HIV; the HIV/EBV-2 coinfected patients were grouped independently of the others and without points of intersection (Figure 6E).

3.4. Late Attendance and Symptomatology Affect the Profile of Immunological and Virological Markers

We calculated the time of late attendance of the HIV-1-infected patients and approximately 40% of multi-infected patients were more than 12 months after the first diagnosis and had not started antiretroviral treatment, a statistically significant proportion when compared to the delay time of the other groups (G test: 27.47; p: 0.001) (Table 1).

We evaluated the profile of immunological markers based on the delay in time of attendance of patients (Figure 7A). We observed a subtle increase in the median HIV viral load in patients with more than 12 months of delay after the first diagnosis (p: 0.9636; med: 125704.5; IQ (25–75%): 74149.25–177259.75). Regarding EBV, there was a linear increase in viral load based on the delay of adherence, with a peak in patients more than 12 months

after the first diagnosis (med: 38.3; IQ (25–75%): 24.17–52.50), but we did not observe significant differences between the medians of sample collections (*p*: 0.7713). The median T helper lymphocyte count remained below 400 cells/ µL at all adherence times, and only the cytotoxic T lymphocyte count changed; it was higher in patients more than 12 months after the first diagnosis (*p*: 0.00034; med: 1870.5; IQ (25–75%): 1383.75–2357.25). The number of DN T lymphocytes was lower in patients with a delay of 7 to 12 months (med: 57.00; IQ (25–75%): 27.00–79.00), but the difference was not statistically significant (*p*: 0.3913). The DP T lymphocyte count was similar between the different times of attendance (*p*: 0.7208).

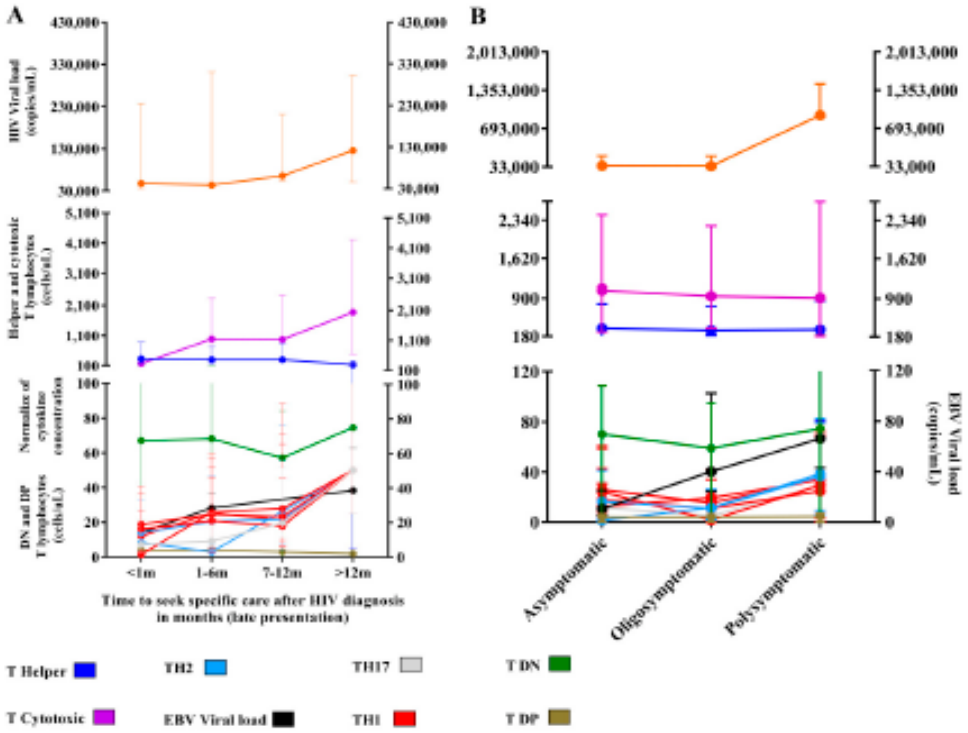


Figure 7. Time of seeking specific care and symptomatology: (**A**) Graph of the evaluation of immunological markers and viral load after different delays in attendance. In total, 129 patients with immediate attendance (<1 m), 105 patients with attendance between 1 to 6 months (1–6 m), 32 patients with attendance between 7 to 12 months (7–12 m) and 4 patients with late attendance (>12 m), were evaluated. (**B**) Graph of the evaluation of immunological markers and viral loads based on symptomatologic category. Totals of 138 asymptomatic, 74 oligosymptomatic, and 56 polysymptomatic patients were evaluated.

There was a prevalence of the TH1 profile in patients with recent presentation, particularly the IFN-γ level (*p*: 0.0004; med: 18.8; IQ (25–75%): 16.30–22.17); the codominance of all profiles was observed in patients 7 to 12 months after the first diagnosis, and greater evidence was obtained for patients more than 12 months after the first diagnosis.

The symptoms were similar among the groups; symptoms such as fever and sore throat were the most frequent (Table 1). We grouped the patients as asymptomatic, oligosymptomatic (two to three symptoms), and polysymptomatic (four or more symptoms). Asymptomatic patients were more frequent, except for multi-infected patients, for whom the

proportion of oligosymptomatic patients was more evident; however, the difference was not significant (G test: 06.09; p: 0.41) (Table 1).

We also analyzed the fluctuations in the biomarkers of HIV-1 and EBV infections based on symptomatologic groups (Figure 7B). The median HIV viral load was higher in polysymptomatic patients (p: 0.04; med: 45,998.5; IQ (25–75%): 12,936.25–173,970.5). The counts of $CD4^+$ T and $CD8^+$ T cells remained similar among all groups, and the median $CD4^+$ T count remained below 310 cells/µL in all groups. The EBV viral load was lower in asymptomatic patients (p: 0.05; med: 10.67; IQ (25–75%): 5–14.67) and gradually increased in oligo- and polysymptomatic patients. We did not observe significant differences in the SD or DN T lymphocyte count (p: 0.5787, p: 0.2335, respectively). We observed a predominance of the TH1 profile in asymptomatic (p: 0.0004) and oligosymptomatic (p: 0.0001) patients, although a decrease in cytokine levels occurred in oligosymptomatic patients, with the exception of IL-10, which was increased in asymptomatic patients ($p < 0.0001$; med: 11.55; IQ (25–75%): 9.71–14.18); in the polysymptomatic group, codominance of all profiles prevailed. We emphasized the increased levels of IL4 ($p < 0.0001$; med: 36.28; IQ (25–75%): 27.06–43.12), IL-10 ($p < 0.0001$; med: 38.4; IQ (25–75%): 32.26–44.12) and IL-17A (p: 0.0019; med: 29.29; IQ (25–75%): 19.73–48.67).

4. Discussion

The low overall prevalence of primary EBV infection was similar to that observed in other developing countries [17,18]. However, we expected that the rate of coinfected patients among the immunocompromised patients would be higher [19]; these divergences may be related to the methodology used. In the present study, the anti-VCA IgM (+) IgG (−) serological panel was used for sample screening; this profile is indicative of primary EBV infection, has high sensitivity, and is applied in the discrimination of false-positive cases [20].

In the surveillance of EBV infection, there is controversy as to the type of blood extract that should be used as a standard in determining viral load. The quantification of different fractions may provide different information that may or may not correspond to the clinical profile of the patient [21]. In the present study, we quantified the viral load of EBV in the buffy coat and blood plasma, and discrepant levels of viral genetic material were observed in the buffy coat samples; however, all associations were established using the viral load in blood plasma.

There is no methodological consensus regarding the best extract in cases of primary infection or infectious mononucleosis. It has been suggested that most of the EBV load is located in the cell fraction and that low detection in plasma is related to the release of viral DNA from apoptotic cells or damage to infected B lymphocytes during the separation of blood compartments [22]. We confirmed that the viral load was high in the buffy coat. However, it is argued that the detection of EBV in plasma is a biomarker of active and replicative infection due to the rare circulation of free viral particles in the plasma; therefore, any change in this parameter is indicative of infection [23,24].

Therefore, we suggest that plasma may be a more promising sample for assessing primary EBV infection, consistent with results from the aforementioned studies. Cell extracts are more convenient for determining clinical profiles when the virus is latent, as in cases of lymphomas associated with infection [25,26].

Regarding the virological interaction, we observed that the EBV-2 viral load was associated with low plasma levels of HIV viral load, with trends toward the maintenance of the helper T lymphocyte count and the control of cytotoxic T lymphocytes. The coinfected group exhibited low levels of plasma cytokines, especially IFN-γ and IL-4, without dominance of effector immunological profiles.

The EBV-2 viral load was inversely associated with IL-6 and IFN-γ levels. Studies have shown that the viral protein LMP2a regulates the production of IL-6 [27] and decreases the capacity of the cellular response to stimulation by IFN-γ [28]. Other gene products interfere

with the transcriptional pathway of factors linked to IFN production and the induction of an antiviral state [29].

IL-4, although at significantly low levels, was related to the EBV-2 viral load, a finding that may have implications both in maintaining an anti-inflammatory microenvironment [30] and in favoring viral persistence [31], and affected cell susceptibility by inducing the expression of surface markers [32]. We suggest that in coinfection, EBV-2 modulates the immune response, leading to a homeostatic threshold that contributes to the regulation of inflammation, which consequently contrasts with the overall inflammatory state of the progression of HIV infection [33], as observed in the present study. It is likely that the positive correlation between T helper and cytotoxic T lymphocyte counts observed herein reflects a marked cellular response that results in partial recovery of T helper lymphocytes [34].

Another noteworthy point is the indirect association of EBV-2 with the number of DN T lymphocytes. In our findings, these cells were strongly associated with IL-10 levels, which may contribute to the modulation of inadequate immune activation and limit the tissue damage characteristic of HIV infection [35]. This could also explain the negative correlation between DN T lymphocytes and cytotoxic T lymphocytes through TNF, which is a factor that promotes clonal expansion and differentiation and the survival of cytotoxic lymphocytes [36]. In simian retrovirus (SIV) infection models, it was found that in the depletion of helper T lymphocytes, DN T lymphocytes emerge as a potential subset of cells capable of performing similar functions [37]. In the present study, both strains were favored by the immunomodulation suggested for EBV-2.

We did not rule out a specific association between EBV-2 and T lymphocytes because this particular genotype is able to infect these cells and use them as a persistent or additional latent reservoir [38], which is also observed in the context of coinfection with HIV [39]. The infection of T cells makes them both a replicative niche supporting primary infection or reactivation and microenvironments conducive to viral latency, leading to the proliferation of infected T lymphocytes with the consequent control of the immune response [40]. It has not been established which T cell lines are most affected by EBV-2 infection; however, we suggest that DN T lymphocytes may be the target cells in this process because they were the lineage with a significantly higher absolute number in the HIV/EBV-2 coinfected group, a finding that has been observed in other clinical profiles associated with EBV [41]. Thus, the supply of T lymphocytes with auxiliary functions affected by EBV-2 favors the immune balance of the anti-HIV response.

From a cellular point of view, studies indicate that in transformed B lymphocyte lines, the regulation of the EBNA2 gene, and probably LMP1, of EBV-2 [42] is associated with low expression of CCR5 [43] and CXCR4 [44,45] and HIV entry receptors in the host cell [46]. Although these results pertain to B cells, the changes induced by herpesvirus were associated with the selection of HIV strains by receptor tropisms [47], and we cannot rule out that these changes may occur at the level of T lymphocytes because EBV-2 can also infect these cells.

Notably, all the likely modifications induced by EBV-2 favor the pathogenesis of the virus. Thus, longitudinal studies of HIV/EBV-2 coinfection are needed to evaluate, over the long term, the repercussions of the viral scenario observed in the present study and whether it can contribute to the typically observed herpesvirus lymphomagenesis [47].

In contrast to what was found in the HIV/EBV-2 group, our results suggest a positive correlation between the EBV-1 infection markers and the HIV-1 infection biomarkers. Certainly, the correlation of the virological markers of both agents suggests that the persistence of coinfection favors the expansion of infected cells and the onset of related malignant diseases [48]. Recent results show that under conditions of impaired immune response, EBV-1-infected B lymphocyte lines are susceptible to HIV-1, which could potentially reside in the latent form in memory B cells and thus constitute a potential additional reservoir for infection [49].

In our findings, the viral interaction in HIV/EBV-1 coinfection may have occurred through a proinflammatory pathway due to the correlation of HIV viral load with IL-6

and IFN-γ, potentially resulting in the plausible depletion of helper T lymphocytes, which is a well-known profile for the attempted control and suppression of the most evident infection in the early stages of the disease [50]. Alternatively, EBV viral load was directly proportional to IL-4 and inversely proportional to IL-10, potentially counteracting the exacerbated and harmful inflammation initiated by the type 1 response [51]. This distorted immune balance contributes to the maintenance of HIV infection, as observed in other clinical profiles associated with infection [52], whose predominance of a proinflammatory response similar to that observed has also been associated with the reactivation of EBV in more severe states of retroviral immunodeficiency [53]. In fact, we observed that HIV alone could illicit a multi-interactive immune control system that culminated in the predominance of an inflammatory profile.

In this study, the DP T lymphocyte count was high only in the HIV/EBV-1 group and had no direct association with the agents themselves, but was a consequence of the change in the number of T helper lymphocytes resulting from the complex interactive immunopathogenic system. DP cell numbers were proportional to the levels of IL-17A and IL-2 in coinfection, a finding that may be indicative of an attempt to maintain the immune response under the imminent instability of T helper lymphocytes considering that the DP line represents a frequent and highly reactive cell population, some of which are IL-2+ subpopulations, in HIV infection [54,55]. This reinforces our hypothesis regarding the activation of a cellular response. In turn, high levels of IL-17A may help control the aggressive immune activation observed in HIV+ patients [56].

The function of DP T lymphocytes in EBV infection is not known. A baseline study detected the presence of a subpopulation with low CD4 surface expression at the onset of acute infection; however, the functions of this subpopulation have not been elucidated [57]. More recent studies have characterized this specific line as constitutively expressing high concentrations of immunological activation markers [58] in different stages of chronic viral infection. It is likely that these cells support the antiviral response through the secretion of proinflammatory cytokines and the conversion to immunological memory profiles [59].

In HIV/EBV-1/2 multi-infection, we observed an immunological profile similar to EBV-1 coinfection. In this group, the viral interaction was stronger and inversely proportional to the number of helper T lymphocytes; as an example, there was a significant increase in the levels of cytokines and a balance in the response profiles. Similarly, HIV viral load was correlated with proinflammatory cytokines, while EBV viral load was correlated with IL-10 levels.

EBV-1 prevails in cases of coinfection by both genotypes, and epidemiological changes may occur through an intense specific-type immune response [60]. Whether the distinct pathological and functionally more aggressive profile of EBV-1 may be associated with its prevalence in several clinical cases of infection has been discussed [61,62]. Recent results have shown similarities in the immunological response against EBV vaccine antigens in children infected by genotype 1 or both genotypes [63].

The heterogeneity between the EBV genotypes is not restricted to the scores in the viral genome but affects the recognition of different types in the development of the immune response [64]. New studies focused on response mechanisms to the intraspecific divergences of EBV may elucidate the pathological profiles of herpesviruses. In this study, we utilized additional data on viral interactions in the context of HIV coinfection.

We observed that the later the attendance of HIV-1-infected patients to adequate medical care, the greater the increase in cytotoxic T lymphocyte count and cytokine levels, which was associated with a slight decline in helper T lymphocytes, whose levels remained below 400 cells/μL in all groups. From the analysis of the delay in patient adherence, it is likely that the patients were in the early stages of HIV infection, which is characterized by a progressive immune response and by the inversion of the $CD4^+/CD8^+$ ratio [65], generating a vigorous cytotoxic cell response correlated with high viral loads [66], which is a viremic trait that persisted in our study.

However, in patients with late presentation, a numerical increase in HIV viral load may reflect the initial cytotoxic response, which, although following a pattern of immunodominance against some viral epitopes, is successfully predicted to some extent [67] because with ongoing escape mutations, HIV evades recognition by the cellular response [68]. Similarly, the EBV viral load tends to slightly increase, even proportional to the increase in cytotoxic T lymphocytes; this suggests that the paradoxical success of viral replication against the sustained immune response may be the result of the actions of multiple products of lytic viral genes that inhibit antigen presentation pathways [69].

The prevalent TH1 profile in patients with immediate adherence is again consistent with early and continuous proinflammatory immune activation at the beginning of coinfection [70,71]. However, long-term delays manifest as immunoregulatory profiles that interfere with the activation of the cellular response at the expense of establishing viral persistence [69,72]. The curious upregulation of all cytokine profiles in patients with longer delays in adherence may be related to the immune balance against active coinfection, which originates from both the host response and viral escape mechanisms [73,74]. Associated with these aspects, a high percentage of multi-infected patients sought specific care only 12 months after the first HIV-1 diagnosis. This highlights our concern because this infectious profile was associated with favoring HIV pathology, and delay in care was detrimental to the maintenance of the antiviral response.

Asymptomatic individuals prevailed in all groups. The symptoms, when present, were similar to both the initial phase of HIV infection and the primary symptomatic EBV infection [34,75]. Particularly with EBV, these symptoms were associated with age groups above 18 years and with the host's compromised immune status [76].

Although we did not associate symptomatology with the groups studied, based on our analyses, we assume that symptomatology was more associated with EBV due to the proportionality of the viral load to the emerging symptoms [77]. These manifestations usually regress in a short time. However, in rare cases, they present heterogeneous conditions that may persist or recur repeatedly due to viral load [78]. In the face of HIV coinfection, our data suggest caution, because these conditions may be intensified by propathological viral interactions [79], which would explain the peak HIV viral load associated with polysymptomatic patients.

The prevalence of the cytotoxic response with a reduced number of circulating helper T lymphocytes was expected. It is argued that EBV favors a TH1 reaction by modulating specific cytokines; consequently, $CD8^+$ T cells are more numerous in the early stages of infection [80], as observed in asymptomatic and oligosymptomatic patients. We suggest that the increase in the levels of anti-inflammatory cytokines in polysymptomatic individuals may reflect the immune-mediated suppression that is characteristic of an ineffective antiviral response [81], which is consistent with the cascade of interactions proposed specifically for coinfection with EBV-1 and in multi-infection. We argue that this complex viral interaction also impacts the maintenance of the HIV viral load in polysymptomatic patients because the significant increase in this marker is a predictor of disease progression and clinical worsening [82], which plays a critical role in the escape of immune recognition via multifaceted mechanisms [83], potentially contributing to the pathological scenario.

5. Conclusions

We conclude that different immunological profiles are associated with the EBV genotype in primary EBV infection in untreated PLHIV. The results suggest that EBV-2 was associated with low levels of HIV-1 viral load, whereas EBV-1 viral load was positively correlated with HIV-1 viral load. These aspects become more worrying with a delayed attendance to specific care and the consequent delay in adherence to antiretroviral treatment, which leads to clinical changes and effects on the maintenance of the immune response. Finally, although the size of our sample of infected patients was small, it is probably a reflection of the real-world prevalence of primary EBV infection in adult PLHIV in the Brazilian Amazon region.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/v14020168/s1, Figure S1: Laboratory report produced by BD Multiset™ Software v3.1 (BD Biosciences, San Jose, CA, USA). Cell quantification is standardized by the manufacturer and is unique to lymphocyte populations with direct relevance to the pathogenesis of HIV infection. There was no need to change the intercept gates of dot plot graphics; Table S1: Correlation of EBV viral load quantified in plasma and whole blood with immunological markers, symptoms and time of adherence to HAART.

Author Contributions: T.A.F.M., O.M., R.C.M.S., E.B.F., I.B.C. (Iran Barros Costa) and A.C.R.V. conceived the project. L.M.S.P., E.d.S.F., A.B.C.F. and F.L.d.P.R. were responsible for sample collection at reference centers. L.M.S.P., E.d.S.F., I.B.C. (Igor Brasil Costa) and I.T.L. developed the methodology for the project. L.M.S.P. applied the statistical analyses and wrote the article. I.B.C. (Igor Brasil Costa) and A.C.R.V. reviewed the article. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by the Health Surveillance Secretariat of the Ministry of Health of the Federative Republic of Brazil and National Council for Scientific and Technological Development (CNPQ-301869/2017-0). We would like to thank the Coordination for the Improvement of Higher Education Personnel (CAPES) for granting the scholarship (process number: 88882.183970/2018-01).

Institutional Review Board Statement: All methods and experimental protocols were carried out in accordance with regulations 466/2012 and 347/05 of the Brazilian National Health Council and were approved by the Human Research Ethics Committee of Instituto Evandro Chagas (Protocol n. 3.121.265; CAAE n. 73927717.3.0000.0019).

Informed Consent Statement: All participants were informed about the study objectives and signed an informed consent form. The collected biological samples were stored in a biorepository until use.

Data Availability Statement: Data supporting the findings of this study are available from Igor Brasil Costa, but restrictions apply to the availability of these data, which were used under license for the present study and therefore are not publicly available. However, data are available from the authors upon reasonable request and with permission from Igor Brasil Costa.

Acknowledgments: We would like to thank all the technical professionals and administrative staff of the participating institutions for helping enroll patients for the project and all patients and blood donors for their trust and consideration during their participation in the project.

Conflicts of Interest: The authors declare that there were no conflict of interest in conducting this project.

References

1. Lacoste, V.; Judde, J.G.; Bestetti, G.; Cadranel, J.; Antoine, M.; Valensi, F.; Delabesse, E.; MacIntyre, E.; Gessain, A. Virological and molecular characterisation of a new B lymphoid cell line, established from an AIDS patient with primary effusion lymphoma, harbouring both KSHV/HHV8 and EBV viruses. *Leuk. Lymphoma* **2000**, *38*, 401–409. [CrossRef] [PubMed]
2. Chao, C.; Silverberg, M.J.; Martinez-Maza, O.; Chi, M.; Abrams, D.I.; Haque, R.; Zha, H.D.; McGuire, M.; Xu, L.; Said, J. Epstein-Barr Virus Infection and Expression of B-cell Oncogenic Markers in HIV-Related Diffuse Large B-cell Lymphoma. *Clin. Cancer Res.* **2012**, *18*, 4702–4712. [CrossRef]
3. Lang, F.; Pei, Y.; Lamplugh, Z.L.; Robertson, E.S. Molecular Biology of EBV in Relationship to HIV/AIDS-Associated Oncogenesis. *Cancer Treat Res.* **2019**, *177*, 81–103. [PubMed]
4. Friis, A.M.C.; Åkerlund, B.; Gyllensten, K.; Aleman, A.; Ernberg, I. Host–Epstein–Barr virus relationship affected by immunostimulation in HIV-infected patients representing distinct progressor profile groups. *Scand. J. Infect. Dis.* **2012**, *44*, 388–392. [CrossRef] [PubMed]
5. Riedel, D.J.; Tang, L.S.; Rositch, A.F. The Role of Viral Co-Infection in HIV-Associated Non-AIDS-Related Cancers. *Curr. HIV/AIDS Rep.* **2015**, *12*, 362–372. [CrossRef] [PubMed]
6. Hernández, D.M.; Valderrama, S.; Gualtero, S.; Hernández, C.; López, M.; Herrera, M.V.; Solano, J.; Fiorentino, S.; Quijano, S. Loss of T-Cell Multifunctionality and TCR-Vβ Repertoire Against Epstein-Barr Virus Is Associated With Worse Prognosis and Clinical Parameters in HIV+ Patients. *Front. Immunol.* **2018**, *9*, 2291. [CrossRef] [PubMed]
7. Balfour, J.H.H.; Verghese, P. Primary Epstein–Barr Virus Infection: Impact of Age at Acquisition, Coinfection, and Viral Load. *J. Infect. Dis.* **2013**, *207*, 1787–1789. [CrossRef] [PubMed]

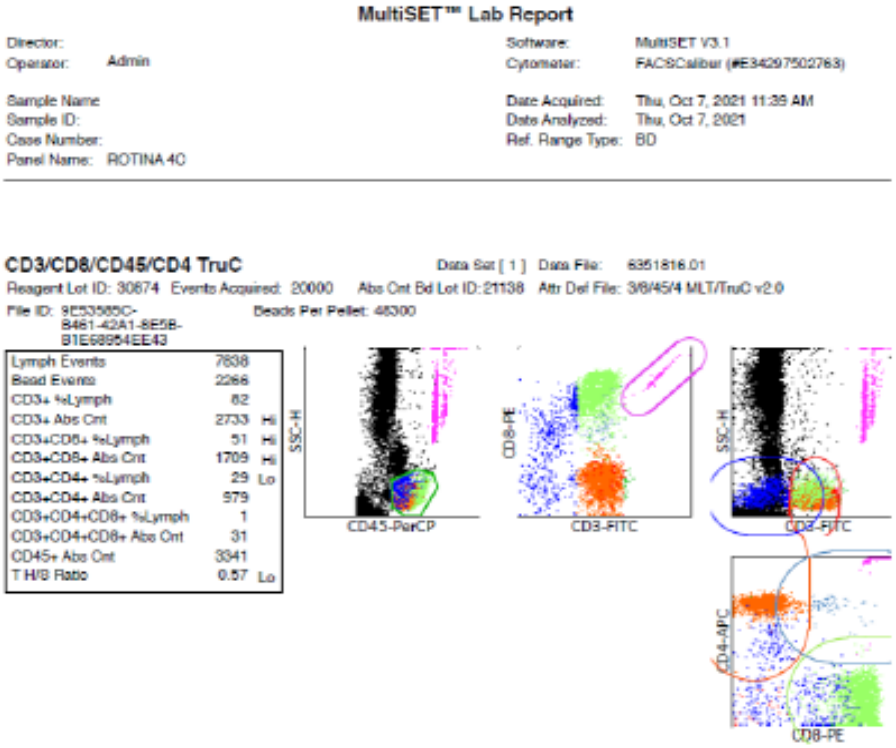
8. Slyker, J.A.; Casper, C.; Tapia, K.; Richardson, B.; Bunts, L.; Huang, M.-L.; Maleche-Obimbo, E.; Nduati, R.; John-Stewart, G. Clinical and Virologic Manifestations of Primary Epstein-Barr Virus (EBV) Infection in Kenyan Infants Born to HIV-Infected Women. *J. Infect. Dis.* **2013**, *207*, 1798–1806. [CrossRef]
9. Petrara, M.R.; Cattelan, A.; Zanchetta, M.; Sasset, L.; Freguja, R.; Gianesin, K.; Cecchetto, M.G.; Carmona, F.; De Rossi, A. Epstein-Barr Virus load and immune activation in Human Immunodeficiency Virus type 1-infected patients. *J. Clin. Virol.* **2012**, *53*, 196–200. [CrossRef]
10. Dos Santos, L.A.S.; De Azevedo, K.M.L.; Silva, L.E.; Oliveira, L.H.S. Epstein-Barr virus in oral mucosa from human immunodeficiency virus positive patients. *Rev. Assoc. Médica Bras.* **2014**, *60*, 262–269. [CrossRef] [PubMed]
11. Traore, L.; Nikiema, O.; Ouattara, A.K.; Compaore, T.R.; Soubeiga, S.T.; Diarra, B.; Obiri-Yeboah, D.; Sorgho, P.A.; Djigma, F.W.; Bisseye, C.; et al. EBV and HHV-6 Circulating Subtypes in People Living with HIV in Burkina Faso, Impact on CD4 T cell count and HIV Viral Load. *Mediterr. J. Hematol. Infect. Dis.* **2017**, *9*, e2017049. [CrossRef] [PubMed]
12. Figueira-Silva, C.M.; Pereira, F.E. Prevalence of Epstein-Barr virus antibodies in healthy children and adolescents in Vitória, State of Espírito Santo, Brazil. *Rev. Soc. Bras. Med. Trop.* **2004**, *37*, 409–412. [CrossRef] [PubMed]
13. Gerpe, N.F.; Vistarop, A.; Moyano, A.; De Matteo, E.; Preciado, M.; Chabay, P. Distinctive EBV infection characteristics in children from a developing country. *Int. J. Infect. Dis.* **2020**, *93*, 139–145. [CrossRef]
14. Lorenzetti, M.A.; Altcheh, J.; Moroni, S.; Moscatelli, G.; Chabay, P.A.; Preciado, M.V. EBNA1 sequences in Argentinean pediatric acute and latent Epstein-Barr virus infection reflect circulation of novel South American variants. *J. Med. Virol.* **2010**, *82*, 1730–1738. [CrossRef]
15. Sample, J.; Young, L.; Martin, B.; Chatman, T.; Kieff, E.; Rickinson, A.; Kieff, E. Epstein-Barr virus types 1 and 2 differ in their EBNA-3A, EBNA-3B, and EBNA-3C genes. *J. Virol.* **1990**, *64*, 4084–4092. [CrossRef]
16. Ayres, M.; Ayres Júnior, M.; Ayres, D.L.; Santos, A.S. *BioEstat 5.0: Aplicações Estatísticas nas Áreas de Ciências Biológicas e Médicas*; Sociedade Civil Mamirauá: Belém, Brazil, 2008; CNPq; 364p.
17. Cui, J.; Yan, W.; Xu, S.; Wang, Q.; Zhang, W.; Liu, W.; Ni, A. Anti-Epstein–Barr virus antibodies in Beijing during 2013–2017: What we have found in the different patients. *PLoS ONE* **2018**, *13*, e0193171. [CrossRef] [PubMed]
18. Beader, N.; Kolarić, B.; Slačanac, D.; Tabain, I.; Vilibić-Čavlek, T. Seroepidemiological Study of Epstein-Barr Virus in Different Population Groups in Croatia. *Isr. Med. Assoc. J. IMAJ* **2018**, *20*, 86–90.
19. Okonko, I.O.; Makinde, T.S.; Okonko, B.J.; Ogbu, O. Immunological and epidemiological evaluation of EBV infections among HIV-1 infected individuals in Abakaliki, Nigeria supports the potential use of neutrophils as a marker of EBV in HIV disease progression and as useful markers of immune activation. *J. Immunoass. Immunochem.* **2020**, *41*, 158–170. [CrossRef]
20. Guerrero-Ramos, A.; Patel, M.; Kadakia, K.; Haque, T. Performance of the Architect EBV Antibody Panel for Determination of Epstein-Barr Virus Infection Stage in Immunocompetent Adolescents and Young Adults with Clinical Suspicion of Infectious Mononucleosis. *Clin. Vaccine Immunol.* **2014**, *21*, 817–823. [CrossRef]
21. Kanakry, J.; Ambinder, R. The Biology and Clinical Utility of EBV Monitoring in Blood. *Curr. Top. Microbiol. Immunol.* **2015**, *391*, 475–499.
22. Stevens, S.J.C.; Pronk, I.; Middeldorp, J.M. Toward Standardization of Epstein-Barr Virus DNA Load Monitoring: Unfractionated Whole Blood as Preferred Clinical Specimen. *J. Clin. Microbiol.* **2001**, *39*, 1211–1216. [CrossRef]
23. Wagner, H.-J.; Fischer, L.; Jabs, W.J.; Holbe, M.; Pethig, K.; Bucsky, P. Longitudinal analysis of Epstein-Barr viral load in plasma and peripheral blood mononuclear cells of transplanted patients by real-time polymerase chain reaction12. *Transplantation* **2002**, *74*, 656–664. [CrossRef]
24. Hara, S.; Kimura, H.; Hoshino, Y.; Tanaka, N.; Nishikawa, K.; Ihira, M.; Yoshikawa, T.; Morishima, T. Detection of Herpesvirus DNA in the Serum of Immunocompetent Children. *Microbiol. Immunol.* **2002**, *46*, 177–180. [CrossRef]
25. Hohaus, S.; Santangelo, R.; Giachelia, M.; Vannata, B.; Massini, G.; Cuccaro, A.; Martini, M.; Cesarini, V.; Cenci, T.; D'Alò, F.; et al. The Viral Load of Epstein–Barr Virus (EBV) DNA in Peripheral Blood Predicts for Biological and Clinical Characteristics in Hodgkin Lymphoma. *Clin. Cancer Res.* **2011**, *17*, 2885–2892. [CrossRef]
26. Tisi, M.C.; Cupelli, E.; Santangelo, R.; Maiolo, E.; Alma, E.; Giachelia, M.; Martini, M.; Bellesi, S.; D'Alò, F.; Voso, M.T.; et al. Whole blood EBV-DNA predicts outcome in diffuse large B-cell lymphoma. *Leuk. Lymphoma* **2016**, *57*, 628–634. [CrossRef]
27. Stewart, S.; Dawson, C.W.; Takada, K.; Curnow, J.; Moody, C.A.; Sixbey, J.W.; Young, L.S. Epstein-Barr virus-encoded LMP2A regulates viral and cellular gene expression by modulation of the NF-kappaB transcription factor pathway. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 15730–15735. [CrossRef] [PubMed]
28. Shah, K.M.; Stewart, S.E.; Wei, W.; Woodman, C.B.J.; O'Neil, J.D.; Dawson, C.W.; Young, L.S. The EBV-encoded latent membrane proteins, LMP2A and LMP2B, limit the actions of interferon by targeting interferon receptors for degradation. *Oncogene* **2009**, *28*, 3903–3914. [CrossRef] [PubMed]
29. Hahn, A.M.; Huye, L.E.; Ning, S.; Webster-Cyriaque, J.; Pagano, J.S. Interferon Regulatory Factor 7 Is Negatively Regulated by the Epstein-Barr Virus Immediate-Early Gene, BZLF-1. *J. Virol.* **2005**, *79*, 10040–10052. [CrossRef] [PubMed]
30. Ho, I.-C.; Miaw, S.-C. Regulation of IL-4 Expression in Immunity and Diseases. *Adv. Exp. Med. Biol.* **2016**, *941*, 31–77.
31. Kis, L.L.; Gerasimčik, N.; Salamon, D.; Persson, E.K.; Nagy, N.; Klein, G.; Severinson, E.; Klein, E. STAT6 signaling pathway activated by the cytokines IL-4 and IL-13 induces expression of the Epstein-Barr virus-encoded protein LMP-1 in absence of EBNA-2: Implications for the type II EBV latent gene expression in Hodgkin lymphoma. *Blood* **2011**, *117*, 165–174. [CrossRef] [PubMed]

32. Malizia, A.P.; Egan, J.J.; Doran, P.P. IL-4 increases CD21-dependent infection of pulmonary alveolar epithelial type II cells by EBV. *Mol. Immunol.* **2009**, *46*, 1905–1910. [CrossRef] [PubMed]
33. De Medeiros, R.M.; Valverde-Villegas, J.M.; Junqueira, D.M.; Gräf, T.; Lindenau, J.D.-R.; De Mello, M.G.; Vianna, P.; Almeida, S.E.M.; Chies, J.A.B. Rapid and Slow Progressors Show Increased IL-6 and IL-10 Levels in the Pre-AIDS Stage of HIV Infection. *PLoS ONE* **2016**, *11*, e0156163. [CrossRef]
34. Braun, D.L.; Kouyos, R.D.; Balmer, B.; Grube, C.; Weber, R.; Günthard, H.F. Frequency and Spectrum of Unexpected Clinical Manifestations of Primary HIV-1 Infection. *Clin. Infect. Dis.* **2015**, *61*, 1013–1021. [CrossRef] [PubMed]
35. Petitjean, G.; Chevalier, M.; Tibaoui, F.; Didier, C.; Manea, M.E.; Liovat, A.-S.; Campa, P.; Müller-Trutwin, M.; Girard, P.-M.; Meyer, L.; et al. Level of double negative T cells, which produce TGF-β and IL-10, predicts CD8 T-cell activation in primary HIV-1 infection. *AIDS* **2012**, *26*, 139–148. [CrossRef]
36. Ye, L.-L.; Wei, X.-S.; Zhang, M.; Niu, Y.-R.; Zhou, Q. The Significance of Tumor Necrosis Factor Receptor Type II in $CD8^+$ Regulatory T Cells and $CD8^+$ Effector T Cells. *Front. Immunol.* **2018**, *9*, 583. [CrossRef]
37. Milush, J.M.; Mir, K.D.; Sundaravaradan, V.; Gordon, S.N.; Engram, J.; Cano, C.A.; Reeves, J.D.; Anton, E.; O'Neill, E.; Butler, E.; et al. Lack of clinical AIDS in SIV-infected sooty mangabeys with significant $CD4^+$ T cell loss is associated with double-negative T cells. *J. Clin. Investig.* **2011**, *121*, 1102–1110. [CrossRef]
38. Coleman, C.B.; Daud, I.I.; Ogolla, S.O.; Ritchie, J.A.; Smith, N.A.; Sumba, P.; Dent, A.E.; Rochford, R. Epstein-Barr Virus Type 2 Infects T Cells in Healthy Kenyan Children. *J. Infect. Dis.* **2017**, *216*, 670–677. [CrossRef] [PubMed]
39. Bekker, V.; Scherpbier, H.; Beld, M.; Piriou, E.; Van Breda, A.; Lange, J.; van Leth, F.; Jurriaans, S.; Alders, S.; Dillen, P.W.; et al. Epstein-Barr Virus Infects B and Non-B Lymphocytes in HIV-1–Infected Children and Adolescents. *J. Infect. Dis.* **2006**, *194*, 1323–1330. [CrossRef] [PubMed]
40. Coleman, C.B.; Lang, J.; Sweet, L.A.; Smith, N.A.; Freed, B.M.; Pan, Z.; Haverkos, B.; Pelanda, R.; Rochford, R. Epstein-Barr Virus Type 2 Infects T Cells and Induces B Cell Lymphomagenesis in Humanized Mice. *J. Virol.* **2018**, *92*, e00813-18. [CrossRef]
41. Bian, Z.; Liu, J.; Xu, L.-P.; Chang, Y.-J.; Wang, Y.; Zhang, X.-H.; Huang, X.-J. Association of Epstein–Barr virus reactivation with the recovery of CD4/CD8 double-negative T lymphocytes after haploidentical hematopoietic stem cell transplantation. *Bone Marrow Transplant.* **2017**, *52*, 264–269. [CrossRef]
42. Lucchesi, W.; Brady, G.; Dittrich-Breiholz, O.; Kracht, M.; Russ, R.; Farrell, P.J. Differential Gene Regulation by Epstein-Barr Virus Type 1 and Type 2 EBNA2. *J. Virol.* **2008**, *82*, 7456–7466. [CrossRef] [PubMed]
43. Iyengar, S.; Schwartz, D.H. Potentiation of EBV-Induced B Cell Transformation by CXCR4-Tropic, But Not CCR5-Tropic, HIV gp120: Implications for HIV-Associated Lymphomagenesis. *AIDS Res. Hum. Retroviruses* **2011**, *27*, 519–523. [CrossRef] [PubMed]
44. Nakayama, T.; Fujisawa, R.; Izawa, D.; Hieshima, K.; Takada, K.; Yoshie, O. Human B Cells Immortalized with Epstein-Barr Virus Upregulate CCR6 and CCR10 and Downregulate CXCR4 and CXCR5. *J. Virol.* **2002**, *76*, 3072–3077. [CrossRef] [PubMed]
45. Ehlin-Henriksson, B.; Mowafi, F.; Klein, G.; Nilsson, A. Epstein-Barr virus infection negatively impacts the CXCR4-dependent migration of tonsillar B cells. *Immunology* **2006**, *117*, 379–385. [CrossRef] [PubMed]
46. Chen, Y.; Maskri, O.; Chaminade, F.; René, B.; Benkaroun, J.; Godet, J.; Mély, Y.; Mauffret, O.; Fossé, P. Structural Insights into the HIV-1 Minus-strand Strong-stop DNA. *J. Biol. Chem.* **2016**, *291*, 3468–3482. [CrossRef]
47. Petrara, M.R.; Freguja, R.; Gianesin, K.; Zanchetta, M.; De Rossi, A. Epstein-Barr virus-driven lymphomagenesis in the context of human immunodeficiency virus type 1 infection. *Front. Microbiol.* **2013**, *4*, 311. [CrossRef]
48. LeGoff, J.; Amiel, C.; Calisonni, O.; Fromentin, D.; Rajoely, B.; Abuaf, N.; Tartour, E.; Rozenbaum, W.; Bélec, L.; Nicolas, J.-C. Early Impairment of $CD8^+$ T Cells Immune Response Against Epstein–Barr Virus (EBV) Antigens Associated with High Level of Circulating Mononuclear EBV DNA Load in HIV Infection. *J. Clin. Immunol.* **2004**, *24*, 125–134. [CrossRef]
49. McHugh, D.; Myburgh, R.; Caduff, N.; Spohn, M.; Kok, Y.L.; Keller, C.W.; Murer, A.; Chatterjee, B.; Rühl, J.; Engelmann, C.; et al. EBV renders B cells susceptible to HIV-1 in humanized mice. *Life Sci. Alliance* **2020**, *3*, e202000640. [CrossRef]
50. Baiyegunhi, O.; Ndlovu, B.; Ogunshola, F.; Ismail, N.; Walker, B.D.; Ndung'U, T.; Ndhlovu, Z.M. Frequencies of Circulating Th1-Biased T Follicular Helper Cells in Acute HIV-1 Infection Correlate with the Development of HIV-Specific Antibody Responses and Lower Set Point Viral Load. *J. Virol.* **2018**, *92*, e00659-18. [CrossRef]
51. Lloyd, C.M.; Snelgrove, R.J. Type 2 immunity: Expanding our view. *Sci. Immunol.* **2018**, *3*, eaat1604. [CrossRef]
52. Xu, L.; Guo, Y.; Zhao, Y.; Xu, Y.; Peng, X.; Yang, Z.; Tao, R.; Huang, Y.; Xu, Y.; Chen, Y.; et al. Chemokine and Cytokine Cascade Caused by Skewing of the Th1-Th2 Balance Is Associated with High Intracranial Pressure in HIV-Associated Cryptococcal Meningitis. *Mediat. Inflamm.* **2019**, *2019*, 2053958. [CrossRef] [PubMed]
53. Tang, J.-T.; Li, Y.-Y.; Zhang, Y.; Liu, J.; Guan, W.; Wang, R.-R.; He, L.-P.; Zhang, J.-B.; Kuang, Y.-Q. HIV infection confers distinct mechanisms in severe drug eruption: Endogenous virus activation with aberrant Th2/Th1 and CD8 + T cells function. *Clin. Exp. Pharmacol. Physiol.* **2020**, *47*, 1005–1013. [CrossRef]
54. Chauhan, N.K.; Vajpayee, M.; Mojumdar, K.; Singh, R.; Singh, A. Study of $CD4^+CD8^+$ Double positive T-lymphocyte phenotype and function in Indian patients infected with HIV-1. *J. Med. Virol.* **2012**, *84*, 845–856. [CrossRef]
55. Frahm, M.A.; Picking, R.A.; Kuruc, J.D.; McGee, K.; Gay, C.; Eron, J.J.; Hicks, C.B.; Tomaras, G.D.; Ferrari, G. $CD4^+CD8^+$ T Cells Represent a Significant Portion of the Anti-HIV T Cell Response to Acute HIV Infection. *J. Immunol.* **2012**, *188*, 4289–4296. [CrossRef] [PubMed]

56. Celis, F.P.; Feria, M.G.; Taborda, N.A.; Rugeles, M.T. A Low Frequency of IL-17-Producing CD8$^+$ T-Cells Is Associated With Persistent Immune Activation in People Living With HIV Despite HAART-Induced Viral Suppression. *Front. Immunol.* **2018**, *9*, 2502. [CrossRef]
57. Ortolani, C.; Forti, E.; Radin, E.; Cibin, R.; Cossarizza, A. Cytofluorometric Identification of Two Populations of Double Positive (CD4$^+$,CD8$^+$) T Lymphocytes in Human Peripheral Blood. *Biochem. Biophys. Res. Commun.* **1993**, *191*, 601–609. [CrossRef]
58. Gonzalez-Mancera, M.S.; Bolaños, N.I.; Salamanca, M.; Orjuela, G.A.; Rodriguez, A.N.; Gonzalez, J.M. Percentages of CD4$^+$CD8$^+$ double-positive T lymphocytes in the peripheral blood of adults from a blood bank in Bogotá, Colombia. *Turk. J. Hematol.* **2020**, *37*, 36–41. [CrossRef]
59. Howe, R.; Dillon, S.; Rogers, L.; Palmer, B.; MaWhinney, S.; Blyveis, N.; Schlichtemeier, R.; D'Souza, M.; Ingoldby, L.; Harwood, J.E.F.; et al. Phenotypic and Functional Characterization of HIV-1-Specific CD4$^+$CD8$^+$ Double-Positive T Cells in Early and Chronic HIV-1 Infection. *JAIDS J. Acquir. Immune Defic. Syndr.* **2009**, *50*, 444–456. [CrossRef]
60. Kim, S.-M.; Kang, S.-H.; Lee, W.-K. Identification of two types of naturally-occurring intertypic recombinants of Epstein-Barr virus. *Mol. Cells* **2006**, *21*, 302–307. [PubMed]
61. Boyle, M.J.; Sculley, T.B.; Penny, R.; Sewell, W.A.; Tschuchnigg, M.; Berger, M.F.; Cooper, D.A. The Role of Epstein-Barr Virus Subtypes in Human Immunodeficiency Virus-Associated Lymphoma. *Leuk. Lymphoma* **1993**, *10*, 17–23. [CrossRef]
62. Tzellos, S.; Farrell, P.J. Epstein-Barr Virus Sequence Variation—Biology and Disease. *Pathogens* **2012**, *1*, 156–174. [CrossRef] [PubMed]
63. Smith, N.A.; Baresel, P.C.; Jackson, C.L.; Ogolla, S.; Toko, E.N.; Heit, S.; Piriou, E.; Sumba, O.P.; Middeldorp, J.; Colbom, K.L.; et al. Differences in the Epstein-Barr Virus gp350 IgA Antibody Response Are Associated With Increased Risk for Coinfection With a Second Strain of Epstein-Barr Virus. *J. Infect. Dis.* **2019**, *219*, 955–963. [CrossRef] [PubMed]
64. Kanda, T.; Yajima, M.; Ikuta, K. Epstein-Barr virus strain variation and cancer. *Cancer Sci.* **2019**, *110*, 1132–1139. [CrossRef]
65. Vergis, E.N.; Mellors, J.W. Natural history of HIV-1 infection. *Infect. Dis. Clin. N. Am.* **2000**, *14*, 809–825. [CrossRef]
66. Ndhlovu, Z.M.; Kamya, P.; Mewalal, N.; Kløverpris, H.; Nkosi, T.; Pretorius, K.; Laher, F.; Ogunshola, F.; Chopera, D.; Shekhar, K.; et al. Magnitude and Kinetics of CD8$^+$ T Cell Activation during Hyperacute HIV Infection Impact Viral Set Point. *Immunity* **2015**, *43*, 591–604. [CrossRef] [PubMed]
67. Appay, V.; Nixon, D.F.; Donahoe, S.M.; Gillespie, G.M.; Dong, T.; King, A.; Ogg, G.S.; Spiegel, H.M.; Conlon, C.; Spina, C.A.; et al. HIV-Specific CD8$^+$ T Cells Produce Antiviral Cytokines but Are Impaired in Cytolytic Function. *J. Exp. Med.* **2000**, *192*, 63–76. [CrossRef] [PubMed]
68. Streeck, H.; Nixon, D. T Cell Immunity in Acute HIV-1 Infection. *J. Infect. Dis.* **2010**, *202* (Suppl. 2), S302–S308. [CrossRef]
69. Ressing, M.E.; Horst, D.; Griffin, B.D.; Tellam, J.; Zuo, J.; Khanna, R.; Rowe, M.; Wiertz, E.J. Epstein-Barr virus evasion of CD8$^+$ and CD4$^+$ T cell immunity via concerted actions of multiple gene products. *Semin. Cancer Biol.* **2008**, *18*, 397–408. [CrossRef]
70. Zaunders, J.J.; Munier, M.L.; Kaufmann, D.E.; Ip, S.; Grey, P.; Smith, D.; Ramacciotti, T.; Quan, D.; Finlayson, R.; Kaldor, J.; et al. Early proliferation of CCR5+ CD38+++ antigen-specific CD4$^+$ Th1 effector cells during primary HIV-1 infection. *Blood* **2006**, *106*, 1660–1667. [CrossRef]
71. Nilsson, J.; Kinloch-De-Loes, S.; Granath, A.; Sönnerborg, A.; Goh, L.-E.; Andersson, J. Early immune activation in gut-associated and peripheral lymphoid tissue during acute HIV infection. *AIDS* **2007**, *21*, 565–574. [CrossRef]
72. Sarikonda, G.; Von Herrath, M.G. Immunosuppressive Mechanisms during Viral Infectious Diseases. *Methods Mol. Biol.* **2011**, *677*, 431–447.
73. Ma, X.; Wang, L.; Zhao, H.; Pang, N.; Zhang, F.; Jiang, T.; Liu, X.; Mamuti, W.; Wen, H.; Ding, J. Th17 cells are associated with the Th1/Th2-cell balance during Echinococcus multilocularis infection. *Mol. Med. Rep.* **2014**, *10*, 236–240. [CrossRef]
74. Ren, Y.; Yang, J.; Li, M.; Huang, N.; Chen, Y.; Wu, X.; Wang, X.; Qiu, S.; Wang, H.; Li, X. Viral IL-10 promotes cell proliferation and cell cycle progression via JAK2/STAT3 signaling pathway in nasopharyngeal carcinoma cells. *Biotechnol. Appl. Biochem.* **2020**, *67*, 929–938. [CrossRef] [PubMed]
75. De Oliveira, R.D.S.M.; Benzaken, A.S.; Saraceni, V.; Sabidó, M. HIV/AIDS epidemic in the State of Amazonas: Characteristics and trends from 2001 to 2012. *Rev. Soc. Bras. Med. Trop.* **2015**, *48*, 70–78. [CrossRef]
76. Dunmire, S.K.; Verghese, P.S.; Balfour, H.H., Jr. Primary Epstein-Barr virus infection. *J. Clin. Virol.* **2018**, *102*, 84–92. [CrossRef] [PubMed]
77. Vouloumanou, E.K.; Rafailidis, P.I.; Falagas, M.E. Current diagnosis and management of infectious mononucleosis. *Curr. Opin. Hematol.* **2012**, *19*, 14–20. [CrossRef] [PubMed]
78. Fujiwara, S.; Nakamura, H. Chronic Active Epstein–Barr Virus Infection: Is It Immunodeficiency, Malignancy, or Both? *Cancers* **2020**, *12*, 3202. [CrossRef]
79. Lupia, T.; Milia, M.G.; Atzori, C.; Gianella, S.; Audagnotto, S.; Imperiale, D.; Mighetto, L.; Pirriatore, V.; Gregori, G.; Lipani, F.; et al. Presence of Epstein–Barr virus DNA in cerebrospinal fluid is associated with greater HIV RNA and inflammation. *AIDS* **2020**, *34*, 373–380. [CrossRef] [PubMed]
80. Massini, G.; Siemer, D.; Hohaus, S. EBV in Hodgkin Lymphoma. *Mediterr. J. Hematol. Infect. Dis.* **2009**, *1*, e2009013. [CrossRef]
81. Lambert, S.L.; Martinez, O.M. Latent Membrane Protein 1 of EBV Activates Phosphatidylinositol 3-Kinase to Induce Production of IL-10. *J. Immunol.* **2007**, *179*, 8225–8234. [CrossRef]

82. McPhee, E.; Grabowski, M.K.; Gray, R.H.; Ndyanabo, A.; Ssekasanvu, J.; Kigozi, G.; Makumbi, F.; Serwadda, D.; Quinn, T.C.; Laeyendecker, O. Short Communication: The Interaction of HIV Set Point Viral Load and Subtype on Disease Progression. *AIDS Res. Hum. Retroviruses* **2019**, *35*, 49–51. [CrossRef] [PubMed]
83. Durantel, D.; Kusters, I.; Louis, J.; Manel, N.; Ottenhoff, T.H.; Picot, V.; Saadatian-Elahi, M. Mechanisms behind TB, HBV, and HIV chronic infections. *Infect. Genet. Evol.* **2017**, *55*, 142–150. [CrossRef] [PubMed]

Supplementary Materials:



MultiSET™ Lab Report

Director:
Operator: Admin

Software: MultiSET V3.1
Cytometer: FACSCalibur (#E34297502783)

Sample Name
Sample ID:
Case Number:
Panel Name: ROTINA 4C

Date Acquired: Thu, Oct 7, 2021 11:39 AM
Date Analyzed: Thu, Oct 7, 2021
Ref. Range Type: BD

CD3/CD8/CD45/CD4 TruC

Data Set [1] Data File: 6351816.01

Reagent Lot ID: 30674 Events Acquired: 20000 Abs Cnt Bd Lot ID: 21138 Attr Def File: 3/8/45/4 MLT/TruC v2.0

File ID: 9E53565C-8461-42A1-8E5B-B1E68954EE43 Beads Per Pellet: 46300

Lymph Events	7638	
Blood Events	2266	
CD3+ %Lymph	82	
CD3+ Abs Cnt	2733	Hi
CD3+CD8+ %Lymph	51	Hi
CD3+CD8+ Abs Cnt	1709	Hi
CD3+CD4+ %Lymph	29	Lo
CD3+CD4+ Abs Cnt	979	
CD3+CD4+CD8+ %Lymph	1	
CD3+CD4+CD8+ Abs Cnt	31	
CD45+ Abs Cnt	3341	
T H/S Ratio	0.57	Lo

Figure S1: Laboratory report produced by BD Multiset™ Software v3.1 (BD Biosciences, San Jose, CA, USA). Cell quantification is standardized by the manufacturer and is unique to lymphocyte populations with direct relevance to the pathogenesis of HIV infection. There was no need to change the intercept gates of dot plot graphics.

Table 51. Correlation of EBV viral load quantified in plasma and whole blood with immunological markers, symptoms and time of adherence to HAART.

		EBV viral load in plasma			EBV viral load in buffy coat (log10)		
Markers	med (IIQ 25%-75%)	med (IIQ 25%-75%)	rs	p valor	med (IIQ 25%-75%)	rs	p valor
IL-4	8.61 (7.01-9.00)	16.67 (7.67-67.00)	0.92	0.037	3.56 (3.23-4.41)	0.26	0.274
Double positive T lymphocytes	4.00 (2.50-7.50)		0.89	0.034		-0.14	0.557
IFN-γ	10.70 (9.90-12.76)		0.88	0.049		-0.08	0.734
IL-10	10.79 (10.20-10.51)		0.79	0.066		0.22	0.374
HIV viral load (log10)	4.38 (3.76-5.54)		0.65	0.003		0.12	0.570
Symptomatology	-		0.55	0.015		0.52	0.022
T helper lymphocytes	296.00 (127.00-434.00)		-0.35	0.085		-0.10	0.680
Cytotoxic T lymphocytes	1015.00 (875.50-2033.00)		-0.24	0.323		-0.23	0.344
IL-2	9.77 (8.84-10.03)		0.18	0.440		0.20	0.407
IL-6	14.43 (11.27-19.29)		0.16	0.521		0.14	0.557
Delay time in HAART adherence	-		0.15	0.532		0.17	0.499
TNF	9.71 (8.63-10.27)		0.11	0.640		0.08	0.740
Double negative T lymphocytes	76.00 (50.50-114.00)		0.08	0.741		-0.004	0.989
IL-17A	26.11 (0-53.69)		0.02	0.945		0.06	0.809

med: Median.
IIQ: Interquartile.
rs: Spearman's Coefficient

2.3 TERCEIRO MANUSCRITO

Título: HLA-B*13, B*35 and B*39 alleles are closely associated with nonresponse to ART in HIV infection: a cohort study in a population of northern Brazil.

Lista de autores: Leonn Mendes Soares Pereira, Eliane dos Santos França, Iran Barros Costa, Erika Vanessa Oliveira Jorge; Patrícia Jeanne de Souza Mendonça Mattos; Igor Tenório Lima, Amaury Bentes Cunha Freire, Francisco Lúzio de Paula Ramos, Talita Antonia Furtado Monteiro, Olinda Macedo, Rita Catarina Medeiros Sousa, Felipe Bonfim Freitas, Igor Brasil Costa & Antonio Carlos Rosário Vallinoto.

Status da publicação: Publicado.

Nome da revista: Frontiers in Immunology.

ISSN: 1664-3224

Fator de impacto: 7.561 (Qualis A1)

DOI: https://doi.org/10.3389/fimmu.2022.829126

frontiers in Immunology

ORIGINAL RESEARCH
published: 16 March 2022
doi: 10.3389/fimmu.2022.829126

HLA-B*13, B*35 and B*39 Alleles Are Closely Associated With the Lack of Response to ART in HIV Infection: A Cohort Study in a Population of Northern Brazil

Leonn Mendes Soares Pereira[†], *Eliane dos Santos França*[2], *Iran Barros Costa*[2], *Erika Vanessa Oliveira Jorge*[3], *Patrícia Jeanne de Souza Mendonça Mattos*[3], *Amaury Bentes Cunha Freire*[4], *Francisco Lúzio de Paula Ramos*[4], *Talita Antonia Furtado Monteiro*[2], *Olinda Macedo*[5], *Rita Catarina Medeiros Sousa*[2,6], *Eduardo José Melo dos Santos*[7,8], *Felipe Bonfim Freitas*[5], *Igor Brasil Costa*[2,8†] *and Antonio Carlos Rosário Vallinoto*[1,2*†]

[1] Virology Laboratory, Institute of Biological Sciences, Federal University of Pará, Belém, Brazil, [2] Epstein-Barr Virus Laboratory, Virology Unit, Evandro Chagas Institute, Ananindeua, Brazil, [3] Department of Immunogenetics, Hemotherapy and Hematology Foundation of the State of Pará, Belém, Brazil, [4] Epidemiology and Surveillance Service, Evandro Chagas Institute, Ananindeua, Brazil, [5] Retrovirus Laboratory, Virology Unit, Evandro Chagas Institute, Ananindeua, Brazil, [6] School of Medicine, Federal University of Pará, Belém, Brazil, [7] Laboratory of Human and Medical Genetics, Institute of Biological Sciences, Federal University of Pará, Belém, Brazil, [8] Graduate Program in Biology of Infectious and Parasitic Agents, Institute of Biological Sciences, Federal University of Pará, Belém, Brazil

Introduction: Immune reconstitution failure after HIV treatment is a multifactorial phenomenon that may also be associated with a single polymorphism of human leukocyte antigen (HLA); however, few reports include patients from the Brazilian Amazon. Our objective was to evaluate the association of the immunogenic profile of the "classical" HLA-I and HLA-II loci with treatment nonresponse in a regional cohort monitored over 24 months since HIV diagnosis.

Materials and Methods: Treatment-free participants from reference centers in the state of Pará, Brazil, were enrolled. Infection screening was performed using enzyme immunoassays (Murex AG/AB Combination DiaSorin, UK) and confirmed by immunoblots (Bio-Manguinhos, FIOCRUZ). Plasma viral load was quantified by real-time PCR (ABBOTT, Chicago, Illinois, USA). $CD4^+$/$CD8^+$ T lymphocyte quantification was performed by immunophenotyping and flow cytometry (BD Biosciences, San Jose, CA, USA). Infection was monitored via test and logistics platforms (SISCEL and SICLOM). Therapeutic response failure was inferred based on $CD4^+$ T lymphocyte quantification after 1 year of therapy. Loci A, B and DRB1 were genotyped using PCR-SSO (One Lambda Inc., Canoga Park, CA, USA). Statistical tests were applied using GENEPOP, GraphPad Prism 8.4.3 and BioEstat 5.3.

Results: Of the 270 patients monitored, 134 responded to treatment ($CD4^+ \geq 500$ cells/μL), and 136 did not respond to treatment ($CD4^+ < 500$ cells/μL). The allele frequencies of the loci were similar to heterogeneous populations. The allelic profile of locus B was statistically

OPEN ACCESS

Reviewed by:
Antonio Bellas,
Transfusion Center of the Community of Madrid, Spain
Leila B. Giron,
Weber Institute, United States

Edited by:
Shokrollah Elahi,
University of Alberta, Canada

***Correspondence:**
Antonio Carlos Rosário Vallinoto
vallinoto@ufpa.br

[†] *These authors have contributed equally to this work and share senior authorship*

Specialty section:
This article was submitted to Viral Immunology, a section of the journal Frontiers in Immunology

Received: *04 December 2021*
Accepted: *23 February 2022*
Published: *16 March 2022*

Citation:
Pereira LMS, França ES, Costa IB, Jorge EVO, Mattos PJSM, Freire ABC, Ramos FLdP, Monteiro TAF, Macedo O, Sousa RCM, Santos EJM, Freitas FB, Costa IB and Vallinoto ACR (2022) HLA-B13, B35 and B39 Alleles Are Closely Associated With the Lack of Response to ART in HIV Infection: A Cohort Study in a Population of Northern Brazil. Front. Immunol. 13:829126. doi: 10.3389/fimmu.2022.829126

associated with treatment nonresponse, and the B*13, B*35 and B*39 alleles had the greatest probabilistic influence. The B*13 allele had the highest risk of treatment nonresponse, and carriers of the allele had a detectable viral load and a CD4+ T lymphocyte count less than 400 cells/μL with up to 2 years of therapy. The B*13 allele was associated with a switch in treatment regimens, preferably to efavirenz (EFZ)-based regimens, and among those who switched regimens, half had a history of coinfection with tuberculosis.

Conclusions: The allelic variants of the B locus are more associated with non-response to therapy in people living with HIV (PLHIV) from a heterogeneous population in the Brazilian Amazon.

Keywords: HIV, therapeutic response, immunogenetics, HLA, B*13

INTRODUCTION

In contrast to what was expected with the introduction of antiretroviral therapy (ART), treatment maintenance fails to restore the $CD4^+$ T lymphocyte count in a portion of people living with HIV (PLHIV) and consequently enables immune dysfunction and observed morbidities when compared to complete immune reconstitution (1).

There is no consensus regarding the definition of "treatment nonresponders". However, the prevailing understanding is that an adequate immune response should include a $CD4^+$ T lymphocyte count greater than 500 cells/μL because the morbidity rate for individuals with this immune profile is similar to that for seronegative individuals (2).

The mechanisms associated with immune reconstitution failure are varied and, in most cases, dependent; these mechanisms manifest as dysfunctions in the balance of lymphocyte maintenance (3, 4). Among the set of associated factors, it is suggested that the genetic profile of key immune response elements may impact recovery failure (5).

The HLA system plays roles in various stages of HIV infection, i.e., the selection of the viral epitope repertoire presented to $CD4^+$ and $CD8^+$ T lymphocytes (6), the induction of antiviral cytotoxicity (7) and the selection of mutations in the virus, thus modulating viral escape kinetics (8).

In an overview, the alleles HLA-A*24 (9), A*36 (10), B*35 (11), B*53 (12), and DRB1*13 (13) are some associated with the progression of HIV infection. In contrast, the HLA-A*02 (14), B*27 (15), B*57 (16) and DRB1*15 (17) are examples associated with infection control. Rare alleles can lead to additional viral evolution compared to the more common alleles, particularly with respect to the HIV-1 *env*, *nef* and *pol* genes (18). On the other hand, heterozygosity is considered a selective advantage against the progression of infection due to the ability of the host to present a larger repertoire of viral antigens, thus developing a broader response (19).

In the maintenance of the therapeutic response, studies suggest that allelic variants of HLA may be associated with post-ART immune failure; for example, having the HLA-B*57 allele impairs the recovery of $CD4^+$ T cells during the first three years after the onset of ART, even though the allele provides a potential mechanism for epidemiological protection against infection (20). However, discussions already point to the inability of ART to reverse the immune escape process of HIV associated with the HLA profile (21); for patients with the B*57 allele, immune escape may be aggravated by a hypersensitivity reaction to some antiretroviral drugs (22).

The allele clusters Bw4 and Bw6, which interact with killer cell immunoglobulin-like receptors (KIR), have been associated with HIV disease progression control as well as with resistance to infection in different populations (23, 24). In an Australian cohort, Bw4 homozygosity seems to predict a compromised recovery of $CD4^+$ T lymphocytes after the start of combination ART (25). In a similar ethnic population, post-ART counts of $CD4^+$ $Bw4^+$ T lymphocytes were associated with the partial normalization of NK-KIR3DL1 cell functionality. However, the reconstitution of immune competence was not associated with the complete restoration of antibody-dependent cellular cytotoxicity (26).

Other class 1 and class 2 molecules are abnormally expressed in patients receiving ART, each related to an aspect of the immune response (27, 28). It is likely that in this context, some alleles may mediate post-ART immune failure (29); however, others are camouflaged by the effects of therapy (30).

In this context, we observed a lack of studies on the relationship between HLA and treatment nonresponse in patients from the Brazilian Amazon and the northern region of Brazil. In Brazil, the frequency of nonresponders tends to vary from 38 to 50% depending on the region and on the methodology used in studies, in which biomarkers of varying etiology have been associated with the observed immunological condition (31–33).

Thus, herein, we evaluate the frequency and possible associations of HLA class 1 (HLA-A and HLA-B) and class 2 (HLA-DRB1) alleles in nonresponder PLHIVs from a northern Brazilian cohort.

MATERIALS AND METHODS

Sampling and Ethical Aspects

This cohort study was created by recruiting patients from *Setor de Atendimento Médico Unificado do Instituto Evandro Chagas* (Unified Medical Service of the Evandro Chagas Institute;

SOAMU-IEC) and *Centro de Atenção à Saúde nas Doenças Infecciosas Adquiridas* (Health Care Center for Acquired Infectious Diseases; CASA DIA) through the weekly selection of peripheral blood samples from prescreened PLHIV or suspected HIV-infected people living in the state of Pará, Brazil, from January 2018 to January 2019.

In compliance with resolutions 466/2012 and 347/05 of the National Health Council, which addresses the guidelines and regulatory standards of research involving human beings, the project was submitted for ethical review and approved by the Human Research Ethics Committee of *Instituto Evandro Chagas* (Protocol: 3.121.265; CAAE: 73927717.3.0000.0019). All participants were informed about the study objectives, and those who agreed to participate signed an informed consent form and answered a sociodemographic questionnaire. Individuals under 18 years of age or who were already receiving ART when first approached were not considered for the study. Participants who died, those who did not have continuous data on monitoring platforms, or those who abandoned the governmental assistance program for PLHIV during the study period were excluded.

Confirmatory Methods, Quantification of Viral Load and CD4$^+$ and CD8$^+$ T Lymphocytes

The suspicion of HIV infection was confirmed by the qualitative detection of p24 antigen and anti-HIV-1 and anti-HIV-2 IgG antibodies by enzyme immunoassays (Murex AG/AB Combination DiaSorin, UK). Serology was confirmed using a rapid Immunoblot DPP HIV-1/2 kit (Bio-Manguinhos, FIOCRUZ) following the manufacturer's protocol.

Plasma HIV viral load was quantified by real-time PCR using an Abbott mSample Preparation System RNA extraction kit and Abbott Real-Time HIV-1 amplification matrix (ABBOTT, Chicago, Illinois, USA).

The quantification of CD4 T ($CD45^{bright}$ $CD3^+$ $CD4^+$ $CD8^-$) and CD8 T ($CD45^{bright}$ $CD3^+$ $CD4^-$ $CD8^+$) lymphocytes was performed by immunophenotyping and flow cytometry using BD FACSCalibur-4 core equipment, FACSCount™ Reagents and Tritest™/TruCount kits (BD Biosciences, San Jose, CA, USA).

Monitoring of Infection and Grouping Into Therapeutic Response Profiles

Access to the monitoring data platforms was obtained through pre-established authorizations declared for ethical review and in accordance with the consent of the participants.

Lymphocyte quantification and viral load data, after the first collection, were obtained from *Sistema de Controle de Exames Laboratoriais da Rede Nacional de Contagem de Linfócitos CD4$^+$/CD8$^+$ e Carga Viral do HIV* (Laboratory Test Control System of the National Network of CD4$^+$/CD8$^+$ Lymphocyte Count and HIV Viral Load; SISCEL) every six months. The quantification methods in each period were the same as previously described. The data regarding the management and distribution of antiretrovirals were obtained from *Sistema de Controle Logístico de Medicamentos* (Medication Logistics Control System; SICLOM) every six months; the initial therapeutic regimens, changes in approach and reasons for changes were determined. The data collection occurred for twenty-four months from the date of inclusion in the study.

In total, 270 PLHIVs participated in the study; after one year of ART, these PLHIVs were stratified based on their immune response to treatment into treatment nonresponders (CD4$^+$ < 500 cells/μL; varied viral load) and treatment responders (CD4$^+$ ≥ 500 cells/μL; varied viral load). We chose this cutoff because a previous analysis showed that for the same period, the viral load itself was not a determining factor in the differentiation of different progression groups. These established groups were evaluated for another year of therapy, totaling 24 months of analysis and data collection.

DNA Extraction and Genotyping of the HLA Locus

Peripheral blood samples were collected from the participants, from which DNA was extracted using a QIAamp DNA Mini Kit (Qiagen, Düsseldorf, Nordrhein-Westfalen, Germany) following the manufacturer's recommendations. The newly extracted DNA samples were quantified using a Qubit spectrofluorometer (Invitrogen, USA), following the manufacturer's recommendations; and the degree of purity was evaluated using a NanoDrop™ 2000/2000c spectrophotometer (Waltham, Massachusetts, USA), in which the elution solution used in the extraction of genetic material was used as a reference standard.

We standardized the following profiles as the ideal range for successful amplification: for loci A and DRB1, a concentration between 10 and 15 μg/ml; for the B locus, a concentration between 10 and 12 μg/mL. The degree of purity for all loci was represented by the ratios 260/280: 1.8-2.0 and 260/230: 1.8-2.2. Samples having concentrations above the established standard were diluted in DNase/RNase-free ultrapure distilled water (Invitrogen, USA). Samples with concentrations below the established standard were reextracted, and those that still had an inadequate profile were discarded from subsequent analyses.

In this sense, of the 270 participants obtained, we were not successful in genotyping some samples, of which only one sample from the responder group was not genotyped for locus A. For locus B, 29 samples from the responder group and 31 samples from the nonresponder group were not genotyped. For the DRB1 locus, only three samples were not genotyped from the nonresponder group.

Loci A, B and DRB1 of HLA classes 1 and 2 were genotyped *via* PCR-SSO (polymerase chain reaction-sequence specific oligonucleotide) methodology using Luminex technology (Luminex Corporation, Austin, TX, USA) and a LABType® kit (One Lambda Inc., Canoga Park, CA, USA).

In the amplification step, the target DNA was amplified by conventional PCR in a reaction mixture containing specific primers for each locus provided by the kit, recombinant Taq (Invitrogen, USA) and D-mix solution provided in the kit. Amplification occurred in a Mastercycler® Thermal Cycler, following the amplification protocol described in the kit. To confirm the amplification, 2.5% agarose gel electrophoresis was performed.

In the hybridization stage, the amplified DNA was denatured and hybridized with a set of specific oligonucleotide probes,

which were immobilized in fluorescently labelled polystyrene microspheres. After the hybridization step, the microspheres were washed and subsequently incubated with a streptavidin-phycoerythrin conjugate (SAPE), which binds to biotinylated amplified DNA.

In the last phase, a Luminex analyzer was used to measure the intensity of phycoerythrin fluorescence in each microsphere, and the data generated were stored to specific extensions. From those data, HLA Fusion software was used to determine the alleles of the HLA genes. The pattern of reactivity of each DNA sample relative to the set of probes conjugated to the microspheres allowed the establishment of the HLA genotype at medium resolution.

Statistical Analysis

To corroborate the proposed groupings, we applied discriminant multivariate analysis that adopted the same definition criteria for the groups of nonresponders and responders; a scatter plot was inferred for group identification.

We applied Cochran's Q test in the paired comparisons of lymphocyte quantification and viral load data obtained during the study; for intergroup analyses, we applied the Kruskal–Wallis test. We chose nonparametric tests due to the degree of normality of the variables studied, as estimated by the Lilliefors test.

For each HLA locus, Hardy-Weinberg equilibrium was measured, selecting as an alternative hypothesis of interest the excess heterozygotes in the groups; the standard parameters of the Markov chain were adopted. The genotypic linkage disequilibrium between the loci was determined by the log probability ratio, also adopting the Markov standard parameters. Allele clusters Bw4 and Bw6 were identified based on the HLA nomenclature database (34).

The parametric proportion (π) of the most frequent allele of each locus was calculated to estimate the confidence interval of the expected global frequency. We applied multivariate cluster analysis to cluster the populations of different ethnic profiles based on allele frequency data available in the "The Allele Frequency Net Database" (35). The allele frequencies of the Caucasoid, Negroid, Asian, Amerindian and heterogeneous populations of different regions of Brazil were collected; the clusters were inferred using the Ward minimum variance clustering method, with Euclidean distance and standardization of the variables.

The expected heterozygosity (*He*) for each HLA locus in the studied population was calculated based on the formula adapted from Nei et al. (36).

The frequency of the HLA alleles was calculated by direct counting and compared between the groups by the G test. For groups with significantly different allelic profiles, the chi-square residual test was applied to determine the probabilistic importance of each of the alleles for each group, followed by the calculation of the odds ratio to measure the advantage or disadvantage of the significant alleles. The comparison of the viral load and lymphocyte count data between the alleles was also performed using Cochran's Q test for paired analysis and the Kruskal–Wallis test for interallelic analyses. Regarding ART, the rates of maintenance of treatment gains between alleles were compared using the G test.

We estimated survival curves using the Kaplan–Meier product-limit method, followed by the log-rank method (Mantel-Cox) to compare the proposed curves. We adopted the proportion of responders to ART as an event of interest in the analysis the 24-month study period as the time variant. The estimates of response to therapy were evaluated among the alleles associated with the response progression profile.

We calculated the Spearman coefficient using a general correlation matrix for all the genetic, immunological, virological and therapeutic factors evaluated. The correlations were categorized as follows: negative and strong when the Spearman coefficient was between -1.0 and -0.5; negative and weak when the Spearman coefficient was between -0.5 and 0; positive and weak when the Spearman coefficient was between 0 and 0.5; and positive and strong when the Spearman coefficient was between 0.5 and 1.0.

For the statistical analyses, we adopted a significance level (α) of 95%, considering a probability of significance (p) less than or equal to 0.05 as a criterion for rejecting the null hypotheses. The estimation of Hardy-Weinberg equilibrium and linkage disequilibrium were calculated in GENEPOP software (37). The other analyses were performed using GraphPad Prism 8.4.3 (San Diego, CA, USA) and BioEstat 5.3 (38).

RESULTS

Sample Characterization

Examining the overall infection scenario, we observed a significant reduction in HIV viral load $\log_{10}$ ($p < 0.0001$), a significant reduction in $CD8^+$ T lymphocyte count ($p < 0.0001$), and an absolute increase in $CD4^+$ T lymphocytes ($p < 0.0001$) after starting ART, over 24 months of observations (**Table 1**; **Figure 1A**).

After 12 months of ART, 134 PLHIVs had a satisfactory immune response (responders), and 136 manifested failures in the restoration of $CD4^+$ T lymphocyte counts (nonresponders). The multivariate analysis clearly showed that responders and nonresponders formed two distinct groups with unique immunological characteristics that did not overlap (**Figure 1B**).

In this context, the viral load $\log_{10}$ remained significantly detectable in nonresponders up to 12 months after initiating ART [(month 0 = $p < 0.0001$), (month 6 = p: 0.0008), (month 12 = p: 0.0016)] (**Table 1**; **Figure 1C**); the median $CD4^+$ T lymphocyte count in nonresponders remained less than 500 cells/μL regardless of their time receiving ART [(month 0 = $p < 0.0001$), (month 6 = $p < 0.0001$), (month 12 = $p < 0.0001$), (month 18 = $p < 0.0001$), (month 24 = $p < 0.0001$)] (**Table 1**; **Figure 1D**); and we observed a tendency for $CD8^+$ T lymphocyte count to be higher in nonresponders up to the first 12 months after initiating ART [(month 0 = p: 0.007), (month 12 = p: 0.0008)] (**Table 1**; **Figure 1E**).

Regarding the epidemiological, sociodemographic and therapeutic factors associated with the therapeutic response, we found that females [p = 0.037; OR (95% CI): 1.94 (1.06-3.54)], those with low education levels [p = 0.042; OR (95% CI): 1.71

TABLE 1 | Analysis of HIV viral load (log 10), CD4+ T lymphocyte count and CD8+ T lymphocyte count over the study period.

TIME BIOMARKERS	month 0 Median (IQR (25%-75%)	month 6 Median (IQR (25%-75%)	month 12 Median (IQR (25%-75%)	month 18 Median (IQR (25%-75%)	month 24 Median (IQR (25%-75%)
Overall infection scenario					
HIV viral load (log_{10})	4.7 (4.04-5.26)	1.6 (0-1.60)	1.6 (0-1.6)	0 (0-1.6)	0 (0-1.60)
$CD8^+$ T lymphocyte	1 077 (759-1464)	1029 (747-1410)	926 (656-1273)	813 (609-1160)	819 (626-999)
$CD4^+$ T lymphocyte	334 (169-506)	477 (306-693)	498 (322-762)	546 (373-767)	591 (384-860)
Responders vs. Nonresponders					
(res.) HIV viral load (log_{10})	4.52 (3.83-4.97)	1.6 (0-1.6)	1.6 (0-1.7)	0 (0-1.6)	0 (0-1.6)
(nonres.) HIV viral load (log_{10})	4.97 (4.47-5.54)	1.6 (0-2.14)	0 (0-1.6)	0 (0-1.6)	0 (0-1.6)
(res.) $CD8^+$ T lymphocyte	1 232 (871-1630)	1029 (759-1452)	1037 (736-1357)	780 (593-1033)	828 (650-1028)
(nonres.) $CD8^+$ T lymphocyte	1 004 (715-1310)	1016 (769-1322)	876 (599-1156)	858 (640-1228)	781 (590-962)
(res.) $CD4^+$ T lymphocyte	462 (350-628)	666 (540-859)	764 (612-917)	704 (571-880)	834 (620-1075)
(nonres.) $CD4^+$ T lymphocyte	169 (80-280)	313 (233-400)	323 (232-400)	415 (286-520)	392 (317-590)
Associated alleles					
HIV viral load (log_{10})					
B*13	4.97 (4.77-5.71)	1.76 (1.2-2.43)	1.6 (1.6-2.46)	1.6 (1.2-2.47)	1.6 (1 -3.4)
B*35	4.8 (4.15-5.28)	1.6 (0-1.65)	1.6 (0 -1.6)	0 (0-1.6)	0 (0-1.6)
B*39	5.1 (4.46-5.55)	1.65 (1.6-2.16	1.6 (0-1.74)	0 (0-1.6)	0 (0-1.6)
Others	4.69 (4.03-5.25)	1.6 (0-1.64)	0 (0-1.6)	0 (0-1.6)	0 (0-1.6)
$CD8^+$ T lymphocyte					
B*13	972 (262-985)	932 (646-1225)	745 (541-1214)	676 (521-1303)	766 (714-1304)
B*35	1 110 (736-1593)	1042 (760-1458)	901 (662-1232)	767 (537-1196)	749 (596-896)
B*39	765 (559-1 103)	795 (611-1400)	698 (664-1406)	902 (662-1130)	926 (630-1206)
Others	1 076 (773-1520)	1029 (755-1520)	984 (672-1277)	832 (640-962)	767 (592-1024)
$CD4^+$ T lymphocyte					
B*13	77 (51-314)	246 (137-340)	272 (134-407)	337 (145-562)	307 (192-457)
B*35	346 (139-512)	473 (323-709)	390 (218-697)	533 (349-709)	679 (383-928)
B*39	248 (103-446)	268 (176-400)	363 (212-598)	391 (262-664)	560 (384-614)
Others	326 (161-503)	521 (282-675)	556 (352-827)	548 (397-753)	581 (399-843)

res., responders; nonres., nonresponders; IQR, interquartile range.

(0.96-3.07)] and heterosexual individuals [p = 0.005; OR (95% CI): 2.36 (1.39-4.02)] had an approximately two times higher risk of nonresponse to ART and that an active sex life was a more frequent factor in responders [p = 0.017; OR (95% CI): 0.54 (0.33-0.89)]. A switch in treatment was the most prominent factor in the first analysis, being approximately 10 times more likely to occur in patients with immune failure after ART [p < 0.0001; OR (95% CI): 9.64 (3.66-25.43)] (**Table 2**).

Based on the multiple logistic regression results, sexual preference [OR (95% CI): 1.70 (1.00-2.89)] and switch in therapy [OR (95% CI): 6.42 (2.12-19.39)] were the factors that remained associated with response failure (**Table 2**).

Allelic Frequency of Loci A, B and DRB1 in the Studied Population

For locus A, the A*02 allele was the most common in the studied population (25.9%; 95% CI: 23.3% < π < 28.5%); for locus B, the B*35 allele was the most common (15.2%; 95% CI: 12.7% < π < 17.8%); and for the DRB1 locus, the DRB1*13 allele was the most common (14.8%; 95% CI: 12.7% < π < 16.9%). The expected heterozygosity was highest for locus B (*He*: 0.93) (**Figure 2A**). All loci were in Hardy-Weinberg equilibrium (locus A, p = 0.777; locus B, p = 0.896; and locus DRB1, p = 0.974). All loci were in linkage equilibrium; therefore, it was assumed that the segregation of the allele pairs was random (locus AB, p = 1.000; locus A-DRB1, p = 0.220; and locus B-DRB1, p = 0.300).

In the cluster analysis, the allelic frequencies of loci A, B and DRB1 in the studied population (Amazon population) were similar to those observed in heterogeneous ethnic populations from different regions of Brazil, more specifically from northern Brazil. The allele frequency for Caucasoids was directly related to the heterogeneous clade; in another segment, we observed a closer relationship between Amerindians and Asians; and Negroes were related to this clade at the second level (**Figure 2B**).

The allelic frequencies of loci A and DRB1, as well as their zygosity, were similar between ART responders and nonresponders (locus A, p = 0.9819; zygosity, p = 0.3561); (locus DRB1, p = 0.1516; zygosity, p = 0.9817) (**Supplementary Table 1**). However, the allelic profile of locus B was statistically associated with therapeutic nonresponse (p = 0.0449) (**Figure 3**). Based on the chi-square residual, alleles B*13, B*35 and B*39 had the greatest probabilistic influence in the analysis; both zygosity (p: 0.6760) and the frequency of clusters Bw4 and Bw6 (p: 0.9386) were not associated with the profile (**Supplementary Table 1**).

The B*13, B*35 and B*39 Alleles Were Associated With Immunological Aspects of the Therapeutic Response

In the odds ratio calculation, the B*13, B*35 and B*39 alleles were associated with the risk of therapeutic nonresponse in PLHIVs, and the odds ratio for nonresponse was approximately twofold for the B*35 allele [OR (95% CI): 1.86 (1.08-3.18)] and threefold for the B*39 allele [OR (95% CI): 3.33 (1.33-8.69)]; the B*13 allele, with the highest ratio, had an odds ratio for nonresponse approximately fourfold higher [OR (95% CI): 4.06 (0.45-36.62)]. We suggest that the extensive confidence interval is a reflection of

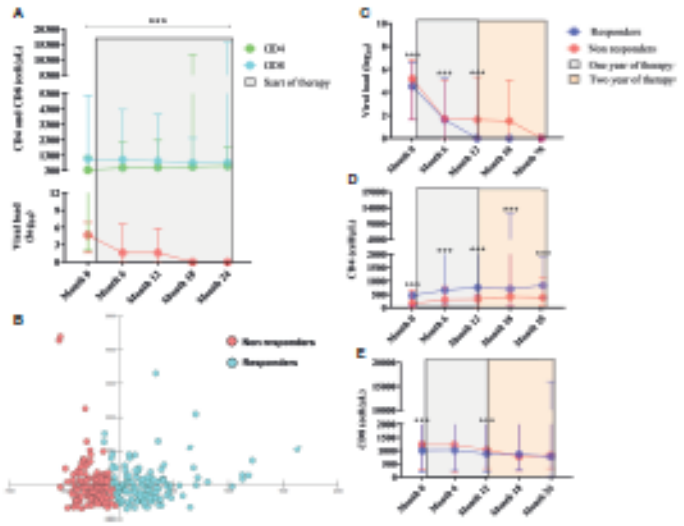


FIGURE 1 | **(A)** Longitudinal immunovirological evaluation of PLHVs before and throughout ART during the two-year treatment period. Viral load is in red. **(B)** Scatter plot of multivariate discriminant analysis of viral load and CD4+ T and CD8+ T lymphocyte counts showing the groups of therapeutic responders and nonresponders categorized based on CD4+ T lymphocyte count ≥ or < 500 cells/μL. **(C)** Longitudinal evaluation of viral load. **(D)** CD4+ T lymphocyte count and **(E)** CD8+ T lymphocyte count between responders and nonresponders before and after the start of therapy. ***: $p < 0.0001$.

the low allele frequency in the groups (**Figure 4A**). The estimated survival curves were significant for the comparison methods used ($\chi^2 = 9.923$; $p = 0.0192$); the B*13 allele had the lowest proportion of ART responders across the study period, with approximately 6.67% of responders at the end of 24 months of analysis, followed by the B*35 allele (16.15%), B* 39 (19.98%) and other alleles (24.05%) (**Figure 4B**).

The viral load was similar among the B locus alleles in the pretreatment collection; however, a threshold of statistical significance was observed at six months of therapy, with carriers of the B*13 allele having the highest viral load (p: 0.0526; **Table 1**). A statistically significant association was observed after 12 months of therapy, during which only carriers of alleles without risk of therapeutic response (other alleles) maintained viral loads below the limit of detection ($p = 0.0237$; **Table 1**). After 12 months of therapy, the viral load for all alleles was statistically similar and close to the limit of detection; carriers of the B*35 allele had very low viral loads at 18 months of therapy, and carriers of the B*39 allele had very low viral loads at 24 months; however, carriers of the B*13 allele still had detectable viral loads even with a long period of treatment (**Table 1**; **Figure 4C**).

The $CD4^+$ T lymphocyte count remained statistically low in the B*13 allele carriers through the first 12 months of observation [(month 0 = p: 0.0001), (month 6 = p: 0.017), (month 12 = p: 0.0032)]. After 12 months of ART, the lymphocyte count was standardized among the alleles; however, the carriers of the B*13 allele had low counts relative to the others (**Table 1**; **Figure 4D**).

The number of $CD8^+$ T lymphocytes was similar among the alleles, regardless of the duration of ART (**Figure 4E**). In a longitudinal analysis, there was a reduction in cellularity in carriers of the B*35 allele (p: 0.005) and carriers of alleles without a response risk (p: 0.0001) over the 24 months of therapy (**Table 1**).

The B*13 Allele May be Associated With Therapy Switching

We evaluated the frequency of ART replacement in the intervals of 0-12 months and 12-24 months of treatment, for which we calculated the rate of regimen switching, the rate of maintaining the previous regimen and the rate of continuing the first therapeutic regimen since the beginning of monitoring. After 24 months of therapy, there was a significant 75% (3 to 4) increase in the rate of regimen switching among carriers of the B*13 allele and a 25% decrease (1 to 4) in the rates of maintaining and continuing previous regimens (p: 0.0344; **Figure 5A**). However, we detected only four carriers of the allele in the present study, which limits more robust conclusions about the relationship between genotype and maintenance of therapy. We describe here only one observed trend.

In the first 12 months of therapy, 75% of carriers of the B*13 allele were treated with the tenofovir + lamivudine and dolutegravir regimen (TDF+3TC and DTG), and only 25% started treatment with the second most common regimen: tenofovir + lamivudine + efavirenz (TDF+3TC+EFZ).

TABLE 2 | Epidemiological, sociodemographic and therapeutic factors associated with nonresponse to ART in PLHVs.

FACTORS	Responders n: 134	Nonresponders n: 136	p	OR[3] CI (95%)	OR[4] CI (95%)
Sex					
Female	21 (15.67)	36 (26.47)	0.037*	1.94	
Male	113 (84.33)	100 (73.53)		(1.06-3.54)	
Age					
18-30	85 (63.43)	73 (53.68)	0.110*		
31-72	49 (36.57)	63 (46.32)			
Education					
Not literate to basic education	98 (73.13)	112 (82.35)	0.042*	1.71	
High school to higher education	36 (26.87)	24 (17.65)		(0.96-3.07)	
Family income					
No income to 3 minimum monthly wages	122 (91.04)	121 (88.97)	0.696*		
4 to >10 minimum monthly wages	12 (08.96)	15 (11.03)			
Alcohol use					
No	12 (08.96)	17 (12.50)	0.433*		
Yes	122 (91.04)	119 (87.50)			
Illicit drugs					
No	94 (70.15)	100 (73.53)	0.569*		
Yes	40 (29.85)	36 (26.47)			
Time of use					
0 to 6 years	23 (57.50)	15 (41.67)	0.258*		
7 to more years	17 (42.50)	21 (58.33)			
Sexual orientation					
Heterosexual	42 (31.34)	69 (50.74)	0.005#	2.36	1.70
Homosexual	69 (51.49)	48 (35.29)		(1.39-4.02)	(1.00-2.89)
Bisexual	23 (17.16)	19 (13.97)			
Active sex life					
Yes	94 (70.15)	76 (55.88)	0.017*	0.54	
No	40 (29.85)	60 (44.12)		(0.33-0.89)	
Permanent partner					
No	65 (48.51)	64 (47.06)	0.903*		
Yes	69 (51.49)	72 (52.94)			
Sexual relations with sex workers					
No	104 (77.61)	103 (75.74)	0.774*		
Yes	30 (22.39)	33 (24.26)			
Use of condoms					
Occasionally	76 (56.72)	75 (55.15)	0.696#		
Never	15 (11.19)	20 (14.71)			
Always	43 (32.09)	41 (30.15)			
Treatment abandonment					
No	0	2 (01.00)	0.498*		
Yes	134 (100.0)	134 (99.00)			
Treatment switch					
No	129 (96.27)	99 (72.79)	<0.0001*	9.64	6.42
Yes	5 (03.73)	37 (27.21)		(3.66-25.43)	(2.12-19.39)

*Fisher's exact test; #Chi-square test; [3]Simple logistic regression; [4]Multiple logistic regression.

However, after 24 months of therapy, we observed an inversion of prevalence, with the TDF+3TC+EFZ regimen becoming the most common (50%); the TDF+3TC and DTG regimen was maintained for 25% of patients, and a new regimen, raltegravir and tenofovir + lamivudine (RAL and TDF+3TC), was administered to 25% of patients (**Figure 5B**). For the other locus B alleles, the TDF+3TC and DTG regimen prevailed regardless of the duration of therapy, with a frequency ranging from 81% to 93% (**Figures 5C–E**).

The reasons for switching therapy were not reported for most patients (50-90%); approximately 66% of carriers of the B*13 allele switched due to coinfection with tuberculosis, and approximately 5% of carriers of the B*35 allele switched therapy due to adverse reactions to the previous regimen. Pregnancy (15%), use of metformin as an antidiabetic agent (4%) and virological failure (4%) were reported only for carriers of other alleles. The frequency of the reasons for switching was not significant among the alleles (G: 15.05; G (Williams): 8.27; p: 0.9124) (**Figure 5F**).

General Correlation Matrix

We generated a correlation matrix for the genetic, immunological and therapeutic factors evaluated in the present study (**Figure 6**).

The B*13 allele was significantly correlated with switching therapy (r: 0.975; p: 0.0210), the TDF+3TC+EFZ regimen (r: 0.451; p: 0.0394), a low $CD4^+$ T lymphocyte count (r: -0.765; p: 0.0265) and increased plasma viral load (r: 0.662; p: 0.0546).

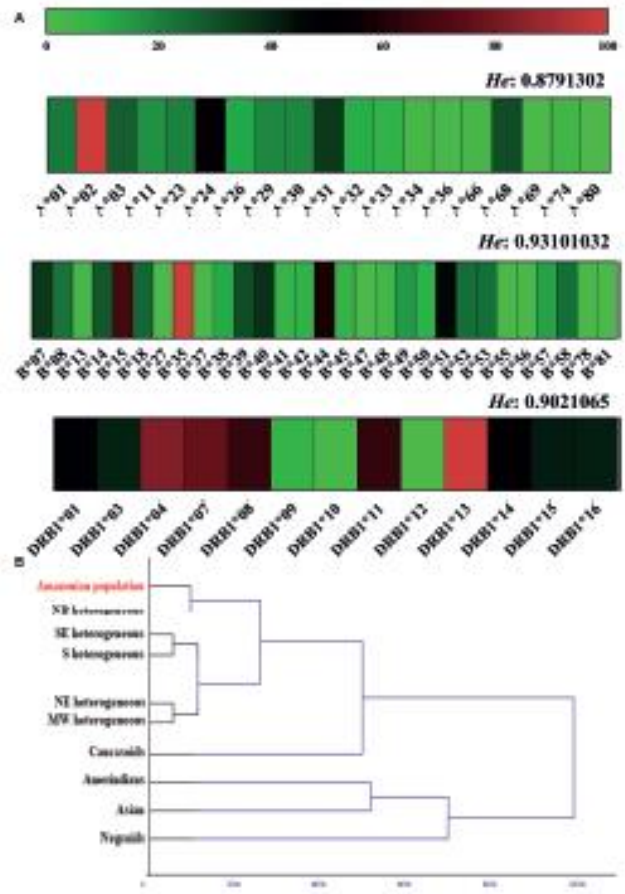


FIGURE 2 | **(A)** Heatmap of the allelic frequency and expected heterozygosity (He) of loci A, B and DRB1 in the general population. **(B)** Dendrogram of the multivariate cluster analysis showing proximity due to allele frequency similarity of the studied population (Amazonian population) with other ethnic groups. NE, Northern Brazil; NE, Northeast Brazil; MW, Brazilian Midwest; SE, Southeast Brazil; S, Southern Brazil.

The B*35 allele was not correlated with the factors evaluated. The B*39 allele was correlated with an increased viral load (r: 0.440; p: 0.0379), a low CD4$^+$ T lymphocyte count (r: -0.747; p: 0.0304) and a high CD8$^+$ T lymphocyte count (r: 0.440; p: 0.0452).

The therapeutic regimens significantly correlated with reductions in viral load were TDF+3TC and DTG (r: -0.667; p< 0.0001) and TDF+3TC+EFZ (r: -0.109; p: 0.0016). The maintenance of the CD4$^+$ T lymphocyte count was correlated only with the TDF+3TC and DTG regimen (r: 0.191; p< 0.0001). A reduction in CD8$^+$ T lymphocyte count was correlated with the TDF+3TC+EFZ regimen (r: -0.071; p: 0.0385). Most patients administered the RAL+TDF+3TC (r: 0.135; p: 0.0001) and AZT+3TC+EFZ (r: 0.107; p: 0.0020) regimens switched therapy during the two years of treatment. The TDF+3TC and DTG (r: 0.923; p: 0.0004) and TDF+3TC+EFZ (r: -0.119; p: 0.0006) regimens were correlated with maintaining the same regimen throughout the two years of treatment.

DISCUSSION

In the present study, 50.37% of the participants were treatment nonresponders, a value within the expected range based on the

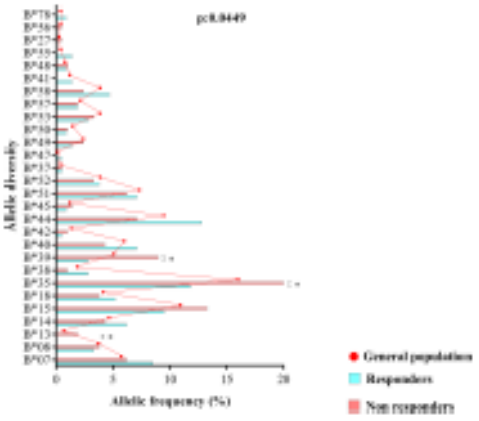


FIGURE 3 | Allele diversity of the HLA-B locus between treatment responders, treatment no responders and the entire study population (general population). The B*13, B*35 and B*39 alleles were more common in nonresponders ($p = 0.0449$).

heterogeneity of studies on the evaluation of suboptimal immune recovery (39).

The duration of treatment required to categorize therapeutic response is also a contrasting factor (40). We suggest that with two years of therapy, the classification of groups was feasible due to the noticeable delineation of the response status. Reports indicate that between one and three years of continuous therapy, the evaluation of biomarkers associated with therapy is functionally able to stratify groups regarding therapeutic response (2, 41, 42).

Among the set of associated factors, the genetic profile of key elements in the immune response may also influence recovery failure (5). Interestingly, our results showed that even before the onset of ART, some markers of infection progression were already different among patients who would be classified based on therapeutic response. This fact supports the hypothesis that hereditary factors play a role in possible susceptibility to failure of immune reconstitution. We validated this hypothesis by demonstrating that the HLA class I B*13, B*35 and B*39 alleles were associated with nonresponders.

Herein, we demonstrated that the B*35 allele was the most prevalent allele in the general population and was associated with the lowest risk of therapeutic response failure; however, the proportion of responders after two years of therapy was

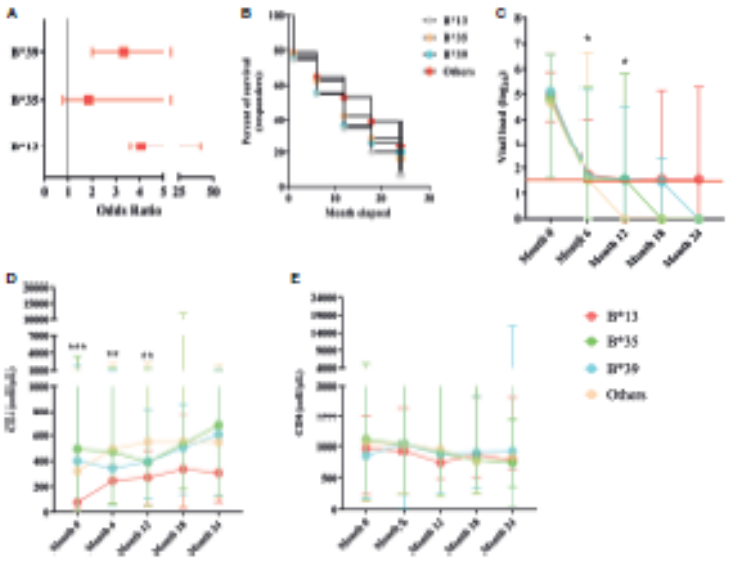


FIGURE 4 | **(A)** Box plot graph showing the odds ratio values for the risk of therapeutic nonresponse associated with the B*13, B*35 and B*39 alleles. The horizontal bars are the statistical confidence interval for each odds ratio. **(B)** Kaplan–Meier and Mantel–Cox survival curve models for alleles B*13, B*35, B*39 and other alleles related to the proportion of ART responders across 24 months of observation; the proposed curves were significant ($\chi^2 = 9.923$; $p = 0.0192$); the B*13 allele occurred in the lowest proportion of responders over the study period (5.67%). **(C)** Longitudinal evaluation of viral load. The red line represents the detection limit of the kit used. **(D)** CD4+ T lymphocyte count and **(E)** CD8+ T lymphocyte count among carriers of alleles B*13, B*35, B*39 and other alleles in the 24-month period; ***: $p < 0.0001$; **: $0.005 \geq p < 0.0001$; *: $0.05 \leq p > 0.0001$.

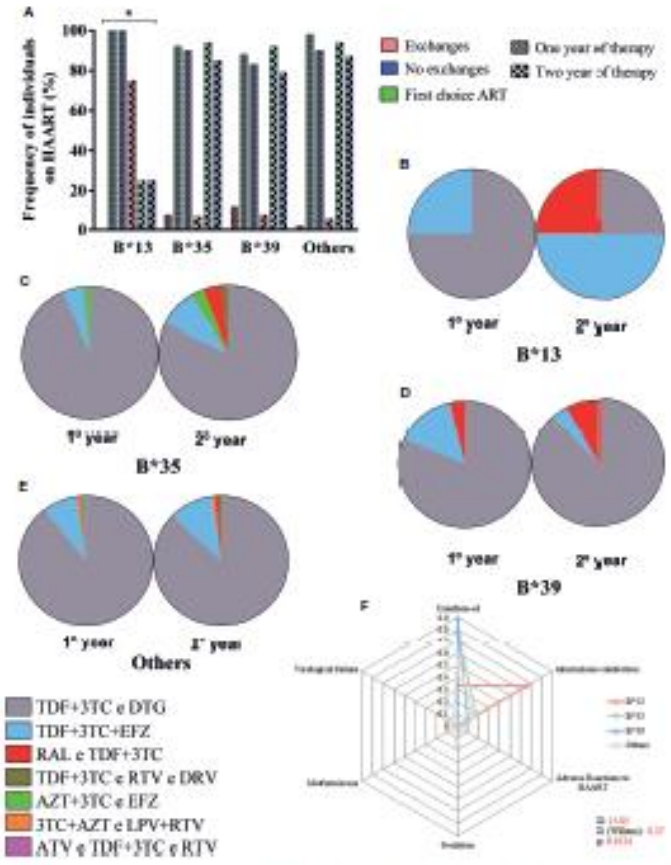


FIGURE 5 | **(A)** Frequency plot of the rate of maintenance of therapeutic regimens among carriers of alleles B*13, B*35, B*39 and other alleles in the 24-month period; the treatment switching rate was associated with allele B*13; *: 0.05 ≤ p > 0.0001. **(B–E)** Diversity of therapeutic regimens administered in 1- and 2-year periods in carriers of alleles B*13 **(B)**, B*35 **(C)**, B*39 **(D)** and other alleles **(E)**. **(F)** Radar chart showing the reasons for switching therapy between carriers of alleles B*13, B*35, B*39 and other alleles; each equiangular spoke represents a reason for switching therapy; approximately 66% of B*13 allele carriers switched treatments due to coinfection with tuberculosis. TDF, tenofovir; 3TC, lamivudine; DTG, dolutegravir; EFZ, efavirenz; RAL, raltegravir; RTV, ritonavir; DRV, darunavir; AZT, zidovudine; LPV, lopinavir.

approximately 16%. Carriers of the allele maintained a detectable viral load up to 12 months after the initiation of therapy, with a decrease in the $CD4^+$ T lymphocyte count during the same period. The B*35 allele is in fact consistently associated with the progression of HIV infection because $CD8^+$ T lymphocytes associated with this allele exhibit a dysfunctional phenotype that cannot elicit an effective response to control viral replication (43); essentially, we found that the concentration of $CD8^+$ T lymphocytes decreased in B*35 allele carriers in the two years of observation. The immune response by B*35+ cells may be robust due to avidity in antigen presentation; however, this may be influenced by epitope type or the dominant viral clade (44, 45).

Subvariants of the B*35 allele that affect antigen presentation are associated with different outcomes regarding the risk of HIV progression (46, 47). In our study, the genotyping resolution allowed us to identify only the allele group of each locus and was not specific enough to distinguish other levels of classification. Therefore, the grouping of functionally distinct variations may have influenced the nonpresentation of more categorical associations of the risk allele with the pathological markers.

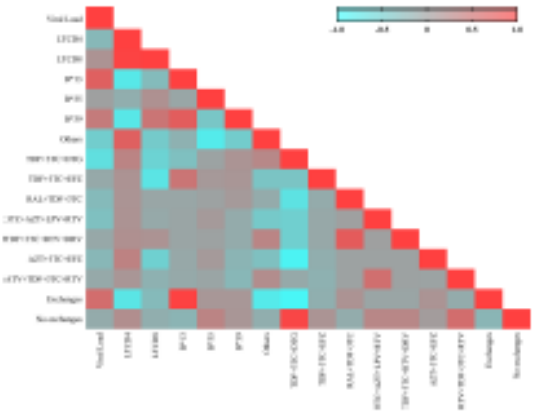


FIGURE 6 | Heatmap showing the matrix of correlations between the immunovirological and genetic variables. Each square represents the Spearman coefficient of the correlation between two variables. Negative and strong correlations have a coefficient between −1.0 and −0.5; negative and weak correlations have a coefficient between −0.5 and 0; positive and weak correlations have a coefficient between 0 and 0.5; and positive and strong correlations have a coefficient between 0.5 and 1.0.

Carriers of the B*39 allele were approximately three times more likely to present therapeutic response failure. The viral load remained detectable after 18 months of continuous therapy; however, there was an increase in the $CD4^+$ T lymphocyte count after 12 months of ART and a positive correlation with $CD8^+$ T lymphocytes. This attempt at an immune rebound is consistent with reports that show an association of B*39 with slower disease progression due to epitopes with fitness costs to the virus (48, 49). A possible justification is that the cellular response restricted to the allele tends to be more robust and substantial, resulting in improved effector activity with impacts on clinical aspects (50, 51).

The B*13 allele was more closely associated with the risk of response failure than were the other alleles, with approximately 7% of responders at the end of two years of therapy and a detectable viral load and $CD4^+$ T lymphocyte count below 400 cells/μL over the same period. These findings contrast with most of the reports that show an association of the B*13 allele with a robust immune response, especially against epitopes originating from the Gag and Nef regions, given that the restricted mutations incur a significant viral fitness cost (52–54). However, the claimed strong effect on disease control is more noticeable in the long term (55) and is probably masked by the noncontinuous administration of antiretrovirals, even for brief periods (56). In contrast, the association of the B*13 allele with mutational resistance variants that hinder immunological recognition and favor therapeutic switches in the short and medium terms has been discussed (57, 58).

In this regard, the treatment switch rate was higher in carriers of the B*13 allele in the second year of treatment, and the allele was correlated with the maintenance of the TDF+3TC+EFZ regimen. According to Brazilian guidelines, for patients beginning treatment, the preferred regimen is a combination of nucleoside reverse transcriptase inhibitors (NRTI), 3TC+TDF, and the integrase inhibitor (INI) DTG (59). Users of 3TC+TDF have a favorable profile regarding virological suppression and $CD4^+$ T lymphocyte response (60); it is an interchangeable regimen with low rates of relative risk and few resistant strains (61) that can also be administered in cases of hepatitis B coinfection (62). The addition of DTG provides a high genetic barrier with few adverse events, especially for treatment-free PLHIVs (63).

However, the exception to this preferred regimen is coinfection with tuberculosis, for which the administration of a nonnucleoside reverse transcriptase inhibitor (NNRTI), EFZ, is indicated as a substitute for DTG (59) because the treatment of tuberculosis can alter the effective concentration of DTG in the body (64). The pharmacological interaction of EFZ with anti-tuberculosis agents is associated with successful virological results (65). This is consistent with our results, i.e., the regimen is correlated with the control of viral load and maintenance of the $CD8^+$ T lymphocyte count.

Studies have discussed the association of the B*13 allele with the risk of developing thoracic tuberculosis and multisystemic sarcoidosis (66, 67). Interestingly, in the present study, we also showed that 50% of B*13 allele carriers who switched treatment regimens in the second year of treatment were coinfected with tuberculosis.

Thus, our dataset suggests that at the beginning of HIV infection, the B*13 allele may be favored after an attempted antiviral response, even in patients undergoing intensive therapy and showing a sustained virological response, which predispose these patients to coinfections that require changes to specific therapeutic regimens. This could be one of the causes of the high incidence of tuberculosis in nonresponders and the direct relationship with $CD4^+$ T count, regardless of virological status (68, 69). In addition, HLA can mediate the loss of $CD4^+$ T lymphocytes in nonresponders due to

the unregulated immune activation in HIV infection, even though ART is being administered (70).

Our findings on the association of the B*13 allele with therapeutic nonresponse, although intriguing, require further investigation, as our sample size may not be satisfactory for accurate conclusions.

This is the first study to associate HLA allele variants with the therapeutic response status in PLHIVs in the Brazilian Amazon region. Our findings indicate that the allele frequencies of HLA-I and HLA-II were similar to those for heterogeneous populations, especially in the northern region of the country, a finding that was expected given the diversified ethnic influence on regional population structure (71). In addition, we noted a similarity in the allele frequencies of Caucasoids with the population studied, a finding also observed in other studies of Amazon ancestry profiles (72). The analyses herein contribute to studies of HLA associations on intra- and interethnic scales and validate our proposal and those of others (73) specifically for HIV infection, i.e., pathological diversification is a continuous process that is influenced by the genetic profile of the ethnic composition of an infected population (74).

Illiterate and heterosexual women were more common in the group of nonresponders; however, we are unsure of the scientific implications of sociodemographic profile in the immune reconstitution of patients. Future studies on that issue may establish whether these findings may in fact be associated with an immunovirological response (75) or specific viral transmissibility routes (76) or a reflection of the social epidemiology of HIV (77).

We conclude that the allelic variants of the B locus are more associated with nonresponse to therapy in PLHIV from a heterogeneous population in the Brazilian Amazon. The sample size may have been a limiting factor in our study; however, we do believe that our findings provide a better understanding of the role of HLA in the context of the antiretroviral therapeutic response in HIV infection.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Human Research Ethics Committee of Instituto Evandro Chagas (Protocol: 3.121.265; CAAE: 73927717.3.0000.0019). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

TAFM, OM, RCMS, FBF, IBC, and ACRV were the creators of the project. LMSP, ESF, ABCF, and FLPR were responsible for sample collection at reference centers. LMSP, ESF and IrBC developed the methodology for the project; specifically, EVOJ and PJSMM conducted the HLA loci genotyping. LMSP conducted the statistical analysis and wrote the article. ESF, FBF, IgBC, and ACRV reviewed the article. All authors contributed to the article and approved the submitted version.

FUNDING

This study was funded by specific incentives from *Secretaria de Vigilância em Saúde do Ministério da Saúde* (Health Surveillance Secretariat of the Ministry of Health), *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (National Council for Scientific and Technological Development; CNPQ) (no. 301869/2017-0) and Fundação Amazônia de Amparo a Estudos e Pesquisa – FAPESPA (ICAAF-60/2020). We thank *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (Brazilian Federal Agency for the Support and Evaluation of Graduate Education; CAPES) for granting a scholarship (process number: 88882.183970/2018-01).

ACKNOWLEDGMENTS

We thank all the technical and administrative staff of the participating institutions for their help in recruiting the study patients as well as all the participants for their confidence and willingness to participate in the study. We thank CAPES for granting a scholarship.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.829126/full#supplementary-material

REFERENCES

1. Yang X, Su B, Zhang X, Liu Y, Wu H, Zhang T. Incomplete Immune Reconstitution in HIV/AIDS Patients on Antiretroviral Therapy: Challenges of Immunological Nonresponders. *J Leukoc Biol* (2020) 107 (4):597-612. doi: 10.1002/JLB.4MR1019-189R
2. Lewden C, Chene G, Morlat P, Raffi F, Dupon M, Dellamonica P, et al. HIV-Infected Adults With a CD4 Cell Count Greater Than 500 Cells/Mm3 on Long-Term Combination Antiretroviral Therapy Reach Same Mortality Rates as the General Population. *J Acquir Immune Defic Syndr* (2007) 46(1):72–7. doi: 10.1097/QAI.0b013e318136257a
3. Tanaskovic S, Fernandez S, French MA, Price RI, Song S, Robins PD, et al. Thymic Tissue Is Not Evident on High-Resolution Computed Tomography and [^{18}F]fluoro-Deoxy-Glucose Positron Emission Tomography Scans of Aviraemic HIV Patients With Poor Recovery of CD4$^+$ T Cells. *AIDS* (2011) 25(9):1235–7. doi: 10.1097/QAD.0b013e3283674155
4. Shive CL, Freeman ML, Younes SA, Kowal CM, Canaday DH, Rodriguez B, et al. Markers of T Cell Exhaustion and Senescence and Their Relationship to

Plasma TGF-β Levels in Treated HIV+ Immune Nonresponders. *Front Immunol* (2021) 12:628010. doi: 10.3389/fimmu.2021.628010

5. Soria A, Guerini FR, Bandera A, Bolognesi E, Uglietti A, Fusco C, et al. NK-HLA Genotypes in HIV-Infected Patients Lacking Immunological Recovery Despite Effective Antiretroviral Therapy. *PloS One* (2011) 6(11):e27349. doi: 10.1371/journal.pone.0027349
6. Chakraborty S, Rahman T, Chakravorty R, Kuchta A, Rabby A, Sahluzzaman M. HLA Super Types Contribute to HIV Type 1 Cytotoxic T Lymphocyte Epitope Clustering in Nefand Gag Proteins. *AIDS Res Hum Retroviruses* (2013) 29(2):270–8. doi: 10.1089/AID.2012.0160
7. Hua S, Lécuroux C, Sáez-Cirión A, Pancino G, Girault I, Versmisse P, et al. Potential Role the HIV-Specific CD38-/HLA-DR+ CD8+ T Cells in Viral Suppression and Cytotoxicity in HIV Controllers. *PloS One* (2014) 9(7): e101920. doi: 10.1371/journal.pone.0101920
8. Chang CH, Kist NC, Stuart Chester TL, Sreenu VB, Herman M, Luo M, et al. HIV-Infected Sex Workers With Beneficial HLA-Variants Are Potential Hubs for Selection of HIV-1 Recombinants That may Affect Disease Progression. *Sci Rep* (2015) 5:11253. doi: 10.1038/srep11253
9. Murakoshi H, Koyanagi M, Akahoshi T, Chikata T, Kuse N, Gatanaga H, et al. Impact of a Single HLA-A*24:02-Associated Escape Mutation on the Detrimental Effect of HLA-B*35:01 in HIV-1 Control. *EBioMedicine* (2018) 36:103–12. doi: 10.1016/j.ebiom.2018.09.022
10. Tang J, Malhotra R, Song W, Brill I, Hu L, Pei F, et al. Human Leukocyte Antigens and HIV Type 1 Viral Load in Early and Chronic Infection: Predominance of Evolving Relationships. *PloS One* (2010) 5(3):e9629. doi: 10.1371/journal.pone.0009629
11. Scorza Smeraldi R, Fabio G, Lazzarin A, Ehera N, Uberti Foppa C, Moroni M, et al. HLA-Associated Susceptibility to AIDS. HLA B35 Is a Major Risk Factor for Italian HIV-Infected Intravenous Drug Addicts. *Hum Immunol* (1988) 22 (2):73–9. doi: 10.1016/0198-8859(88)90088-9
12. Dorrell L, Wilcox BE, Jones EY, Gillespie G, Njai H, Sabally S, et al. Cytotoxic T Lymphocytes Recognize Structurally Diverse, Clade-Specific and Cross-Reactive Peptides in Human Immunodeficiency Virus Type-1 Gag Through HLA-B53. *Eur J Immunol* (2001) 31(6):1767–76. doi: 10.1002/1521-4141(200106)31:6<1767::aid-immu1767>3.0.co;2-l
13. Ortiz-Tordera B, Llano A, Ganoza C, Cate S, Hildebrand W, Sanchez J, et al. Impact of HLA-DRB1 Allele Polymorphisms on Control of HIV Infection in a Peruvian MSM Cohort. *HLA* (2017) 90(4):234–7. doi: 10.1111/tan.13085
14. Li WX, Xia J, Zhou X, Ma Y, Shen G, Qiu FW. Association of HLA Alleles (A, B, DRB1) and HIV-1 Infection in the Han Population of Hubei, China. *J Huazhong Univ Sci Technol Med Sci* (2017) 37(1):131–9. doi: 10.1007/s11596-017-1706-x
15. Kaslow RA, Carrington M, Apple R, Park L, Munoz A, Saah AJ, et al. Influence of Combinations of Human Major Histocompatibility Complex Genes on the Course of HIV-1 Infection. *Nat Med* (1996) 2:405–11. doi: 10.1038/nm0496-405
16. Schneidewind A, Tang Y, Brockman MA. Maternal Transmission of HIV Escape Mutations Subverts HLA-B57 Immunodominance But Facilitates Viral Control in the Haploidentical Infant. *J Virol* (2009) 83:8616–27. doi: 10.1128/JVI.00730-09
17. Ranasinghe S, Cutler S, Davis I, Lu R, Soghoian DZ, Qi Y, et al. Association of HLA-DRB1-Restricted CD8+ T Cell Responses With HIV Immune Control. *Nat Med* (2013) 19(7):930–3. doi: 10.1038/nm.3229
18. Rousseau CM, Lockhart DW, Listgarten J. Rare HLA Driven Additional HIV Evolution Compared to More Frequent Alleles. *AIDS Res Hum Retroviruses* (2009) 25:297–303. doi: 10.1089/aid.2008.0208
19. Fenton TA, Kimani J, Wachihi C, Bielawny T, Mendoza I, Thavaneswaran S, et al. HLA Class I Associations With Rate of HIV-1 Seroconversion and Disease Progression in the Pumwani Sex Worker Cohort. *Tissue Antigens* (2013) 81(2):93–107. doi: 10.1111/tan.12051
20. Nakajima T, Kimura A. Genetic Factors That Confer Sensitivity to TARV in HIV-Infected Subjects: Implication of a Benefit of an Earlier Initiation of TARV. *Pharmacogenomics* (2008) 9(9):1367–81. doi: 10.2217/14622416.9.9.1347
21. Knapp DJ, Brumme ZL, Huang SY, Wynhoven B, Dong WW, Mo T, et al. Increasingly Successful Highly Active Antiretroviral Therapy Delays the Emergence of New HLA Class I-Associated Escape Mutations in HIV-1. *Clin Infect Dis* (2012) 54(11):1652–9. doi: 10.1093/cid/cis253
22. Peter J, Chosh P, Lehloenya RJ. Drug Hypersensitivity in HIV Infection. *Curr Opin Allergy Clin Immunol* (2019) 19(4):272–82. doi: 10.1097/ACI.0000000000000545
23. Qing M, Li T, Han Y, Qiu Z, Jiao Y. Accelerating Effect of Human Leukocyte Antigen-Bw6 Homozygosity on Disease Progression in Chinese HIV-1-Infected Patients. *J Acquir Immune Defic Syndr* (2006) 41(2):137–9. doi: 10.1097/01.qai.0000195607.25262.92
24. Maruthamuthu S, Rajalingam R, Pandian K, Madasamy S, Manoharan M, Pitchai L, et al. Inhibitory Natural Killer Cell Receptor NKSDL1 With Its Ligand Bw4 Constraints HIV-1 Disease Among South Indians. *AIDS* (2018) 32(18):2679–88. doi: 10.1097/QAD.0000000000002028
25. Rauch A, Nolan D, Furrer H, McKinnon E, John M, Mallal S, et al. HLA-Bw4 Homozygosity Is Associated With an Impaired CD4 T Cell Recovery After Initiation of Antiretroviral Therapy. *Clin Infect Dis* (2008) 46(12):1921–5. doi: 10.1086/588479
26. Parsons MS, Wren L, Isitman G, Navis M, Stratov I, Bernard NF, et al. HIV Infection Abrogates the Functional Advantage of Natural Killer Cells Educated Through NKSDL1/HLA-Bw4 Interactions to Mediate Anti-HIV Antibody-Dependent Cellular Cytotoxicity. *J Virol* (2012) 86(18):9688–95. doi: 10.1128/JVI.06113-11
27. Cabello A, Rivero A, García MJ, Lozano JM, Torre-Cisneros J, González R, et al. TARV Induces the Expression of HLA-G on Peripheral Monocytes in HIV-1 Infected Individuals. *Hum Immunol* (2003) 64(11):1045–9. doi: 10.1016/j.humimm.2003.08.353
28. Lichtfuss GF, Hoy J, Rajasuriar R, Kramski M, Crowe SM, Lewin SR. Biomarkers of Immune Dysfunction Following Combination Antiretroviral Therapy for HIV Infection. *Biomark Med* (2011) 5(2):171–86. doi: 10.2217/bmm.11.15
29. El-Beali M, Al-Mahroogi SH, Youssef RM, Zadjali F, Balkhair A, Al-Balushi MS, et al. HLA-A68 and HLA-B15 Alleles Correlate With Poor Immune Response Among AIDS Patients on Combined Antiretroviral Therapy. *Hum Immunol* (2016) 77(6):490–7. doi: 10.1016/j.humimm.2016.06.009
30. Ferre AL, Hunt PW, McConnell DH, Morris MM, Garcia JC, Pollard RB, et al. HIV Controllers With HLA-DRB1*13 and HLA-DQB1*06 Alleles Have Strong, Polyfunctional Mucosal CD4+ T-Cell Responses. *J Virol* (2010) 84 (21):11020–9. doi: 10.1128/JVI.00980-10
31. Scarpellini S, Zanoni M, Sucupira MC, Truong HM, Janini LM, Segurado ID, et al. Plasma Metabolomics Biosignature According to HIV Stage of Infection, Pace of Disease Progression, Viremia Level and Immunological Response to Treatment. *PloS One* (2016) 11(12):e0161920. doi: 10.1371/journal.pone.0161920
32. Coelho AVC, Moura RR, Guimarães RL, Brandão LAC, Crovella S. Antiretroviral Therapy Immunologic Nonresponse in a Brazilian Population: Association Study Using Pharmaco- and Immunogenetic Markers. *Braz J Infect Dis* (2018) 22(5):392–401. doi: 10.1016/j.bjid.2018.09.002
33. Andrade-Santos JL, Carvalho-Silva WHV, Coelho AVC, Souto FO, Crovella S, Brandão LAC, et al. IL18 Gene Polymorphism and Its Influence on CD4+ T-Cell Recovery in HIV-Positive Patients Receiving Antiretroviral Therapy. *Infect Genet Evol* (2019) 75:103997. doi: 10.1016/j.meegid.2019.103997
34. Robinson J, Halliwell JA, Hayhurst JD, Flicek P, Parham P, Marsh SG. The IPD and IMGT/HLA Database: Allele Variant Databases. *Nucleic Acids Res* (2015) 43(Database issue):D423–31. doi: 10.1093/nar/gku1161
35. González-Galarza FF, McCabe A, Santos EJ, Jones J, Takeshita LY, Ortega-Rivera ND, et al. Allele Frequency Net Database (AFND) 2020 Update: Gold-Standard Data Classification, Open Access Genotype Data and New Query Tools. *Nucleic Acids Res* (2020) 48(D1):D783–D788. doi: 10.1093/nar/gkz1029
36. Nei M, Roychoudhury AK. Sampling Variances of Heterozygosity and Genetic Distance. *Genetics* (1974) 76(2):379–90. doi: 10.1093/genetics/76.2.379
37. Raymond M, Rousset F. GENEPOP (Version 1.2): Software De Genética Populacional Para Testes Exatos E Ecumenismo. *J Hered* (1995) 86:248–9. doi: 10.1093/oxfordjournals.jhered.a111573
38. Ayres M, JrM A, D'L A, Santos AS. BioEstat 5.0: Aplicações Estatísticas Nas Áreas De Ciências Biológicas E Médicas. In: *Belém*. Brasília: CNPq: Sociedade Civil Mamirauá (2008).
39. Ernest S, Ondoa P, Kityo CM, Siwale M, Akanmu S, Wellington M, et al. Suboptimal Immune Recovery During Antiretroviral Therapy With Sustained

HIV Suppression in Sub-Saharan Africa. *AIDS* (2018) 32(8):1049–51. doi: 10.1097/QAD.0000000000001801

40. Gaardbo JC, Hartling HJ, Gerstoft J, Nielsen SD. Incomplete Immune Recovery in HIV Infection: Mechanisms, Relevance for Clinical Care, and Possible Solutions. *Clin Dev Immunol* (2012) 2012:670957. doi: 10.1155/2012/670957
41. Robbins GK, Spritzler JG, Chan ES, Asmuth DM, Gandhi RT, Rodriguez BA, et al. Incomplete Reconstitution of T Cell Subsets on Combination Antiretroviral Therapy in the AIDS Clinical Trials Group Protocol 384. *Clin Infect Dis* (2009) 48(3):350–61. doi: 10.1086/595888
42. Engsig FN, Gerstoft J, Kronborg G, Larsen CS, Pedersen G, Rage E, et al. Long-Term Mortality in HIV Patients Virally Suppressed for More Than Three Years With Incomplete CD4 Recovery: A Cohort Study. *BMC Infect Dis* (2010) 10:318. doi: 10.1186/1471-2334-10-318
43. Habi S, Shahbaz S, Houston S. Selective Upregulation of CTLA-4 on CD8+ T Cells Restricted by HLA-B*35Px Renders Them to an Exhausted Phenotype in HIV-1 Infection. *PLoS Pathog* (2020) 16(8):e1008696. doi: 10.1371/journal.ppat.1008696
44. Friedrich D, Jalbert E, Dingus WL, Sidney J, Sette A, Huang Y, et al. Vaccine-Induced HIV-Specific CD8+ T Cells Utilize Preferential HLA Alleles and Target-Specific Regions of HIV-1. *J Acquir Immune Defic Syndr* (2011) 58(3):248–52. doi: 10.1097/QAI.0b013e318228f992
45. Matthews PC, Koyanagi M, Klenerman P, Harndahl M, Stryhn A, Akahoshi T, et al. Differential Clade-Specific HLA-B*3501 Association With HIV-1 Disease Outcome is Linked to Immunogenicity of a Single Gag Epitope. *J Virol* (2012) 86(23):12640–54. doi: 10.1128/JVI.01581-12
46. Olvera A, Gatell JM, Peiró-Álvarez S, Hildebrand W, Sanchez J, Brander C. HLA-B*35-PX and HLA-B*35-PY Subtype Differentiation Does Not Predict Observed Differences in Level of HIV Control in a Peruvian MSM Cohort. *AIDS* (2014) 28(15):2283–5. doi: 10.1097/QAD.0000000000000403
47. Juarez-Molina CI, Valenzuela-Ponce H, Avila-Rios S, Garrido-Rodriguez D, Garcia-Tellez T, Soto-Nava M, et al. Impact of HLA-B*35 Subtype Differences on HIV Disease Outcome in Mexico. *AIDS* (2014) 28(11):1687–90. doi: 10.1097/QAD.0000000000000322
48. Ntale RS, Chopera DR, Ngandu NK, Anta de Rosa D, Zembe L, Gamieldien H, et al. Temporal Association of HLA-B*81:01- and HLA-B*39:10-Mediated HIV-1 P24 Sequence Evolution With Disease Progression. *J Virol* (2012) 86(22):12013–24. doi: 10.1128/JVI.00539-12
49. Valenzuela-Ponce H, Alva-Hernández S, Garrido-Rodríguez D, Soto-Nava M, García-Téllez T, Bacamilla-Gómez T, et al. Novel HLA Class I Associations With HIV-1 Control in a Unique Genetically Admixed Population. *Sci Rep* (2018) 8(1):6111. doi: 10.1038/s41598-018-23849-7
50. Schloss J, Ali R, Racine JJ, Chapman HD, Serreze DV, DiLorenzo TP. HLA-B*39:06 Efficiently Mediates Type 1 Diabetes in a Mouse Model Incorporating Reduced Thymic Insulin Expression. *J Immunol* (2018) 200(10):3353–63. doi: 10.4049/jimmunol.1701652
51. Yeo L, Pujol-Autonell I, Baptista R, Eichmann M, Ernstsen-Vermeer D, Heck S, et al. Circulating β Cell-Specific CD8+ T Cells Restricted by High-Risk HLA Class I Molecules Show Antigen Experience in Children With and at Risk of Type 1 Diabetes. *Clin Exp Immunol* (2020) 199(3):263–77. doi: 10.1111/cei.13391
52. Honeyborne I, Prendergast A, Pereyra F, Leslie A, Crawford H, Payne R, et al. Control of Human Immunodeficiency Virus Type 1 is Associated With HLA-B*13 and Targeting of Multiple Gag-Specific CD8+ T-Cell Epitopes. *J Virol* (2007) 81(7):3667–72. doi: 10.1128/JVI.02689-06
53. Prado JG, Honeyborne I, Brierley I, Puertas MC, Martinez-Picado J, Goulder PJ. Functional Consequences of Human Immunodeficiency Virus Escape From an HLA-B*13-Restricted CD8+ T-Cell Epitope in P1 Gag Protein. *J Virol* (2009) 83(2):1018–25. doi: 10.1128/JVI.01882-08
54. Shahid A, Olvera A, Avinole G, Kuang XT, Cotton LA, Plana M, et al. Consequences of HLA-B*13-Associated Escape Mutations on HIV-1 Replication and Nef Function. *J Virol* (2015) 89(22):11557–71. doi: 10.1128/JVI.01955-15
55. Antoni G, Guergnon J, Meaudre C, Samri A, Bouhassira F, Goujard C, et al. MHC-Driven HIV-1 Control on the Long Run Is Not Systematically Determined at Early Times Post-HIV-1 Infection. *AIDS* (2013) 27(11):1707–16. doi: 10.1097/QAD.0b013e32836a6bd
56. Harrer EG, Bergmann S, Hamann K, Rittmaier M, Goldwich A, Müller SM, et al. A Conserved HLA B13-Restricted Cytotoxic T Lymphocyte Epitope in Nef Is a Dominant Epitope in HLA B13-Positive HIV-1-Infected Patients. *AIDS* (2005) 19(7):754–5. doi: 10.1097/01.aids.0000166099.36638.56
57. Dikmer M, Averkiev S, Aragón SS, Kartashev V, Popłavskija T, Klein R, et al. Short Communication: Selection of Thymidine Analog Resistance Mutational Patterns in Children Infected From a Common HIV Type 1 Subtype G Source. *AIDS Res Hum Retroviruses* (2010) 26(3):275–8. doi: 10.1089/aid.2009.0233
58. Verheyen J, Schweitzer F, Harrer EG, Knops E, Mueller SM, Dikmer M, et al. Analysis of Immune Selection as a Potential Cause for the Presence of Cleavage Site Mutation 431 V in Treatment-Naive HIV Type-1 Isolates. *Antivir Ther* (2010) 15(6):907–12. doi: 10.3851/IMP1640
59. Brasil, Ministério da Saúde. *Protocolo Clínico E Diretrizes Terapêuticas Para Manejo Da Infecção Pelo HIV Em Adultos. Brasília: Departamento De Vigilância, Prevenção E Controle Das Infecções Sexualmente Transmissíveis, do HIV/Aids E Das Hepatites Virais - Brasília Ministério da Saúde*. (2018). p. 416.
60. Gallant JE, DeJesus E, Arribas JR, Pozniak AL, Gazzard B, Campo RE, et al. Emtricitabine and Efavirenz vs. Zidovudine, Lamivudine, and efavirenz for HIV. *N Engl J Med* (2006) 354(3):251–60. doi: 10.1056/NEJMoa051871
61. Waters L, Mehta V, Gogtay J, Boffito M. The Evidence for Using Tenofovir Disoproxil Fumarate Plus Lamivudine as a Nucleoside Analog Backbone for the Treatment of HIV. *J Virus Erad* (2021) 7(1):100028. doi: 10.1016/j.jve.2021.100028
62. Lee YB, Jung EU, Kim BH, Lee JH, Cho H, Ahn H, et al. Tenofovir Monotherapy Versus Tenofovir Plus Lamivudine or Telbivudine Combination Therapy in Treatment of Lamivudine-Resistant Chronic Hepatitis B. *Antimicrob Agents Chemother* (2015) 59(2):972–8. doi: 10.1128/AAC.04454-14
63. Cahn P, Madero JS, Arribas JR, Antinori A, Ortiz R, Clarke AE, et al. Durable Efficacy of Dolutegravir Plus Lamivudine in Antiretroviral Treatment-Naive Adults With HIV-1 Infection: 96-Week Results From the GEMINI-1 and GEMINI-2 Randomised Clinical Trials. *J Acquir Immune Defic Syndr* (2020) 85(3):310–8. doi: 10.1097/QAI.0000000000002275
64. Tornheim JA, Dooley KE. Challenges of TB and HIV Cotreatment: Updates and Insights. *Curr Opin HIV AIDS* (2018) 13(6):486–91. doi: 10.1097/COH.0000000000000495
65. Jiang HY, Zhang MN, Chen HJ, Yang Y, Deng M, Ruan B. Nevirapine Versus Efavirenz for Patients Coinfected With HIV and Tuberculosis: A Systematic Review and Meta-Analysis. *Int J Infect Dis* (2014) 25:150–5. doi: 10.1016/j.ijid.2014.04.020
66. Dubaniewicz A, Szczerkowska Z, Hoppe A. Comparative Analysis of HLA Class I Antigens in Pulmonary Sarcoidosis and Tuberculosis in the Same Ethnic Group. *Mayo Clin Proc* (2003) 78(4):436–42. doi: 10.4065/78.4.436
67. Kettaneh A, Seng L, Tiev KP, Tolédano C, Fabre B, Cabane J. Human Leukocyte Antigens and Susceptibility to Tuberculosis: A Meta-Analysis of Case-Control Studies. *Int J Tuberc Lung Dis* (2006) 10(7):717–25.
68. Martín-Echevarría E, Rodríguez-Zapata M, Torralba M, Fernández JM, Moreno A, Casado JL, et al. Incidence of Tuberculosis in HIV-Infected Patients Receiving TARV: Interaction Between TST and CD4 Count. *Int J Tuberc Lung Dis* (2011) 15(10):1347–52. doi: 10.5588/ijtld.10.0686
69. Martín-Echevarría E, Serrano-Villar S, Sainz T, Moreno A, Casado JL, Dronda F, et al. Development of Tuberculosis in Human Immunodeficiency Virus Infected Patients Receiving Antiretroviral Therapy. *Int J Tuberc Lung Dis* (2014) 18(9):1080–6. doi: 10.5588/ijtld.13.0757
70. Cobos Jiménez V, Wit FW, Joerink M, Maurer I, Hamann AM, Schouten J, et al. T-Cell Activation Independently Associates With Immunosenescence in HIV-Infected Recipients of Long-Term Antiretroviral Treatment. *J Infect Dis* (2016) 214(2):216–25. doi: 10.1093/infdis/jiw146
71. Salzano FM, Sans M. Interethnic Admixture and the Evolution of Latin American Populations. *Genet Mol Biol* (2014) 37(1 Suppl):151–70. doi: 10.1590/s1415-47572014000200003
72. da Silva EM, Fernandes MR, de Carvalho DC, Lobato LPC, Cavalcante GC, Pereira EEB, et al. Effect of Genetic Ancestry to the Risk of Susceptibility to Gastric Cancer in a Mixed Population of the Brazilian Amazon. *BMC Res Notes* (2017) 10(1):646. doi: 10.1186/s13104-017-2963-4

73. Stephens HA. HIV-1 Diversity Versus HLA Class I Polymorphism. *Trends Immunol* (2005) 26(1):41–7. doi: 10.1016/j.it.2004.11.001
74. Kiepiela P, Leslie AJ, Honeyborne I, Ramduth D, Thobakgale C, Chetty S, et al. Dominant Influence of HLA-B in Mediating the Potential Co-Evolution of HIV and HLA. *Nature* (2004) 432(7018):769–75. doi: 10.1038/nature03113
75. Kloverpris HN, Karaca-Griffin S, Chopra A, Chetty S, Martinez-Picado J, Prado J, et al. HIV Control Mediated by HLA-B*27 Requires Dual Recognition of Conserved Gag Residues. *J Virol* (2012) 86(15):7958–68. doi: 10.1128/JVI.00188-12
76. Kloverpris HN, Leslie A, Goulder P. Role of Human Leukocyte Antigen Class I Subtype Associations With HIV Disease Progression in Early and Chronic Infection. *Immunol Rev* (2010) 239(1):17–30. doi: 10.1111/j.1600-065X.2010.00972.x
77. Kloverpris HN, Stryhn A, Harndahl M, Haahr S, Hviid A, Buus S, et al. Human Leukocyte Antigen-B Signal Peptide Binding Groups Predict Progression Rate in Early HIV-1 Subtype A and C Infection. *AIDS* (2012) 26(8):933–43. doi: 10.1097/QAD.0b013e3283521d1e
78. Kloverpris HN, Karaca-Griffin S, Stryhn A, Harndahl M, Ruhwald M, Ostergaard L, et al. Protective HLA-B*27-Restricted CD8+ T-Cell Responses Against HIV-1 Gag Are Associated With Low Frequencies of Memory Precursors. *J Virol* (2012) 86(15):7969–77. doi: 10.1128/JVI.00189-12
79. Kloverpris HN, Payne RP, Ladell K, Stryhn A, Harndahl M, Ruhwald M, et al. Antigen Presentation Level Influences CD8+ T Cell Differentiation and Bias in Human Immunodeficiency Virus Type 1 Infection. *J Virol* (2014) 88(15):8344–54. doi: 10.1128/JVI.00722-14
80. Kloverpris HN, Payne RP, Ladell K, Stryhn A, Harndahl M, Ruhwald M, et al. Antigen Presentation Level Influences CD8+ T Cell Differentiation and Bias in Human Immunodeficiency Virus Type 1 Infection. *J Virol* (2014) 88(15):8344–54. doi: 10.1128/JVI.00722-14
81. Kloverpris HN, Payne RP, Ladell K, Stryhn A, Harndahl M, Ruhwald M, et al. Antigen Presentation Level Influences CD8+ T Cell Differentiation and Bias in Human Immunodeficiency Virus Type 1 Infection. *J Virol* (2014) 88(15):8344–54. doi: 10.1128/JVI.00722-14
82. Kloverpris HN, Payne RP, Ladell K, Stryhn A, Harndahl M, Ruhwald M, et al. Antigen Presentation Level Influences CD8+ T Cell Differentiation and Bias in Human Immunodeficiency Virus Type 1 Infection. *J Virol* (2014) 88(15):8344–54. doi: 10.1128/JVI.00722-14
83. Kloverpris HN, Payne RP, Ladell K, Stryhn A, Harndahl M, Ruhwald M, et al. Antigen Presentation Level Influences CD8+ T Cell Differentiation and Bias in Human Immunodeficiency Virus Type 1 Infection. *J Virol* (2014) 88(15):8344–54. doi: 10.1128/JVI.00722-14
84. Kloverpris HN, Payne RP, Ladell K, Stryhn A, Harndahl M, Ruhwald M, et al. Antigen Presentation Level Influences CD8+ T Cell Differentiation and Bias in Human Immunodeficiency Virus Type 1 Infection. *J Virol* (2014) 88(15):8344–54. doi: 10.1128/JVI.00722-14
85. Kloverpris HN, Payne RP, Ladell K, Stryhn A, Harndahl M, Ruhwald M, et al. Antigen Presentation Level Influences CD8+ T Cell Differentiation and Bias in Human Immunodeficiency Virus Type 1 Infection. *J Virol* (2014) 88(15):8344–54. doi: 10.1128/JVI.00722-14
86. Kloverpris HN, Payne RP, Ladell K, Stryhn A, Harndahl M, Ruhwald M, et al. Antigen Presentation Level Influences CD8+ T Cell Differentiation and Bias in Human Immunodeficiency Virus Type 1 Infection. *J Virol* (2014) 88(15):8344–54. doi: 10.1128/JVI.00722-14
87. Kloverpris HN, Payne RP, Ladell K, Stryhn A, Harndahl M, Ruhwald M, et al. Antigen Presentation Level Influences CD8+ T Cell Differentiation and Bias in Human Immunodeficiency Virus Type 1 Infection. *J Virol* (2014) 88(15):8344–54. doi: 10.1128/JVI.00722-14
88. Kloverpris HN, Payne RP, Ladell K, Stryhn A, Harndahl M, Ruhwald M, et al. Antigen Presentation Level Influences CD8+ T Cell Differentiation and Bias in Human Immunodeficiency Virus Type 1 Infection. *J Virol* (2014) 88(15):8344–54. doi: 10.1128/JVI.00722-14
89. Kloverpris HN, Payne RP, Ladell K, Stryhn A, Harndahl M, Ruhwald M, et al. Antigen Presentation Level Influences CD8+ T Cell Differentiation and Bias in Human Immunodeficiency Virus Type 1 Infection. *J Virol* (2014) 88(15):8344–54. doi: 10.1128/JVI.00722-14
90. Kloverpris HN, Payne RP, Ladell K, Stryhn A, Harndahl M, Ruhwald M, et al. Antigen Presentation Level Influences CD8+ T Cell Differentiation and Bias in Human Immunodeficiency Virus Type 1 Infection. *J Virol* (2014) 88(15):8344–54. doi: 10.1128/JVI.00722-14
91. Kloverpris HN, Payne RP, Ladell K, Stryhn A, Harndahl M, Ruhwald M, et al. Antigen Presentation Level Influences CD8+ T Cell Differentiation and Bias in Human Immunodeficiency Virus Type 1 Infection. *J Virol* (2014) 88(15):8344–54. doi: 10.1128/JVI.00722-14
92. Kloverpris HN, Payne RP, Ladell K, Stryhn A, Harndahl M, Ruhwald M, et al. Antigen Presentation Level Influences CD8+ T Cell Differentiation and Bias in Human Immunodeficiency Virus Type 1 Infection. *J Virol* (2014) 88(15):8344–54. doi: 10.1128/JVI.00722-14
93. Kloverpris HN, Payne RP, Ladell K, Stryhn A, Harndahl M, Ruhwald M, et al. Antigen Presentation Level Influences CD8+ T Cell Differentiation and Bias in Human Immunodeficiency Virus Type 1 Infection. *J Virol* (2014) 88(15):8344–54. doi: 10.1128/JVI.00722-14
94. Kloverpris HN, Payne RP, Ladell K, Stryhn A, Harndahl M, Ruhwald M, et al. Antigen Presentation Level Influences CD8+ T Cell Differentiation and Bias in Human Immunodeficiency Virus Type 1 Infection. *J Virol* (2014) 88(15):8344–54. doi: 10.1128/JVI.00722-14
95. Kloverpris HN, Payne RP, Ladell K, Stryhn A, Harndahl M, Ruhwald M, et al. Antigen Presentation Level Influences CD8+ T Cell Differentiation and Bias in Human Immunodeficiency Virus Type 1 Infection. *J Virol* (2014) 88(15):8344–54. doi: 10.1128/JVI.00722-14
96. Kloverpris HN, Payne RP, Ladell K, Stryhn A, Harndahl M, Ruhwald M, et al. Antigen Presentation Level Influences CD8+ T Cell Differentiation and Bias in Human Immunodeficiency Virus Type 1 Infection. *J Virol* (2014) 88(15):8344–54. doi: 10.1128/JVI.00722-14
97. Kloverpris HN, Payne RP, Ladell K, Stryhn A, Harndahl M, Ruhwald M, et al. Antigen Presentation Level Influences CD8+ T Cell Differentiation and Bias in Human Immunodeficiency Virus Type 1 Infection. *J Virol* (2014) 88(15):8344–54. doi: 10.1128/JVI.00722-14
98. Kloverpris HN, Payne RP, Ladell K, Stryhn A, Harndahl M, Ruhwald M, et al. Antigen Presentation Level Influences CD8+ T Cell Differentiation and Bias in Human Immunodeficiency Virus Type 1 Infection. *J Virol* (2014) 88(15):8344–54. doi: 10.1128/JVI.00722-14
99. Kloverpris HN, Payne RP, Ladell K, Stryhn A, Harndahl M, Ruhwald M, et al. Antigen Presentation Level Influences CD8+ T Cell Differentiation and Bias in Human Immunodeficiency Virus Type 1 Infection. *J Virol* (2014) 88(15):8344–54. doi: 10.1128/JVI.00722-14
100. Kloverpris HN, Payne RP, Ladell K, Stryhn A, Harndahl M, Ruhwald M, et al. Antigen Presentation Level Influences CD8+ T Cell Differentiation and Bias in Human Immunodeficiency Virus Type 1 Infection. *J Virol* (2014) 88(15):8344–54. doi: 10.1128/JVI.00722-14

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Pereira, Franca, Costa, Jorge, Matias, Pinto, Ramos, Monteiro, Macedo, Sousa, Santos, Freitas, Costa and Valdeiosta. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

2.4 QUARTO MANUSCRITO

Título: DRB1 locus alleles of HLA class II are associated with modulation of the immune response in different phases of epstein-barr virus infection

Lista de autores: Leonn Mendes Soares Pereira, Eliane dos Santos França, Iran Barros Costa, Erika Vanessa Oliveira Jorge; Patrícia Jeanne de Souza Mendonça Mattos; Igor Tenório Lima, Amaury Bentes Cunha Freire, Francisco Lúzio de Paula Ramos, Talita Antonia Furtado Monteiro, Olinda Macedo, Rita Catarina Medeiros Sousa, Felipe Bonfim Freitas, Igor Brasil Costa & Antonio Carlos Rosário Vallinoto.

Status da publicação: Não submetido.

Nome da revista: Molecular Medicine.

ISSN: 1528-3658

Fator de impacto: 6.354 (Qualis A1)

DRB1 LOCUS ALLELES OF HLA CLASS II ARE ASSOCIATED WITH MODULATION OF THE IMMUNE RESPONSE IN DIFFERENT PHASES OF EPSTEIN-BARR VIRUS INFECTION

Leonn Mendes Soares Pereira[1]; Eliane dos Santos França[2]; Iran Barros Costa[2]; Igor Tenório Lima[2]; Erika Vanessa Oliveira Jorge[3]; Patrícia Jeanne de Souza Mendonça Mattos[3]; Amaury Bentes Cunha Freire[4]; Francisco Lúzio de Paula Ramos[4]; Talita Antonia Furtado Monteiro[2]; Olinda Macedo[5]; Rita Catarina Medeiros Sousa[2,6]; Felipe Bonfim Freitas[5]; Igor Brasil Costa[2,7†;] Antonio Carlos Rosário Vallinoto[1,7†*].

1. Virology Laboratory; Institute of Biological Sciences; Federal University of Pará, Belém, Pará, Brazil.
2. Epstein-Barr Virus Laboratory; Virology Unit; Evandro Chagas Institute, Ananindeua, Pará, Brazil.
3. Department of Immunogenetics; Hemotherapy and Hematology Foundation of the State of Pará.
4. Epidemiology and Surveillance Service; Evandro Chagas Institute, Ananindeua, Pará, Brazil.
5. Retrovirus Laboratory; Virology Unit; Evandro Chagas Institute, Ananindeua, Pará, Brazil.
6. School of Medicine; Federal University of Pará, Belém, Pará, Brazil.
7. Laboratory of Human and Medical Genetics; Institute of Biological Sciences; Federal University of Pará, Belém, Pará, Brazil.

† - These authors contributed equally to this study and share co-senior authorship.

*. Corresponding author: vallinoto@ufpa.br

Abstract

Background: Epstein–Barr virus (EBV) infection involves different clinical phases that have distinct immunological and virological characteristics. Studies suggest that alleles of human leukocyte antigen (HLA) may be associated with infection profiles, mainly for class I loci. In the present study, we evaluated the frequency of alleles of loci A, B and DRB1 of HLA classes I and II in different serological profiles of EBV infection.

Methods: We recruited 19 patients with primary infection (IgM+/IgG- anti-viral capsid antigen [anti-VCA]), 91 in serological transition (IgM+/IgG+ anti-VCA) and 470 with past infection (IgM-/IgG+ anti-VCA). Sociodemographic and clinical data were extracted from

questionnaires and medical reports. EBV viral load was quantified by real-time PCR, and viral genotyping was performed by Polymerase Chain Reaction (PCR) using described primers. T lymphocyte quantification and cytokine level analysis were performed by flow cytometry and HLA locus genotyping by medium-resolution PCR-SSO.

Results: The three serological groups differed in terms of EBV viral load and CD4(+) and CD8(+) T lymphocyte counts. All patients with primary infection had a history of coinfection. The DRB1*09 allele was associated with primary infection, and carriers of the allele showed changes in EBV viral load, in CD8(+) and double-positive T lymphocyte counts, and in IL-4 and TNF levels . This allele was also frequent in HIV-coinfected individuals, and was related to HIV viral load and CD8(+) T lymphocyte count. The DRB1*03 allele correlated with serological transition, and carriers exhibited EBV viral load, CD4(+), T CD8(+) and double-negative lymphocyte count, and IL-4 and IL-6 level changes. Past infection was related to the DRB1*16 allele, with carriers displaying IgG level, CD4(+) T lymphocyte count, and suggestively, CD8(+) T count alterations. In EBV genotyping, DRB1*16 allele was more frequent among nonamplified samples.

Conclusions: Our results suggest that HLA class II alleles are associated with modulation of the immune response in different profiles of EBV infection.

Key words: EBV, infection profile, HLA class II, DRB1 locus.

Introduction

In the natural history of Epstein–Barr virus (EBV) infection, primary infection is predominantly asymptomatic and silent when acquired in childhood. However, EBV infection in adolescents and adults can result in infectious mononucleosis, which is characterized by significant clinical variation and can progress to atypical manifestations in approximately 15% of young adults (Son e Shin, 2011; Bolis et al., 2016). Although the prevalence of primary infection varies between early childhood and early adulthood, it is assumed that on a global scale, approximately 90% of the population will come into contact with the virus before the age of 30 years old (Dunmire et al., 2018; Dunmire et al., 2015).

Serum levels of IgM anti-viral capsid antigen (VCA) antibody reach their serological peak within the first 5 days of disease onset, a period in which there is an increase in clinical severity and EBV viral load in the oral mucosa and peripheral blood. Seroconversion to IgG anti-VCA antibodies occurs later while clinical symptoms are still present and tends to remain

high and persistent even with a decrease in viral load in the bloodstream (Paschale et al., 2012; Grimm et al., 2016).

After the end of the active phase of EBV infection, the latent phase begins, in which the viral DNA remains a closed circular plasmid in the memory B lymphocytes of the host. Coordinated expression of EBV proteins stimulates different patterns of latency that can either induce viral persistence without causing cellular transformation or favor specific lymphoproliferative disorders (Münz, 2019; Kerr, 2019).

It has been argued that a hereditary basis for risk of EBV infection is as relevant as other known risk factors (Balfour et al., 2013). For human leukocyte antigen (HLA), most of the associations observed are related to class I loci.

The genetic profile *A* and *B* loci of class I have been associated with a polyclonal T lymphocyte response in primary EBV infection, and the specificity of the response can differ slightly even between similar allelic groups (Khanna et al., 1997). In an ex vivo study, maintenance of a balanced anti-EBV response was associated with the *HLA-B*08* allele, with restriction to the early BZLF1 protein (Silins et al., 1997); however, the restricted response of the *HLA-A*02:01* allele to epitopes of lytic phase proteins appears to be unstable and associated with the expression of cell death markers (Sauce et al., 2009). Other studies have shown that microsatellite markers *D6S510* and *D6S265* and polymorphisms *rs2530388* and *rs6457110* in *HLA* class I loci are associated with changes in viral load and immune cell counts in patients with mononucleosis (McAulay et al., 2007). The strict relationship between EBV and *HLA* class I is proposed to be due to blockage of the synthesis of the TAP transporter by the viral gene BNLF2a, leading to interference of antigen presentation (Horst et al., 2009).

Regarding *HLA* class II, one study showed that the viral protein EBNA2 downregulates HLA expression through a CIITA transcription factor-dependent pathway (Su et al., 2021).

Taken together, the aforementioned findings show that *HLA* variability is associated with the elaboration of relevant immune responses that indicate specific outcomes in EBV infection. In the present study, we evaluated the allelic frequency of the *A, B* and *DRB1* loci of *HLA* classes I and II and their association with immunological and viral biomarkers in patients with primary EBV infection, in those during serological class transition (interprofiles) and in those with past infection. Our objective was to identify immunogenetic markers associated with the complex natural history of EBV.

Materials and methods

Sampling and ethical aspects

This was a descriptive, cross-sectional and analytical study. We recruited patients from the Setor de Atendimento Médico Unificado do Instituto Evandro Chagas (SOAMU-IEC) and the Centro de Atenção à Saúde nas Doenças Infecciosas Adquiridas (CASA DIA) and from among voluntary blood donors at the Fundação Centro de Hematologia e Hemoterapia do estado do Pará (HEMOPA). From January 2018 to January 2020, peripheral blood samples were selected weekly.

A total of 580 participants were included, of whom 19 had serology consistent with primary EBV infection (P.I.) (anti-VCA IgM (+), anti-VCA IgG (-)), 91 had a class serological transition profile (S.T.) (anti-VCA IgM (+), anti-VCA IgG (+)) and 470 had serology consistent with past EBV infection (Past I.) (anti-VCA IgM (-), anti-VCA IgG(+)).

In compliance with resolutions 466/2012 and 347/05 of the National Health Council, which addresses guidelines and regulatory standards for research involving humans, the project was submitted for ethical consideration and approved by the Ethics Committee in Research with Human Beings of the IEC (Protocol: 3.121.265; CAAE: 73927717.3.0000.0019). All participants were informed about the research objectives, and those who agreed signed a consent form. Individuals under 18 years of age or who were using any specific therapy (antivirals, antiretrovirals and immunosuppressants) were excluded.

Sociodemographic and clinical data

Sociodemographic and clinical data related to symptoms and history of infections were obtained through a questionnaire specific to the project applied during participant interviews and from medical record databases accessed through authorization protocoled by the institutions where the study was carried out. All access and disclosures of the participants' data were included in the ethical opinion of the study.

Screening and viral load

EBV infection was screened by semiquantitative detection of anti-VCA IgM and IgG class antibodies by enzyme immunoassay (Dia. Pro Diagnostic Bioprobes EBV VCA, Italy). Identification of EBV genotypes was performed by nested PCR targeting the EBNA-3C gene using primers described by Lorenzetti et al., 2010 (1° round) (F: 5'-AGATGGTGAGCCTGACGTG-3'/R: 5'-GCATCCTTCAAAACCTCAGC-3') and Sample et al., 1990 (2° round) (F: 5'-AGAAGGGGAGCGGTGTGTTGT-3'/R: 5'-GGCTGTTTTTGACGTCGGC-3') following appropriate recommendations. The reaction mixture and program were as follows: 10 pmol/µL of primers, MgCl2 (50 mM), dNTP (10

mM), and Taq (5 U/µL); cycling 1° round- 1 cycle of 95° C/3', 20 cycles of (94°C/45"; 56°C/45"; 72°C/45"), and 1 cycle of 72°C/7'; cycling 2° round- 1 cycle of 95° C/3'; 35 cycles of (94°C/45"; 56°C/45"; 72°C/45"); and 1 cycle of 72°C/7'. The presence of a 153-bp fragment was considered positive for EBV-1; a 246-bp fragment was considered positive for EBV-2.

To quantify the EBV viral load, we used blood plasma samples from patients with positive serology for anti-VCA IgM in a real-time PCR estimation matrix following the protocol of the XGEN MASTER EBV kit (Mobius Life Science, Pinhais, PR, Brazil).

Cytokine level and cell quantification

Plasma concentrations of the cytokines IL-17A, IFN-ɣ, TNF, IL-10, IL-6, IL-4 and IL-2 were determined using a Cytometric Bead Array (CBA) using BD FACSCanto™ II and BD™CBA Human Th1/Th2/Th17 Cytokine kits (BD Biosciences, San Jose, CA, USA).

Quantification of T CD4(+), T CD8(+), T CD4(+)CD8(+) (double-positive) and T CD4(-)CD8(-) (double-negative) lymphocytes was performed by immunophenotyping and flow cytometry using BD FACSCalibur-4 colors and monitoring kits FACSCount™ Reagents and TriTEST™/TruCount (BD Biosciences, San Jose, CA, USA).

DNA extraction and genotyping of the HLA locus

Peripheral blood samples were collected from the participants, in which, DNA was extracted using a QiaAmp DNA Mini Kit (Qiagen, Düsseldorf, Nordrhein-Westfalen, Germany) following the manufacturer's recommendations. The newly extracted DNA samples were quantified by spectrofluorimetry using the Qubit equipment (Invitrogen, USA), following the manufacturer's recommendations; and the degree of purity was evaluated using the NanoDrop™ 2000/2000c Spectrophotometers equipment (Waltham, Massachusetts, USA), in which the elution solution used in the extraction of genetic material was used as a reference standard, as described in recent studies (Pereira et al., 2022).

We standardized the following profiles as the ideal range for successful amplification: for loci A and DRB1, concentration between 10 to 15 ug/ml; for the B locus, concentrations between 10 to 12 ug/mL. The degree of purity for all loci was represented by the ratios 260/280: 1.8-2.0 and 260/230: 1.8-2.2. Samples outside the established standard were diluted in ultra pure distilled water free of DNase/RNase (Invitrogen, USA), when in concentrations above the expected. Samples with concentrations below the expected were re-extracted, those that remained with an inadequate profile were discarded from subsequent analyses, as described in recent studies (Pereira et al., 2022).

In this sense, of the 580 participants obtained, we amplify all samples to the A locus. For locus B, we amplify all samples from the group with primary infection, 64 samples from the serological transition group and 302 samples from the group with past infection. For the DRB1 locus, we amplify all samples from the group with primary infection, 90 samples from the serological transition group and 467 samples from the group with past infection.

Genotyping of *HLA* class I and II loci *A, B* and *DRB1* was performed using medium resolution PCR-SSO methodology (Polymerase Chain Reaction – Sequence Specific Oligonucleotide) with Luminex technology (Luminex Corporation, Austin, TX, USA) and the kit LABType® (One Lambda Inc., Canoga Park, CA, USA), as described in recent studies (Pereira et al., 2022).

Statistical analysis

We evaluated the arrangement of the groups in a scatter plot, as based on discriminant multivariate analysis of the variables viral load, cytokine level, cell quantification and semiquantitative antibody level. We applied principal component analysis to indicate which predictive variables were significant in discriminating the groups.

We calculated the Spearman coefficient through a matrix of general correlations between the immunological and virological variables in each of the three groups.

The frequency of HLA alleles was calculated by direct counting and compared between groups using the G test. For groups with significantly different allelic profiles, the chi-square residual test was applied to determine the probabilistic importance of each of the alleles, followed by the calculation of the odds ratio to determine the advantage or disadvantage of significant alleles.

We also applied the G test to compare sociodemographic and clinical data between the groups. To assess significance, the squared residual and logistic regression were calculated, with the EBV serological profile as the dependent variable and the analyzed data as independent variables.

For each *HLA* locus, the Hardy-Weinberg equilibrium was measured, selecting as an alternative hypothesis of interest an excess of heterozygotes in the groups; standard Markov chain parameters were adopted. Genotypic linkage disequilibrium between loci was determined by the log odds ratio, also adopting standard Markov parameters, as described in recent studies (Pereira et al., 2022). Allelic groupings Bw4 and Bw6 were determined according to the *HLA* nomenclature database (Robinson et al., 2015). These parameters were calculated using the software GENEPOP (Raymond e Rousset, 1995).

We applied the Mann–Whitney test in two-by-two comparisons of quantitative and semiquantitative data between the groups and between *HLA* alleles. We opted for nonparametric tests due to the degree of normality of the variables in question, which was estimated by the Lilliefors test.

We adopted a significance level (α) of 95%, considering the probability of significance (p) less than or equal to 0.05 as a criterion for rejecting the null hypotheses, for the statistical analyses. The analyses were carried out using GraphPad Prism 8.4.3 (San Diego, CA, USA) and Bioestat 5.3 (Ayres et al., 2008).

Results

Characterization of the groups

With regard to overall prevalence, 3.28% of our subjects had primary EBV infection, 15.69% were in class transition, and 81.03% had past EBV infection (Figure 1A).

In multivariate analysis, we observed an overlap between the serological transition groups and the past EBV infection group, and the primary infection group tended to cluster in coordinates further away from the others (Figure 1B). Viral load and CD4(+) and CD8(+) T lymphocyte counts were the most relevant components, with an accumulated variance rate of 47.83% (Figure 1C); the primary infection group had a higher viral load [p< 0.00001 (P.I.= med: 15.33; IIQ (25%-75%): 6.50-78.50); (S.T.= med: 0; IIQ (25%-75%): 0-2.0)] (Figure 1D) and a lower CD4(+) T lymphocyte count [p: 0.0068 (P.I.= med: 319.50; IIQ (25%-75%): 140-443.25); (S.T.= med: 475; IIQ (25%-75%): 245-764); (Past I.= med: 525.50; IIQ (25%-75%): 280.75-938.25)] (Figure 1E), but higher CD8(+) T lymphocyte count than the other groups [p< 0.0001 (P.I.= med: 1024.50; IIQ (25%-75%): 886.25-2136.75); (S.T.= med: 1140; IIQ (25%-75%): 582-1735); (Past I.= med: 807; IIQ (25%-75%): 553-1235)] (Figure 1F).

Based on correlation matrices for the three EBV infection serological profiles (Figure 2), IgM dosage correlated negatively with viral load (p: 0.0334, r: -0.568) and positively with the CD4(+) T lymphocyte count (p: 0.0006, r: 0.7004), IL-6 level (p: 0.0457, r: 0.1767), TNF level (p: 0.0522, r: 0.2019) and IL-4 level (p: 0.0517, r: 0.1860) In the group with primary EBV infection (Figure 2A), viral load correlated negatively with the CD4(+) T lymphocyte count (p: 0.0526, r: -0.1874) and IL-6 level (p: 0.0525, r: -0.1915) but positively with the IL-4 level (p: 0.0427, r: 0.6976). In addition, the CD4(+) T lymphocyte count correlated positively with IL-17A (p: 0.0494, r: 0.1250) and TNF (p: 0.0513, r: 0.1367) levels. The CD8(+) T lymphocyte count correlated negatively with the CD4(+) T lymphocyte count (p: 0.0497, r: -0.1612) and IL-4 level (p: 0.0475, r: -0.1748) and positively with the double-negative T lymphocyte count

(p: 0.0002, r: 0.7431). The double-positive T lymphocyte count also correlated positively with the IL-17A level (p: 0.0016, r: 0.6584), and cytokine levels correlated positively with each other (r: 0.1051-0.8297).

In the serological transition group (Figure 2 B), viral load correlated negatively with the CD4(+) T lymphocyte count (p: 0.0569, r: -0.1791) and positively with the IL-6 level (p: 0.0017, r: 0.3312). The CD4(+) T lymphocyte count correlated negatively with the IL-6 level (p: 0.0021, r: -0.6256), though the double-positive T lymphocyte count correlated positively with the CD4(+) T lymphocyte count (p< 0.0001, r: 0.6521) and negatively with the IL-6 level (p: 0.0177, r: -0.2536). Additionally, cytokine levels correlated positively (r: 0.4998-0.9343).

Regarding the group with past EBV infection (Figure 2C), the IgG level correlated negatively with CD4(+) T lymphocyte (p< 0.0001, r: -0.3256) and double-positive T lymphocyte (p: 0.0401, r: -0.1108) counts and positively with the CD8(+) T lymphocyte count (p: 0.0016, r: 0.1712). However, the CD4(+) T lymphocyte count correlated negatively with the IL-6 level (p: 0.0476, r: -0.1069) and positively with TNF (p: 0.0535, r: 0.1042) and IL-4 (p: 0.0510, r: 0.1053) levels and with double-negative T lymphocyte (p< 0.0001, r: 0.3555) and double-positive T lymphocyte (p< 0.0001, r: 0.4532) counts. The CD8(+) T lymphocyte count correlated positively with double-positive T lymphocyte (p: 0.0219, r: 0.1238) and double-negative T lymphocyte (p< 0.0001, r: 0.2279) count, and the double-negative T lymphocyte count correlated positively with IL-2 (p: 0.0368, r: 0.1128), TNF (p: 0.0041, r: 0.1554) and IL-4 (p: 0.0175, r: 0.1283) levels. As in the other groups, cytokine levels correlated positively with each other (r: 0.0913-0.8608).

Among sociodemographic characteristics, male sex, age between 18 and 28 years, completion of elementary school and family income between 1 and 3 salaries were most common. There were also higher rates of nonsmokers, those who consumed alcohol and those without a history of illicit drug use among the participants as well as heterosexuals and those with an active sexual life, with a steady partner, without relationships with sex professionals and who reported condom use in their relationships. Regarding stress and cancer history, individuals who had no sleeping difficulties but with variable rest times and without cancer diagnosis or family history were most common. All individuals in the group with primary EBV infection had a history of coinfection (100%; p: 0.004), with a prevalence of HIV (61.29%) and bacterial STDs (32.26%). Specifically, the absence of coinfection was considered a protective factor against primary EBV infection, being 8.5-fold more frequent in the serological transition and past EBV infection groups, though symptomatology varied between the groups. All these characteristics are provided in Table 1.

Frequency of *HLA class I* and *class II* alleles

For the A locus, the A*24 and A*02 alleles were most frequent in the three groups evaluated, with no significant differences (p: 0.7096). The frequency of heterozygosity for these loci was similar in all three groups, with no significant differences (p: 0.3122) (Supplementary data 1).

Regarding the B locus, the B*35 allele was most frequent, with no significant differences between the groups (p: 9098). The BW6 cluster was also the most frequent, with no significant differences (p: 0.9528). B locus heterozygosity was most frequent, with no significant differences (p: 0.9966) (Supplementary data 1).

The HLA-A and HLA-B loci were in linkage disequilibrium in the group with primary infection (Table 02). We organized the haplotype sequence according to frequency in the general population; and we did not observe significant differences between haplotypes between the groups studied (Supplementary data 2).

The DRB1*13 allele of the DRB1 locus was most frequent; however, we observed significant differences between the groups (p: 0.0477), whereby the DRB1*09 allele showed greater probabilistic relevance in the group with primary EBV infection. The DRB1*03 allele was more relevant in the serological transition group and the DRB1*16 allele in the group with past EBV infection (Figure 3A; Supplementary data 1). The rate of heterozygosity was high in the three groups, with no significant differences between them (p: 0.1817) (Supplementary data 1).

All loci were in Hardy-Weinberg equilibrium in all groups studied (Table 02).

The DRB1*09 allele was associated with primary EBV infection

The DRB1*09 allele was approximately 4-fold more frequent in the group with primary EBV infection than in the other groups. (p: 0.054, OR: 4.235, CI 95%: 1.21-14.8) (Figure 3B).

Although the anti-VCA IgM titer was not associated with DRB1*09 (Figure 4B), EBV viral load was higher in carriers of this allele [p: 0.0485; (*09= med: 47.33, IIQ (25%-75%): 40.33-80.67); (Others= med: 9.33, IIQ (25%-75%): 3.75-18.33)] (Figure 4A).

The CD8(+) T lymphocyte count was higher [p: 0.0206; (*09= med: 2844, IIQ (25%-75%): 2401.5-3054); (Others= med: 975.5, IIQ (25%-75%): 795.5-1179.5)] (Figure 5B), such as double positive T lymphocyte count [p: 0.0093; (*09= med: 27, IIQ (25%-75%): 21-29); (Others= med: 3.5, IIQ (25%-75%): 2.75-7)] in DRB1*09 allele carriers (Figure 5D).

Levels of TNF [p: 0.0161; (*09= med: 11.64, IIQ (25%-75%): 11.07-12.38); (Others= med: 9.52, IIQ (25%-75%): 8.72-10.24)] and IL-4 [p: 0.0464; (*09= med: 11.78, IIQ (25%-75%): 10.3-12.04); (Others= med: 8.52, IIQ (25%-75%): 6.67-8.85)] were also higher in DRB1*09 allele carriers (Figure 6C and D).

The DRB1*03 allele was associated with the serological transition profile

The DRB1*03 allele was approximately 2-fold more frequent in patients undergoing a serological transition (p: 0.001, OR: 2.1560, IC95%: 1.27-3.67) (Figure 3C).

Moreover, the EBV viral load was higher in DRB1*03 allele carriers [p: 0.0015; (*03= med: 20, IIQ (25%-75%): 0-52.98); (Others= med: 0, IIQ (25%-75%): 0-0)] (Figure 4A). In contrast, anti-VCA IgM and IgG titers were not associated with this allele (Figures 4B e C).

The CD4(+) T lymphocyte counts [p: 0.0112; (*03= med: 272, IIQ (25%-75%): 83.25-450.25); (Others= med: 477, IIQ (25%-75%): 223-743.5)] (Figure 5A) and double-negative [p: 0.0540; (*03= med: 50, IIQ (25%-75%): 39.25-90); (Others= med: 75, IIQ (25%-75%): 45.5-116.5)] (Figure 5C) were lower in carriers of the DRB1*03 allele, but the CD8(+) T lymphocyte count was higher [p: 0.0260; (*03= med: 1749, IIQ (25%-75%): 1426-2367.25); (Others= med: 1111, IIQ (25%-75%): 620-1687.5)] (Figure 5B).

Additionally, the IL-4 dosage levels was lower in carriers of this allele [p: 0.0478; (*03= med: 8.89, IIQ (25%-75%): 7.09-11.34); (Others= med: 10.10, IIQ (25%-75%): 8.45-14.1)] (Figure 6A), but the IL-6 level was higher [p: 0.0175; (*03= med: 14.96, IIQ (25%-75%): 11.22-26); (Others= med: 12.46, IIQ (25%-75%): 9.44-16.62)] (Figure 6B).

The DRB1*16 allele was associated with past EBV infection

In this study, the DRB1*16 allele was statistically associated with past EBV infection (p: 0.012; OR: 0.2724; CI 95%: 0.10-0.76) (Figure 3C), being up to 7-fold more frequent in this group.

Anti-VCA IgG titer was lower in DRB1*16 allele carriers [p: 0.0020; (*16= med: 5.41, IIQ (25%-75%): 3.76-7.63); (Others= med: 6.90, IIQ (25%-75%): 4.93-8.39)] (Figure 4C).

The CD4(+) T lymphocyte count was higher in carriers of this allele [p: 0.0116; (*16= med: 541, IIQ (25%-75%): 361-980); (Others= med: 480, IIQ (25%-75%): 250.75-891.25)] (Figure 5A). Counts of other cells were not associated with the allele; however, CD8(+) T lymphocytes displayed a tendency toward lower counts in carriers of the allele [p: 0.0602; (*16= med: 744, IIQ (25%-75%): 595-1106); (Others= med: 853, IIQ (25%-75%): 595-1273)] (Figure 5B).

A history of HIV coinfection was associated with the DRB1*09 allele in patients with primary EBV infection

As history of coinfection was the only factor associated with the serological profiles of EBV infection, with a higher frequency in the group with primary infection (Table 1), we subsequently evaluated whether this aspect was also associated with DRB1 locus alleles. We found that 17 (89.47%) individuals carrying the DRB1*09 allele had a history of coinfection and that only 2 (10.53%) carriers had no history, a difference that was statistically significant (p: 0.041). In addition, DRB1*03 and DRB1*16 allele frequencies were statistically similar between patients with and without a history of coinfection {[*03= (history: 66 (66.67%)); (no history: 33 (33.33%))]; [*16= (history: 41 (43.16%)); (no history: 54 (56.84%))]; [Others= (history: 126 (31.27%)); (no history: 277 (68.73%))]} (Figure 7A).

Among the 17 DRB1*09 allele carriers with a history of coinfection, 76.47% were at least coinfected with HIV (Figure 7B). We subsequently assessed whether the allele frequency among HIV/EBV-coinfected individuals varied between the different EBV infection serological profiles and found that DRB1*09 was statistically more frequent in coinfected individuals with primary EBV infection (Figure 7C).

Finally, we evaluated whether the DRB1*09 allele was also associated with markers of HIV infection. Overall, the log10 HIV viral load [p: 0.0476; (*09= med: 5.72, IIQ (25%-75%): 5.55-5.73) (Others= med: 4.45, IIQ (25%-75%): 3.85-5.33)] and CD8(+) T lymphocyte count [p: 0.0446; (*09= med: 2844, IIQ (25%-75%): 2401.5-3054); (Others= med: 1025.5, IIQ (25%-75%): 834.5-2136.75)] were higher in carriers (Figure 7D and F).

HIV viral load was associated with the serological profile and EBV viral load

Our next step was to assess whether HIV viral load was associated with serological profiles of EBV infection and viral load and found that the log10 HIV viral load was lower in patients with past EBV infection [p: 0.0060; (P. I.= med: 4.96, IIQ (25%-75%): 4.34-5.67); (S. T.= med: 5.06, IIQ (25%-75%): 4.49-5.52); (Past I.= med: 4.68, IIQ (25%-75%): 3.99-5.21)] (Figure 8A).

Furthermore, positive linear regression was inferred between viral load in patients with primary EBV infection (p: 0.0412; R2: 0.22). There was also a negative trend between viral loads in patients in serological transition, though without statistical significance (p: 0.1214; R2: 0.03) (Figure 8B).

Detection of EBV was associated with the serological profile and allelic profile

We searched for viral genotypes in 110 samples from the primary infection and serological transition groups. Of these, amplification occurred for only 23 samples (3.95%), of which 9 (7.96%) were EBV-1, 9 (7.96%) EBV-2 and 5 (4.42%) EBV-1/2. Viral genetic material was not amplified in the other 87 samples (79.65%) (Figure 9A). We did not investigate the group with past infection in this regard due to the biological characteristics of EBV in this infection profile (Thorley-Lawson et al., 2014).

In total, 18 amplified samples (78.26%) were from the primary infection group and 5 (21.74%) from the serological transition group (Figure 9B). Among the nonamplified samples, 2 (2.22%) were from the primary infection group and 85 (97.78%) the serological transition group (Figure 9C). This difference was statistically significant ($p< 0.0001$).

We evaluated whether detection of EBV genotype was also associated with DRB1 locus alleles. Among the amplified samples, 5 (41.67%) cases involved DRB1*03 allele carriers and 3 (25%) DRB1*09 allele carriers; 1 (8.33%) case involved a carrier of the DRB1*16 allele, and 3 (25%) cases involved other alleles. Among the unamplified samples, 16 (18.39%) cases involved DRB1*03 allele carriers, 1 (1.15%) a DRB1*09 allele carrier, and 4 (4.60%) DRB1*16 allele carries; 64 (75.86%) cases involved carriers of other alleles. These differences were statistically significant (p: 0.0049) (Figure 9D).

Discussion

There are still few epidemiological reports on EBV infection in the Brazilian Amazon region. In the present study, we report a primary infection prevalence of 3.26% in a population of young adults in a state in the region; 96.73% already had positive serology for anti-VCA IgG, but only 80.58% showed complete seroconversion indicative of past infection. These data are similar to recent reports of observations in different age groups in the same region, suggesting a trend toward an increase in the number of cases according to age, regardless of the current or previous clinical condition of the patients (Pereira et al., 2021; de Castro et al., 2022).

We stratified the population into three serological profiles, which were also immunologically distinguished in terms of EBV viral load and CD4(+) and CD8(+) T lymphocyte counts. In patients with primary infection, viral biosynthesis was more active and statistically productive, as we observed CD8(+) T lymphocyte count maintenance and CD4(+) T lymphocyte depletion. This seems to be in agreement with studies showing differential kinetics of T lymphocytes in primary EBV infection, in which the CD4(+) T lymphocyte response appears early, with a rapid decrease, though the CD8(+) T lymphocyte response tends

to be more robust and durable (Precopio et al., 2003; Piriou et al., 2006). This complex response reflects the immunodominance of the proinflammatory cytokine profile in primary infection (Wada et al.,2013); nevertheless, the presence of anti-inflammatory cytokines suggests immunomodulation of EBV (Jabs et al., 2000), which could also justify our findings regarding the correlation matrix between immunological factors and cytokines in different profiles.

In this context, we observed that the HLA class II DRB1*09 allele frequency was associated with primary EBV infection. In fact, the association of this allele with the incidence of infection and, mainly, autoimmune diseases in populations with different ethnic profiles has been discussed, and it has been suggested that the allele favors inflammation related to the establishment of the pathological condition (Du et al., 2007; Williams et al., 2008; Mackie et al., 2011; Tian et al., 2018; Zhang et al., 2019). Recently, a study carried out in Japanese individuals showed an association of the DRB1*09 allele with severe cases of COVID-19 (Anzurez et al., 2021), which, from an immunological point of view, is characterized by a systemic inflammatory state (Anka et al., 2020). Our results appear to be in agreement with this hypothesis, as carriers of the DRB1*09 allele had high CD8(+) and double-positive T lymphocyte counts and TNF levels, which may be indicative of an attempt to maintain a proinflammatory antiviral response to active EBV infection (Pereira et al., 2022), as represented by high viral load in carriers. On the other hand, high IL-4 levels in the same group may be associated with viral persistence (Malizia et al., 2009; Kis et al., 2011), which is also in line with our results of a positive correlation of cytokines with EBV viral load.

However, the negative correlation between EBV viral load and CD4(+) T lymphocyte count was a finding that conflicts with the literature. This suggested that the lymphopenia observed may, in part, not be specific to EBV infection (Sutkowski et al., 2001). Interestingly, all patients with primary infection had a history of coinfection, with HIV being the most frequent, and the HIV viral load was high and directly proportional to that of EBV. We consider that the presence of HIV might be closely related to changes in lymphocyte counts, as expected in a typical infection (Margolick et al., 2006), favoring expansion of EBV (Sánchez-Ponce et al., 2021). In fact, in a recent study involving patients with primary infection and a history of coinfection, we showed that in HIV/EBV coinfection, retroviral pathology benefits at the expense of host immune response maintenance (Pereira et al., 2022).

Here, we highlight that the DRB1*09 allele frequency is associated with HIV/EBV coinfection, specifically primary EBV infection, and both the HIV viral load and CD8(+) T lymphocyte count were elevated in these patients. Again, probable immunological modulation by this allele is related to an attempt to maintain the response against viral coinfection. Although

we did not assess the predominance of response specificity, a consistent response against HIV can trigger immune activation against EBV (Doisne et al., 2004) and may be different in terms of magnitude and polyfunctionality (Figueiredo et al., 2014). Regardless, there is a strict relationship between the viral load of each (Petrara et al., 2012).

Patients in serological transition were characterized by a low EBV viral load and maintenance of CD4(+) and CD8(+) T lymphocyte counts; cytokines correlated strongly with each other, but without a clear immunodominance profile. It is likely that this scenario reflects sustainable immunological control of the active phase of the infection and consequent management of the homeostatic balance of an expected response in the latent phase (Eligio et al., 2010). In this case, establishment of a viral memory pool with qualitatively different responses to lytic and latent virus antigens is also observed (Taylor et al., 2015).

In general, patients in serological transition coinfected with HIV maintained a high HIV viral load, even though there was no association with an EBV viral load. Hence, the attempt at immune reboot observed in the transition group, which is expected due to the characteristics of EBV in the latent phase (Ressing et al., 2008; Nagy et al., 2012), may assist in the response to HIV. The fact that the HIV viral load was lower in the group with past infection compared to the other groups may indicate that this process of immunological control occurs in the long term. Nonetheless, we cannot ignore the close relationship between these viruses in the stimulation of carcinogenic conditions (Shindiapina et al., 2020), which requires a detailed assessment of long-term aspects of the pathogenesis of coinfection. Most patients in the present study had no cancer diagnosis or family history of cancer.

The DRB1*03 allele was associated with serological transition; however, contrary to the immunological tendency observed in the group, carriers of this allele had a high viral load and a suggestive proinflammatory profile, as represented by an increase in CD8(+) T lymphocytes and IL-6 and low IL-4. Such immune modulation has been associated with the DRB1*03 allele in the context of autoimmune diseases and therapy-induced platelet disorders in different populations (Shankarkumar et al., 2003; Liphaus et al., 2007; Dittmar et al., 2008; van Gerven et al., 2015; Sayeh et al., 2017; Zhang et al., 2019). We propose that the persistence of viral activation specifically in carriers of the DRB1*03 allele can induce a more effective immune counterresponse for controlling the infection. A longitudinal study evaluating whether this immunological and virological profile of DRB1*03 allele carriers is maintained in the long term, even after complete seroconversion, would be enlightening, especially in cases of active chronic EBV infection (CAEBV) (Fujiwara et al., 2020).

Patients with past EBV infection had high counts of CD4(+) T lymphocytes but low counts of CD8(+) T lymphocytes. Nonetheless, a certain degree of immunological activity was observed, mainly by the correlation of CD4(+) and double-negative T lymphocytes with proinflammatory cytokines, which may be a reflection of the immune response to HIV infection that was present in approximately 53% of this group.

The DRB1*16 allele was associated with past infection, anti-VCA IgG titer regulation and a high CD4(+) T lymphocyte but suggestive low CD8(+) T count. Our findings appear to contradict studies that relate this allele to proinflammatory profiles in autoimmune diseases in different ethnic groups, some of which were acquired after viral infections (Sepúlveda-Delgado et al., 2018; Shu et al., 2019). However, we showed that DRB1*16 allele carriers tended to exhibit control of the immune response consistent with the profile of patients with past EBV infection.

We were unable to genotype most of the peripheral blood samples; approximately 2% and 98% were from patients with primary infection and those in serological transition, respectively. For samples that did produce an amplification product, approximately 78% and 22% were from patients with primary infection and those in serological transition, respectively. These findings were expected, as the virus spreads to the oropharyngeal mucosa and peripheral blood in primary infection, whereas the virus tends to remain in germinal centers and can be reactivated and recirculated under specific conditions during conversion to the latent phase (Thorley-Lawson et al., 2014). Nevertheless, our results suggest that DRB1 locus heterozygosity may be related to nonamplification of viral genetic material in blood samples.

Hypothetically, the results may be related to the heterozygote advantage initially suggested by Doherty and Zinkernagel, in which the presumed ability of heterozygous carriers to present a wide range of pathogen-derived peptides increases the probability of inducing a targeted response (Doherty and Zinkernagel, 1975). This might explain the low levels of EBV viral load in carriers of different alleles in both primary infection and serological transition phases.

Conclusions

We conclude that the DRB1*09, *03 and *16 alleles are associated with immunological modulation in different serological profiles of EBV infection in young adult patients from the Brazilian Amazon region. To the best of our knowledge, this is the first report that relates these alleles to viral infection, with most studies to date focusing on autoimmune diseases. As EBV

is one of the factors associated with autoimmunity, this interrelation should not be neglected (Houen et al., 2021).

List of abbreviations

CASA DIA: Centro de Atenção à Saúde nas Doenças Infecciosas Adquiridas; EBV: Epstein-Barr Virus; HEMOPA: Fundação Centro de Hematologia e Hemoterapia do estado do Pará; HIV: Human immunodeficiency virus; HLA: human leukocyte antigen; CI: confidence interval; P.I.: primary EBV infection; Past I.: past EBV infection; Med.: median; SOAMU-IEC: Setor de Atendimento Médico Unificado do Instituto Evandro Chagas; S.T.: serological transition; VCA: viral capsid antigen.

Ethical approval and consent

In compliance with resolutions 466/2012 and 347/05 of the National Health Council, which addresses the guidelines and regulatory standards for research involving humans, the project was submitted for ethical consideration and approved by the Ethics Committee in Research with Human Beings (CEP) of the IEC (Protocol: 3.121.265; CAAE: 73927717.3.0000.0019). All participants were informed about the research objectives, and those who agreed signed the consent form.

Data availability

Data supporting the results of this study may be obtained from the corresponding author, but restrictions apply to the availability of these data, which were used under license for the current study and are therefore not publicly available. However, the data are made available by the authors upon reasonable request and with the permission of the corresponding author.

Conflicts of interest

The authors declare that the research was carried out in the absence of any commercial or financial relationships that could be interpreted as a potential conflict of interest.

Author contributions

TAFM, OM, RCMS, FBF, IBC and ACRV conceived of the project. LMSP, ESF, ABCF and FLPR were responsible for collecting the samples at the reference centers. LMSP ESF, IBC and ITL developed the project's methodological activities; specifically, EVOJ and

PJSMM conducted the genotyping of the HLA loci. LMSP carried out the statistical analysis and wrote the article. EJMS, FBF, IBC and ACRV reviewed the article.

Financing

This study was funded by specific incentives from Secretaria de Vigilância em Saúde do Ministério da Saúde and from Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPQ (n° 301869/2017-0). We thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for granting the scholarship (process number: 88882.183970/2018-01).

Acknowledgments

We thank the entire technical and administrative staff of the participating institutions for their help in recruiting patients for the study, as well as all the participants for their trust and availability to participate in the study.

References

Anka AU, Tahir MI, Abubakar SD, Alsabbagh M, Zian Z, Hamedifar H, Sabzevari A, Azizi G. Coronavirus disease 2019 (COVID-19): An overview of the immunopathology, serological diagnosis and management. Scandinavian Journal of Immunology. 2021;93(4):e12998. doi: 10.1111/sji.12998.

Anzurez A, Naka I, Miki S, Nakayama-Hosoya K, Isshiki M, Watanabe Y, Nakamura-Hoshi M, Seki S, Matsumura T, Takano T, Onodera T, Adachi Y, Moriyama S, Terahara K, Tachikawa N, Yoshimura Y, Sasaki H, Horiuchi H, Miyata N, Miyazaki K, Koga M, Ikeuchi K, Nagai H, Saito M, Adachi E, Yotsuyanagi H, Kutsuna S, Kawashima A, Miyazato Y, Kinoshita N, Kouno C, Tanaka K, Takahashi Y, Suzuki T, Matano T, Ohashi J, Kawana-Tachikawa A. Association of HLA-DRB1*09:01 with severe COVID-19. HLA. 2021;98(1):37-42. doi: 10.1111/tan.14256.

Ayres M, Ayres JrM, Ayres DL, Santos AS. BioEstat 5.0: Aplicações Estatísticas nas Áreas de Ciências Biológicas e Médicas. Belém: Sociedade Civil Mamirauá; Brasília: CNPq, 2008.

Balfour HH Jr, Sifakis F, Sliman JA, Knight JA, Schmeling DO, Thomas W. Age-specific prevalence of Epstein-Barr virus infection among individuals aged 6-19 years in the United States and factors affecting its acquisition. J Infect Dis. 2013;208(8):1286-93. doi: 10.1093/infdis/jit321.

572 Bolis V, Karadedos C, Chiotis I, Chaliasos N, Tsabouri S. Atypical manifestations of Epstein-
573 Barr virus in children: a diagnostic challenge. Jornal de Pediatria (Rio J). 2016;92(2):113-
574 21. doi: 10.1016/j.jped.2015.06.007.
575 de Castro Alves CE, Pinheiro-Silva R, Silva MT, Galvão TF, de Melo Silva J, Moura Neto JP,
576 Pontes GS. Seroprevalence of Epstein-Barr virus and cytomegalovirus infections in
577 Presidente Figueiredo, Amazonas, Brazil. Journal of Immunoassay & Immunochemistry.
578 2022;43(1):67-77. doi: 10.1080/15321819.2021.1942043.
579 De Paschale M, Clerici P. Serological diagnosis of Epstein-Barr vírus infection: Problems and
580 solutions. De Paschale, M., Clerici, P. World Jornal of Virology; 2012;1(1):31-43.
581 doi:10.5501/wjv.v1.i1.31.
582 Dittmar M, Ide M, Wurm M, Kahaly GJ. Early onset of polyglandular failure is associated with
583 HLA-DRB1*03. Europena Journal of Endocrinology. 2008;159(1):55-60. doi:
584 10.1530/EJE-08-0082.
585 Doisne JM, Urrutia A, Lacabaratz-Porret C, Goujard C, Meyer L, Chaix ML, Sinet M, Venet
586 A. CD8+ T cells specific for EBV, cytomegalovirus, and influenza virus are activated
587 during primary HIV infection. Journal of Immunology. 2004;173(4):2410-8. doi:
588 10.4049/jimmunol.173.4.2410.
589 Doherty PC, Zinkernagel RM. Enhanced immunological surveillance in mice heterozygous at
590 the H-2 gene complex. Nature. 1975; 256 (5512): 50-2. doi: 10.1038/256050a0.
591 Du J, Liu J, Gu J, Zhu P. HLA-DRB1*09 is associated with increased incidence of
592 cytomegalovirus infection and disease after allogeneic hematopoietic stem cell
593 transplantation. Biol Blood Marrow Transplant. 2007;13(12):1417-21. doi:
594 10.1016/j.bbmt.2007.09.003.
595 Dunmire SK, Hogquist KA, Balfour HH. Infectious Mononucleosis. Dunmire, S. K., Hogquist,
596 K. A., & Balfour, H. H. (2015). Infectious Mononucleosis. Current topics in microbiology
597 and immunology, 390 (Pt 1): 211-240. doi:10.1007/978-3-319-22822-8_9
598 Dunmire SK, Verghese PS, Balfour HH Jr. Primary Epstein-Barr vírus infection. Journal of
599 Clinical Virology. 2018;102:84-92. doi: 10.1016/j.jcv.2018.03.001.
600 Eligio P, Delia R, Valeria G. EBV Chronic Infections. Mediterranean Journal of Hematology
601 Infectious Diseases. 2010;2(1):e2010022. doi: 10.4084/MJHID.2010.022.
602 Figueiredo S, Charmeteau B, Surenaud M, Salmon D, Launay O, Guillet JG, Hosmalin A,
603 Gahery H. Memory CD8(+) T cells elicited by HIV-1 lipopeptide vaccines display similar
604 phenotypic profiles but differences in term of magnitude and multifunctionality compared

with FLU- or EBV-specific memory T cells in humans. Vaccine. 2014 Jan 16;32(4):492-501. doi: 10.1016/j.vaccine.2013.11.052.

Fujiwara S, Nakamura H. Chronic Active Epstein-Barr Virus Infection: Is It Immunodeficiency, Malignancy, or Both? Cancers (Basel). 2020;12(11):3202. doi: 10.3390/cancers12113202.

Grimm JM, Schmeling DO, Dunmire SK, Knight JA, Mullan BD, Ed JA, Brundage RC, Hogquist KA, Balfour HH Jr. Prospective studies of infectious mononucleosis in university students. Grimm, J. M., Schmeling, D. O., Dunmire, S. K., Knight, J. A., Mullan, B. D., Ed, J. A., Brundage, R. C., Hogquist, K. A., & Balfour, H. H., Jr. Prospective studies of infectious mononucleosis in university students. Clinical & translational immunology, 5(8), e94. https://doi.org/10.1038/cti.2016.48. 2016;5(8):e94. doi: 10.1038/cti.2016.48.

Horst D, van Leeuwen D, Croft NP, Garstka MA, Hislop AD, Kremmer E, Rickinson AB, Wiertz EJ, Ressing ME. Specific targeting of the EBV lytic phase protein BNLF2a to the transporter associated with antigen processing results in impairment of HLA class I-restricted antigen presentation. J Immunol. 2009 Feb 15;182(4):2313-24. doi: 10.4049/jimmunol.0803218.

Houen G, Trier NH. Epstein-Barr Virus and Systemic Autoimmune Diseases. Front Immunol. 2021 Jan 7;11:587380. doi: 10.3389/fimmu.2020.587380.

Jabs WJ, Wagner HJ, Schlenke P, Kirchner H. The primary and memory immune response to Epstein-Barr virus infection in vitro is characterized by a divergent production of IL-1beta/IL-6 and IL-10. Scandinavian Journal of Immunology. 2000;52(3):304-8. doi: 10.1046/j.1365-3083.2000.00776.x.

Kerr JR. Epstein-Barr virus (EBV) reactivation and therapeutic inhibitors. J Clin Pathol. 2019;72(10):651-658. doi: 10.1136/jclinpath-2019-205822.

Khanna R, Burrows SR, Neisig A, Neefjes J, Moss DJ, Silins SL. Hierarchy of Epstein-Barr virus-specific cytotoxic T-cell responses in individuals carrying different subtypes of an HLA allele: implications for epitope-based antiviral vaccines. J Virol. 1997 Oct;71(10):7429-35. doi: 10.1128/JVI.71.10.7429-7435.1997. Erratum in: J Virol 1997 Dec;71(12):9859. PMID: 9311821; PMCID: PMC192088.

Kis LL, Gerasimcik N, Salamon D, Persson EK, Nagy N, Klein G, Severinson E, Klein E. STAT6 signaling pathway activated by the cytokines IL-4 and IL-13 induces expression of the Epstein-Barr virus-encoded protein LMP-1 in absence of EBNA-2: implications for the type II EBV latent gene expression in Hodgkin lymphoma. Blood. 2011 Jan 6;117(1):165-74. doi: 10.1182/blood-2010-01-265272.

Liphaus BL, Kiss MH, Goldberg AC. HLA-DRB1 alleles in juvenile-onset systemic lupus erythematosus: renal histologic class correlations. Brazilian Journal of Medical and Biological Research. 2007;40(4):591-7. doi: 10.1590/s0100-879x2007000400019.

Lorenzetti MA, Altcheh J, Moroni S, Moscatelli G, Chabay PA, Preciado MV. EBNA1 sequences in Argentinean pediatric acute and latent Epstein-Barr virus infection reflect circulation of novel South American variants. Journal of Medical Virology. 2010; 82 (10): 1730-8. doi: 10.1002/jmv.21871.

Mackie SL, Taylor JC, Martin SG; YEAR Consortium; UKRAG Consortium, Wordsworth P, Steer S, Wilson AG, Worthington J, Emery P, Barrett JH, Morgan AW. A spectrum of susceptibility to rheumatoid arthritis within HLA-DRB1: stratification by autoantibody status in a large UK population. Genes and Immunity. 2012;13(2):120-8. doi: 10.1038/gene.2011.60.

Malizia AP, Egan JJ, Doran PP. IL-4 increases CD21-dependent infection of pulmonary alveolar epithelial type II cells by EBV. Mol Immunol. 2009 May;46(8-9):1905-10. doi: 10.1016/j.molimm.2009.01.002.

Margolick JB, Gange SJ, Detels R, O'Gorman MR, Rinaldo CR Jr, Lai S. Impact of inversion of the CD4/CD8 ratio on the natural history of HIV-1 infection. Journal of Acquired Immune Deficiency Syndrome. 2006;42(5):620-6. doi: 10.1097/01.qai.0000223028.55080.9d.

McAulay KA, Higgins CD, Macsween KF, Lake A, Jarrett RF, Robertson FL, Williams H, Crawford DH. HLA class I polymorphisms are associated with development of infectious mononucleosis upon primary EBV infection. J Clin Invest. 2007 Oct;117(10):3042-8. doi: 10.1172/JCI32377.

Münz C. Latency and lytic replication in Epstein-Barr virus-associated oncogenesis. Nat Rev Microbiol. 2019 Nov;17(11):691-700. doi: 10.1038/s41579-019-0249-7.

Nagy N, Adori M, Rasul A, Heuts F, Salamon D, Ujvári D, Madapura HS, Leveau B, Klein G, Klein E. Soluble factors produced by activated CD4+ T cells modulate EBV latency. Proc Natl Acad Sci U S A. 2012 Jan 31;109(5):1512-7. doi: 10.1073/pnas.1120587109.

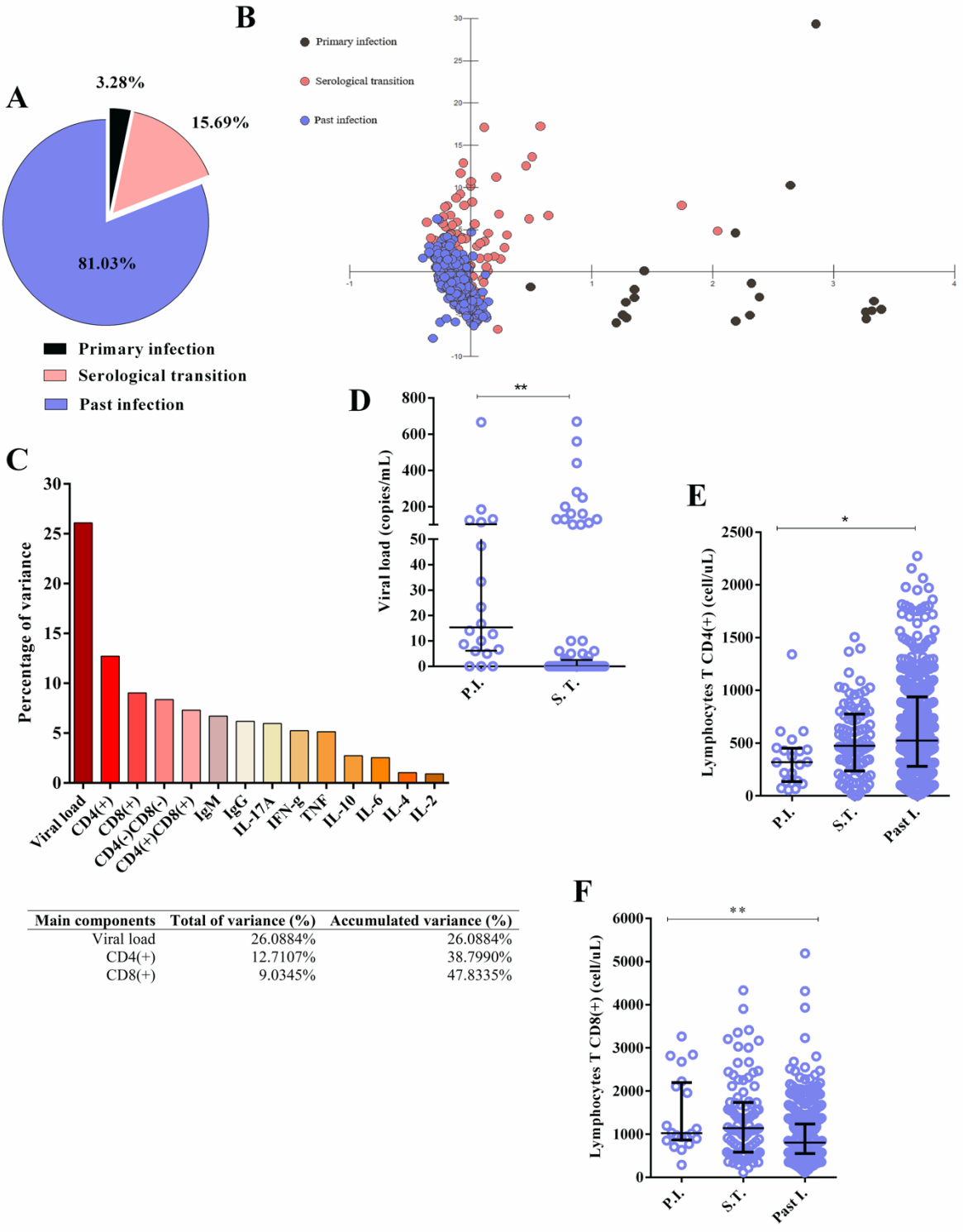
Pereira LMS, Dos Santos França E, Costa IB, Lima IT, Freire ABC, de Paula Ramos FL, Monteiro TAF, Macedo O, Sousa RCM, Freitas FB, Costa IB, Vallinoto ACR. Epidemiological risk factors associated with primary infection by Epstein-Barr virus in HIV-1-positive subjects in the Brazilian Amazon region. Scientific Reports. 2021;11(1):18476. doi: 10.1038/s41598-021-97707-4.

672 Pereira LMS, França EdS, Costa IB, Lima IT, Freire ABC, Ramos FLdP, Monteiro TAF,
673 Macedo O, Sousa RCM, Freitas FB, Brasil Costa I, Vallinoto ACR. Epstein–Barr Virus
674 (EBV) Genotypes Associated with the Immunopathological Profile of People Living with
675 HIV-1: Immunological Aspects of Primary EBV Infection. Viruses. 2022; 14(2):168.
676 https://doi.org/10.3390/v14020168.
677 Petrara MR, Cattelan AM, Zanchetta M, Sasset L, Freguja R, Gianesin K, Cecchetto MG,
678 Carmona F, De Rossi A. Epstein-Barr virus load and immune activation in human
679 immunodeficiency virus type 1-infected patients. J Clin Virol. 2012 Mar;53(3):195-200.
680 doi: 10.1016/j.jcv.2011.12.013.
681 Piriou ER, van Dort K, Weel JF, Bemelman FJ, Gamadia LE, van Oers MH, van Baarle D.
682 Detailed kinetics of EBV-specific CD4+ and CD8+ T cells during primary EBV infection
683 in a kidney transplant patient. Clinical Immunology. 2006;119(1):16-20. doi:
684 10.1016/j.clim.2005.11.009.
685 Precopio ML, Sullivan JL, Willard C, Somasundaran M, Luzuriaga K. Differential kinetics and
686 specificity of EBV-specific CD4+ and CD8+ T cells during primary infection. Journal of
687 Immunology. 2003;170(5):2590-8. doi: 10.4049/jimmunol.170.5.2590.
688 Raymond M, Rousset F, 1995. GENEPOP (versão 1.2): population genetics software for
689 accurate testing and ecumenism. J. Heredity, 86: 248-249.
690 Ressing ME, Horst D, Griffin BD, Tellam J, Zuo J, Khanna R, Rowe M, Wiertz EJ. Epstein-
691 Barr virus evasion of CD8(+) and CD4(+) T cell immunity via concerted actions of
692 multiple gene products. Semin Cancer Biol. 2008 Dec;18(6):397-408. doi:
693 10.1016/j.semcancer.2008.10.008.
694 Sample J, Young L, Martin B, Chatman T, Kieff E, Rickinson A, Kieff E. Epstein-Barr virus
695 types 1 and 2 differ in their EBNA-3A, EBNA-3B, and EBNA-3C genes. Journal of
696 Virology. 1990;64(9):4084-92. doi: 10.1128/JVI.64.9.4084-4092.1990.
697 Sánchez-Ponce Y, Fuentes-Pananá EM. The Role of Coinfections in the EBV-Host Broken
698 Equilibrium. Viruses. 2021;13(7):1399. doi: 10.3390/v13071399.
699 Sauce D, Larsen M, Abbott RJ, Hislop AD, Leese AM, Khan N, Papagno L, Freeman GJ,
700 Rickinson AB. Upregulation of interleukin 7 receptor alpha and programmed death 1 marks
701 an epitope-specific CD8+ T-cell response that disappears following primary Epstein-Barr
702 virus infection. J Virol. 2009 Sep;83(18):9068-78. doi: 10.1128/JVI.00141-09.
703 Sayeh A, Ben Cheikh C, Mardessi A, Mrad M, Nsiri B, Oumaya A, Fekih-Mrissa N. HLA
704 DRB1*03 as a possible common etiology of schizophrenia, Graves' disease, and type 2
705 diabetes. Annals of General Psychiatry. 2017;16:7. doi: 10.1186/s12991-017-0128-4.

706 Sepúlveda-Delgado J, Danis-Lozano R, Ocaa-Sibilla MJ, Ramirez-Valdespino JC, Cetina-Díaz
707 JH, Bulos-Rodriguez P, Hernández-Doo S, Ruiz-Gómez D, García R, Juárez-Nicolás F,
708 Tevera-Gamboa MG, Vera-Lastra OL, Jara LJ, Canseco-Avila LM, Dominguez-
709 Arrevillaga S, Trujillo-Murillo K, Julio Granados J. Role of HLA DRB1*15 and HLA
710 DRB1*16 alleles in the genetic susceptibility to develop systemic lupus erythematosus
711 (SLE) after Chikungunya and Zika viruses infection in México. Asia-Pacific Journal of
712 Blood Types and Genes. 2018; 2(4):233-236. doi:10.46701/APJBG.2018042018127.
713 Shankarkumar U, Ghosh K, Badakere SS, Mohanty D. HLA-DRB1*03 and DQB1*0302
714 associations in a subset of patients severely affected with systemic lupus erythematosus
715 from western India. Annals of the Rheumatic Disease. 2003;62(1):92-3. doi:
716 10.1136/ard.62.1.92.
717 Shindiapina P, Ahmed EH, Mozhenkova A, Abebe T, Baiocchi RA. Immunology of EBV-
718 Related Lymphoproliferative Disease in HIV-Positive Individuals. Front Oncol. 2020 Sep
719 30;10:1723. doi: 10.3389/fonc.2020.01723.
720 Shu Y, Qiu W, Zheng J, Sun X, Yin J, Yang X, Yue X, Chen C, Deng Z, Li S, Yang Y, Peng
721 F, Lu Z, Hu X, Petersen F, Yu X. HLA class II allele DRB1*16:02 is associated with anti-
722 NMDAR encephalitis. J Neurol Neurosurg Psychiatry. 2019 Jun;90(6):652-658. doi:
723 10.1136/jnnp-2018-319714.
724 Silins SL, Cross SM, Elliott SL, Pye SJ, Burrows JM, Moss DJ, Misko IS. Selection of a diverse
725 TCR repertoire in response to an Epstein-Barr virus-encoded transactivator protein BZLF1
726 by CD8+ cytotoxic T lymphocytes during primary and persistent infection. Int Immunol.
727 1997 Nov;9(11):1745-55. doi: 10.1093/intimm/9.11.1745.
728 Son KH, Shin MY. Clinical features of Epstein-Barr virus-associated infectious mononucleosis
729 in hospitalized Korean children. Korean Journal of Pediatrics. 2011;54(10):409-13. doi:
730 10.3345/kjp.2011.54.10.409.
731 Strang G, Rickinson AB. In vitro expansion of Epstein-Barr virus-specific HLA-restricted
732 cytotoxic T cells direct from the blood of infectious mononucleosis patients. Immunology.
733 1987;62(4):647-54. PMID: 2828228; PMCID: PMC1454146.
734 Su C, Lu F, Soldan SS, Lamontagne RJ, Tang HY, Napoletani G, Farrell PJ, Tempera I,
735 Kossenkov AV, Lieberman PM. EBNA2 driven enhancer switching at the CIITA-DEXI
736 locus suppresses HLA class II gene expression during EBV infection of B-lymphocytes.
737 PLoS Pathog. 2021;17(8):e1009834. doi: 10.1371/journal.ppat.1009834.

738 Sutkowski N, Conrad B, Thorley-Lawson DA, Huber BT. Epstein-Barr virus transactivates the
739 human endogenous retrovirus HERV-K18 that encodes a superantigen. Immunity.
740 2001;15(4):579-89. doi: 10.1016/s1074-7613(01)00210-2.
741 Taylor GS, Long HM, Brooks JM, Rickinson AB, Hislop AD. The immunology of Epstein-
742 Barr virus-induced disease. Annual Review of Immunology. 2015;33:787-821. doi:
743 10.1146/annurev-immunol-032414-112326.
744 Thorley-Lawson DA, Hawkins JB, Tracy SI, Shapiro M. The pathogenesis of Epstein-Barr
745 virus persistent infection. Current Opinion in Virology. 2013 Jun;3(3):227-32. doi:
746 10.1016/j.coviro.2013.04.005.
747 Tian L, Hou L, Wang L, Xu H, Xiao J, Ying B. HLA-DRB1*09:01 allele is associated with
748 anti-E immunization in a Chinese population. Transfusion. 2018;58(6):1536-1539. doi:
749 10.1111/trf.14568.
750 van Gerven NM, de Boer YS, Zwiers A, Verwer BJ, Drenth JP, van Hoek B, van Erpecum KJ,
751 Beuers U, van Buuren HR, den Ouden JW, Verdonk RC, Koek GH, Brouwer JT,
752 Guichelaar MM, Vrolijk JM, Coenraad MJ, Kraal G, Mulder CJ, van Nieuwkerk CM,
753 Bloemena E, Verspaget HW, Kumar V, Zhernakova A, Wijmenga C, Franke L, Bouma G;
754 Dutch Autoimmune Hepatitis Study Group. HLA-DRB1*03:01 and HLA-DRB1*04:01
755 modify the presentation and outcome in autoimmune hepatitis type-1. Genes and
756 Immunity. 2015;16(4):247-52. doi: 10.1038/gene.2014.82.
757 Wada T, Muraoka M, Yokoyama T, Toma T, Kanegane H, Yachie A. Cytokine profiles in
758 children with primary Epstein-Barr virus infection. Pediatric Blood & Cancer.
759 2013;60(7):E46-8. doi: 10.1002/pbc.24480.
760 Williams AJ, Aitken RJ, Chandler MA, Gillespie KM, Lampasona V, Bingley PJ.
761 Autoantibodies to islet antigen-2 are associated with HLA-DRB1*07 and DRB1*09
762 haplotypes as well as DRB1*04 at onset of type 1 diabetes: the possible role of HLA-DQA
763 in autoimmunity to IA-2. Diabetologia. 2008;51(8):1444-8. doi: 10.1007/s00125-008-
764 1047-3.
765 Zhang M, Lin S, Yuan X, Lin Z, Huang Z. HLA-DQB1 and HLA-DRB1 Variants Confer
766 Susceptibility to Latent Autoimmune Diabetes in Adults: Relative Predispositional Effects
767 among Allele Groups. Genes (Basel). 2019;10(9):710. doi: 10.3390/genes10090710.
768 Zhang R, Duffy BF, Lange V, Eby CS, Liu C. Association between the HLA-DRB1*03:01-
769 DQB1*02:01 haplotype and PF4/heparin antibodies. Blood Advances. 2019;3(20):3136-
770 3142. doi: 10.1182/bloodadvances.2019000311.
771

Figures



Main components	Total of variance (%)	Accumulated variance (%)
Viral load	26.0884%	26.0884%
CD4(+)	12.7107%	38.7990%
CD8(+)	9.0345%	47.8335%

Figure 1 - Immunological characterization of the studied groups. (A) General frequency of those with active infection, serological transition and past infection by EBV. (B) Discriminant scatter plot of the groups based on immunological and virological aspects. Patients in serological transition and with past infection tended to overlap; patients with primary infection grouped the part. (C) Principal component plot showing the relative significance of predictive immunological and virological variables. Viral load and CD4(+) and CD8(+) T lymphocyte counts were factors with the highest variance. (D-F) Dot plot graphs showing differences in viral load quantification (D), CD4(+) T lymphocyte count (E) and CD8(+) T lymphocyte count (F) between the groups. *= p: 0.005-0.0001; **= p< 0.0001.

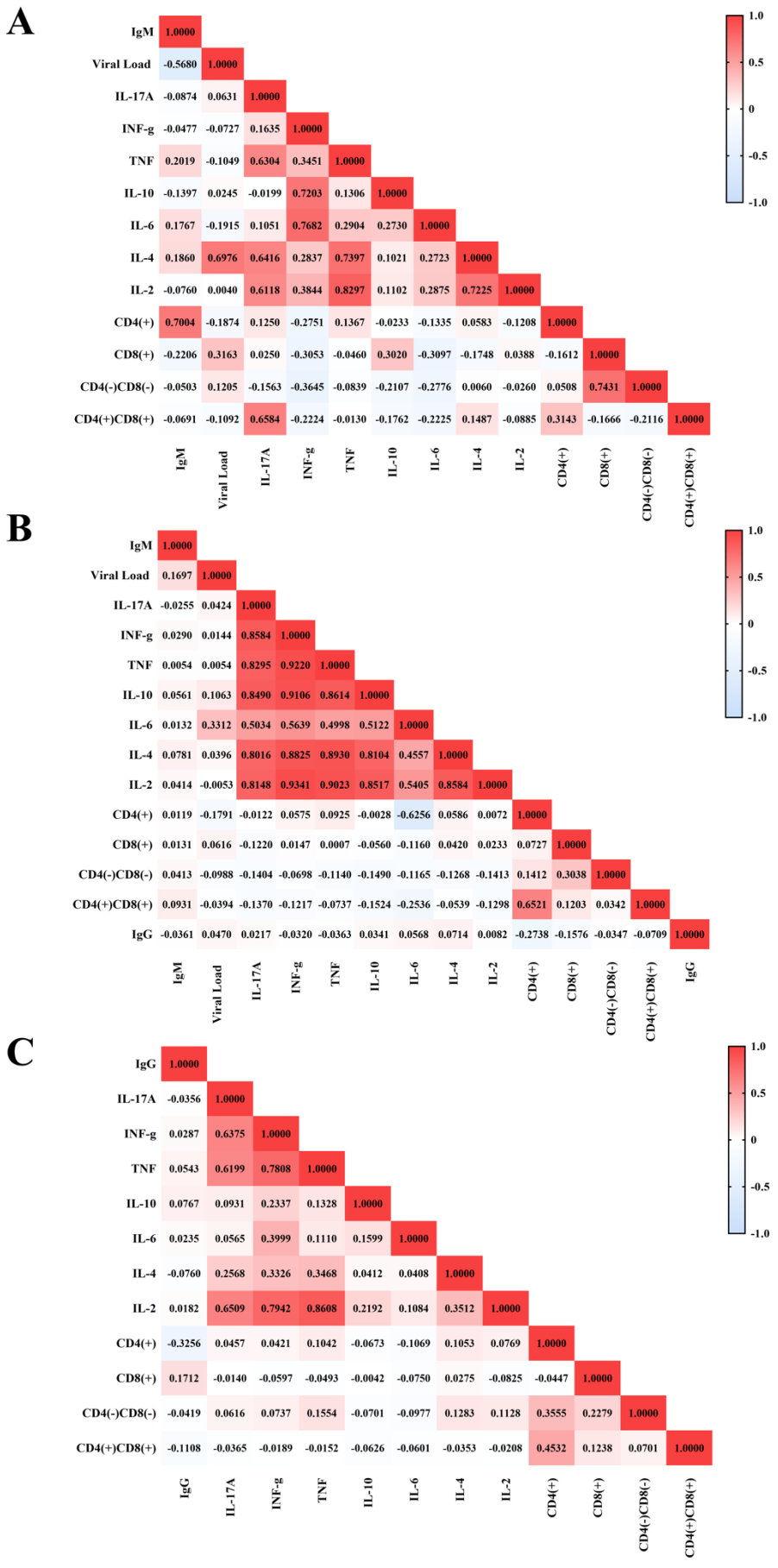

A
IgM 1.0000
Viral Load -0.5680 1.0000
IL-17A -0.0874 0.0631 1.0000
INF-g -0.0477 -0.0727 0.1635 1.0000
TNF 0.2019 -0.1049 0.6304 0.3451 1.0000
IL-10 -0.1397 0.0245 -0.0199 0.7203 0.1306 1.0000
IL-6 0.1767 -0.1915 0.1051 0.7682 0.2904 0.2730 1.0000
IL-4 0.1860 0.6976 0.6416 0.2837 0.7397 0.1021 0.2723 1.0000
IL-2 -0.0760 0.0040 0.6118 0.3844 0.8297 0.1102 0.2875 0.7225 1.0000
CD4(+) 0.7004 -0.1874 0.1250 -0.2751 0.1367 -0.0233 -0.1335 0.0583 -0.1208 1.0000
CD8(+) -0.2206 0.3163 0.0250 -0.3053 -0.0460 0.3020 -0.3097 -0.1748 0.0388 -0.1612 1.0000
CD4(-)CD8(-) -0.0503 0.1205 -0.1563 -0.3645 -0.0839 -0.2107 -0.2776 0.0060 -0.0260 0.0508 0.7431 1.0000
CD4(+)CD8(+) -0.0691 -0.1092 0.6584 -0.2224 -0.0130 -0.1762 -0.2225 0.1487 -0.0885 0.3143 -0.1666 -0.2116 1.0000
IgM Viral Load IL-17A INF-g TNF IL-10 IL-6 IL-4 IL-2 CD4(+) CD8(+) CD4(-)CD8(-) CD4(+)CD8(+)
1.0 0.5 0 -0.5 -1.0
B
IgM 1.0000
Viral Load 0.1697 1.0000
IL-17A -0.0255 0.0424 1.0000
INF-g 0.0290 0.0144 0.8584 1.0000
TNF 0.0054 0.0054 0.8295 0.9220 1.0000
IL-10 0.0561 0.1063 0.8490 0.9106 0.8614 1.0000
IL-6 0.0132 0.3312 0.5034 0.5639 0.4998 0.5122 1.0000
IL-4 0.0781 0.0396 0.8016 0.8825 0.8930 0.8104 0.4557 1.0000
IL-2 0.0414 -0.0053 0.8148 0.9341 0.9023 0.8517 0.5405 0.8584 1.0000
CD4(+) 0.0119 -0.1791 -0.0122 0.0575 0.0925 -0.0028 -0.6256 0.0586 0.0072 1.0000
CD8(+) 0.0131 0.0616 -0.1220 0.0147 0.0007 -0.0560 -0.1160 0.0420 0.0233 0.0727 1.0000
CD4(-)CD8(-) 0.0413 -0.0988 -0.1404 -0.0698 -0.1140 -0.1490 -0.1165 -0.1268 -0.1413 0.1412 0.3038 1.0000
CD4(+)CD8(+) 0.0931 -0.0394 -0.1370 -0.1217 -0.0737 -0.1524 -0.2536 -0.0539 -0.1298 0.6521 0.1203 0.0342 1.0000
IgG -0.0361 0.0470 0.0217 -0.0320 -0.0363 0.0341 0.0568 0.0714 0.0082 -0.2738 -0.1576 -0.0347 -0.0709 1.0000
IgM Viral Load IL-17A INF-g TNF IL-10 IL-6 IL-4 IL-2 CD4(+) CD8(+) CD4(-)CD8(-) CD4(+)CD8(+) IgG
1.0 0.5 0 -0.5 -1.0
C
IgG 1.0000
IL-17A -0.0356 1.0000
INF-g 0.0287 0.6375 1.0000
TNF 0.0543 0.6199 0.7808 1.0000
IL-10 0.0767 0.0931 0.2337 0.1328 1.0000
IL-6 0.0235 0.0565 0.3999 0.1110 0.1599 1.0000
IL-4 -0.0760 0.2568 0.3326 0.3468 0.0412 0.0408 1.0000
IL-2 0.0182 0.6509 0.7942 0.8608 0.2192 0.1084 0.3512 1.0000
CD4(+) -0.3256 0.0457 0.0421 0.1042 -0.0673 -0.1069 0.1053 0.0769 1.0000
CD8(+) 0.1712 -0.0140 -0.0597 -0.0493 -0.0042 -0.0750 0.0275 -0.0825 -0.0447 1.0000
CD4(-)CD8(-) -0.0419 0.0616 0.0737 0.1554 -0.0701 -0.0977 0.1283 0.1128 0.3555 0.2279 1.0000
CD4(+)CD8(+) -0.1108 -0.0365 -0.0189 -0.0152 -0.0626 -0.0601 -0.0353 -0.0208 0.4532 0.1238 0.0701 1.0000
IgG IL-17A INF-g TNF IL-10 IL-6 IL-4 IL-2 CD4(+) CD8(+) CD4(-)CD8(-) CD4(+)CD8(+)
1.0 0.5 0 -0.5 -1.0

Figure 2 - Correlation between immunological and virological factors in the studied groups. Heatmap graphs showing correlation matrices between immunological and virological factors in patients with active infection (A), patients in serological transition (B) and patients with past EBV infection (C).

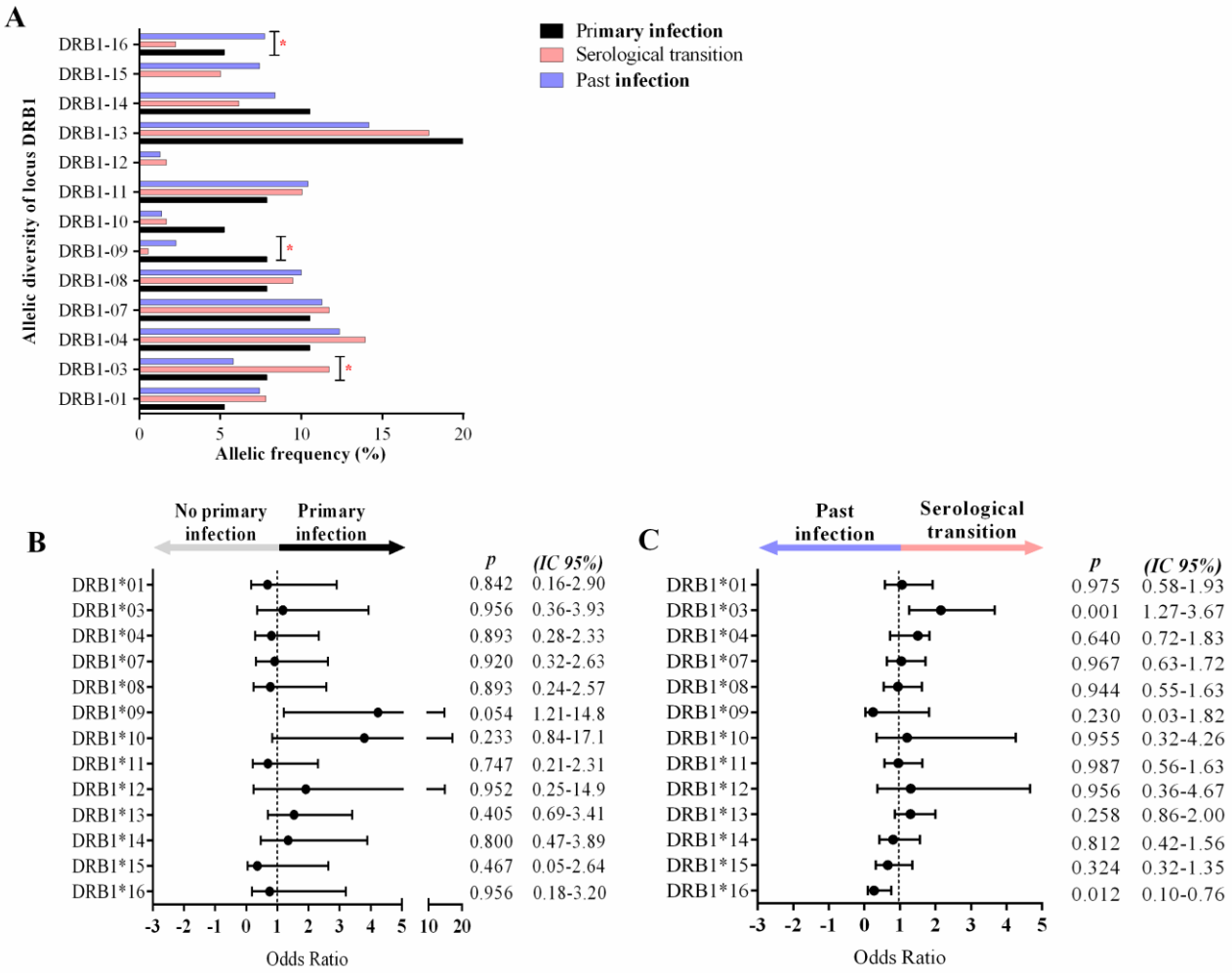


Figure 3 - Alleles associated with EBV infection profiles. (A) Column graph showing the frequency of alleles at the HLA class II DRB1 locus. The DRB1*09, *03 and *16 alleles were associated with primary infection, serological transition and past EBV infection, respectively. *= p: 0.005-0.0001. (B) Dot plot graph showing the odds ratio and confidence interval (CI 95%) of DRB1 alleles in primary EBV infection. The DRB1*09 allele was approximately 4-fold more frequent in the group . (C) Dot plot graph showing the odds ratio and confidence interval (CI 95%) of DRB1 alleles at serologic transition and past EBV infection. The DRB1*03 allele was approximately 2-fold more frequent in the serological transition group. The DRB1*16 allele was less frequent in the serological transition group (OR: 0.2724) and approximately 7-fold more frequent in the group with past infection.

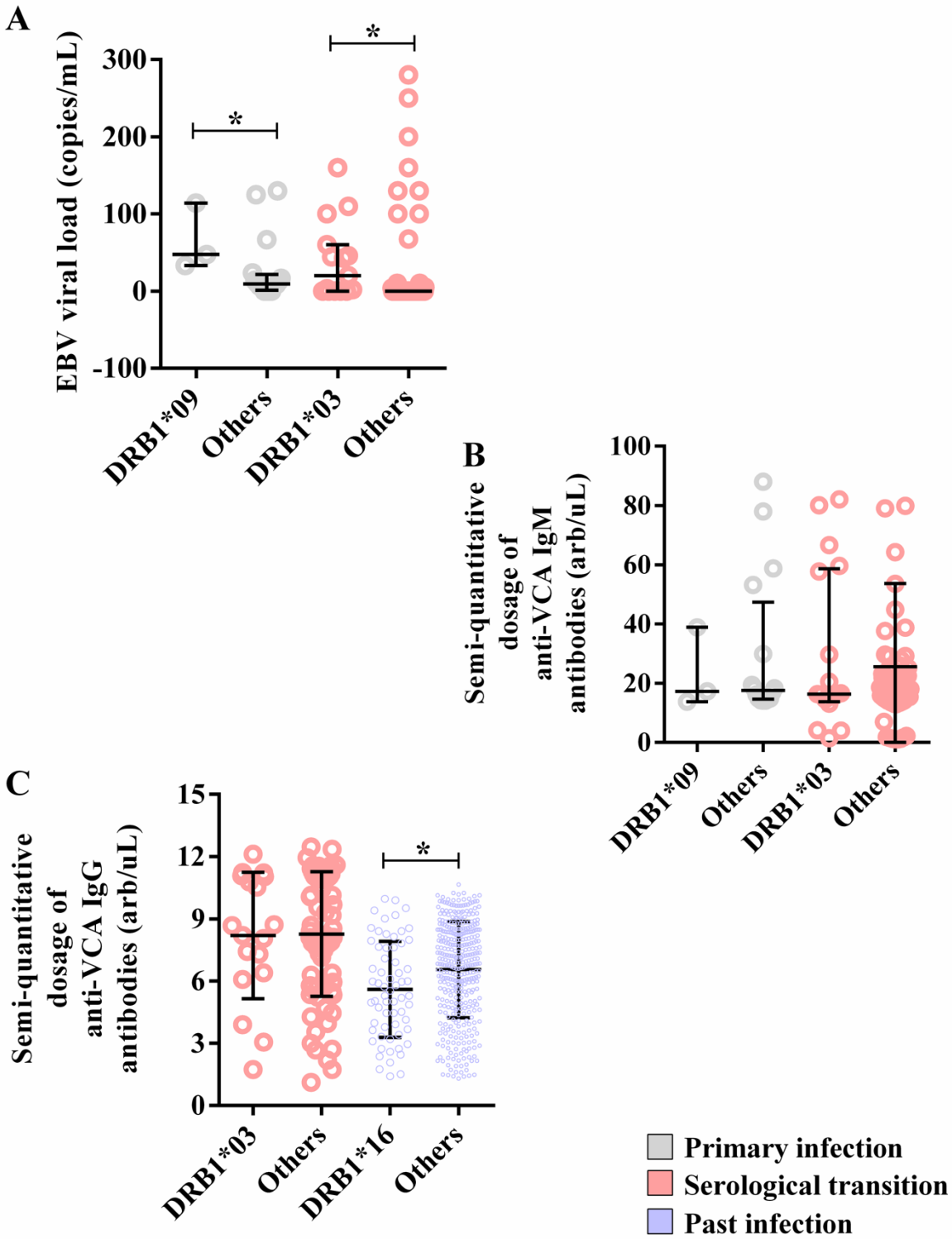


Figure 4 - Alleles associated with EBV viral load and anti-VCA IgM and IgG antibody levels. (A) Dot plot graph showing the association of DRB1*03 and DRB1*09 alleles with EBV viral load. (B) Dot plot graph showing the no association of DRB1*03 and DRB1*09 alleles with semiquantitative IgM antibody titers. (C) Dot plot graph showing the association of the DRB1*16 allele with semiquantitative IgG antibody titers. *= p: 0.005-0.0001.

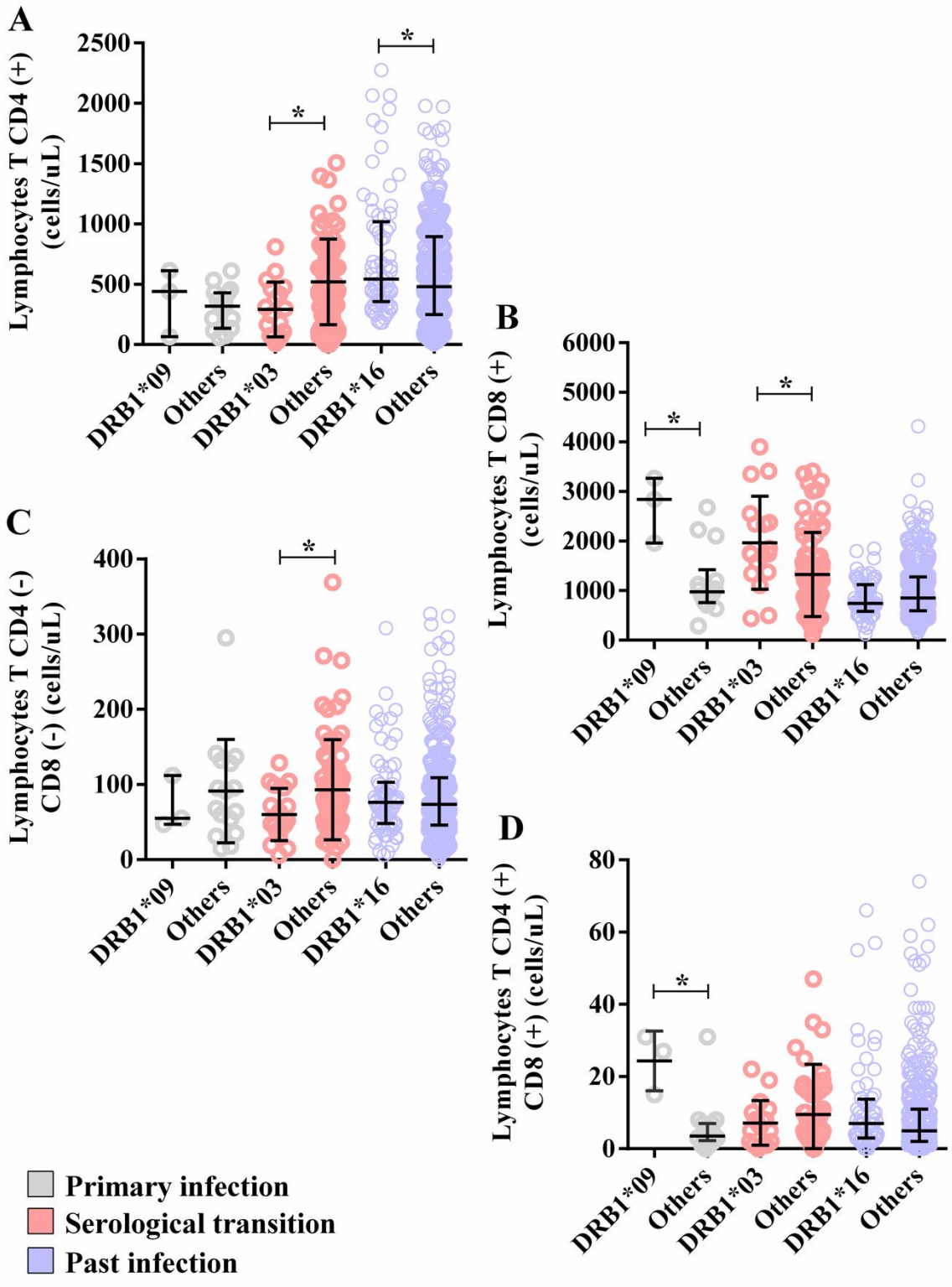


Figure 5 - Alleles associated with T lymphocyte count. (A) Dot plot graph showing the association of DRB1*03 and DRB1*16 alleles with the CD4(+) T lymphocyte count. (B) Dot plot graph showing the association of DRB1*03 and DRB1*09 alleles with the CD8(+) T lymphocyte count. (C) Dot plot graph showing the association of the DRB1*03 allele with the double-negative T lymphocyte count (CD8-CD4-). (D) Dot plot graph showing the association of the DRB1*09 allele with the double-positive T lymphocyte count (CD8+CD4+). *= p: 0.005-0.0001.

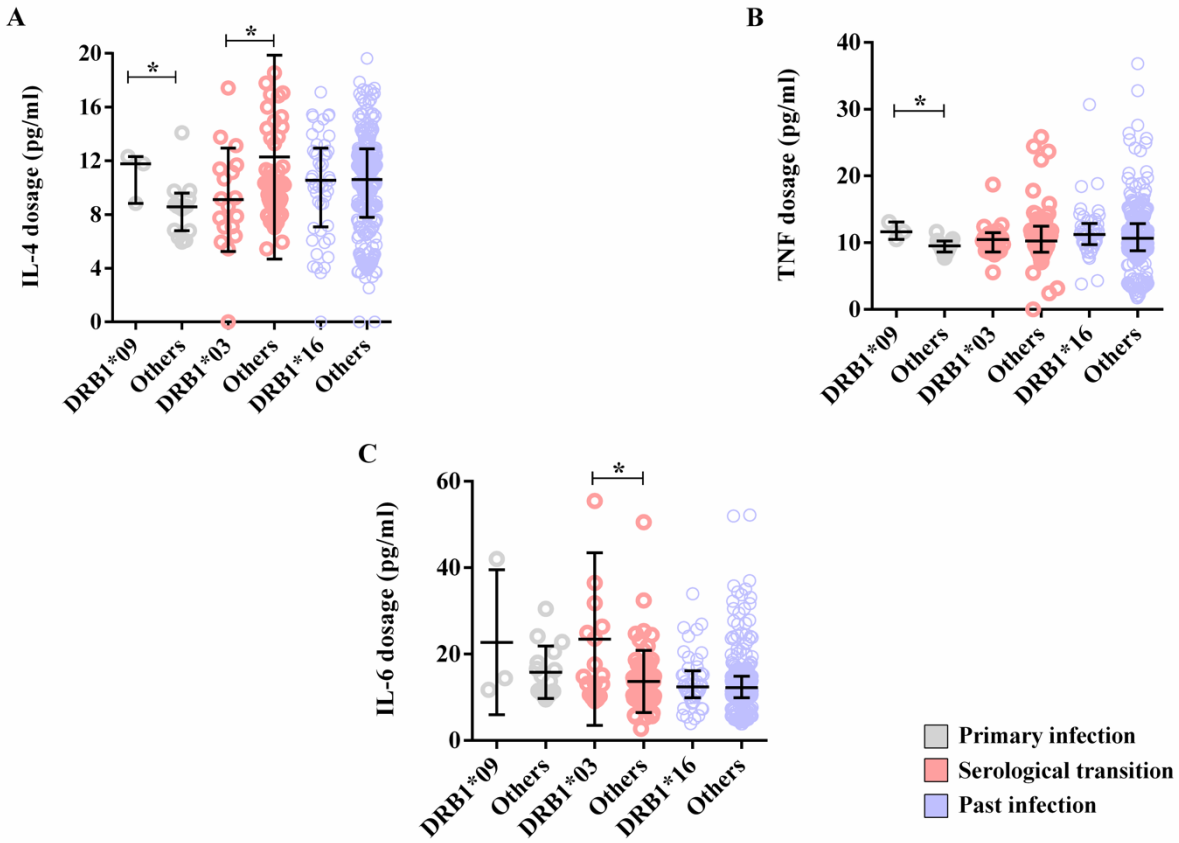
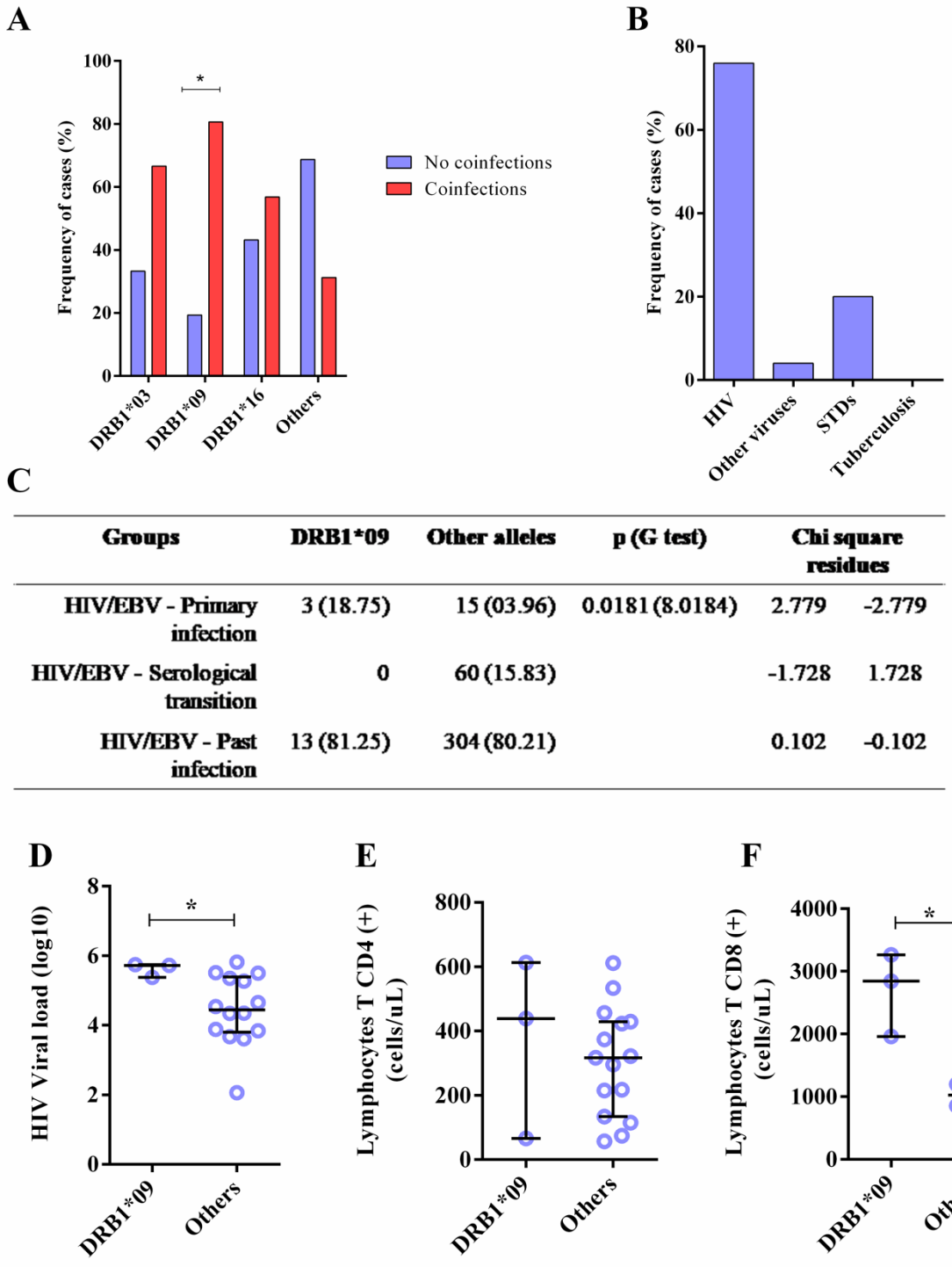


Figure 6 - Alleles associated with cytokine dosage. (A) Dot plot graph showing the association of DRB1*03 and DRB1*09 alleles with the IL-4 level. (B) Dot plot showing the association of the DRB1*09 allele with the TNF level. (C) Dot plot showing the association of the DRB1*03 allele with the IL-6 level. *= p: 0.005-0.0001.



C

Groups	DRB1*09	Other alleles	p (G test)	Chi square residues	
HIV/EBV - Primary infection	3 (18.75)	15 (03.96)	0.0181 (8.0184)	2.779	-2.779
HIV/EBV - Serological transition	0	60 (15.83)		-1.728	1.728
HIV/EBV - Past infection	13 (81.25)	304 (80.21)		0.102	-0.102

Figure 7 - The DRB1*09 allele was associated with HIV/EBV coinfection. (A) Column graph showing the frequency of the DRB1*09, *03, *16 and other alleles in patients with and without a history of coinfection. The DRB1*09 allele was more frequent in patients with a history of coinfection. (B) Column graph showing the diversity of coinfections in carriers of the DRB1*09 allele. HIV coinfection was more frequent. (C) Table showing the frequency of the DRB1*09 allele compared to other alleles in HIV^{+} patients among the three serological profiles of EBV infection. The DRB1*09 allele was associated with primary HIV/EBV coinfection. (D) Dot plot showing the association of the DRB1*09 allele with HIV viral load. (C) Dot plot graph showing the no association of the DRB1*09 allele with the CD4(+) T lymphocyte count. (D)

Dot plot graph showing the association of the DRB1*09 allele with the CD8(+) T lymphocyte count. *= p: 0.005-0.0001.

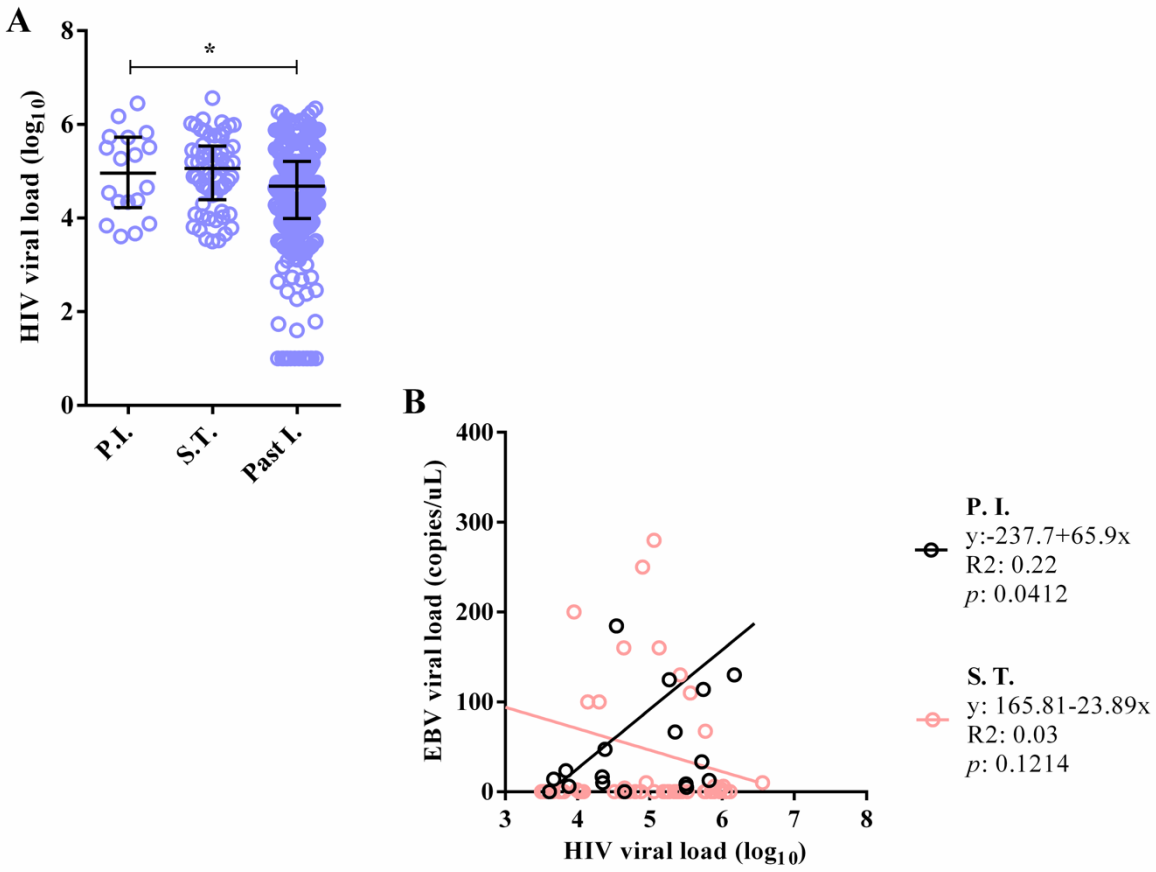
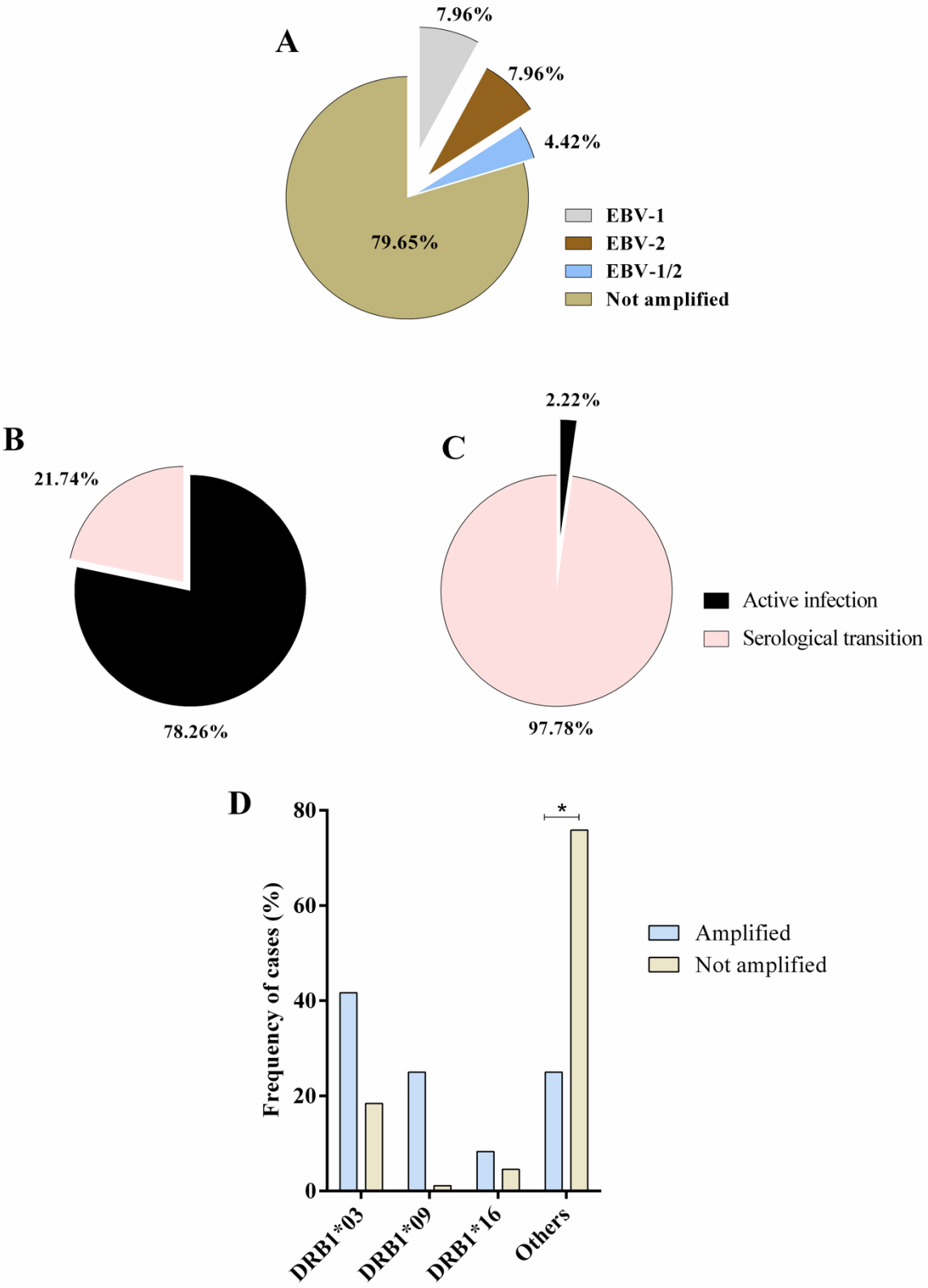


Figure 8 - HIV viral load was associated with infection profiles and EBV viral load. (A) Dot plot graph showing that HIV viral load was elevated in groups with primary EBV infection and in serologic transition. *= p: 0.005-0.0001. (B) Linear regression between the viral loads of both viruses. HIV viral load correlated positively with EBV viral load in patients with primary EBV infection.



850

851 **Figure 9 -** EBV genotyping and association with DRB1 locus heterozygosity. (A) General
852 frequency of EBV genotypes and unamplified samples. (B) Prevalence of EBV infection
853 profiles among amplified samples, with primary infection being most frequent. (C) Prevalence
854 of EBV infection profiles among unamplified samples, with serological transition being most

855 frequent. (D) Column graph showing the frequency of amplified and unamplified samples
856 among DRB1*09, *03, *16 and other alleles. There was a tendency toward samples from
857 carriers of “other” alleles to show no amplification.

858 **Table 1 –** Multifactorial comparison of sociodemographic, behavioral and clinical aspects between patients with primary infection, in serological
859 transition and past EBV infection.

GROUPS	Primary	Transition	Past		residues x^2 (α:1.96)				L.R.
FACTORS	**n: 19**	**n: 91**	**n: 470**	***p***	**Primary**	**Transition**	**Past**	***p***	**OR (IC 95%)**
Sex									
Female	6 (31.6)	26 (28.57)	108 (22.98)	0.4093					
Male	13 (68.4)	65 (71.43)	362 (77.02)						
Age									
18-28	10 (52.6)	40 (43.96)	217 (46.17)	0.4440					
29-39	7 (36.8)	27 (29.67)	129 (27.45)						
40-50	1 (05.3)	18 (19.78)	85 (18.09)						
51-61	1 (05.3)	3 (03.30)	35 (07.45)						
62-72	0	3 (03.30)	4 (00.85)						
Complete education									
Illiterate	0	0	1 (00.21)	0.2505					
Literate	2 (10.5)	7 (07.69)	44 (09.36)						

Elementary School 1	6 (31.6)	12 (13.19)	63 (13.41)	
Elementary School 2	9 (47.4)	47 (51.65)	170 (36.17)	
High school	2 (10.5)	20 (21.98)	127 (27.02)	
University education	0	5 (05.49)	65 (13.83)	
Family income				
No fixed salary	1 (05.3)	7 (07.69)	19 (04.04)	0.2562
(<1) salary	8 (42.1)	14 (15.38)	72 (15.31)	
(1-3) salary	10 (52.6)	57 (62.64)	310 (65.96)	
(4-6) salary	0	7 (07.69)	43 (09.15)	
(7-10) salary	0	4 (04.40)	17 (03.62)	
(>10) salary	0	2 (02.20)	9 (01.91)	
Smoking				
Historic				
No	10 (52.6)	49 (53.85)	238 (50.64)	0.8500

	Yes	9 (47.4)	42 (46.15)	232 (49.36)	
Current usage					
	No	6 (66.7)	32 (76.19)	182 (78.45)	0.7162
	Yes	3 (33.3)	10 (23.81)	50 (21.55)	
Alcoholism					
Historic					
	No	2 (10.5)	12 (13.19)	47 (10.00)	0.6904
	Yes	17 (89.5)	79 (86.81)	423 (90.00)	
Current usage					
	No	10 (58.8)	46 (50.55)	182 (43.03)	0.2189
	Yes	7 (41.2)	45 (49.45)	241 (56.97)	
Illicit drugs					
Historic					
	No	14 (73.7)	68 (74.73)	361 (76.81)	0.8814
	Yes	5 (26.3)	23 (25.27)	109 (23.19)	

Usage time				
<5 years	3 (60.00)	8 (34.78)	58 (53.21)	0.2663
≥5 years	2 (40.00)	15 (65.22)	51 (46.79)	
Sexual orientation				
Heterosexual	9 (47.4)	49 (53.85)	278 (59.15)	0.7480
Homosexual	7 (36.8)	30 (32.98)	143 (30.43)	
Bisexual	3 (15.9)	12 (13.19)	49 (10.43)	
Active sex life				
No	8 (42.1)	30 (32.97)	137 (29.15)	0.4187
Yes	11 (57.9)	61 (67.03)	333 (70.85)	
Fixed partner				
No	5 (26.3)	41 (45.05)	178 (37.87)	0.2342
Yes	14 (73.7)	50 (54.95)	292 (62.13)	
Relationships with sex workers				
No	16 (84.2)	67 (73.63)	359 (76.38)	0.5931

	Yes	3 (15.8)	24 (26.37)	111 (23.62)	
Use of condoms					
	No	4 (21.05)	15 (16.48)	105 (22.34)	0.4505
	Yes	15 (78.95)	76 (83.52)	365 (77.66)	
Difficulty sleeping					
	No	14 (73.68)	50 (54.95)	288 (61.28)	0.2616
	Yes	5 (26.32)	41 (45.05)	182 (38.72)	
Daily rest hours					
	<8 hours	8 (42.11)	60 (65.93)	262 (55.74)	0.0843
	≥8 hours	11 (57.89)	31 (34.07)	208 (44.26)	
Cancer diagnosis					
	No	19 (100.0)	91 (100.0)	468 (99.57)	0.8475
	Yes	0	0	2 (00.43)	
Family history of cancer					
	No	12 (63.2)	53 (58.24)	264 (56.17)	0.7938

Yes	7 (36.8)	38 (41.76)	206 (43.83)						
Degree of kinship									
first-degree relatives	1 (14.29)	8 (21.05)	41 (19.90)	0.9904					
second-degree relatives	3 (42.86)	14 (36.84)	73 (35.43)						
third-degree relatives	3 (42.86)	16 (42.11)	92 (44.66)						
History of co-infections									
No history	0	31 (24.41)	136 (22.48)	0.0433	-3.0090	0.7529	0.7740	0.0158	0.12 (0.01-0.81)
HIV	19 (61.29)	61 (48.03)	320 (52.89)		1.0091	-1.0858	0.5064	0.4091	1.46 (0.70-3.05)
Other viruses	0	7 (05.51)	28 (04.63)		-1.2464	0.5456	0.1058	0.9742	0.66 (0.08-5.01)
bacterial STD’s	10 (32.26)	23 (18.11)	115 (19.01)		1.8489	-0.4017	-0.5316	0.1059	2.05 (0.94-4.45)
Tuberculosis	2 (06.45)	4 (03.15)	6 (00.99)		2.2291	1.5644	-2.5241	0.0666	6.88 (1.33-35.6)
Fungi	0	1 (00.79)	0		-0.2059	2.2393	-1.9581	0.1325	24.4 (1.49-400)
Symptomatology									
Asymptomatic	9 (47.37)	44 (48.35)	256 (54.47)	0.4976					
Symptomatic	10 (52.63)	47 (51.65)	214 (45.53)						

Symptoms				
Adenomegaly	0	1 (01.56)	0	0.9312
Fever	9 (37.50)	19 (29.69)	69 (29.87)	
Sore throat	7 (29.17)	19 (29.69)	77 (33.33)	
Arthralgia	2 (08.33)	5 (07.81)	23 (09.96)	
Headache	4 (16.67)	14 (21.88)	51 (22.08)	
Lymphadenopathy	0	1 (01.56)	0	
Myalgia	2 (08.33)	5 (07.81)	11 (04.76)	

L.R.: Logistic regression; OR: Odds ratio; CI: Confidence interval

869 **Table 2 -** Estimation of population genetic parameters of groups with primary infection, serological transition and past infection with EBV

Hardy-Weinberg	**Loci**	***p***	**standard error**
Primary infection	locus A	0.8476	0.0236
	locus B	0.6548	0.0383
	locus DRB1	0.9158	0.0138
Serological transition	locus A	0.5180	0.0126
	locus B	0.5442	0.0418
	locus DRB1	0.7626	0.0218
Past infection	locus A	0.7658	0.0279
	locus B	0.9367	0.0188
	locus DRB1	0.8574	0.0197

Linkage disequilibrium	**Locus 01**	**Locus 02**	***p***	**standard error**
Primary infection	locus A	locus B	0.01832	0.012944
	locus A	locus DRB1	1	0
	locus B	locus DRB1	1	0
Serological transition	locus A	locus B	1	0

	locus A	locus DRB1	0.488270	0.488270
	locus B	locus DRB1	1	0
	locus A	locus B	1	0
Past infection	locus A	locus DRB1	0.362660	0.046918
	locus B	locus DRB1	0.068190	0.023208

885 **Supplementary data 1** - Allelic frequency of loci *A, B* and *DRB1* of *HLA class I* and *II* in groups with primary infection, serological transition
886 and past infection with EBV.

	Primary		Transition		Past				Primary	Transition	Past
Alleles	**N**	**%**	**N**	**%**	**N**	**%**	***p***	**G test**	**residues x^2 (α: 0.05): 1.96**	**residues x^2 (α: 0.05): 1.96**	**residues x^2 (α: 0.05): 1.96**
A*01	2	5.00	12	6.59	48	5.06	0.7096	30.9006	-0.0859	0.8482	-0.7442
A02	11	27.50	48	26.37	243	25.63			0.2482	0.1884	-0.2892
A03	3	7.50	7	3.85	80	8.44			-0.0464	-2.1190	1.9802
A11	1	2.50	6	3.30	42	4.43			-0.5423	-0.6532	0.8551
A23	1	2.50	17	9.34	46	4.85			-0.8406	2.4989	-1.9203
A24	12	30.00	25	13.74	122	12.87			3.0818	0.0628	-1.4863
A26	1	2.50	5	2.75	27	2.85			-0.1246	-0.0650	0.1178
A29	0	0.00	5	2.75	50	5.27			-1.4293	-1.3550	1.9149
A30	2	5.00	8	4.40	49	5.17			-0.0126	-0.4342	0.4071
A31	4	10.00	18	9.89	85	8.97			0.1908	0.3793	-0.4391
A32	0	0.00	5	2.75	25	2.64			-1.0440	0.1701	0.3266
A33	1	2.50	2	1.10	19	2.00			0.2936	-0.8446	0.6446
A34	0	0.00	2	1.10	6	0.63			-0.5340	0.7396	-0.4362
A36	0	0.00	1	0.55	4	0.42			-0.4216	0.2748	-0.0586
A66	0	0.00	0	0.00	11	1.16			-0.6270	-1.4302	1.6126

A68	1	2.50	17	9.34	78	8.23			-1.3378	0.6074	0.0585
A69	0	0.00	1	0.55	2	0.21			-0.3263	0.8507	-0.6351
A74	1	2.50	2	1.10	10	1.05			0.8527	-0.0171	-0.3794
A80	0	0.00	1	0.55	1	0.11			-0.2663	1.3452	-1.1200
Homozygous	1	5.00	14	15.38	59	12.55	0.3810	1.9297	-1.0084	0.9704	-0.4498
Heterozygous	18	95.00	77	84.61	411	87.45			1.0084	-0.9704	0.4498
B07	1	2.50	9	7.09	33	5.48	0.9098	42.4280	-0.9546	0.8153	-0.2011
B08	2	5.00	5	3.94	21	3.49			0.3697	0.2045	-0.3875
B13	0	0.00	0	0.00	5	0.83			-0.5448	-0.9954	1.1921
B14	0	0.00	3	2.36	33	5.48			-1.4924	-1.3455	2.0297
B15	3	7.5	11	8.66	73	12.13			-0.9161	-1.0168	1.4167
B18	1	2.50	8	6.30	24	3.99			-0.6502	1.2340	-0.7442
B27	0	0.00	0	0.00	2	0.33			-0.3439	-0.6283	0.7525
B35	11	27.50	18	14.17	92	15.28			1.8390	-0.5088	-0.5626
B37	0	0.00	2	1.57	1	0.17			-0.4215	2.3506	-1.8697
B38	1	2.50	1	0.79	11	1.83			0.3365	-0.8591	0.5823
B39	3	7.5	6	4.72	35	5.81			0.3718	-0.5186	0.2582
B40	3	7.5	5	3.94	34	5.65			0.4571	-0.8172	0.4782
B41	0	0.00	0	0.00	9	1.50			-0.7329	-1.3390	1.6036

B42	0	0.00	1	0.79	9	1.50			-0.7730	-0.5538	0.9233
B44	2	5.00	17	13.39	53	8.80			-1.0849	1.7211	-0.9393
B45	2	5.00	1	0.79	6	1.00			2.1911	-0.4346	-0.8238
B47	0	0.00	0	0.00	1	0.17			-0.2430	-0.4440	0.5318
B48	1	2.50	1	0.79	4	0.66			1.1898	0.0143	-0.6713
B49	2	5.00	3	2.36	15	2.49			0.8753	-0.1773	-0.3258
B50	2	5.00	5	3.94	6	1.00			1.5562	2.1589	-2.7927
B51	2	5.00	6	4.72	46	7.64			-0.6201	-1.0975	1.3250
B52	2	5.00	5	3.94	24	3.99			0.2185	-0.0493	-0.0768
B53	2	5.00	6	4.72	22	3.65			0.2672	0.5349	-0.6264
B56	0	0.00	0	0.00	3	0.50			-0.4215	-0.7701	0.9222
B55	0	0.00	1	0.79	2	0.33			-0.4215	0.7903	-0.4738
B57	1	2.50	4	3.15	11	1.83			0.1199	0.9321	-0.9003
B58	1	2.50	9	7.09	23	3.82			-0.6502	1.7139	-1.1735
B78	1	2.50	0	0.00	2	0.33			2.1009	-0.7701	-0.4738
B81	0	0.00	0	0.00	2	0.33			-0.3439	-0.6283	0.7525
Bw4	1	5.00	3	4.84	15	4.98	0.9767	1.2042	0.0083	-0.0484	0.0389
Bw4/Bw6	1	5.00	5	8.06	16	5.32			-0.1469	0.8577	-0.6905
Bw6	14	70.00	37	59.68	188	62.46			0.7206	-0.4838	0.0436

No Bw4/6	4	20.00	17	27.42	82	27.24			-0.7141	0.1021	0.2956
Homozygous	2	10.00	6	9.38	29	9.60	0.9897	0.0207	0.1359	-0.0171	-0.0452
Heterozygous	17	90.00	58	90.62	273	90.40			-0.1359	0.0171	0.0452
DRB1-01	2	5.26	14	7.82	69	7.42	0.0477	34.3380	-0.5140	0.2283	0.0233
DRB1-03	3	7.89	21	11.73	54	5.81			0.2725	2.8528	-2.7680
DRB1-04	4	10.53	25	13.97	115	12.37			-0.3837	0.6206	-0.3997
DRB1-07	4	10.53	21	11.73	105	11.29			-0.1597	0.1828	-0.0964
DRB1-08	3	7.89	17	9.50	93	10.00			-0.4117	-0.1733	0.3487
DRB1-09	3	7.89	1	0.56	21	2.26			2.4538	-1.6167	0.3768
DRB1-10	2	5.26	3	1.68	13	1.40			1.8632	0.1250	-0.9673
DRB1-11	3	7.89	18	10.06	97	10.43			-0.4938	-0.1111	0.3286
DRB1-12	0	0.00	3	1.68	12	1.29			-0.7217	0.4720	-0.1076
DRB1-13	8	21.05	32	17.88	132	14.19			1.0636	1.1754	-1.5751
DRB1-14	4	10.53	11	6.15	78	8.39			0.5554	-1.0473	0.7166
DRB1-15	0	0.00	9	5.03	69	7.42			-1.6934	-1.0253	1.7239
DRB1-16	2	5.26	4	2.23	72	7.74			-0.3828	-2.6412	2.6223
Homozygous	1	5.26	16	17.78	54	11.56	0.1817	3.4109	-0.9568	1.8375	-1.2797
Heterozygous	18	94.74	74	82.22	413	88.44			0.9568	-1.8375	1.2797

887

888

889 **Supplementary data 2 -** Haplotypes frequency of *HLA-A* and *HLA-B* gene in groups with primary infection, in serological transition and with
890 past infection with EBV.

Haplotypes	General population		Primary		Transition		Past			Primary	Transition	Past
	N	%	N	%	N	%	N	%	*p*	residues x^2 (α: 0.05): 1.96	residues x^2 (α: 0.05): 1.96	residues x^2 (α: 0.05): 1.96
Frequency ≥ 1% of the general population												
A*02B*07	17	1.48	0	0.00	3	1.63	14	1.55	0.80	-0.9667	0.3124	0.2479
A*02B*14	18	1.57	0	0.00	2	1.09	16	1.77		-0.9960	-0.4728	0.9648
A*02B*15	41	3.57	1	1.69	4	2.17	36	3.97		-0.7942	-0.9926	1.3188
A*02B*18	14	1.22	0	0.00	4	2.17	10	1.10		-0.8738	1.4477	-0.8162
A*02B*35	55	4.79	5	8.47	8	4.35	42	4.64		1.4989	-0.1017	-0.7260
A*02B*39	19	1.65	0	0.00	5	2.72	14	1.55		-1.0246	1.4154	-0.7052
A*02B*40	25	2.18	3	5.08	1	0.54	21	2.32		1.6586	-1.5908	0.5164
A*02B*44	34	2.96	1	1.69	8	4.35	25	2.76		-0.5758	1.4561	-0.9861
A*02B*51	27	2.35	1	1.69	1	0.54	**25**	2.76		-0.3200	-1.7023	1.6940
A*02B*52	12	1.04	2	3.39	4	2.17	6	0.66		1.8828	1.8059	-2.6379
A*03B*35	15	1.31	1	1.69	1	0.54	13	1.43		0.3019	-0.9213	0.6580
A*03B*44	12	1.04	0	0.00	1	0.54	11	1.21		-0.8069	-0.6567	1.0259
A*24B*15	15	1.31	1	1.69	2	1.09	12	1.32		0.3019	-0.1843	0.0000

A*24B*35	25	2.18	2	3.39	5	2.72	18	1.99		0.7108	0.7231	-1.0328
A*24B*39	13	1.13	0	0.00	3	1.63	10	1.10		-0.8409	0.8292	-0.2820
A*24B*51	14	1.22	1	1.69	1	0.54	12	1.32		0.3745	-0.8381	0.5441
A*31B*35	17	1.48	1	1.69	3	1.63	13	1.43		0.1706	0.3124	-0.3718
A*31B*44	15	1.31	1	1.69	2	1.09	12	1.32		0.3019	-0.1843	0.0000
A*68B*35	12	1.04	0	0.00	2	1.09	10	1.10		-0.8069	0.1642	0.2931
Frequency < 1% of the general population												
A*01B*07	6	0.52	1	1.69	3	1.63	2	0.22	0.70	1.2686	2.2130	-2.6764
A*01B*08	5	0.44	1	1.69	1	0.54	3	0.33		1.4939	0.2079	-0.9916
A*01B*13	1	0.09	0	0.00	0	0.00	1	0.11		-0.2345	-0.4457	0.5278
A*01B*14	2	0.17	0	0.00	0	0.00	2	0.22		-0.3319	-0.6308	0.7469
A*01B*15	7	0.61	1	1.69	2	1.09	4	0.44		1.0863	0.8594	-1.3589
A*01B*18	6	0.52	0	0.00	2	1.09	4	0.44		-0.5764	1.1102	-0.6897
A*01B*35	9	0.78	0	0.00	2	1.09	7	0.77		-0.7074	0.4602	-0.0336
A*01B*37	1	0.09	0	0.00	1	0.54	0	0.00		-0.2345	2.2466	-1.8973
A*01B*38	2	0.17	0	0.00	0	0.00	2	0.22		-0.3319	-0.6308	0.7469
A*01B*39	2	0.17	0	0.00	0	0.00	2	0.22		-0.3319	-0.6308	0.7469
A*01B*40	5	0.44	0	0.00	1	0.54	4	0.44		-0.5258	0.2079	0.0958
A*01B*41	1	0.09	0	0.00	0	0.00	1	0.11		-0.2345	-0.4457	0.5278

A*01B*44	4	0.35	1	1.69	1	0.54	2	0.22	1.7866	0.4556	-1.3723
A*01B*49	4	0.35	0	0.00	2	1.09	2	0.22	-0.4700	1.8045	-1.3723
A*01B*51	3	0.26	0	0.00	1	0.54	2	0.22	-0.4068	0.7834	-0.4867
A*01B*52	3	0.26	0	0.00	0	0.00	3	0.33	-0.4068	-0.7730	0.9153
A*01B*53	3	0.26	0	0.00	1	0.54	2	0.22	-0.4068	0.7834	-0.4867
A*01B*55	2	0.17	0	0.00	1	0.54	1	0.11	-0.3319	1.2742	-0.9691
A*01B*57	4	0.35	0	0.00	3	1.63	1	0.11	-0.4700	3.1533	-2.5873
A*01B*58	5	0.44	0	0.00	0	0.00	5	0.55	-0.5258	-0.9993	1.1833
A*02B*08	10	0.87	1	1.69	2	1.09	7	0.77	0.6868	0.2950	-0.6356
A*02B*13	2	0.17	0	0.00	0	0.00	2	0.22	-0.3319	-0.6308	0.7469
A*02B*27	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*02B*38	3	0.26	0	0.00	0	0.00	3	0.33	-0.4068	-0.7730	0.9153
A*02B*41	4	0.35	0	0.00	0	0.00	4	0.44	-0.4700	-0.8932	1.0576
A*02B*42	4	0.35	0	0.00	1	0.54	3	0.33	-0.4700	0.4556	-0.1573
A*02B*45	5	0.44	1	1.69	1	0.54	3	0.33	1.4939	0.2079	-0.9916
A*02B*48	3	0.26	1	1.69	1	0.54	1	0.11	2.1972	0.7834	-1.8887
A*02B*49	9	0.78	0	0.00	1	0.54	8	0.88	-0.7074	-0.4421	0.7791
A*02B*50	10	0.87	1	1.69	6	3.26	3	0.33	0.6868	3.7212	-3.7217
A*02B*53	10	0.87	2	3.39	2	1.09	6	0.66	2.1198	0.2950	-1.4071

A*02B*56	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*02B*57	8	0.70	1	1.69	2	1.09	5	0.55	0.9335	0.6461	-1.0846
A*02B*58	9	0.78	0	0.00	1	0.54	8	0.88	-0.7074	-0.4421	0.7791
A*02B*78	3	0.26	1	1.69	0	0.00	2	0.22	2.1972	-0.7730	-0.4867
A*02B*81	2	0.17	0	0.00	0	0.00	2	0.22	-0.3319	-0.6308	0.7469
A*03B*07	2	0.17	0	0.00	0	0.00	2	0.22	-0.3319	-0.6308	0.7469
A*03B*08	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*03B*13	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*03B*14	4	0.35	0	0.00	0	0.00	4	0.44	-0.4700	-0.8932	1.0576
A*03B*15	6	0.52	0	0.00	0	0.00	6	0.66	-0.5764	-1.0955	1.2971
A*03B*18	2	0.17	0	0.00	0	0.00	2	0.22	-0.3319	-0.6308	0.7469
A*03B*27	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*03B*38	2	0.17	1	1.69	0	0.00	1	0.11	2.8551	-0.6308	-0.9691
A*03B*39	3	0.26	0	0.00	1	0.54	2	0.22	-0.4068	0.7834	-0.4867
A*03B*40	2	0.17	0	0.00	0	0.00	2	0.22	-0.3319	-0.6308	0.7469
A*03B*42	2	0.17	0	0.00	0	0.00	2	0.22	-0.3319	-0.6308	0.7469
A*03B*45	2	0.17	1	1.69	0	0.00	1	0.11	2.8551	-0.6308	-0.9691
A*03B*49	3	0.26	0	0.00	0	0.00	3	0.33	-0.4068	-0.7730	0.9153
A*03B*50	2	0.17	1	1.69	0	0.00	1	0.11	2.8551	-0.6308	-0.9691

A*03B*51	9	0.78	0	0.00	0	0.00	9	0.99	-0.7074	-1.3444	1.5918
A*03B*52	5	0.44	0	0.00	0	0.00	5	0.55	-0.5258	-0.9993	1.1833
A*03B*53	4	0.35	0	0.00	0	0.00	4	0.44	-0.4700	-0.8932	1.0576
A*03B*57	2	0.17	0	0.00	0	0.00	2	0.22	-0.3319	-0.6308	0.7469
A*03B*58	5	0.44	0	0.00	1	0.54	4	0.44	-0.5258	0.2079	0.0958
A*03B*78	1	0.09	1	1.69	0	0.00	0	0.00	4.2696	-0.4457	-1.8973
A*11B*07	1	0.09	0	0.00	1	0.54	0	0.00	-0.2345	2.2466	-1.8973
A*11B*08	2	0.17	0	0.00	1	0.54	1	0.11	-0.3319	1.2742	-0.9691
A*11B*14	4	0.35	0	0.00	1	0.54	3	0.33	-0.4700	0.4556	-0.1573
A*11B*15	9	0.78	0	0.00	0	0.00	9	0.99	-0.7074	-1.3444	1.5918
A*11B*35	4	0.35	1	1.69	1	0.54	2	0.22	1.7866	0.4556	-1.3723
A*11B*37	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*11B*38	3	0.26	0	0.00	0	0.00	3	0.33	-0.4068	-0.7730	0.9153
A*11B*39	4	0.35	0	0.00	0	0.00	4	0.44	-0.4700	-0.8932	1.0576
A*11B*40	2	0.17	0	0.00	0	0.00	2	0.22	-0.3319	-0.6308	0.7469
A*11B*41	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*11B*44	8	0.70	0	0.00	0	0.00	8	0.88	-0.6665	-1.2666	1.4998
A*11B*47	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*11B*49	1	0.09	1	1.69	0	0.00	0	0.00	4.2696	-0.4457	-1.8973

A*11B*50	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*11B*51	10	0.87	0	0.00	3	1.63	7	0.77	-0.7461	1.1516	-0.6356
A*11B*52	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*11B*56	3	0.26	0	0.00	0	0.00	3	0.33	-0.4068	-0.7730	0.9153
A*11B*57	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*11B*58	1	0.09	0	0.00	1	0.54	0	0.00	-0.2345	2.2466	-1.8973
A*23B*07	3	0.26	0	0.00	1	0.54	2	0.22	-0.4068	0.7834	-0.4867
A*23B*08	5	0.44	0	0.00	0	0.00	5	0.55	-0.5258	-0.9993	1.1833
A*23B*13	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*23B*14	3	0.26	0	0.00	0	0.00	3	0.33	-0.4068	-0.7730	0.9153
A*23B*15	6	0.52	1	1.69	0	0.00	5	0.55	1.2686	-1.0955	0.3037
A*23B*18	1	0.09	0	0.00	1	0.54	0	0.00	-0.2345	2.2466	-1.8973
A*23B*35	6	0.52	0	0.00	0	0.00	6	0.66	-0.5764	-1.0955	1.2971
A*23B*37	1	0.09	0	0.00	1	0.54	0	0.00	-0.2345	2.2466	-1.8973
A*23B*38	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*23B*39	4	0.35	0	0.00	0	0.00	4	0.44	-0.4700	-0.8932	1.0576
A*23B*40	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*23B*41	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*23B*42	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278

A*23B*44	2	0.17	0	0.00	0	0.00	2	0.22	-0.3319	-0.6308	0.7469
A*23B*45	2	0.17	0	0.00	1	0.54	1	0.11	-0.3319	1.2742	-0.9691
A*23B*48	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*23B*49	1	0.09	1	1.69	0	0.00	0	0.00	4.2696	-0.4457	-1.8973
A*23B*50	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*23B*51	3	0.26	0	0.00	1	0.54	2	0.22	-0.4068	0.7834	-0.4867
A*23B*52	3	0.26	0	0.00	0	0.00	3	0.33	-0.4068	-0.7730	0.9153
A*23B*53	4	0.35	0	0.00	0	0.00	4	0.44	-0.4700	-0.8932	1.0576
A*23B*57	2	0.17	0	0.00	1	0.54	1	0.11	-0.3319	1.2742	-0.9691
A*23B*58	7	0.61	0	0.00	3	1.63	4	0.44	-0.6230	1.8811	-1.3589
A*23B*81	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*24B*07	4	0.35	0	0.00	1	0.54	3	0.33	-0.4700	0.4556	-0.1573
A*24B*08	3	0.26	0	0.00	0	0.00	3	0.33	-0.4068	-0.7730	0.9153
A*24B*13	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*24B*14	4	0.35	0	0.00	0	0.00	4	0.44	-0.4700	-0.8932	1.0576
A*24B*18	5	0.44	1	1.69	0	0.00	4	0.44	1.4939	-0.9993	0.0958
A*24B*38	3	0.26	1	1.69	0	0.00	2	0.22	2.1972	-0.7730	-0.4867
A*24B*40	11	0.96	1	1.69	2	1.09	8	0.88	0.5841	0.1462	-0.4462
A*24B*41	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278

A*24B*44	10	0.87	0	0.00	2	1.09	8	0.88	-0.7461	0.2950	0.1360
A*24B*45	1	0.09	1	1.69	0	0.00	0	0.00	4.2696	-0.4457	-1.8973
A*24B*48	1	0.09	1	1.69	0	0.00	0	0.00	4.2696	-0.4457	-1.8973
A*24B*49	6	0.52	1	1.69	1	0.54	4	0.44	1.2686	0.0074	-0.6897
A*24B*50	3	0.26	1	1.69	1	0.54	1	0.11	2.1972	0.7834	-1.8887
A*24B*52	10	0.87	1	1.69	2	1.09	7	0.77	0.6868	0.2950	-0.6356
A*24B*53	9	0.78	0	0.00	1	0.54	8	0.88	-0.7074	-0.4421	0.7791
A*24B*55	1	0.09	0	0.00	1	0.54	0	0.00	-0.2345	2.2466	-1.8973
A*24B*56	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*24B*57	4	0.35	0	0.00	1	0.54	3	0.33	-0.4700	0.4556	-0.1573
A*24B*58	6	0.52	2	3.39	1	0.54	3	0.33	3.1136	0.0074	-1.6830
A*24B*78	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*26B*07	2	0.17	0	0.00	0	0.00	2	0.22	-0.3319	-0.6308	0.7469
A*26B*14	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*26B*18	4	0.35	0	0.00	0	0.00	4	0.44	-0.4700	-0.8932	1.0576
A*26B*35	3	0.26	1	1.69	0	0.00	2	0.22	2.1972	-0.7730	-0.4867
A*26B*38	3	0.26	0	0.00	1	0.54	2	0.22	-0.4068	0.7834	-0.4867
A*26B*39	3	0.26	0	0.00	1	0.54	2	0.22	-0.4068	0.7834	-0.4867
A*26B*40	3	0.26	0	0.00	1	0.54	2	0.22	-0.4068	0.7834	-0.4867

A*26B*44	3	0.26	0	0.00	1	0.54	2	0.22	-0.4068	0.7834	-0.4867
A*26B*45	2	0.17	0	0.00	0	0.00	2	0.22	-0.3319	-0.6308	0.7469
A*26B*49	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*26B*50	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*26B*51	2	0.17	1	1.69	0	0.00	1	0.11	2.8551	-0.6308	-0.9691
A*26B*52	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*26B*53	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*26B*55	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*29B*07	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*29B*15	4	0.35	0	0.00	0	0.00	4	0.44	-0.4700	-0.8932	1.0576
A*29B*18	2	0.17	0	0.00	1	0.54	1	0.11	-0.3319	1.2742	-0.9691
A*29B*35	3	0.26	0	0.00	0	0.00	3	0.33	-0.4068	-0.7730	0.9153
A*29B*38	2	0.17	0	0.00	0	0.00	2	0.22	-0.3319	-0.6308	0.7469
A*29B*39	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*29B*39	2	0.17	0	0.00	0	0.00	2	0.22	-0.3319	-0.6308	0.7469
A*29B*40	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*29B*42	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*29B*44	5	0.44	0	0.00	2	1.09	3	0.33	-0.5258	1.4152	-0.9916
A*29B*48	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278

A*29B*49	2	0.17	0	0.00	1	0.54	1	0.11	-0.3319	1.2742	-0.9691
A*29B*50	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*29B*51	4	0.35	0	0.00	0	0.00	4	0.44	-0.4700	-0.8932	1.0576
A*29B*52	4	0.35	0	0.00	1	0.54	3	0.33	-0.4700	0.4556	-0.1573
A*29B*53	6	0.52	0	0.00	3	1.63	3	0.33	-0.5764	2.2130	-1.6830
A*29B*57	2	0.17	0	0.00	0	0.00	2	0.22	-0.3319	-0.6308	0.7469
A*29B*58	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*30B*07	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*30B*08	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*30B*14	2	0.17	0	0.00	0	0.00	2	0.22	-0.3319	-0.6308	0.7469
A*30B*15	4	0.35	0	0.00	1	0.54	3	0.33	-0.4700	0.4556	-0.1573
A*30B*18	3	0.26	0	0.00	1	0.54	2	0.22	-0.4068	0.7834	-0.4867
A*30B*35	9	0.78	1	1.69	1	0.54	7	0.77	0.8021	-0.4421	-0.0336
A*30B*38	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*30B*39	2	0.17	0	0.00	0	0.00	2	0.22	-0.3319	-0.6308	0.7469
A*30B*40	3	0.26	0	0.00	1	0.54	2	0.22	-0.4068	0.7834	-0.4867
A*30B*42	2	0.17	0	0.00	1	0.54	1	0.11	-0.3319	1.2742	-0.9691
A*30B*44	4	0.35	0	0.00	1	0.54	3	0.33	-0.4700	0.4556	-0.1573
A*30B*45	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278

A*30B*48	2	0.17	0	0.00	0	0.00	2	0.22	-0.3319	-0.6308	0.7469
A*30B*49	3	0.26	1	1.69	0	0.00	2	0.22	2.1972	-0.7730	-0.4867
A*30B*50	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*30B*51	6	0.52	0	0.00	1	0.54	5	0.55	-0.5764	0.0074	0.3037
A*30B*52	3	0.26	0	0.00	0	0.00	3	0.33	-0.4068	-0.7730	0.9153
A*30B*53	4	0.35	0	0.00	2	1.09	2	0.22	-0.4700	1.8045	-1.3723
A*30B*57	2	0.17	0	0.00	1	0.54	1	0.11	-0.3319	1.2742	-0.9691
A*30B*58	3	0.26	0	0.00	0	0.00	3	0.33	-0.4068	-0.7730	0.9153
A*31B*07	5	0.44	0	0.00	0	0.00	5	0.55	-0.5258	-0.9993	1.1833
A*31B*08	2	0.17	0	0.00	1	0.54	1	0.11	-0.3319	1.2742	-0.9691
A*31B*14	4	0.35	0	0.00	0	0.00	4	0.44	-0.4700	-0.8932	1.0576
A*31B*15	6	0.52	0	0.00	0	0.00	6	0.66	-0.5764	-1.0955	1.2971
A*31B*18	3	0.26	0	0.00	0	0.00	3	0.33	-0.4068	-0.7730	0.9153
A*31B*27	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*31B*39	10	0.87	0	0.00	5	2.72	5	0.55	-0.7461	2.8647	-2.1786
A*31B*40	6	0.52	0	0.00	1	0.54	5	0.55	-0.5764	0.0074	0.3037
A*31B*41	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*31B*42	4	0.35	0	0.00	0	0.00	4	0.44	-0.4700	-0.8932	1.0576
A*31B*49	2	0.17	0	0.00	0	0.00	2	0.22	-0.3319	-0.6308	0.7469

A*31B*50	1	0.09	0	0.00	1	0.54	0	0.00	-0.2345	2.2466	-1.8973
A*31B*51	9	0.78	1	1.69	1	0.54	7	0.77	0.8021	-0.4421	-0.0336
A*31B*52	7	0.61	0	0.00	2	1.09	5	0.55	-0.6230	0.8594	-0.4386
A*31B*53	4	0.35	1	1.69	1	0.54	2	0.22	1.7866	0.4556	-1.3723
A*31B*55	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*31B*56	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*31B*57	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*31B*58	3	0.26	0	0.00	0	0.00	3	0.33	-0.4068	-0.7730	0.9153
A*32B*08	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*32B*14	1	0.09	0	0.00	1	0.54	0	0.00	-0.2345	2.2466	-1.8973
A*32B*15	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*32B*35	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*32B*39	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*32B*44	4	0.35	0	0.00	1	0.54	3	0.33	-0.4700	0.4556	-0.1573
A*32B*45	2	0.17	0	0.00	0	0.00	2	0.22	-0.3319	-0.6308	0.7469
A*32B*47	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*32B*51	4	0.35	0	0.00	1	0.54	3	0.33	-0.4700	0.4556	-0.1573
A*32B*52	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*32B*53	3	0.26	0	0.00	1	0.54	2	0.22	-0.4068	0.7834	-0.4867

A*32B*58	2	0.17	0	0.00	0	0.00	2	0.22	-0.3319	-0.6308	0.7469
A*32B*81	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*33B*15	2	0.17	0	0.00	0	0.00	2	0.22	-0.3319	-0.6308	0.7469
A*33B*35	2	0.17	0	0.00	1	0.54	1	0.11	-0.3319	1.2742	-0.9691
A*33B*48	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*33B*49	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*33B*50	1	0.09	1	1.69	0	0.00	0	0.00	4.2696	-0.4457	-1.8973
A*33B*51	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*33B*52	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*33B*53	3	0.26	0	0.00	0	0.00	3	0.33	-0.4068	-0.7730	0.9153
A*33B*57	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*33B*57	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*33B*58	2	0.17	0	0.00	0	0.00	2	0.22	-0.3319	-0.6308	0.7469
A*34B*18	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*34B*35	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*34B*40	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*34B*41	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*34B*44	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*34B*53	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278

A*36B*14	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*36B*15	2	0.17	0	0.00	2	1.09	0	0.00	-0.3319	3.1793	-2.6850
A*66B*35	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*66B*39	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*66B*41	2	0.17	0	0.00	0	0.00	2	0.22	-0.3319	-0.6308	0.7469
A*66B*52	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*66B*57	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*68B*07	4	0.35	0	0.00	0	0.00	4	0.44	-0.4700	-0.8932	1.0576
A*68B*08	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*68B*14	2	0.17	0	0.00	0	0.00	2	0.22	-0.3319	-0.6308	0.7469
A*68B*15	6	0.52	0	0.00	1	0.54	5	0.55	-0.5764	0.0074	0.3037
A*68B*18	5	0.44	0	0.00	1	0.54	4	0.44	-0.5258	0.2079	0.0958
A*68B*39	5	0.44	0	0.00	0	0.00	5	0.55	-0.5258	-0.9993	1.1833
A*68B*40	3	0.26	0	0.00	0	0.00	3	0.33	-0.4068	-0.7730	0.9153
A*68B*41	2	0.17	0	0.00	0	0.00	2	0.22	-0.3319	-0.6308	0.7469
A*68B*42	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*68B*44	6	0.52	0	0.00	2	1.09	4	0.44	-0.5764	1.1102	-0.6897
A*68B*49	2	0.17	0	0.00	0	0.00	2	0.22	-0.3319	-0.6308	0.7469
A*68B*51	4	0.35	0	0.00	0	0.00	4	0.44	-0.4700	-0.8932	1.0576

A*68B*52	2	0.17	0	0.00	0	0.00	2	0.22	-0.3319	-0.6308	0.7469
A*68B*53	4	0.35	1	1.69	2	1.09	1	0.11	1.7866	1.8045	-2.5873
A*68B*57	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*68B*58	8	0.70	0	0.00	4	2.17	4	0.44	-0.6665	2.5588	-1.9460
A*68B*78	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*69B*58	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*74B*07	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*74B*14	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*74B*35	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*74B*35	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*74B*40	1	0.09	0	0.00	0	0.00	1	0.11	-0.2345	-0.4457	0.5278
A*74B*44	2	0.17	0	0.00	1	0.54	1	0.11	-0.3319	1.2742	-0.9691
A*74B*50	2	0.17	0	0.00	1	0.54	1	0.11	-0.3319	1.2742	-0.9691
A*74B*57	1	0.09	1	1.69	0	0.00	0	0.00	4.2696	-0.4457	-1.8973
A*74B*58	2	0.17	0	0.00	0	0.00	2	0.22	-0.3319	-0.6308	0.7469
A*80B*18	1	0.09	0	0.00	1	0.54	0	0.00	-0.2345	2.2466	-1.8973

891

892

3. CONSIDERAÇÕES FINAIS

O conjunto dos resultados apresentados gerou conclusões nas áreas da epidemiologia, imunologia e imunogenética da infecção por HIV e EBV.

Mostramos que no período do estudo a prevalência de novos casos de infecção por HIV tendeu a estabilidade, enquanto a infecção primária por EBV mostrou um notório crescimento, principalmente em pacientes coinfectados por HIV, sendo o genótipo EBV-1 o mais frequente tanto na população geral quanto nos coinfectados. Na progressão da infecção por HIV observamos a proporcionalidade de casos de respondedores e não respondedores terapêuticos. E dentre os diferentes perfis sorológicos da infecção por EBV, a baixa prevalência da infecção primária estava dentro do esperado para o perfil etário da população do estudo.

Ratificamos que o consumo de drogas ilícitas, baixa escolaridade e renda familiar, o não uso de preservativos, a orientação homoafetiva, ter múltiplos parceiros sexuais e o histórico de DST's continuam sendo indidicadores relacionados ao risco da infecção por HIV e à coinfecção HIV/EBV. Quando enfatizamos no EBV, fatores epidemiológicos não aparentaram estar influenciando nos perfis de infecção, apenas o histórico de coinfecções foi associado ao quadro primário.

Associamos os genótipos do EBV a diferentes perfis imunológicos na coinfecção por HIV. Enquanto o EBV-2 prevaleceu em pacientes que apresentavam uma boa resposta no controle do HIV, no outro extremo, o EBV-1 foi relacionado a um perfil de sobressaliência do HIV e depleção imunológica. Nesse contexto, pacientes com apresentação tardia e polissintomáticos possuiam alterações significativas na carga viral de ambos os vírus e nos marcadores imunológicos estudados.

Observamos uma transição do perfil imunológico ativo na infecção primária por EBV para um perfil imunológico balanceado nos pacientes com infecção passada, e alguns desses achados imunológicos foram relacionados a coinfecção por HIV presente na maioria dos pacientes.

Por fim, sugerimos que os alelos *HLA-B*13, B*35* e *B*39* foram associados a não resposta terapêutica na infecção por HIV e os alelos *HLA-DRB1*03, DRB1*09* e *DRB1*16* a manutenção da resposta imunológica em diferentes fases da infecção por EBV.

O presente estudo abrange aspectos multifatoriais da infecção por HIV e EBV no estado do Pará. Ao que consta, em alguns pontos contribuimos de forma inédita com os conhecimentos até então publicados sobre as temáticas, o que o torna ainda mais relevante se levarmos em consideração a carência de estudos do mesmo perfil na região amazônica brasileira.

4. REFERÊNCIAS BIBLIOGRÁFICAS

ABBOTT RJ, PACHNIO A, PEDROZA-PACHECO I, LEESE AM, BEGUM J, LONG HM, CROOM-CARTER D, STACEY A, MOSS PAH, HISLOP AD, BORROW P, RICKINSON AB, BELL AI. Asymptomatic Primary Infection with Epstein-Barr Virus: Observations on Young Adult Cases. **Journal of Virology 91 (21)**: 23, 2017.

ADAMSON AL, DARR D, HOLLEY-GUTHRIE E, JOHNSON RA, MAUSER A, SWENSON J, KENNEY S. Epstein-Barr virus immediate-early proteins BZLF1 and BRLF1 activate the ATF2 transcription factor by increasing the levels of phosphorylated p38 and c-Jun N-terminal kinases. **Journal of Virology 74 (3)**: 1224-1243, 2000.

ADLER B, SCHAADT E, KEMPKES B, ZIMBER-STROBL U, BAIER B, BORNKAMM GW. Control of Epstein-Barr virus reactivation by activated CD40 and viral latent membrane protein 1.**Proceedings of the National Academy of Sciences of the United States of America 99 (1)**: 437-442, 2002.

AGOSTINI S, MANCUSO R, GUERINI FR, D'ALFONSO S, AGLIARDI C, HERNIS A, ZANZOTTERA M, BARIZZONE N, LEONE MA, CAPUTO D, ROVARIS M, CLERICI M. HLA alleles modulate EBV viral load in multiple sclerosis. **Journal of Translational Medicine 16 (1)**: 80, 2018.

AGUILAR LK, ROONEY CM, HESLOP HE. Lymphoproliferative disorders involving Epstein-Barr virus after hemopoietic stem cell transplantation. **Current Opinion in Oncology 11 (2)**: 96-101, 1999.

ALFIERI C, TANNER J, CARPENTIER L, PERPÊTE C, SAVOIE A, PARADIS K, DELAGE G, JONCAS J. Epstein-Barr virus transmission from a blood donor to an organ transplant recipient with recovery of the same virus strain from the recipient's blood and oropharynx. **Blood 87 (2)**: 812-817, 1996.

AL-JABRI AA. How does HIV-1 infect a susceptible human cell?: Current thinking. **Journal for Scientific Research. Medical Sciences 5 (1-2)**: 31-44, 2003.

ALTER G, TEIGEN N, AHERN R, STREECK H, MEIER A, ROSENBERG ES, ALTFELD M. Evolution of innate and adaptive effector cell functions during acute HIV-1 infection. **The Journal of Infectious Diseases 195 (10)**: 1452-1460, 2007.

ALTFELD M, VAN LUNZEN J, FRAHM N, YU XG, SCHNEIDER C, ELDRIDGE RL, FEENEY ME, MEYER-OLSON D, STELLBRINK HJ, WALKER BD. Expansion of pre-existing, lymph node-localized CD8+ T cells during supervised treatment interruptions in chronic HIV-1 infection. **The Journal of Clinical Investigation 109 (6)**: 837-843, 2002.

ALEXAKI A, LIU Y, WIGDAHL B. Cellular reservoirs of HIV-1 and their role in viral persistence. **Current HIV Research 6 (5): 388-400**, 2008.

ALLDAY, M.J., CRAWFORD, D.H. Role of epithelium in EBV persistence and pathogenesis of B-cell tumours. **Lancet 1(8590)**:855-857, 1988

AMBINDER R.F. &MANNR.B. Detection and characterization of Epstein-Barr virus in clinical specimens. **The American Journal of Pathology 145(2)**:239-252, 1994.

ANDRADE TA, EVANGELISTA AF, CAMPOS AH, POLES WA, BORGES NM, CAMILLO CM, SOARES FA, VASSALLO J, PAES RP, ZERBINI MC, SCAPULATEMPO C, ALVES AC, YOUNG KH, COLLEONI GW.A microRNA signature profile in EBV+ diffuse large B-cell lymphoma of the elderly. **Oncotarget 5 (23)**: 11813-11826, 2014.

ANDREWS, D.M., ANDONIOU, C.E., FLEMING, P., SMYTH, M.J., DEGLI-ESPOSTI, M.A. The early kinetics of cytomegalovirus-specific CD8+ T-cell responses are not affected by antigen load or the absence of perforin or gamma interferon. **Journal of Virology 82(10)**:4931-4937, 2008.

APOLLONI A & SCULLEY TB. Detection of A-type and B-type Epstein-Barr virus in throat washings and lymphocytes. **Virology 202 (2)**: 978-981, 1994.

ARVIN A, CAMPADELLI-FIUME G, MOCARSKI E, MOORE PS, ROIZMAN B, WHITLEY R, YAMANISHI K. **Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis**. Cambridge, Cambridge University Press, 2007.

AYOUBA A, AKOUA-KOFFI C, CALVIGNAC-SPENCER S, ESTEBAN A, LOCATELLI S, LI H, LI Y, HAHN BH, DELAPORTE E, LEENDERTZ FH, PEETERS M. Evidence for continuing cross-species transmission of SIVsmm to humans: characterization of a new HIV-2 lineage in rural Côte d'Ivoire. **AIDS 27 (15)**: 2488-2491, 2013.

AYO CM, DA SILVEIRA CAMARGO AV, XAVIER DH, BATISTA MF, CARNEIRO OA, BRANDÃO DE MATTOS CC, RICCI O JR, DE MATTOS LC. Frequencies of allele groups HLA-A, HLA-B and HLA-DRB1 in a population from the northwestern region of São Paulo State, Brazil. **International Journal of Immunogenetics 42 (1)**: 19-25, 2015.

AYRES M, AYRES JRM, AYRES DL, SANTOS AS. BioEstat 5.0: Aplicações Estatísticas nas Áreas de Ciências Biológicas e Médicas. Belém: Sociedade Civil Mamirauá; Brasília: CNPq, 2008.

BALFOUR HH JR, SIFAKIS F, SLIMAN JA, KNIGHT JA, SCHMELING DO, THOMAS W. Age-specific prevalence of Epstein-Barr virus infection among individuals aged 6-19 years in the United States and factors affecting its acquisition. **The Journal of Infectious Diseases 208 (8)**: 1286-1293, 2013.

BALLANA E, RUIZ-DE ANDRES A, MOTHE B, RAMIREZ DE ARELLANO E, AGUILAR F, BADIA R, GRAU E, CLOTET B, DEL VAL M, BRANDER C, ESTÉ JA.Differential prevalence of the HLA-C -- 35 CC genotype among viremic long term non-progressor and elite controller HIV+ individuals. **Immunobiology 217 (9)**: 889-894, 2012.

BARRÉ-SINOUSSI F, CHERMANN JC, REY F, NUGEYRE MT, CHAMARET S, GRUEST J, DAUGUET C, AXLER-BLIN C, VÉZINET-BRUN F, ROUZIOUX C, ROZENBAUM W, MONTAGNIER L. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS).**Science 220 (4599):** 868-871, 1983.

BARROS RS, MATTOS PJSM, CABRAL LJB, PARENTE JSC, FILHO JSB, PORCHERA DCR, FILHO ESM, JORGE EVO, PALMEIRA MK, PEREIRA LMCM, GUERREIRO JF. Evaluation of the allelic and haplotypic frequency of the HLA system for HLA-A, HLA-B e HLA-DRB1 loci of renal receptors and living donors from the state of Pará, Brazil. **Brazilian Journal of Development 7 (2)**: 18826-18843, 2021.

BASHAW JM&YATES JL.Replication from oriP of Epstein-Barr virus requires exact spacing of two bound dimers of EBNA1 which bend DNA. **Journal of Virology 75(22)**: 10603-10611, 2001.

BASMACIOGULLARI S & PIZZATO M. The activity of Nef on HIV-1 infectivity. **Frontiers in Microbiology 5**: 232, 2014.

BASTOS MS, LESSA N, NAVECA FG, MONTE RL, BRAGA WS, FIGUEIREDO LT, RAMASAWMY R, MOURÃO MP. Detection of Herpesvirus, Enterovirus, and Arbovirus infection in patients with suspected central nervous system viral infection in the Western Brazilian Amazon. **Journal of Medical Virology 86 (9)**: 1522-1527, 2014.

BASU S & SRIVASTAVA PK. Calreticulin, a peptide-binding chaperone of the endoplasmic reticulum, elicits tumor- and peptide-specific immunity. **The Journal of Experimental Medicine 189 (5)**: 797-802, 1999.

BELOUKAS A, PSARRIS A, GIANNELOU P, KOSTAKI E, HATZAKIS A, PARASKEVIS D. Molecular epidemiology of HIV-1 infection in Europe: An overview. **Infection, Genetics and Evolution 46**: 180-189, 2016.

BELLOWS DS, HOWELL M, PEARSON C, HAZLEWOOD SA, HARDWICK JM. Epstein-Barr virus BALF1 is a BCL-2-like antagonist of the herpesvirus antiapoptotic BCL-2 proteins. **Journal of Virology 76 (5)**: 2469-2479, 2002.

BLACKARD JT, COHEN DE, MAYER KH. Human immunodeficiency virus superinfection and recombination: current state of knowledge and potential clinical consequences. **Clinical Infectious Diseases 34 (8)**: 1108-1114, 2002.

BLACKWELL JM, JAMIESON SE, BURGNER D.HLA and infectious diseases. **Clinical Microbiology Reviews 22 (2)**: 370-385, 2009.

BLISSENBAGH M, GREWE B, HOFFMANM B, BRANDT S, ÜBERLA K. Nuclear RNA Export and Packaging Functions of HIV-1 Rev Revisited. **Journal of Virology 84 (13)**: 6598-6604, 2010.

BLOSS TA & SUGDEN B. Optimal lengths for DNAs encapsidated by Epstein-Barr virus. **Journal of Virology 68(12)**:8217-8222, 1994.

BOCKER JF, TIEDEMANN KH, BORNKAMM GW, ZUR HAUSEN H. Characterization of an EBV-like virus from African green monkey lymphoblasts. **Virology 101 (1)**: 291-295, 1980.

BODMER JG, MARSH SG, ALBERT ED, BODMER WF, BONTROP RE, DUPONT B, ERLICH HA, HANSEN JA, MACH B, MAYR WR, PARHAM P, PETERSDORF EW, SASAZUKI T, SCHREUDER GM, STROMINGER JL, SVEJGAARD A, TERASAKI PI. Nomenclature for factors of the HLA system, 1998.**Vox Sanguinis 77 (3)**: 164-191, 1999.

BOLIS V, KARADEDOS C, CHIOTIS I, CHALIASOS N, TSABOURI S. Atypical manifestations of Epstein–Barr virus in children: a diagnostic challenge. **Jornal de Pediatria 92 (2)**: 113-121, 2016.

BOQUETT JA, BISSO-MACHADO R, ZAGONEL-OLIVEIRA M, SCHÜLER-FACCINI L, FAGUNDES NJR. HLA diversity in Brazil. **HLA 95 (1)**: 3-14, 2020.

BORZA CM, MORGAN AJ, TURK SM, HUTT-FLETCHER LM. Use of gHgL for attachment of Epstein-Barr virus to epithelial cells compromises infection. **Journal of Virology 78 (10)**: 5007-5014, 2004.

BOUVARD V, BAAN R, STRAIF K, GROSSE Y, SECRETAN B, EL GHISSASSI F, BENBRAHIM-TALLAA L, GUHA N, FREEMAN C, GALICHET L, COGLIANO V; WHO INTERNATIONAL AGENCY FOR RESEARCH ON CANCER MONOGRAPH WORKING GROUP.A review of human carcinogens--Part B: biological agents. **The Lancet Oncology 10 (4)**: 321-322, 2009.

BRAUN DL, KOUYOS RD, BALMER B, GRUBE C, WEBER R, GÜNTHARD HF. Frequency and Spectrum of Unexpected Clinical Manifestations of Primary HIV-1 Infection. **Clinical Infectious Diseases 61 (6): 1013-1021**, 2015.

BRASIL. Ministério da Saúde. **Boletim Epidemiológico HIV AIDS 2021**. Brasília: Departamento de DST, AIDS e Hepatites virais, 72p., 2021.

BRENNAN CA, BODELLE P, COFFEY R, DEVARE SG, GOLDEN A, HACKETT J JR, HARRIS B, HOLZMAYER V, LUK KC, SCHOCHETMAN G, SWANSON P, YAMAGUCHI J, VALLARI A, NDEMBI N, NGANSOP C, MAKAMCHE F, MBANYA D, GÜRTLER LG, ZEKENG L, KAPTUÉ L. The prevalence of diverse HIV-1 strains was stable in Cameroonian blood donors from 1996 to 2004. **Journal of Acquired Immune Deficiency Syndromes 49 (4)**: 432-439, 2008.

BRENNAN RM, BURROWS SR. A mechanism for the HLA-A*01-associated risk for EBV+ Hodgkin lymphoma and infectious mononucleosis. **Blood 112 (6)**: 2589-90, 2008..

BRIGGS JAG, WILK T, WELKER R, KRÄUSSLICH H-G, FULLER S. Structural organization of authentic, mature HIV-1 virions and cores. **The EMBO Journal 22 (7)**: 1707-1715, 2003.

BRIGGS JAG, RICHES JD, GLASS B, BARTONOVA V, ZANETTI G, KRÄUSSLIC HG. Structure and assembly of immature HIV. **Proceedings of the National Academy of Sciences of the United States of America 106 (27)**: 11090-11095, 2009.

BUHLER S, SANCHEZ-MAZAS A. HLA DNA sequence variation among human populations: molecular signatures of demographic and selective events. **PLoS One 6 (2)**: e14643, 2011.

BURKITT D & O'CONOR GT. Malignant lymphoma in African children. I. A clinical syndrome. **Cancer 14**: 258-269, 1961.

BURKITT D. Etiology of Burkitt's lymphoma--an alternative hypothesis to a vectored virus. **Journal of the National Cancer Institute 42 (1)**: 19-28, 1969.

BURGDORF S, KAUTZ A, BÖHNERT V, KNOLLE PA, KURTS C. Distinct pathways of antigen uptake and intracellular routing in CD4 and CD8 T cell activation. **Science 316 (5824)**: 612-616, 2007.

BUZON V, NATRAJAN G, SCHIBLI D, CAMPELO F, KOZLOV MM, WEISSENHORN W. Crystal structure of HIV-1 gp41 including both fusion peptide and membrane proximal external regions. **PLOS Pathogens 6 (5)**: 7, 2010.

CAI X, SCHÄFER A, LU S, BILELLO JP, DESROSIERS RC, EDWARDS R, RAAB-TRAUB N, CULLEN BR. Epstein-Barr virus microRNAs are evolutionarily conserved and differentially expressed. **Plos Pathogens 2 (3)**: 236-247, 2006.

CAMPBELL SM, CROWE SM, MAK J. Lipid rafts and HIV-1: from viral entry to assembly of progeny virions. **Journal of Clinical Virology 22 (3)**: 217-227, 2001.

CAMPOS AHJFM, MOREIRA A, RIBEIRO KB, PAES RP, ZERBINI MC, ALDRED V, DE SOUZA CA, NETO CS, SOARES FA, VASSALLO J. Frequency of EBV associated classical Hodgkin lymphoma decreases over a 54-year period in a Brazilian population. **Scientific Reports 8 (1)**: 1849, 2018.

CANAAN A, HAVIV I, URBAN AE, SCHULZ VP, HARTMAN S, ZHANG Z, PALEJEV D, DEISSEROTH AB, LACY J, SNYDER M, GERSTEIN M, WEISSMAN SM.EBNA1 regulates cellular gene expression by binding cellular promoters. **Proceedings of the National Academy of Sciences of the United States of America 106 (52)**: 224221-22426, 2009.

CAO K, MOORMANN AM, LYKE KE, MASABERG C, SUMBA OP, DOUMBO OK, KOECH D, LANCASTER A, NELSON M, MEYER D, SINGLE R, HARTZMAN RJ, PLOWE CV, KAZURA J, MANN DL, SZTEIN MB, THOMSON G, FERNÁNDEZ-VIÑA MA. Differentiation between African populations is evidenced by the diversity of alleles and haplotypes of HLA class I loci. **Tissue Antigens 63 (4)**: 293-325, 2004.

CARDOZO DM, MARANGON AV, SELL AM, VISENTAINER JEL, SOUZA CA. **HLA and Infectious Diseases**. Disponível em: <https://www.intechopen.com/books/hla-and-associated-important-diseases/hla-and-infectious-diseases>. Acessado em: 04/07/2019.

CARRINGTON, M., NELSON, G.W., MARTIN, M.P., KISSNER, T., VLAHOV, D., GOEDERT, J.J., KASLOW, R., BUCHBINDER, S., HOOTS, K., O´BRIEN, S.J. HLA and HIV-1: heterozygote advantage and B*35-Cw*04 disadvantage. **Science 283**: 1748-1752, 1999.

CENTERS OF DISEASE CONTROL (CDC). Current trends revision of the case definition of Acquired Immunodeficiency Syndrome for national reporting—United States. **Morbidity and Mortality Weekly Report (MMWR) 34 (25):** 373–375, 1985.

CENTERS OF DISEASE CONTROL (CDC). Pneumocystis pneumonia--Los Angeles. **Mobbidity & Mortality Weekly Report (MMWR) 30 (21):** 250-252, 1981.

CHAKRABARTI L, GUYADER M, ALIZON M, DANIEL MD, DESROSIERS RC, TIOLLAIS P, SONIGO P. Sequence of simian immunodeficiency virus from macaque and its relationship to other human and simian retroviruses. **Nature 328:** 543–547, 1987.

CHAKRABORTY, S., RAHMAN, T., CHAKRAVORTY, R., KUCHTA, A., RABBY, A., SAHIUZZAMAN, M. HLA supertypes contribute in HIV type 1 cytotoxic T lymphocyte epitope clustering in Nef and Gag proteins. **AIDS Research and Human Retroviruses 29(2)**:270-278, 2013.

CHAMBUSO R, RAMESAR R, KAAMBO E, DENNY L, PASSMORE JA, WILLIAMSON AL, GRAY CM. Human Leukocyte Antigen (HLA) Class II -DRB1 and -DQB1 Alleles and the Association with Cervical Cancer in HIV/HPV Co-Infected Women in South Africa. **Journal of Cancer 10 (10)**: 2145-2152, 2019.

CHAN, S.H., DAY, N.E., KUNARATNAM, N., CHIA, K.B., SIMONS, M.J. HLA and nasopharyngeal carcinoma in Chinese-a further study. **International Journal of Cancer 32(2)**:171-176, 1983.

CHANG, C.H., KIST, N.C., STUART CHESTER, T.L., SREENU, V.B., HERMAN, M., LUO, M., LUNN, D., BELL, J., PLUMMER, F.A., BALL, T.B., KATZOURAKIS, A., IVERSEN, A.K. HIV-infected sex workers with beneficial HLA-variants are potential hubs for selection of HIV-1 recombinants that may affect disease progression. **Scientific Report 5**: 11253, 2015.

CHANG CM, YU KJ, MBULAITEYE SM, HILDESHEIM A, BHATIA K. The extent of genetic diversity of Epstein-Barr virus and its geographic and disease patterns: a need for reappraisal. **Virus Research 143 (2)**: 209-221, 2009.

CHAO C, SILVERBERG MJ, MARTÍNEZ-MAZA O, CHI M, ABRAMS DI, HAQUE R, ZHA HD, MCGUIRE M, XU L, SAID J. Epstein-Barr virus infection and expression of B-cell oncogenic markers in HIV-related diffuse large B-cell Lymphoma. **Clinical Cancer Research 18 (17)**:4702-4712, 2012.

CHEN DY, BALAMURUGAN A, NG HL, CUMBERLAND WG, YANG OO. Epitope targeting and viral inoculum are determinants of Nef-mediated immune evasion of HIV-1 from cytotoxic T lymphocytes. **Blood 120 (1)**: 100-11, 2012.

CHEN WG, CHEN YY, BACCHI MM, BACCHI CE, ALVARENGA M, WEISS LM. Genotyping of Epstein-Barr virus in Brazilian Burkitt's lymphoma and reactive lymphoid tissue. Type A with a high prevalence of deletions within the latent membrane protein gene. **The American Journal of Pathology 148 (1)**: 17-23, 1996.

CHEN Y, MASKRI O, CHAMINADE F, RENÉ B, BENKAROUN J, GODET J, MÉLY Y, MAUFFRET O, FOSSÉ P. Structural Insights into the HIV-1 Minus-strand Strong-stop DNA. **The Journal of Biological Chemistry 291 (7)**: 3468/3482, 2016.

CHESNOKOVA LS & HUTT-FLETCHER LM. Epstein-Barr virus infection mechanisms. **Chinese Journal of Cancer 33 (11)**: 545-548, 2014.

CHIKATA T, MURAKOSHI H, KOYANAGI M, HONDA K, GATANAGA H, OKA S, TAKIGUCHI M. Control of HIV-1 by an HLA-B*52:01-C*12:02 Protective Haplotype. **The Journal of Infectious Diseases 216 (11)**: 1415-1424, 2017.

CHIKATA T, TRAN GV, MURAKOSHI H, AKAHOSHI T, QI Y, NARANBHAI V, KUSE N, TAMURA Y, KOYANAGI M, SAKAI S, NGUYEN DH, NGUYEN DT, NGUYEN HT, NGUYEN TV, OKA S, MARTIN MP, CARRINGTON M, SAKAI K, NGUYEN KV, TAKIGUCHI M.HLA Class I-Mediated HIV-1 Control in Vietnamese Infected with HIV-1 Subtype A/E. **Journal of Virology 92 (5)**: 11, 2018.

CHIN YM, MUSHIRODA T, TAKAHASHI A, KUBO M, KRISHNAN G, YAP LF, TEO SH, LIM PV, YAP YY, PUA KC, KAMATANI N, NAKAMURA Y, SAM CK, KHOO AS, GRUPO DE ESTUDOS DE NPC DA MALÁSIA, NG CC.HLA-A SNPs and amino acid variants are associated with nasopharyngeal carcinoma in Malaysian Chinese. **International Journal of Cancer 136 (3)**: 678-687, 2015.

CHO Y, RAMER J, RIVAILLER P, QUINK C, GARBER RL, BEIER DR, WANG F. An Epstein-Barr-related herpesvirus from marmoset lymphomas. **Proceedings of the National Academy of Sciences of the United States of America 98 (3)**: 1224-1229, 2001.

CHUN TW, CARRUTH L, FINZI D, SHEN X, DIGIUSEPPE JA, TAYLOR H, HERMANKOVA M, CHADWICK K, MARGOLICK J, QUINN TC, KUO YH, BROOKMEYER R, ZEIGER MA, BARDITCH-CROVO P, SILICIANO RF. Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. **Nature 387 (6629): 183-188**, 1997.

CIUFFI A, LLANO M, POESCHLA E, HOFFMANN C, LEIPZIG J, SHINN P, ECKER JR, BUSHMAN F.A role for LEDGF/p75 in targeting HIV DNA integration. **Nature Medicine 11 (12)**: 1287-1289, 2005.

CLAVEL F, GUÉTARD D, BRUN-VÉZINET F, CHAMARET S, REY MA, SANTOS-FERREIRA MO, LAURENT AG, DAUGUET C, KATLAMA C, ROUZIOUX C. Isolation of a new human retrovirus from West African patients with AIDS. **Science 233 (4761)**: 343-346, 1986.

COHEN GB, GANDHI RT, DAVIS DM, MANDELBOIM O, CHEN BK, STROMINGER JL, BALTIMORE D. The selective downregulation of class I major histocompatibility complex proteins by HIV-1 protects HIV-infected cells from NK cells. **Immunity 10 (6)**: 661-71, 1999.

COFFIN J. HAASE A. LEVY JA. MONTAGNIER L. OROSZLAN S. TEICH N. TEMIN H. What to call the AIDS virus? **Nature 321:** 10, 1986.

CORREA RM, FELLNER MD, ALONIO LV, DURAND K, TEYSSIÉ AR, PICCONI MA. Epstein-barr virus (EBV) in healthy carriers: Distribution of genotypes and 30 bp deletion in latent membrane protein-1 (LMP-1) oncogene. **Journal of Medical Virology 73 (4)**: 583-588, 2004.

COSTA LJ, ARRUDA LB, MENDONÇA LM, LUCAS CGO. Síndrome da Imunodeficiência Adquirida/AIDS. In: **Virologia Humana**. Santos NSO, Romanos MTV, Wigg AD. 3ª eds. Rio de Janeiro: Guanabara Koogan, 2015. P. 1085-1162.

CRAIGIE R. The molecular biology of HIV integrase. **Future Virology 7 (7)**: 679-686, 2012.

CRAWFORD DH, SWERDLOW AJ, HIGGINS C, MCAULAY K, HARRISON N, WILLIAMS H, BRITTON K, MACSWEEN KF. Sexual history and Epstein-Barr virus infection. **The Journal of Infectious Diseases 186 (6)**: 731-736, 2002.

CREARY LE, GANGAVARAPU S, MALLEMPATI KC, MONTERO-MARTÍN G, CAILLIER SJ, SANTANIELLO A, HOLLENBACH JA, OKSENBERG JR, FERNÁNDEZ-VIÑA MA. Next-generation sequencing reveals new information about HLA allele and haplotype diversity in a large European American population. **Human Immunology 80 (10)**: 807-822, 2019.

CRUZ-TAPIAS P, CASTIBLANCO J, ANAYA J-M.Major histocompatibility complex: Antigen processing and presentation. In: **Autoimmunity: From Bench to Bedside**. Anaya J-M, Shoenfeld Y, Rojas-Villarraga A, Levy RA, Cervera R. Bogota (Colombia), El Rosario University Press, 2013.

DALMAU, J.; PUERTAS, M.C.; AZUARA, M. Contribution of immunological and virological factors to extremely severe primary HIV type 1 infection. **Clinical Infectious Diseases 48**: 229-238, 2009.

DAMTIE D, YISMAW G, WOLDEYOHANNES D, ANAGAW B. Common opportunistic infections and their CD4 cell correlates among HIV-infected patients attending at antiretroviral therapy clinic of Gondar University Hospital, Northwest Ethiopia. **BMC Research Notes 6**: 7, 2013.

DAW MA, EL-BOUZEDI A, AHMED MO, DAU AA, LIBYAN STUDY GROUP OF HEPATITIS & HIV. Molecular and epidemiological characterization of HIV-1 subtypes among Libyan patients. **BMC Research Notes 10 (1)**: 170, 2017.

DEACON EM, PALLESEN G, NIEDOBITEK G, CROCKER J, BROOKS L, RICKINSON AB, YOUNG LS. Epstein-Barr virus and Hodgkin's disease: transcriptional analysis of virus latency in the malignant cells. **The Journal of Experimental Medicine 177 (2)**: 339-349, 1993.

DELAMARRE L, PACK M, CHANG H, MELLMAN I, TROMBETTA ES. Differential lysosomal proteolysis in antigen-presenting cells determines antigen fate. **Science 307 (5715)**: 1630-1634, 2005.

DELATORRE E, COUTO-FERNANDEZ JC, BELLO G. HIV-1 Genetic Diversity in Northeastern Brazil: High Prevalence of Non-B Subtypes. **AIDS Research and Human Retroviruses 33 (7)**: 639-647, 2017.

DELELIS O, CARAYON K, SAÏB A, DEPREZ E, MOUSCADET JF. Integrase and integration: biochemical activities of HIV-1 integrase. **Retrovirology 5**: 13, 2008.

DELGADO E, THOMSON MM, VILLAHERMOSA ML, SIERRA M, OCAMPO A, MIRALLES C, RODRÍGUEZ-PÉREZ R, DIZ-AREN J, OJEA-DE CASTRO R, LOSADA E, CUEVAS MT, VÁZQUEZ-DE PARGA E, CARMONA R, PÉREZ-ALVAREZ L, MEDRANO L, CUEVAS L, TABOADA JA, NÁJERA R. Identification of a newly characterized HIV-1 BG intersubtype circulating recombinant form in Galicia, Spain, which exhibits a pseudotype-like virion structure. **Journal of Acquired Immune Deficiency Syndromes 29 (5)**: 536-543, 2002.

DI D, SANCHEZ-MAZAS A. HLA variation reveals genetic continuity rather than population group structure in East Asia. **Immunogenetics 66 (3)**: 153-60, 2014.

DIAO B, DU J, LIU Y, LUO F, HOU W. The association of HLA-DRB1 alleles and drug use with HIV infection in a Chinese Han Cohort. **The Brazilian Journal of Diseases 18 (1)**: 82-87, 2014.

DIEPSTRA, A., NIENS, M., MEERMAN, G.J., POPPEMA, S., VAN DEN BERG, A. Genetic susceptibility to Hodgkin's lymphoma associated with the human leukocyte antigen region. **European Journal of Haematology (66)**:34-41, 2005.

DOLAN BP, GIBBS KD JR, OSTRAND-ROSENBERG S. Dendritic cells cross-dressed with peptide MHC class I complexes prime CD8+ T cells. **Journal of Immunology 177 (9)**: 6018-6024, 2006.

DORRELL, L., WILLCOX, B.E., JONES, E.Y., GILLESPIE, G., NJAI, H., SABALLY, S., JAYE, A., DEGLERIA, K., ROSTRON, T., LEPIN, E., MCMICHAEL, A., WHITTLE, H., ROWLAND-JONES, S. Cytotoxic T lymphocytes recognize structurally diverse, clade-specific and cross-reactive peptides in human immunodeficiency virus type-1 gag through HLA-B53. **European Journal of Immunology 31(6)**:1747-1756, 2001.

DOUEK DC, ROEDERER M, KOUP RA. Emerging concepts in the immunopathogenesis of AIDS. **Annual Review of Medicine 60**: 471-484, 2009.

DRAGON AC, ZIMMERMANN K, NERRETER T, SANDFORT D, LAHRBERG J, KLÖß S, KLOTH C, MANGARE C, BONIFACIUS A, TISCHER-ZIMMERMANN S, BLASCZYK R, MAECKER-KOLHOFF B, UCHANSKA-ZIEGLER B, ABKEN H, SCHAMBACH A, HUDECEK M, EIZ-VESPER B. CAR-T cells and TRUCKs that recognize an EBNA-3C-derived epitope presented on HLA-B*35 control Epstein-Barr virus-associated lymphoproliferation. **Journal of Immunotherapy of Cancer 8 (2)**: e000736, 2020.

DUROVIC B, GASSER O, GUBSER P, SIGLE J, HIRSCH HH, STERN M, BUSER A, HESS C. Epstein-Barr virus negativity among individuals older than 60 years is associated with HLA-C and HLA-Bw4 variants and tonsillectomy. **Journal of Virology 87 (11)**: 6526-9, 2013.

EDWARDS JM &WOODROOF M.EB virus-specific IgA in serum of patients with infectious mononucleosis and of healthy people of different ages. **Journal of Clinical Pathology 32 (10)**: 1036-1040, 1979.

EHLERS B, SPIESS K, LEENDERTZ F, PEETERS M, BOESCH C, GATHERER D, MCGEOCH DJ. Lymphocryptovirus phylogeny and the origins of Epstein-Barr virus. **The Journal of General Virology 91**: 630-642, 2010.

EMBGENBROICH M & BURGDORF S. Current Concepts of Antigen Cross-Presentation. **Frontiers in Immunology 9 (1643)**: 10, 2018.

ENBOM M, STRAND A, FALK KI, LINDE A. Detection of Epstein-Barr virus, but not human herpesvirus 8, DNA in cervical secretions from Swedish women by real-time polymerase chain reaction. **Sexually Transmitted Diseases 28 (5)**: 300-306, 2001.

EPSTEIN A, ACHONG BG, BARR YM. Virus Particles In Cultured Lymphoblasts From Burkitt's Lymphoma. **Lancet 1 (7335)**: 702-703, 1964.

EPSTEIN A. On the discovery of Epstein-Barr virus: a memoir. **Epstein Barr Virus Report 6**: 58-63, 1999.

FALK KI & ERNBERG I. Demethylation of the Epstein-barr virus origin of lytic replication and of the immediate early gene BZLF1 is DNA replication independent. Brief report. **Archives of Virology 144 (11)**: 2219-2227, 1999.

FAN H, & GULLEY ML. Epstein-Barr viral load measurement as a marker of EBV-related disease. **Molecular Diagnosis 6 (4)**:279-289, 2001.

FASSONE L, CINGOLANI A, MARTINI M, MIGLIARETTI G, ORESTE PL, CAPELLO D, GLOGHINI A, VIVENZA D, DOLCETTI R, CARBONE A, ANTINORI A, GAIDANO G, LAROCCA LM. Characterization of Epstein-Barr virus genotype in AIDS-related non-Hodgkin's lymphoma. **AIDS Research and Human Retroviruses 18 (1)**: 19-26, 2002.

FEEDERLE R, KOST M, BAUMANN M, JANZ A, DROUET E, HAMMERSCHMIDT W, DELECLUSE HJ. The Epstein-Barr virus lytic program is controlled by the co-operative functions of two transactivators. **The EMBO Journal 19 (12)**: 3080-3089, 2000.

FERNANDEZ-CRUZ E, DESCO M, GARCIA MONTES M, LONGO N, GONZALEZ B, ZABAY J M. Immunological and serological markers predictive of progression to AIDS in a cohort of HIV-infected drug users. **Journal of AIDS 4:** 987–994, 1990.

FETTIG J, SWAMINATHAN M., MURRILL CS, KAPLAN JE. Global epidemiology of HIV. **Infectious Disease Clinics of North America 28 (3)**: 323-337, 2014.

FIGUEIRA-SILVA CM & PEREIRA FEL. Prevalence of Epstein-Barr virus antibodies in healthy children and adolescents in Vitória, State of Espírito Santo, Brazil. **Revista da Sociedade Brasileira de Medicina Tropical 37 (5)**: 409-412, 2004.

FIGUEIREDO LTM. Mononucleose infecciosa. In: **Tratado de clínica médica**. LOPES, A.C., 3ª ed. São Paulo: Editora Roca; 2009. p. 3862-4.

FINGEROTH JD, DIAMOND ME, SAGE DR, HAYMAN J, YATES JL.CD21-Dependent infection of an epithelial cell line, 293, by Epstein-Barr virus. **Journal of Virology 73 (3)**: 2115-2125, 1999.

FLEISHER G & BOLOGONESE R. Infectious mononucleosis during gestation: report of three women and their infants studied prospectively. **Pediatric Infectious Disease 3 (4)**: 308-311, 1984.

FRAPPIERL. EBNA1 and host factors in Epstein-Barr virus latent DNA replication. **Currente Opinion in Virology 2(6)**:733-739, 2012.

FRIIS AM, ÅKERLUND B, GYLLENSTEN K, ALEMAN A, ERNBERG I. Host-Epstein-Barr virus relationship affected by immunostimulation in HIV-infected patients representing distinct progressor profile groups. **Scandinavian Journal of Infectious Disease 44 (5)**:388-392, 2012.

FRIIS AM, GYLLENSTEN K, ALEMAN A, ERNBERG I, AKERLUND B. The Effect of Antiretroviral Combination Treatment on Epstein-Barr Virus (EBV) Genome Load in HIV-Infected Patients. **Viruses 2 (4)**: 867-879, 2010.

FUKUDA M & KAWAGUCHI Y. Role of the immunoreceptor tyrosine-based activation motif of latent membrane protein 2A (LMP2A) in Epstein-Barr virus LMP2A-induced cell transformation. **Journal of Virology 88 (9)**: 5188-5194, 2014.

GAO F, YUE L, WHITE AT, PAPPAS PG, BARCHUE J, HANSON AP, GREENE BM, SHARP PM, SHAW GM, HAHN BH. Human infection by genetically diverse SIVSM-related HIV-2 in west Africa. **Nature 358 (6386):** 495-499, 1992.

GAO F, VIDAL N, LI Y, TRASK SA, CHEN Y, KOSTRIKIS LG, HO DD, KIM J, OH MD, CHOE K, SALMINEN M, ROBERTSON DL, SHAW GM, HAHN BH, PEETERS M. Evidence of two distinct subsubtypes within the HIV-1 subtype A radiation. **AIDS Research and Human Retroviruses 17 (8)**: 675-688, 2001.

GAO M, MATUSICK-KUMAR L, HURLBURT W, DITUSA SF, NEWCOMB WW, BROWN JC, MCCANN PJ 3RD, DECKMAN I, COLONNO RJ. The protease of herpes simplex virus type 1 is essential for functional capsid formation and viral growth**. Journal of Virology 68 (6)**: 3702-3712, 1994.

GAO X., BASHIROVA A., IVERSEN A.K., PHAIR J., GOEDERT J.J., BUCHBINDER S., HOOTS K., VLAHOV D., ALTFELD M., O'BRIEN S.J., CARRINGTON M. AIDS restriction HLA allotypes target distinct intervals of HIV-1 pathogenesis. **Nature Medicine 11(12)**:1290-1292, 2005.

GARTLAND AJ, LI S, MCNEVIN J, TOMARAS GD, GOTTARDO R, JANES H, FONG Y, MORRIS D, GERAGHTY DE, KIJAK GH, EDLEFSEN PT, FRAHM N, LARSEN BB, TOVANABUTRA S, SANDERS-BUELL E, DECAMP AC, MAGARET CA, AHMED H, GOODRIDGE JP, CHEN L, KONOPA P, NARIYA S, STODDARD JN, WONG K, ZHAO H, DENG W, MAUST BS, BOSE M, HOWELL S, BATES A, LAZZARO M, O'SULLIVAN A, LEI E, BRADFIELD A, IBITAMUNO G, ASSAWADARACHAI V, O'CONNELL RJ, DESOUZA MS, NITAYAPHAN S, RERKS-NGARM S, ROBB ML, SIDNEY J, SETTE A, ZOLLA-PAZNER S, MONTEFIORI D, MCELRATH MJ, MULLINS JI, KIM JH, GILBERT PB, HERTZ T. Analysis of HLA A*02 association with vaccine efficacy in the RV144 HIV-1 vaccine trial. **Journal of Virology 88 (15)**: 8242-55, 2014.

GERMAN ADVISORY COMMITTEE BLOOD (ARBEITSKREIS BLUT), SUBGROUP ‘ASSESSMENT OF PATHOGENS TRANSMISSIBLE BY BLOOD’. Human Immunodeficiency Virus (HIV). **Transfusion Medicine and Hemoterapy 43 (3)**: 203-222, 2016.

GERMI R, EFFANTIN G, GROSSI L, RUIGROK RW, MORAND P, SCHOEHN G. Three-dimensional structure of the Epstein-Barr virus capsid. **The Journal of General Virology 93**: 1769-1773, 2012.

GFELLER D & BASSANI-STERNBERG M. Predicting Antigen Presentation-What Could We Learn From a Million Peptides? **Frontiers in Immunology 9**: 17, 2018.

GIANELLA S, GINOCCHIO CC, DAAR ES, DUBE MP, MORRIS SR. Genital Epstein Barr Virus is associated with higher prevalence and persistence of anal human papillomavirus in HIV-infected men on antiretroviral therapy. **BMC Infectious Diseases 16**: 24, 2016.

GIBSON W, ROIZMAN B. Compartmentalization of spermine and spermidine in the herpes simplex virion. **Proceedings of the National Academy of Sciences of the United States of America 68(11)**:2818-2821, 1971.

GIODINI A & CRESSWELL P.Hsp90-mediated cytosolic refolding of exogenous proteins internalized by dendritic cells. **The EMBO Journal 27 (1)**: 201-211, 2008.

GIRON LB, RAMOS DA SILVA S, BARBOSA AN, MONTEIRO DE BARROS ALMEIDA RA, ROSÁRIO DE SOUZA LD, ELGUI DE OLIVEIRA D. Impact of Epstein-Barr virus load, virus genotype, and frequency of the 30 bp deletion in the viral BNLF-1 gene in patients harboring the human immunodeficiency virus. **Journal of Medical Virology 85 (12)**:2110-2118, 2013.

GOMES STM, GOMES ÉR, DOS SANTOS MB, LIMA SS, QUEIROZ MAF, MACHADO LFA, CAYRES-VALLINOTO IMV, VALLINOTO ACR, DE O GUIMARÃES ISHAK M, ISHAK R. Immunological and virological characterization of HIV-1 viremia controllers in the North Region of Brazil. **BMC Infectious Diseases 17 (1)**: 381, 2017.

GONDOIS-REY F, BIANCOTTO A, PION M, CHENINE AL, GLUSCHANKOF P, HOREJSI V, TAMALET C, VIGNE R, HIRSCH I. Production of HIV-1 by resting memory T lymphocytes. **AIDS 15 (15): 1931-1940**, 2001.

GONZALEZ SM, TABORDA NA, CORREA LA, CASTRO GA, HERNANDEZ JC, MONTOYA CJ, RUGELES MT. Particular activation phenotype of T cells expressing HLA-DR but not CD38 in GALT from HIV-controllers is associated with immune regulation and delayed progression to AIDS. **Immunologic Research 64 (3)**: 765-774, 2016.

GONZALEZ-GALARZA FF, MCCABE A, SANTOS EJ, JONES J, TAKESHITA LY, ORTEGA-RIVERA ND, DEL CID-PAVON GM, RAMSBOTTOM K, GHATTAORAYA GS, ALFIREVIC A, MIDDLETON D, JONES AR. Allele frequency net database (AFND) 2020 update: gold-standard data classification, open access genotype data and new query tools **Nucleic Acid Research 2020 48**:D783-8.

GOULDER, P.J.; BRANDER, C.; TANG. Evolution and transmission of stable CTL escape mutations in HIV infection**. Nature 412**: 334-338, 2001.

GOULDER PJ, WALKER BD. HIV and HLA class I: an evolving relationship. **Immunity 37 (3)**: 426-40, 2012.

GRANDO LJ, MACHADO DC, SPITZER S, NACHMAN S, FERGUSON F, BERENTSEN B, YURGEL LS. Viral coinfection in the oral cavity of HIV-infected children: relation among HIV viral load, CD4+ T lymphocyte count and detection of EBV, CMV and HSV. **Brazilian Oral Research 19 (3)**:228-234, 2005.

GREENSPAN JS, GREENSPAN D, LENNETTE ET, ABRAMS DI, CONANT MA, PETERSEN V, FREESE UK. Replication of Epstein-Barr virus within the epithelial cells of oral "hairy" leukoplakia, an AIDS-associated lesion. **The New England Journal of Medicine 13 (25)**: 1564-1571, 1985.

GRUFFAT H, KADJOUF F, MARIAMÉ B, MANET E. The Epstein-Barr virus BcRF1 gene product is a TBP-like protein with an essential role in late gene expression. **Journal of Virology 86 (11)**: 6023-6032, 2012.

GRUNEWALD V, BONNET M, BOUTIN S, YIP T, LOUZIR H, LEVRERO M, SEIGNEURIN JM, RAPHAEL M, TOUITOU R, MARTEL-RENOIR D, COCHET C, DURANDY A, ANDRE P, LAU W, ZENG Y, JOAB I. Amino-acid change in the Epstein-Barr-virus ZEBRA protein in undifferentiated nasopharyngeal carcinomas from Europe and North Africa. **International Journal of Cancer 75 (4)**: 497-503, 1998.

GUARNER J, DEL RIO C, CARR D, HENDRIX LE, ELEY JW, UNGER ER. Non-Hodgkin's lymphomas in patients with human immunodeficiency virus infection. Presence of Epstein-Barr virus by in situ hybridization, clinical presentation, and follow-up. **Cancer 68 (11)**:2460-2465, 1991

GUIMARÃES ML, MARQUES BC1, BERTONI N2, TEIXEIRA SL1, MORGADO MG1, BASTOS FI2; BRAZILIAN MULTICITY STUDY GROUP ON DRUG MISUSE. Assessing the HIV-1 Epidemic in Brazilian Drug Users: A Molecular Epidemiology Approach. **Plos One 10 (11)**: 17, 2015.

GUTIÉRREZ MI, IBRAHIM MM, DALE JK, GREINER TC, STRAUS SE, BHATIA K. Discrete alterations in the BZLF1 promoter in tumor and non-tumor-associated Epstein-Barr virus. **Journal of the National Cancer Institute 94 (23)**: 1757-1763, 2002.

GULLEY ML. Molecular diagnosis of Epstein-Barr virus-related diseases. **The Journal of Molecular Diagnostics 3(1)**:10, 2001.

GULZAR N & COPELAND KF.CD8+ T-cells: function and response to HIV infection. **Current HIV Research 2 (1)**: 23-37, 2004.

HAQUE T, WILKIE GM, JONES MM, HIGGINS CD, URQUHART G, WINGATE P, BURNS D, MCAULAY K, TURNER M, BELLAMY C, AMLOT PL, KELLY D, MACGILCHRIST

A, GANDHI MK, SWERDLOW AJ, CRAWFORD DH. Allogeneic cytotoxic T-cell therapy for EBV-positive posttransplantation lymphoproliferative disease: results of a phase 2 multicenter clinical trial. **Blood 110 (4)**: 1123-1131, 2007.

HAMMERSCHMIDT W & SUGDEN B. Replication of Epstein-Barr viral DNA. **Cold Spring Harbor Perspectives in Biology 5 (1)**: 13, 2013.

HANNA DB, GUPTA LS, JONES LE, THOMPSON DM, KELLERMAN SE, SACKOFF JE.AIDS-defining opportunistic illnesses in the HAART era in New York City. **AIDS Care 19 (2)**: 264-272, 2007.

HARRINGTON WJJr. New therapeutic approaches for HIV and EBV related lymphomas. **Brazilian Journal of Infectious Diseases 9 (5)**: 436-437, 2005.

HASSAN R, KLUMB CE, FELISBINO FE, GUIRETTI DM, WHITE LR, STEFANOFF CG, BARROS MH, SEUÁNEZ HN, ZALCBERG IR. Clinical and demographic characteristics of Epstein-Barr virus-associated childhood Burkitt's lymphoma in Southeastern Brazil: epidemiological insights from an intermediate risk region. **Haematologica 93 (5)**: 780-783, 2008.

HAZUDA DJ, HASTINGS JC, WOLFE AL, EMINI EA.A novel assay for the DNA strand-transfer reaction of HIV-1 integrase. **Nucleic Acids Research 22 (6)**: 1121-1122, 1994.

HEMELAAR J, GOUWS E, GHYS PD, OSMANOV S. Global and regional distribution of HIV-1 genetic subtypes and recombinants in 2004. **AIDS 20 (16)**: 13-23, 2006.

HENLE W, DIEHL V, KOHN G, ZUR HAUSEN H, HENLE G. Herpes-type virus and chromosome marker in normal leukocytes after growth with irradiated Burkitt cells. **Science 157 (3792)**: 1064-1065, 1967.

HENLE G, HENLE W, DIEHL V. Relation of Burkitt's tumor-associated herpes-ytpe virus to infectious mononucleosis. **Proceedings of the National Academy of Sciences of the United States of America 59 (1)**: 94-101, 1968.

HENLE G, HENLE W, CLIFFORD P, DIEHL V, KAFUKO GW, KIRYA BG, KLEIN G, MORROW RH, MUNUBE GM, PIKE P, TUKEI PM, ZIEGLER JL. Antibodies to Epstein-Barr virus in Burkitt's lymphoma and control groups. **Journal of the National Cancer Institute 43 (5)**: 1147-1157, 1969.

HENSON BW, PERKINS EM, COTHRAN JE, DESAI P. Self-assembly of Epstein-Barr virus capsids. **Journal of Virology 83 (8)**: 3877-3890, 2009.

HJALGRIM H, FRIBORG J, MELBYE M. The epidemiology of EBV and its association with malignant disease. In: **Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis**. Cambridge, Cambridge University Press, 2007.

HJALGRIM, H., ROSTGAARD, K., JOHNSON, P.C.D., LAKE, A., SHIELD, L., LITTLE, A.M., EKSTROM-SMEDBY, K., ADAMI, H.O., GLIMELIUS, B., HAMILTON-DUTOIT, S., KANE, E., TAYLOR, G.M., MCCONNACHIE, A., RYDER, L.P., SUNDSTROM, C., ANDERSEN, P.S., CHANG, E.T., ALEXANDER, F.E., MELBYE, M., JARRETT, R.F. HLAA alleles and infectious mononucleosis suggest a critical role for cytotoxic T-cell response in EBV-related Hodgkin lymphoma. **Proceedings of the National Academy of Sciences 107**: 6400–6405, 2010.

HISLOP AD, TAYLOR GS, SAUCE D, RICKINSON AB. Cellular responses to viral infection in humans: lessons from Epstein-Barr virus. **Annual Review of Immunology 25**: 587-617, 2007.

HLADIK F, MCELRATH MJ. Setting the stage: host invasion by HIV**. Nature Reviews Immunology 8 (6)**: 447-457, 2008.

HSU JL & GLASER SL. Epstein-barr virus-associated malignancies: epidemiologic patterns and etiologic implications. **Critical Reviews in Oncology Hematology 34 (1)**: 27-53, 2000.

HU LF, ZABAROVSKY ER, CHEN F, CAO SL, ERNBERG I, KLEIN G, WINBERG G. Isolation and sequencing of the Epstein-Barr virus BNLF-1 gene (LMP1) from a Chinese nasopharyngeal carcinoma. **The Journal of General Virology 72**: 2399-2409, 1991.

HUANG X, LING H, MAO W, DING X, ZHOU Q, HAN M, WANG F, CHENG L, XIONG H. Association of HLA-A, B, DRB1 alleles and haplotypes with HIV-1 infection in Chongqing, China. **BMC Infectious Diseases 9**: 201, 2009.

HUANG X, KUSHEKHAR K, NOLTE I, KOOISTRA W, VISSER L, BOUWMAN I, KOUPRIE N, VEENSTRA R, VAN IMHOFF G, OLVER B, HOULSTON RS, POPPEMA S, DIEPSTRA A, HEPKEMA B, VAN DEN BERG A. Multiple HLA class I and II associations in classical Hodgkin lymphoma and EBV status defined subgroups. **Blood 118 (19)**: 5211-7, 2011.

HUTT-FLETCHER LM. Epstein-Barr virus entry. **Journal of Virology 81 (15)**: 7825-7832, 2007.

IARC (International Agency for Research on Cancer). Disponível em< https://www.iarc.fr/index.php>. Acessado em: 14/05/2019.

INOTAI D, SZILVASI A, BENKO S, BOROS-MAJOR A, ILLES Z, BORS A, KISS KP, RAJCZY K, GELLE-HOSSÓ A, BUHLER S, NUNES JM, SANCHEZ-MAZAS A, TORDAI A. HLA genetic diversity in Hungarians and Hungarian Gypsies: complementary differentiation patterns and demographic signals revealed by HLA-A, -B and -DRB1 in Central Europe. **Tissue Antigens 86 (2)**: 115-21, 2015.

INTERNATIONAL COMMITTEE ON TAXONOMY OF VIRUSES (ICTV). Disponível em: <https://talk.ictvonline.org/taxonomy/>. Acessado em: 03/04/2019.

International HIV Controllers Study, PEREYRA F, JIA X, MCLAREN PJ, TELENTI A, DE BAKKER PI, WALKER BD, RIPKE S, BRUMME CJ, PULIT SL, CARRINGTON M, KADIE CM, CARLSON JM, HECKERMAN D, GRAHAM RR, PLENGE RM, DEEKS SG, GIANNINY L, CRAWFORD G, SULLIVAN J, GONZALEZ E, DAVIES L, CAMARGO A, MOORE JM, BEATTIE N, GUPTA S, CRENSHAW A, BURTT NP, GUIDUCCI C, GUPTA N, GAO X, QI Y, YUKI Y, PIECHOCKA-TROCHA A, CUTRELL E, ROSENBERG R, MOSS KL, LEMAY P, O'LEARY J, SCHAEFER T, VERMA P, TOTH I, BLOCK B, BAKER B, ROTHCHILD A, LIAN J, PROUDFOOT J, ALVINO DM, VINE S, ADDO MM, ALLEN TM, ALTFELD M, HENN MR, LE GALL S, STREECK H, HAAS DW, KURITZKES DR, ROBBINS GK, SHAFER RW, GULICK RM, SHIKUMA CM, HAUBRICH R, RIDDLER S, SAX PE, DAAR ES, RIBAUDO HJ, AGAN B, AGARWAL S, AHERN RL, ALLEN BL, ALTIDOR S, ALTSCHULER EL, AMBARDAR S, ANASTOS K, ANDERSON B, ANDERSON V, ANDRADY U, ANTONISKIS D, BANGSBERG D, BARBARO D, BARRIE W, BARTCZAK J, BARTON S, BASDEN P, BASGOZ N, BAZNER S, BELLOS NC, BENSON AM, BERGER J, BERNARD NF, BERNARD AM, BIRCH C, BODNER SJ, BOLAN RK, BOUDREAUX ET, BRADLEY M, BRAUN JF, BRNDJAR JE, BROWN SJ, BROWN K, BROWN ST, BURACK J, BUSH LM, CAFARO V, CAMPBELL O, CAMPBELL J, CARLSON RH, CARMICHAEL JK, CASEY KK, CAVACUITI C, CELESTIN G, CHAMBERS ST, CHEZ N, CHIRCH LM, CIMOCH PJ, COHEN D, COHN LE, CONWAY B, COOPER DA, CORNELSON B, COX DT, CRISTOFANO MV, CUCHURAL G JR, CZARTOSKI JL, DAHMAN JM, DALY JS, DAVIS BT, DAVIS K, DAVOD SM, DEJESUS E, DIETZ CA, DUNHAM E, DUNN ME, ELLERIN TB, ERON JJ, FANGMAN JJ, FAREL CE, FERLAZZO H, FIDLER S, FLEENOR-FORD A, FRANKEL R, FREEDBERG KA, FRENCH NK, FUCHS JD, FULLER JD, GABERMAN J, GALLANT JE, GANDHI RT, GARCIA E, GARMON D, GATHE JC JR, GAULTIER CR, GEBRE W, GILMAN FD, GILSON I, GOEPFERT PA, GOTTLIEB MS, GOULSTON C, GROGER RK, GURLEY TD, HABER S, HARDWICKE R, HARDY WD, HARRIGAN PR, HAWKINS TN, HEATH S, HECHT FM, HENRY WK, HLADEK M, HOFFMAN RP, HORTON JM, HSU RK, HUHN GD, HUNT P, HUPERT MJ, ILLEMAN ML, JAEGER H, JELLINGER RM, JOHN M, JOHNSON JA, JOHNSON KL, JOHNSON H, JOHNSON K, JOLY J, JORDAN WC, KAUFFMAN CA, KHANLOU H, KILLIAN RK, KIM AY, KIM DD, KINDER CA, KIRCHNER JT, KOGELMAN L, KOJIC EM, KORTHUIS PT, KURISU W, KWON DS, LAMAR M, LAMPIRIS H, LANZAFAME M, LEDERMAN MM, LEE DM, LEE JM, LEE MJ, LEE ET, LEMOINE J, LEVY JA, LLIBRE JM, LIGUORI MA, LITTLE SJ, LIU AY, LOPEZ AJ, LOUTFY MR, LOY D, MOHAMMED DY, MAN A, MANSOUR MK, MARCONI VC, MARKOWITZ M, MARQUES R, MARTIN JN, MARTIN HL JR, MAYER KH, MCELRATH MJ, MCGHEE TA, MCGOVERN BH, MCGOWAN K, MCINTYRE D, MCLEOD GX, MENEZES P, MESA G, METROKA CE, MEYER-OLSON D, MILLER AO, MONTGOMERY K, MOUNZER KC, NAGAMI EH, NAGIN I, NAHASS RG, NELSON MO, NIELSEN C, NORENE DL, O'CONNOR DH, OJIKUTU BO, OKULICZ J, OLADEHIN OO, OLDFIELD EC 3RD, OLENDER SA, OSTROWSKI M, OWEN WF JR, PAE E, PARSONNET J, PAVLATOS AM, PERLMUTTER AM, PIERCE MN, PINCUS JM, PISANI L, PRICE LJ, PROIA L, PROKESCH RC, PUJET HC, RAMGOPAL M, RATHOD A, RAUSCH M, RAVISHANKAR J, RHAME FS, RICHARDS CS, RICHMAN DD, RODES B, RODRIGUEZ M, ROSE RC 3RD, ROSENBERG ES, ROSENTHAL D, ROSS PE, RUBIN DS, RUMBAUGH E, SAENZ L, SALVAGGIO MR, SANCHEZ WC, SANJANA VM, SANTIAGO S, SCHMIDT W, SCHUITEMAKER H, SESTAK PM, SHALIT P, SHAY W, SHIRVANI VN, SILEBI VI, SIZEMORE JM JR, SKOLNIK PR, SOKOL-ANDERSON M,

SOSMAN JM, STABILE P, STAPLETON JT, STARRETT S, STEIN F, STELLBRINK HJ, STERMAN FL, STONE VE, STONE DR, TAMBUSSI G, TAPLITZ RA, TEDALDI EM, TELENTI A, THEISEN W, TORRES R, TOSIELLO L, TREMBLAY C, TRIBBLE MA, TRINH PD, TSAO A, UEDA P, VACCARO A, VALADAS E, VANIG TJ, VECINO I, VEGA VM, VEIKLEY W, WADE BH, WALWORTH C, WANIDWORANUN C, WARD DJ, WARNER DA, WEBER RD, WEBSTER D, WEIS S, WHEELER DA, WHITE DJ, WILKINS E, WINSTON A, WLODAVER CG, VAN'T WOUT A, WRIGHT DP, YANG OO, YURDIN DL, ZABUKOVIC BW, ZACHARY KC, ZEEMAN B, ZHAO M. The major genetic determinants of HIV-1 control affect HLA class I peptide presentation. **Science 330 (6010)**: 1551-7, 2010.

JANEWAY CAJr. How the immune system protects the host from infection. **Microbes and Infection 3 (13)**: 1167-1171, 2001.

JANSSEN EM, LEMMENS EE, WOLFE T, CHRISTEN U, VON HERRATH MG, SCHOENBERGER SP. CD4+ T cells are required for secondary expansion and memory in CD8+ T lymphocytes. **Nature 421 (6925)**: 852-856, 2003.

JOHNSON PC, MCAULAY KA, MONTGOMERY D, LAKE A, SHIELD L, GALLAGHER A, LITTLE AM, SHAH A, MARSH SG, TAYLOR GM, JARRETT RF. Modeling HLA associations with EBV-positive and -negative Hodgkin lymphoma suggests distinct mechanisms in disease pathogenesis. **International Journal of Cancer 137 (5)**: 1066-75, 2015.

JONES K, WOCKNER L, BRENNAN RM, KEANE C, CHATTOPADHYAY PK, ROEDERER M, PRICE DA, COLE DK, HASSAN B, BECK K, GOTTLIEB D, RITCHIE DS, SEYMOUR JF, VARI F, CROOKS P, BURROWS SR, GANDHI MK. The impact of HLA class I and EBV latency-II antigen-specific CD8(+) T cells on the pathogenesis of EBV(+) Hodgkin lymphoma. **Clinical & Experimental Immunology 183 (2)**: 206-20, 2016

JULG B, MOODLEY ES, QI Y, RAMDUTH D, REDDY S, MNCUBE Z, GAO X, GOULDER PJ, DETELS R, NDUNG'U T, WALKER BD, CARRINGTON M. Possession of HLA class II DRB1*1303 associates with reduced viral loads in chronic HIV-1 clade C and B infection. **The Journal of Infectious Diseases 203 (6)**: 803-9, 2011.

KANG MS & KIEFF E. Epstein-Barr virus latent genes. **Experimental & Molecular Medicine 47**: 16, 2015.

KAPRANOS N, PETRAKOU E, ANASTASIADOU C, KOTRONIAS D. Detection of herpes simplex virus, cytomegalovirus, and Epstein-Barr virus in the semen of men attending an infertility clinic. **Fertility and Sterility 3**: 1566-1570, 2003.

KASLOW, R.A., CARRINGTON, M., APPLE, R., PARK, L., MUNOZ, A., SAAH, A.J., GOEDERT, J.J., WINKLER, C., O´BRIEN, S.J., RINALDO, C. Influence of combinations of human major histocompatibility complex genes on the course of HIV-infection. **Nature Medicine 2**: 405-411, 1996.

KAUR, G., MEHRA, N. Genetic determinants of HIV-1 infection and progression to AIDS: immune response genes. **Tissue Antigens, 74**: 373-395, 2009.

KAUSHIK N, TALELE TT, MONEL R, PALUMBO P, PANDEY VN. Destabilization of tRNA3 (Lys) from the primer-binding site of HIV-1 genome by anti-A loop polyamide nucleotide analog. **Nucleic Acids Research 29 (24)**: 5099-5106, 2001.

KEELE BF, VAN HEUVERSWYN F, LI Y, BAILES E, TAKEHISA J, SANTIAGO ML, BIBOLLET-RUCHE F, CHEN Y, WAIN LV, LIEGEOIS F, LOUL S, NGOLE EM, BIENVENUE Y, DELAPORTE E, BROOKFIELD JF, SHARP PM, SHAW GM, PEETERS M, HAHN BH. Chimpanzee reservoirs of pandemic and nonpandemic HIV-1. **Science 313 (5786):**523–526, 2006.

KENNEY SC, KAMINE J, HOLLEY-GUTHRIE E, LIN JC, MAR EC, PAGANO J. The Epstein-Barr virus (EBV) BZLF1 immediate-early gene product differentially affects latent versus productive EBV promoters. **Journal of Virology 63 (4)**: 1729-1736, 1989.

KENNEY SC. Reactivation and lytic replication of EBV. In: **Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis**. Cambridge, Cambridge University Press, 2007

KHAN G & HASHIM MJ. Global burden of deaths from Epstein-Barr virus attributable malignancies 1990-2010.**Infectious Agents and Cancer 9 (1)**: 38, 2014

KIEPIELA, P.; LESLIE, A.J.; HONEYBORNE, I.; RAMDUTH, D.; THOBAKGALE, C.; CHETTY, S.; RATHNAVALU, P.; MOORE, C.; PFAFFEROTT, K.J.; HILTON, L.; ZIMBWA, P.; MOORE, S.; ALLEN, T.; BRANDER, C.; ADDO, M.M.; ALTFELD, M.; JAMES, I.; MALLAL, S.; BUNCE, M.; BARBER, L.D.; SZINGER, J.; DAY, C.; KLENERMAN, P.; MULLINS, J.; KORBER, B.; COOVADIA, H.M.; WALKER, B.D.; GOULDER PJ. Dominant influence of HLA-B in mediating the potential of coevolution of HIV and HLA. **Nature 432**:769-774, 2004.

KIEPIELA P, NGUMBELA K, THOBAKGALE C, RAMDUTH D, HONEYBORNE I, MOODLEY E, REDDY S, DE PIERRES C, MNCUBE Z, MKHWANAZI N, BISHOP K, VAN DER STOK M, NAIR K, KHAN N, CRAWFORD H, PAYNE R, LESLIE A, PRADO J, PRENDERGAST A, FRATER J, MCCARTHY N, BRANDER C, LEARN GH, NICKLE D, ROUSSEAU C, COOVADIA H, MULLINS JI, HECKERMAN D, WALKER BD, GOULDER P. CD8+ T-cell responses to different HIV proteins have discordant associations with viral load. **Nature Medicine 13 (1)**: 46-53, 2007.

KIM RB, FROMM MF, WANDEL C, LEAKE B, WOOD AJ, RODEN DM, WILKINSON GR. The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors. **The Journal of Clinical Investigation 101 (2): 289-294**, 1998.

KIMURA H, KAWADA J, ITO Y. Epstein-Barr virus-associated lymphoid malignancies: the expanding spectrum of hematopoietic neoplasms. **Nagoya Journal of Medical Science 75 (3-4):** 169-179, 2013.

KIJAK GH, TOVANABUTRA S, SANDERS-BUELL E, WATANAVEERADEJ V, DE SOUZA MS, NELSON KE, KETSARARAT V, GULGOLGARN V, WERA-ARPACHAI M, SRIPLIENCHAN S, KHAMBOONRUENG C, BIRX DL, ROBB ML, MCCUTCHAN FE. Distinguishing molecular forms of HIV-1 in Asia with a high-throughput, fluorescent genotyping assay, MHAbce v.2**. Virology 348 (1)**: 178-191, 2007.

KIRCHHOFF LV, EVANS AS, MCCLELLAND KE, CARVALHO RPS, PANNUT CS. A case-control study of hodgkin's disease in Brazil: I. Epidemiologic aspects. **American Journal of Epidemiology 112 (5)**: 595-608, 1980.

KLEMENC P, MARIN J, SOBA E, GALE N, KOREN S, STROJAN P. Distribution of Epstein-Barr virus genotypes in throat washings, sera, peripheral blood lymphocytes and in EBV positive tumor biopsies from Slovenian patients with nasopharyngeal carcinoma. **Journal of Medical Virology 78 (8)**: 1083-1090, 2006

KNECHT H, BERGER C, ROTHENBERGER S, ODERMATT BF, BROUSSET P. The role of Epstein-Barr virus in neoplastic transformation. **Oncology 60 (4)**: 289-302, 2001.

KOEHLER RN, WALSH AM, SAATHOFF E, TOVANABUTRA S, ARROYO MA, CURRIER JR, MABOKO L, HOELSCHER M, ROBB ML, MICHAEL NL, MCCUTCHAN FE, KIM JH, KIJAK GH. Class I HLA-A*7401 is associated with protection from HIV-1 acquisition and disease progression in Mbeya, Tanzania. **The Journal of Infectious Diseases 202 (10)**: 1562-6, 2010.

KOGAN M & RAPPAPORT J. HIV-1 Accessory Protein Vpr: Relevance in the pathogenesis of HIV and potential for therapeutic intervention. **Retrovirology 8**: 25, 2011.

KOPPENSTEINER H, BRACK-WERNER R, SCHINDLER M. Macrophages and their relevance in Human Immunodeficiency Virus Type I infection. **Retrovirology 9: 82**, 2012.

KOVACS AAZ, KONO N, WANG CH, WANG D, FREDERICK T, OPERSKALSKI E, TIEN PC, FRENCH AL, MINKOFF H, KASSAYE S, T GOLUB E, AOUIZERAT BE, KUNIHOLM MH, MILLSTEIN J. Association of HLA Genotype With T-Cell Activation in Human Immunodeficiency Virus (HIV) and HIV/Hepatitis C Virus-Coinfected Women. **The Journal of Infectious Diseases 221 (7)**: 1156-1166, 2020.

KRAUS RJ, MIROCHA SJ, STEPHANY HM, PUCHALSKI JR, MERTZ JE. Identification of a novel element involved in regulation of the lytic switch BZLF1 gene promoter of Epstein-Barr virus. **Journal of Virology 75 (2)**: 867-877, 2001.

KROPSHOFER H, VOGT AB, MOLDENHAUER G, HAMMER J, BLUM JS, HÄMMERLING GJ. Editing of the HLA-DR-peptide repertoire by HLA-DM. **The EMBO Journal 15 (22)**: 6144-6154, 1996.

KUSUHARA K, TAKABAYASHI A, UEDA K, HIDAKA Y, MINAMISHIMA I, TAKE H, FUJIOKA K, IMAI S, OSATO T. Breast milk is not a significant source for early Epstein-Barr virus or human herpesvirus 6 infection in infants: a seroepidemiologic study in 2 endemic areas of human T-cell lymphotropic virus type I in Japan. **Microbiology and Immunology 41 (4)**: 309-312, 1997.

KVANSAKUL M, WEI AH, FLETCHER JI, WILLIS SN, CHEN L, ROBERTS AW, HUANG DC, COLMAN PM. Structural basis for apoptosis inhibition by Epstein-Barr virus BHRF1.**Plos Pathogens 6 (12)**: 10, 2010.

LACOSTE V, JUDDE JG, BESTETT G, CADRANEL J, ANTOINE M, VALENSI F, DELABESSE E, MACINTYRE E, GESSAIN A. Virological and molecular characterisation of a new B lymphoid cell line, established from an AIDS patient with primary effusion lymphoma, harbouring both KSHV/HHV8 and EBV viruses. **Leukemia & Lymphoma 38 (3-4)**:401-409, 2000.

LAI PK, MACKAY-SCOLLAY EM, ALPERS MP. Epidemiological studies of Epstein-Barr herpesvirus infection in Western Australia. **The Journal of Hygiene 74 (3)**: 329-337, 1975.

LAICHALK LL & THORLEY-LAWSON DA. Terminal differentiation into plasma cells initiates the replicative cycle of Epstein-Barr virus in vivo. **Journal of Virology 79 (2)**: 1296-1307, 2005.

LAMBOTTE O, TAOUFIK Y, DE GOËR MG, WALLON C, GOUJARD C, DELFRAISSY JF. Detection of infectious HIV in circulating monocytes from patients on prolonged highly active antiretroviral therapy. **Journal of Acquired immune Deficiency Syndromes 23 (2): 114-119**, 2000.

LANDERS, R.J., O'LEARY, J.J., CROWLEY, M., HEALY, I., ANNIS, P., BURKE, L., O'BRIEN, D., HOGAN, J., KEALY, W.F., LEWIS, F.A., DOYLE, C.T. Epstein-Barr virus in normal, pre-malignant, and malignant lesions of the uterine cervix. **Journal of Clinical Pathology 46(10)**:931-935, 1993.

LANDON JC, ELLIS LB, ZEVE VH, FABRIZIO DP. Herpes-type virus in cultured leukocytes from chimpanzees. **Journal of the National Cancer Institute 40 (1)**: 181-192, 1968.

LAZAR DC, MORRIS KV, SAAYMAN SM. The emerging role of long non-coding RNAs in HIV infection. **Virus Research 212: 114-126**, 2016.

LÁZARO AM, MORAES ME, MARCOS CY, MORAES JR, FERNÁNDEZ-VIÑA MA, STASTNY P. Evolution of HLA-class I compared to HLA-class II polymorphism in Terena, a South-American Indian tribe. **Human Immunology 60 (11)**: 1138-49, 1999.

LEE ES, LOCKER J, NALESNIK M, REYES J, JAFFE R, ALASHARI M, NOUR B, TZAKIS A, DICKMAN, P.S. The association of Epstein-Barr virus withs mooth-muscle tumors

occurring after organ transplantation. **The New England Journal of Medicine 332(1)**:19-25, 1995.

LEE E, BACCHETTI P, MILUSH J, SHAO W, BORITZ E, DOUEK D, FROMENTIN R, LIEGLER T, HOH R, DEEKS SG, HECHT FM, CHOMONT N, PALMER S. Memory CD4 + T-Cells Expressing HLA-DR Contribute to HIV Persistence During Prolonged Antiretroviral Therapy. **Frontiers in Microbiology 10**: 2214, 2019.

LEITE TC, CAMPOS DP, COELHO AB, TEIXEIRA SL, VELOSO V, MORGADO MG, GUIMARÃES ML. Impact of HIV-1 Subtypes on AIDS Progression in a Brazilian Cohort. **AIDS Research and Human Retroviruses 33 (1)**: 41-48, 2017.

LERENA C, CALLIGARIS SD, COLOMBO MI. Autophagy: for better or for worse, in good times or in bad times. **Current Molecular Medicine 8 (2)**: 92-101, 2008.

LESKO CR & BENGTSON AM. HIV and COVID-19: Intersecting Epidemics With Many Unknowns. **American Journal of Epidemiology 190 (1)**: 10-16, 2021.

LI WX, XIA JA, ZHOU X, MA Y, SHEN G, QIU FW. Association of HLA alleles (A, B, DRB1) and HIV-1 infection in the Han population of Hubei, China. **Journal of Huazhong University of Science and Technology Medical Sciences 37 (1)**: 131-139, 2017.

LI, X., FASANO, R., WANG, E., YAO, K.T., MARINCOLA, F.M. HLA associations with nasopharyngeal carcinoma. **Current Molecular Medicine 9(6)**:751-765, 2009.

LIBRELOTTO CS, GRÄF T, SIMON D, DE ALMEIDA SE, LUNGE VR.HIV-1 epidemiology and circulating subtypes in the countryside of South Brazil. **Revista da Sociedade Brasileira de Medicina Tropical 48 (3)**: 249-257, 2015.

LIMA MAP & RABENHORST SHB. Associação do vírus Epstein-Barr (EBV) com tumores sólidos. **Revista Brasileira de Cancerologia 52(1)**: 87-96, 2006.

LOUBSER S, PAXIMADIS M, GENTLE NL, PUREN A, TIEMESSEN CT. Human leukocyte antigen class I (A, B, C) and class II (DPB1, DQB1, DRB1) allele and haplotype variation in Black South African individuals. **Human Immunology 81 (1)**: 6-7, 2020.

LORÉ K, SMED-SÖRENSEN A, VASUDEVAN J, MASCOLA JR, KOUP RA. Myeloid and plasmacytoid dendritic cells transfer HIV-1 preferentially to antigen-specific CD4+ T cells. **The Journal of Experimental Medicine 201 (12):** 2023-2033, 2005.

LORENZETTI MA, ALTCHEH J, MORONI S, MOSCATELLI G, CHABAY PA, PRECIADO MV. EBNA1 sequences in Argentinean pediatric acute and latent Epstein-Barr virus infection reflect circulation of novel South American variants. **Journal of Medical Virology 82 (10)**: 1730-1738, 2010.

LUCAS, S. & NELSON, A.M. HIV and the spectrum of human disease. **The Journal of Pathology 235 (2)**:229-241, 2015.

LV Z, CHU Y, WANG Y. HIV protease inhibitors: a review of molecular selectivity and toxicity. **HIV/AIDS 7**: 95-104, 2015.

MACHADO LF, VALLINOTO AC, SOUZA MI, AZEVEDO VN, ISHAK MO, ISHAK R. Serological and molecular typing of HIV type 1 infection in the Tiriyo tribe, a native Indian community of the Amazon region of Brazil. **AIDS Research and Human Retroviruses 22 (12)**: 1267-1270, 2006.

MACHADO LF, ISHAK MO, VALLINOTO AC, LEMOS JA, AZEVEDO VN, MOREIRA MR, SOUZA MI, FERNANDES LM, SOUZA LL, ISHAK R. Molecular epidemiology of HIV type 1 in northern Brazil: identification of subtypes C and D and the introduction of CRF02_AG in the Amazon region of Brazil. **AIDS Research and Human Retroviruses 25 (10)**: 961-966, 2009.

MACHADO LF, COSTA IB, FOLHA MN, DA LUZ AL, VALLINOTO AC, ISHAK R, ISHAK MO. Lower genetic variability of HIV-1 and antiretroviral drug resistance in pregnant women from the state of Pará, Brazil. **BMC Infectious Diseases 17 (1)**: 270, 2017.

MADEC Y, BOUFASSA F, AVETTAND-FENOEL V, HENDOU S, MELARD A, BOUCHERIT S, SURZYN J, MEYER L, ROUZIOUX C; ANRS SEROCO/HEMOCO STUDY GROUP. Early control of HIV-1 infection in long-term nonprogressors followed since diagnosis in the ANRS SEROCO/HEMOCO cohort. **Journal of Acquired Immune Deficiency Syndromes 50 (1)**: 19-26, 2009.

MAGRATH IT. African Burkitt's lymphoma. History, biology, clinical features, and treatment. **The American Journal of Pediatric Hematology/Oncology 13 (2)**: 222-246, 1991.

MAHITI, M., BRUMME, Z.L., JESSEN, H., BROCKMAN, M.A., UENO, T. Dynamic range of Nef-mediated evasion of HLA class II-restricted immune responses in early HIV-1 infection. **Biochemical and Biophysical Research Communications 463(3)**:248-254, 2015.

MAHNKE K, GUO M, LEE S, SEPULVEDA H, SWAIN SL, NUSSENZWEIG M, STEINMAN RM. The dendritic cell receptor for endocytosis, DEC-205, can recycle and enhance antigen presentation via major histocompatibility complex class II-positive lysosomal compartments. **The Journal of Cell Biology 151 (3)**: 673-684, 2000.

MANCAO C & HAMMERSCHMIDT W. Epstein-Barr virus latent membrane protein 2A is a B-cell receptor mimic and essential for B-cell survival. **Blood 110 (10)**: 3715-3721, 2007.

MANGEAT B, TURELLI P, CARON G, FRIEDLI M, PERRIN L, TRONO D. Broad antiretroviral defence by human APOBEC3G through lethal editing of nascent reverse transcripts. **Nature 424 (6944)**: 99-103, 2003.

MARSH SG, ALBERT ED, BODMER WF, BONTROP RE, DUPONT B, ERLICH HA, FERNÁNDEZ-VIÑA M, GERAGHTY DE, HOLDSWORTH R, HURLEY CK, LAU M, LEE KW, MACH B, MAIERS M, MAYR WR, MÜLLER CR, PARHAM P, PETERSDORF EW, SASAZUKI T, STROMINGER JL, SVEJGAARD A, TERASAKI PI, TIERCY JM, TROWSDALE J. Nomenclature for factors of the HLA system, 2010.**Tissue Antigens 75 (4)**: 291-455, 2010.

MARTIN DR, YURYEV A, KALLI KR, FEARON DT, AHEARN JM. Determination of the structural basis for selective binding of Epstein-Barr virus to human complement receptor type 2. The Journal of Experimental Medicine 174 (6): 1299-1311, 1991.

MARTINI M, CAPELLO D, SERRAINO D, NAVARRA A, PIERCONTI F, CENCI T, GAIDANO G, LAROCCA LM. Characterization of variants in the promoter of EBV gene BZLF1 in normal donors, HIV-positive patients and in AIDS-related lymphomas. **The Journal of Infection 54 (3)**: 298-306, 2007.

MCAULAY, K.A.; HIGGINS, C.D.; MACSWEEN, K.F.; LAKE, A.; JARRETT, R.F.; ROBERTSON, F.L.; WILLIAMS, HILARY.; CRAWFORD, D.H. HLA class I polymorphisms are associated with development of infectious mononucleosis upon primary EBV infection. **Journal of Clinical Investigation 117**: 3042–3048, 2007.

MCCLAIN, K.L., LEACH, C.T., JENSON, H.B., JOSHI, V.V., POLLOCK, B.H., PARMLEY, R.T., DICARLO, F.J., CHADWICK, E.G., MURPHY, S.B. Association of Epstein-Barr virus with leiomyosarcomas in young people with AIDS. **The New England Journal of Medicine 332(1)**:12-18, 1995.

MCLAREN PJ, RIPKE S, PELAK K, WEINTROB AC, PATSOPOULOS NA, JIA X, ERLICH RL, LENNON NJ, KADIE CM, HECKERMAN D, GUPTA N, HAAS DW, DEEKS SG, PEREYRA F, WALKER BD, DE BAKKER PI; International HIV Controllers Study. Fine-mapping classical HLA variation associated with durable host control of HIV-1 infection in African Americans. **Human Molecular Genetics 21 (19)**: 4334-47, 2012.

MCGEOCH DJ, RIXON F, DAVISON AJ. Topics in herpesvirus genomics and evolution. **Virus Research 117 (1)**: 90-114, 2006.

MCCUNE JM, RABIN LB, FEINBERG MB, LIEBERMAN M, KOSEK JC, REYES GR, WEISSMAN IL. Endoproteolytic cleavage of gp160 is required for the activation of human immunodeficiency virus. **Cell 53 (1)**: 5-67, 1988.

MEKUE LM, NKENFOU CN, NDUKONG E, YATCHOU L, DAMBAYA B, NGOUFACK MN, KAMENI JK, KUIATÉ JR, NDJOLO A. HLA A*32 is associated to HIV acquisition while B*44 and B*53 are associated with protection against HIV acquisition in perinatally exposed infants. **BMC Pediatrics 19 (1)**: 249, 2019

MELHUISH A & LEWTHWAITE P. Natural history of HIV and AIDS. **Medicine 46 (6):** 356-361, 2018.

MERZOUGUI N, KRATZER R, SAVEANU L, VAN ENDERT P.A proteasome-dependent, TAP-independent pathway for cross-presentation of phagocytosed antigen. **EMBO Reports 12 (12)**: 1257-1264, 2011.

McMICHAEL AJ & ROWLAND-JONES SL. Cellular immune responses to HIV. **Nature 410 (6831)**: 980-987, 2001.

MIRANDA AE, PINTO VM, MCFARLAND W, PAGE K. HIV infection among young parturient women in Brazil: prevalence and associated risk factors. **AIDS and Behavior 18 (1)**: 50-52, 2014.

MIRANDA BKB, DE SÁ KSG, DA SILVA ANR, FEITOSA RNM, CAYRES-VALLINOTO IMV, ISHAK R, VALLINOTO ACR.GBV-C/HIV-1 coinfection is associated with low HIV-1 viral load and high CD4+ T lymphocyte count. **Archives of Virology 162 (11)**: 3431-3438, 2017.

MOLESWORTH SJ, LAKE CM, BORZA CM, TURK SM, HUTT-FLETCHER LM. Epstein-Barr virus gH is essential for penetration of B cells but also plays a role in attachment of virus to epithelial cells. **Journal of Virology 74 (14)**: 6324-6332, 2000.

MOORE MD & HU WS.HIV-1 RNA dimerization: It takes two to tango. **AIDS Review 11 (2)**: 91-102, 2009.

MORBIDITY & MORTALITY WEEKLY REPORT. Kaposi's sarcoma and pneumocystis pneumonia among homosexual men - New York City and California. **Morbidity & Mortality Weekly Report (MMWR) 30:** 305– 308, 1981.

MORBIDITY & MORTALITY WEEKLY REPORT. Epidemiologic notes and reports: possible transfusion-associated acquired immune deficiency syndrome, AIDS - California. **Morbidity & Mortality Weekly Report (MMWR) 31:** 652– 654, 1982.

MOTTA P, MARINIC K, SORRENTINO A, LÓPEZ R, ILIOVICH E, HABEGGER DE SORRENTINO A. Association of HLA-DQ and HLA-DR alleles with susceptibility or resistance to HIV-1 infection among the population of Chaco Province, Argentina. **Medicina (B Aires) 62 (3)**: 245-8, 2002.

MOTOMURA K, CHEN J, HU WS. Genetic recombination between human immunodeficiency virus type 1 (HIV-1) and HIV-2, two distinct human lentiviruses. **Journal of Virology 82 (4)**: 1923-1933, 2008.

MOTOZONO C, MILES JJ, HASAN Z, GATANAGA H, MERIBE SC, PRICE DA, OKA S, SEWELL AK, UENO T. CD8(+) T cell cross-reactivity profiles and HIV-1 immune escape

towards an HLA-B35-restricted immunodominant Nef epitope. **PLoS One 8 (6)**: e66152, 2013.

MÜHE J & WANG F. Non-human Primate Lymphocryptoviruses: Past, Present, and Future. **Current Topics in Microbiology and Immunology 391**: 385-405, 2015.

MULLEN MM, HAAN KM, LONGNECKER R, JARDETZKY TS. Structure of the Epstein-Barr virus gp42 protein bound to the MHC class II receptor HLA-DR1. **Molecular Cell 9 (2)**: 375-385, 2002.

MURAKOSHI H, KOYANAGI M, AKAHOSHI T, CHIKATA T, KUSE N, GATANAGA H, ROWLAND-JONES SL, OKA S, TAKIGUCHI M. Impact of a single HLA-A*24:02-associated escape mutation on the detrimental effect of HLA-B*35:01 in HIV-1 control. **EBioMedicine 36**: 103-112, 2018.

NANBO A, NODA T, OHBA Y. Epstein-Barr Virus Acquires Its Final Envelope on Intracellular Compartments With Golgi Markers. **Frontiers in Microbiology 16**: 13, 2018.

NEEFJES J, JONGSMA ML, PAUL P, BAKKE O. Towards a systems understanding of MHC class I and MHC class II antigen presentation. **Nature Reviews 11 (12)**: 823-836, 2011.

NEIJSSEN J, HERBERTS C, DRIJFHOUT JW, REITS E, JANSSEN L, NEEFJES J. Cross-presentation by intercellular peptide transfer through gap junctions. **Nature 434 (7029)**: 83-88, 2005.

NEWCOMB WW, HOMA FL, THOMSEN DR, BOOY FP, TRUS BL, STEVEN AC, SPENCER JV, BROWN JC. Assembly of the herpes simplex virus capsid: characterization of intermediates observed during cell-free capsid formation. **Journal of Molecular Biology 263 (3)**: 432-446, 1996.

NIEDERMAN JC, MILLER G, PEARSON HA, PAGANO JS, DOWALIBY JM. Infectious mononucleosis. Epstein-Barr-virus shedding in saliva and the oropharynx. **The New England Journal of Medicine 294 (25)**: 1355-1359, 1976.

NIEDOBITEK, G., AGATHANGGELOU, A., HERBST, H., WHITEHEAD, L., WRIGHT, D.H., YOUNG, L.S. Epstein-Barr virus (EBV) infection in infectious mononucleosis: virus latency, replication and phenotype of EBV-infected cells. **The Journal of Pathology 182(2)**:151-159, 1997.

NIENS, M., JARRETT, R.F., HEPKEMA, B., NOLTE, I.M., DIEPSTRA, A., PLATTEEL, M., KOUPRIE, N., DELURY, C.P., GALLAGHER, A., VISSER, L., POPPEMA, S., MEERMAN, G.J., VAN DEN BERG, A. HLA-A*02 is associated with a reduced risk and HLA-A*01 with an increased risk of developing EBV+ Hodgkin lymphoma. **Blood 110 (9)**:3310-3315, 2007.

NIIRO H, OTSUKA T, ABE M, SATOH H, OGO T, NAKANO T, FURUKAWA Y, NIHO Y. Epstein-Barr virus BCRF1 gene product (viral interleukin 10) inhibits superoxide anion production by human monocytes**. Lymphokine and Cytokine Research 11 (5)**: 209-214, 1992.

NUNES K, PIOVEZAN B, TORRES MA, PONTES GN, KIMURA L, CARNAVALLI JE, MINGRONI NETTO RC, MORAES ME, MEYER D. Population variation of HLA genes in rural communities in Brazil, the Quilombos from the Vale do Ribeira, São Paulo - Brazil. **Human Immunology 77 (6)**: 447-8, 2016.

O'CONOR GT. Malignant lymphoma in African children. II. A pathological entity. **Cancer 14**: 270-283, 1961.

OGINO, T., MORIAI, S., ISHIDA, Y., ISHII, H., KATAYAMA, A., MIYOKAWA, N., HARABUCHI, Y., FERRONE, S. Association of immunoescape mechanisms with Epstein-Barr virus infection in nasopharyngeal carcinoma. **International Journal of Cancer 120(11)**:2401-2410, 2007.

OGUNSHOLA F, ANMOLE G, MILLER RL, GOERING E, NKOSI T, MUEMA D, MANN J, ISMAIL N, CHOPERA D, NDUNG'U T, BROCKMAN MA, NDHLOVU ZM. Dual HLA B*42 and B*81-reactive T cell receptors recognize more diverse HIV-1 Gag escape variants. **Nature Communications 9 (1)**: 5023, 2018.

OHGA S, NOMURA A, TAKADA H, HARA T. Immunological aspects of Epstein-Barr virus infection. **Critical reviews in Oncology/Hematology 44(3)**:203-215, 2002.

OKANO M. Haematological associations of Epstein-Barr virus infection. Bailliere's Best Practice & Research 13(2):199-214, 2000.

OKULICZ JF, MARCONI VC, LANDRUM ML, WEGNER S, WEINTROB A, GANESAN A, HALE B, CRUM-CIANFLONE N, DELMAR J, BARTHEL V, QUINNAN G, AGAN BK, DOLAN MJ; INFECTIOUS DISEASE CLINICAL RESEARCH PROGRAM (IDCRP) HIV WORKING GROUP. Clinical outcomes of elite controllers, viremic controllers, and long-term nonprogressors in the US Department of Defense HIV natural history study. **The Journal of Infectious Diseases 200 (11)**: 1714-1723, 2009.

OLIVEIRA RSM, BENZAKEN AS, SARACENI V, SABIDÓ M. HIV/AIDS epidemic in the State of Amazonas:characteristics and trends from 2001 to 2012. **Revista da Sociedade Brasileira de Medicina Tropical 48 (1)**: 70-78, 2015.

OLIVEIRA JL, FREITAS RT, ARCURI LJ, GOMES AP, VITORINO RR, RODRIGUES DC, PAULA SO, SANTANA LA, SIQUEIRA-BATISTA R. O vírus Epstein-Barr e a mononucleose infecciosa. **Revista da Sociedade Brasileira de Clínica Médica 10 (6)**: 535-543, 2012.

ORIOL-TORDERA B, LLANO A, GANOZA C, CATE S, HILDEBRAND W, SANCHEZ J, CALLE ML, BRANDER C, OLVERA A. Impact of HLA-DRB1 allele polymorphisms on control of HIV infection in a Peruvian MSM cohort. **HLA 90 (4)**: 234-237, 2017.

OTT E, NORIO P, RITZI M, SCHILDKRAUT C, SCHEPERS A. The dyad symmetry element of Epstein-Barr virus is a dominant but dispensable replication origin. **Plos One 6 (5)**: 13, 2011.

PAGANO JS. Is Epstein-Barr Virus Transmitted Sexually?. **The Journal of Infectious Diseases 195 (4)**: 469-470, 2007.

PALSER AL, GRAYSON NE, WHITE RE, CORTON C, CORREIA S, BA ABDULLAH MM, WATSON SJ, COTTEN M, ARRAND JR, MURRAY PG, ALLDAY MJ, RICKINSON AB, YOUNG LS, FARRELL PJ, KELLAM P. Genome diversity of Epstein-Barr virus from multiple tumor types and normal infection. **Journal of Virology 89 (10)**: 5222-5237, 2015.

PANIKKAR A, SMITH C, HISLOP A, TELLAM N, DASARI V, HOGQUIST KA, WYKES M, MOSS DJ, RICKINSON A, BALFOUR HH JR, KHANNA R. Cytokine-Mediated Loss of Blood Dendritic Cells During Epstein-Barr Virus-Associated Acute Infectious Mononucleosis: Implication for Immune Dysregulation. **The Journal of Infectious Diseases 212 (12)**: 1957-1961, 2015.

PAXIMADIS M, MATHEBULA TY, GENTLE NL, VARDAS E, COLVIN M, GRAY CM, TIEMESSEN CT, PUREN A. Human leukocyte antigen class I (A, B, C) and II (DRB1) diversity in the black and Caucasian South African population. **Human Immunology 73 (1)**: 80-92, 2012.

PARSONS, M.S., WREN, L., ISITMAN, G., NAVIS, M., STRATOV, I., BERNARD, N.F., KENT, S.J. HIV infection abrogates the functional advantage of natural killer cells educated through KIR3DL1/HLA-Bw4 interactions to mediate anti-HIV antibody-dependent cellular cytotoxicity. **Journal of Virology 86(8)**:4488-4495, 2012.

PEEL S, MACHEBOEUF P, MARTINELLI N, WEISSENHORN W. Divergent pathways lead to ESCRT-III-catalyzed membrane fission. **Trends in Biochemical Sciences 36 (4)**: 199-210, 2011.

PEI Y, HUANG H, LI H, CHEN J, WU G. Allelic and haplotype diversity of HLA-A, HLA-B and HLA-DRB1 gene at high resolution in the Nanning Han population. **International Jouirnal of Immunogenetics 45 (4)**: 201-209, 2018.

PERELSON AS, NEUMANN AU, MARKOWITZ M, LEONARD JM, HO DD.HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span, and viral generation time. **Science 271 (5255)**: 1582-1586, 1996.

PEREYRA F, ADDO MM, KAUFMANN DE, LIU Y, MIURA T, RATHOD A, BAKER B, TROCHA A, ROSENBERG R, MACKEY E, UEDA P, LU Z, COHEN D, WRIN T, PETROPOULOS CJ, ROSENBERG ES, WALKER BD. Genetic and immunologic

heterogeneity among persons who control HIV infection in the absence of therapy. **The Journal of infectious Diseases 197 (4)**: 563-571, 2008.

PETERSON, T.A., KIMANI, J., WACHIHI, C., BIELAWNY, T., MENDOZA, L., THAVANESWARAN, S., NARAYANSINGH, M.J., KARIRI, T., LIANG, B., BALL, T.B., NGUGI, E.N., PLUMMER, F.A., LUO, M. HLA class I associations with rates of HIV-1 seroconversion and disease progression in the Pumwani Sex Worker Cohort. **Tissue Antigens 81 (2)**:93-107, 2013.

PETOSA C, MORAND P, BAUDIN F, MOULIN M, ARTERO JB, MÜLLER CW. Structural basis of lytic cycle activation by the Epstein-Barr virus ZEBRA protein. **Molecular Cell 21 (4)**: 565-572, 2006.

PETRARA MR, PENAZZATO M, MASSAVON W, NABACHWA S, NANNYONGA M, MAZZA A, GIANESIN K, DEL BIANCO P, LUNDIN R, SUMPTER C, ZANCHETTA M, GIAQUINTO C, DE ROSSI A. Epstein-Barr virus load in children infected with human immunodeficiency virus type 1 in Uganda. **The Journal of Infectious Diseases 210(3)**:392-399, 2014.

PIEKNA-PRZYBYLSKA D & BAMBARA RA. Requirements for efficient minus strand strong-stop DNA transfer in human immunodeficiency virus 1. **RNA Biology 8 (2)**: 230.236, 2011.

PLANTIER JC, LEOZ M, DICKERSON JE, DE OLIVEIRA F, CORDONNIER F, LEMÉE V, DAMOND F, ROBERTSON DL, SIMON F.A new human immunodeficiency virus derived from gorillas. **Nature Medicine 15 (8)**: 871-872, 2009.

POIESZ, B. J. RUSCETTI, F. W. GAZDAR, A. F. BUNN, P. A. MINNA, J. D. AND GALLO, R. C. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. **Proc. Natl. Acad. Sci. USA 77:** 7415– 7419, 1980.

POLZ D, PODSIADLO L, STEC A, POLZ-DACEWICZ M. Prevalence of EBV genotypes in Polish, Taiwanese and Arabic healthy students and association between genotypes and 30-bp deletion in the LMP-1 gene phylogenetic analysis**. Polish Journal of Microbiology 63 (1)**: 105-109, 2014.

POPOOLA TD & AWODELE O. Interplay between antiretroviral therapy and oxidative stress in HIV seropositive patients. **African Journal of Medicine and Medical Sciences 45 (1)**: 5-21, 2016.

POPOVIC M, SARNGADHARAN MG, READ E, GALLO RC. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. **Science 224 (4648):** 497-500, 1984.

POROPATICH K. & SULLIVAN D.J.J. Human immunodeficiency virus type 1 long-term non progressors: the viral, genetic and immunological basis for disease non-progression. **Journal of General Virology 92**: 247-268, 2011.

PORTAL D, ZHAO B, CALDERWOOD MA, SOMMERMANN T, JOHANNSEN E, KIEFF E.EBV nuclear antigen EBNALP dismisses transcription repressors NCoR and RBPJ from enhancers and EBNA2 increases NCoR-deficient RBPJ DNA binding. **Proceedings of the National Academy of Sciences of the United States of America 108 (19)**: 7808-7813, 2011.

QING M, LI T, HAN Y, QIU Z, JIAO Y. Accelerating effect of human leukocyte antigen-Bw6 homozygosity on disease progression in Chinese HIV-1-infected patients. **Journal of Acquired Immune Deficient Syndromes 41 (2)**: 137-9, 2006.

RAAB-TRAUB, N. Epstein-Barr virus in the pathogenesis of NPC. **Seminars in Cancer Biology 12(6)**:431-441, 2002.

RALLÓN N, RESTREPO C, VICARIO JL, DEL ROMERO J, RODRÍGUEZ C, GARCÍA-SAMANIEGO J, GARCÍA M, CABELLO A, GÓRGOLAS M, BENITO JM. Human leucocyte antigen (HLA)-DQB1*03:02 and HLA-A*02:01 have opposite patterns in their effects on susceptibility to HIV infection. **HIV Medicine 18 (8)**: 587-594, 2017.

RAMDUTH D, CHETTY P, MNGQUANDANISO NC, NENE N, HARLOW JD, HONEYBORNE I, NTUMBA N, GAPPOO S, HENRY C, JEENA P, ADDO MM, ALTFELD M, BRANDER C, DAY C, COOVADIA H, KIEPIELA P, GOULDER P, WALKER B. Differential immunogenicity of HIV-1 clade C proteins in eliciting CD8+ and CD4+ cell responses. **The Journal of Infectious Diseases 192 (9)**: 1588-1896, 2005.

RANASINGHE S, CUTLER S, DAVIS I, LU R, SOGHOIAN DZ, QI Y, SIDNEY J, KRANIAS G, FLANDERS MD, LINDQVIST M, KUHL B, ALTER G, DEEKS SG, WALKER BD, GAO X, SETTE A, CARRINGTON M, STREECK H. Association of HLA-DRB1-restricted $CD4^+$ T cell responses with HIV immune control. **Nature Medicine 19 (7)**: 930-933, 2013.

RANASINGHE, S., LAMOTHE, P.A., SOGHOIAN, D.Z., KAZER, S.W., COLE, M.B., SHALEK, A.K., YOSEF, N., JONES, R.B., DONAGHEY, F., NWONU, C., JANI, P., CLAYTON, G.M., CRAWFORD, F., WHITE, J., MONTOYA, A., POWER, K., ALLEN, T.M., STREECK, H., KAUFMANN, D.E., PICKER, L.J., KAPPLER, J.W., WALKER, B.D. Antiviral CD8+ T Cells Restricted by Human Leukocyte Antigen Class II Exist during Natural HIV Infection and Exhibit Clonal Expansion. **Immunity 45(4)**:917-930, 2016.

RAUCH A, NOLAN D, FURRER H, MCKINNON E, JOHN M, MALLAL S, GAUDIERI S, GÜNTHARD HF, SCHMID P, BATTEGAY M, HIRSCHEL B, TELENTI A, JAMES I; Swiss HIV Cohort Study. HLA-Bw4 homozygosity is associated with an impaired CD4 T cell recovery after initiation of antiretroviral therapy. **Clinical Infectious Diseases 46 (12)**: 1921-5, 2008.

RAVAZZI-GAUCH C, BAJAY MM, CALDAS HC, ABBUD-FILHO M. HLA-A, -B, and -DRB1 allele and haplotype diversity in a cohort of Brazilian renal transplant candidates. **Human Immunology 77 (6)**: 464-9, 2016.

REGNELL SE & LERNMARK Å. Early prediction of autoimmune (type 1) diabetes. **Diabetologia 60 (8)**: 1370-1381, 2017.

RENZETTE N, SOMASUNDARAN M, BREWSTER F, CODERRE J, WEISS ER, MCMANUS M, GREENOUGH T, TABAK B, GARBER M, KOWALIK TF, LUZURIAGA K. Epstein-Barr virus latent membrane protein 1 genetic variability in peripheral blood B cells and oropharyngeal fluids**. Journal of Virology 88 (7)**: 3744-3755, 2014.

RESHEF R, LUSKIN MR, KAMOUN M, VARDHANABHUTI S, TOMASZEWSKI JE, STADTMAUER EA, PORTER DL, HEITJAN DF, TSAI DE E. Association of HLA polymorphisms with post-transplant lymphoproliferative disorder in solid-organ transplant recipients. **American Journal of Transplantation 11 (4)**: 817-25, 2011.

RICE AP. The HIV-1 Tat protein: mechanism of action and target for HIV-1 cure strategies. **Current Pharmaceutical Desing 23 (28)**: 4098-4102, 2017.

RIST MJ, HIBBERT KM, CROFT NP, SMITH C, NELLER MA, BURROWS JM, MILES JJ, PURCELL AW, ROSSJOHN J, GRAS S, BURROWS SR. T Cell Cross-Reactivity between a Highly Immunogenic EBV Epitope and a Self-Peptide Naturally Presented by HLA-B*18:01+ Cells. **Journal of Immunology 194 (10)**: 4668-75, 2015.

ROBERTSON ES, LIN J, KIEFF E. The amino-terminal domains of Epstein-Barr virus nuclear proteins 3A, 3B, and 3C interact with RBPJ(kappa).**Journal of Virology 70 (5)**: 3068-3074, 1996.

ROBINSON J, HALLIWELL JA, HAYHURST JD, FLICEK P, PARHAM P, MARSH SG. The IPD and IMGT/HLA database: allele variant databases. **Nucleic Acids Research 43**: 423-431, 2015.

ROCK KL, FARFÁN-ARRIBAS DJ, SHEN L. Proteases in MHC class I presentation and cross-presentation. **Journal of Immunology 184 (1)**: 9-15, 2010.

ROSENBERG ES, ALTFELD M, POON SH, PHILLIPS MN, WILKES BM, ELDRIDGE RL, ROBBINS GK, D'AQUILA RT, GOULDER PJ, WALKER BD. Immune control of HIV-1 after early treatment of acute infection. **Nature 407 (6803)**: 23-526, 2000.

ROUSSEAU, C.M.; LOCKHART, D.W.; LISTGARTEN, J. Rare HLA driven additional HIV evolution compared to more frequent alleles. **AIDS Research and Human Retroviruses 25**: 297-303, 2009.

ROUSSET, F. GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. **Molecular Ecology Resources 8**: 103–106, 2008.

RUBICZ, ROHINA.; YOLKEN, R.; DRIGALENKO, EUGENE.; CARLESS, M.A.; DYER, T.D.; BAUMAN, L.; MELTON, P.E.; KENT JR, J.W.; HARLEY, J.B.; CURRAN, J.E.; JOHNSON, M.P.; COLE, S.A.; ALMASY, L.; MOSES, E.K.; DHURANDHAR, N.V.; KRAIG, E.; BLANGERO, J.; LEACH, C.T.; GORING, H.H.H. A Genome-Wide Integrative Genomic Study Localizes Genetic Factors Influencing Antibodies against Epstein- Barr Virus Nuclear Antigen 1 (EBNA-1). **PLOS Genetics 9(1)**: 17, 2013.

SABIN CA, LUNDGREN JD. The natural history of HIV infection. **Currente Opinion in HIV and AIDS 8 (4)**: 311-317, 2013.

SAKURAGI J-I. Morphogenesis of the Infectious HIV-1 Virion. **Frontiers in Microbiology 2: 242**, 2011.

SAMPLE J, YOUNG L, MARTIN B, CHATMAN T, KIEFF E, RICKINSON A, KIEFF E. Epstein-Barr virus types 1 and 2 differ in their EBNA-3A, EBNA-3B, and EBNA-3C genes. **Journal of Virology 64 (9)**: 4084-4092, 1990.

SANDERS-BUELL E, SAAD MD, ABED AM, BOSE M, TODD CS, STRATHDEE SA, BOTROS BA, SAFI N, EARHART KC, SCOTT PT, MICHAEL N, MCCUTCHAN FE.A nascent HIV type 1 epidemic among injecting drug users in Kabul, Afghanistan is dominated by complex AD recombinant strain, CRF35_AD.**AIDS Research and Human Retroviruses 23 (6)**: 834-839, 2007.

SANTILLI V, MORA N, AQUILANI A, TCHIDJOU HK, PONTRELLI G, DE VITO R, LOMBARDI A, BERNARDI S, PALMA P. Burkitt's lymphoma mimicking EBV disease as first sign of vertical HIV infection in an adolescent. **Italian Journal of Pediatrics 36**: 34, 2010.

SANTOS AF & SOARES MA.HIV Genetic Diversity and Drug Resistance. **Viruses 2 (2)**: 503-531, 2010.

SANTOS NSO & SILVA RC. Vírus Epstein-Barr. In: **Virologia Humana**. Santos NSO, Romanos MTV, Wigg AD. 3ª eds. Rio de Janeiro: Guanabara Koogan, 2015. P. 1085-1162.

SANTOS L, AZEVEDO K, SILVA L, OLIVEIRA L. Epstein-Barr virus in oral mucosa from human immunodeficiency virus positive patients. **Revista da Associação Medica Brasileira 60 (3)**:262-269, 2014.

SARAFIANOS SG, MARCHAND B, DAS K, HIMMEL DM, PARNIAK MA, HUGHES SH, ARNOLD E. Structure and function of HIV-1 reverse transcriptase: molecular mechanisms of polymerization and inhibition. **Journal of Molecular Biology 385 (3)**: 693-713, 2009.

SARIC T, CHANG SC, HATTORI A, YORK IA, MARKANT S, ROCK KL, TSUJIMOTO M, GOLDBERG AL. An IFN-gamma-induced aminopeptidase in the ER, ERAP1, trims precursors to MHC class I-presented peptides. **Nature Immunology 3 (12)**: 1169-1176, 2002.

SAUTER D, UNTERWEGER D, VOGL M, USMANI SM, HEIGELE A, KLUGE SF, HERMKES E, MOLL M, BARKER E, PEETERS M, LEARN GH, BIBOLLET-RUCHE F, FRITZ JV, FACKLER OT, HAHN BH, KIRCHHOFF F. Human tetherin exerts strong selection pressure on the HIV-1 group N Vpu protein. **PLOS Pathogens 8 (12): 17**, 2012.

SAVINA A, JANCIC C, HUGUES S, GUERMONPREZ P, VARGAS P, MOURA IC, LENNON-DUMÉNIL AM, SEABRA MC, RAPOSO G, AMIGORENA S.NOX2 controls phagosomal pH to regulate antigen processing during crosspresentation by dendritic cells. **Cell 126 (1)**: 205-218, 2006.

SAW WY, LIU X, KHOR CC, TAKEUCHI F, KATSUYA T, KIMURA R, NABIKA T, OHKUBO T, TABARA Y, YAMAMOTO K, YOKOTA M; JAPANESE GENOME VARIATION CONSORTIUM, TEO YY, KATO N. Mapping the genetic diversity of HLA haplotypes in the Japanese populations. **Scientific Reports 9 (5)**: 17855, 2015.

SCHEENSTRA R, VERSCHUUREN EA, DE HAAN A, SLOOFF MJ, THE TH, BIJLEVELD CM, VERKADE HJ. The value of prospective monitoring of Epstein-Barr virus DNA in blood samples of pediatric liver transplant recipients. **Transplant Infectious Disease 6 (1)**: 15-22, 2004.

SCHIRALLI LESTER GM & HENDERSON AJ. Mechanisms of HIV Transcriptional Regulation and Their Contribution to Latency. **Molecular Biology International 2012**: 12, 2012.

SCHNEIDEWIND, A.; TANG, Y.; BROCKMAN, M.A. Maternal transmission of HIV escape mutations subverts HLA-B57 immunodominance but facilitates viral control in the haploidentical infant. **Journal of Virology 83**: 8616-8627, 2009.

SCHOOLEY, R. Infecção pelo vírus Epstein Barr. In: **Cecil: Tratado de medicina interna**. GOLDMAN, L., SCHAFER, A.I. Rio de Janeiro: Elsevier; 2009. p. 2900-3.

SCHUITEMAKER H & B. VAN'T WOUT A. Viral and Host Determinants of HIV-1 Disease Progression. In: **Global HIV/AIDS Medicine.** Volberding PA, Sande MA, Lange JMA, Greene WC. 1ª eds. Saunders. 2008.

SCORZA SMERALDI, R., FABIO, G., LAZZARIN, A., EISERA, N., UBERTI FOPPA, C., MORONI, M., ZANUSSI, C. HLA-associated susceptibility to AIDS: HLA B35 is a major risk factor for Italian HIV-infected intravenous drug addicts. **Human Immunology 22(2)**:73-79, 1988.

SEEDAT F, JAMES I, LOUBSER S, WAJA Z, MALLAL SA, HOFFMANN C, TIEMESSEN CT, CHAISSON RE, MARTINSON NA. Human leukocyte antigen associations with protection against tuberculosis infection and disease in human immunodeficiency virus-1 infected individuals, despite household tuberculosis exposure and immune suppression. **Tuberculosis (Edinb) 126**: 102023, 2021.

SETO E, MOOSMANN A, GRÖMMINGER S, WALZ N, GRUNDHOFF A, HAMMERSCHMIDT W. Micro RNAs of Epstein-Barr virus promote cell cycle progression and prevent apoptosis of primary human B cells. **Plos Pathogenes 6 (8)**: 16, 2010.

SELIN, L.K., BREHM, M.A., NAUMOV, Y.N., CORNBERG, M., KIM, S.K., CLUTE, S.C., WELSH, R.M. Memory of mice and men: CD8+ T-cell cross-reactivity and heterologous immunity. **Immunology Reviews 211**:164-181, 2006.

SHAH K, GUPTA S, MISHRA H, SHARMA PK, JAYASWAL A. Examining structural analogs of elvitegravir as potential inhibitors of HIV-1 integrase. **Archives of Virology 159 (8)**: 2069-2080, 2014.

SHAH S, ALEXAKI A, PIRRONE V, DAHIYA S, NONNEMACHER MR, WIGDAHL B. Functional properties of the HIV-1 long terminal repeat containing single-nucleotide polymorphisms in Sp site III and CCAAT/enhancer binding protein site I. **Virology Journal 11**: 16, 2014.

SHARP PM, ROBERTSON DL, GAO F, HAHN BH. Origins and diversity of human immunodeficiency viruses. **AIDS 8 (1):** S27-S42,1994.

SHIMAKAGE M, SASAGAWA T. Detection of Epstein--Barr virus-determined nuclear antigen-2 mRNA by in situ hybridization. **Journal of Virological Methods 93(1-2)**:23-32, 2001.

SIMBIRI KO, SMITH NA, OTIENO R, WOHLFORD EE, DAUD II, ODADA SP, MIDDLETON F, ROCHFORD R. Epstein-Barr virus genetic variation in lymphoblastoid cell lines derived from Kenyan pediatric population. **Plos One 10 (5)**: 3744-3755, 2014.

SINGH R & CRESSWELL P. Defective cross-presentation of viral antigens in GILT-free mice. **Science 328 (5984)**: 1394-1398, 2010.

SIXBEY JW & YAO QY. Immunoglobulin A-induced shift of Epstein-Barr virus tissue tropism. **Science 25 (5051)**: 1578-1580, 1992.

SLYKER JA, CASPER C, TAPIA K, RICHARDSON B, BUNTS L, HUANG ML, MALECHE-OBIMBO E, NDUATI R, JOHN-STEWART G. Clinical and virologic manifestations of primary Epstein-Barr virus (EBV) infection in Kenyan infants born to HIV-infected women. **The Journal of Infectious Diseases 207 (12)**:1798-1806, 2013.

SMATTI MK, YASSINE HM, ABUODEH R, ALMARAWANI A, TALEB SA, ALTHANI AA, NASRALLAH GK. Prevalence and molecular profiling of Epstein Barr virus (EBV) among healthy blood donors from different nationalities in Qatar. **Plos One 12 (12)**: e0189033. https://doi.org/10.1371/journal.pone.0189033, 2017.

SMATTI MK, AL-SADEQ DW, ALI NH, PINTUS G, ABOU-SALEH H, NASRALLAH GK. Epstein-Barr Virus Epidemiology, Serology, and Genetic Variability of LMP-1 Oncogene Among Healthy Population: An Update. **Frontiers in Oncology 8**: 16, 2018.

SON KH & SHIN MY. Clinical features of Epstein-Barr virus-associated infectious mononucleosis in hospitalized Korean children. **Korean Journal of Pediatric 54 (10)**: 409-413, 2011.

SOUZA JC, CRISPIM MAE, ABRAHIM C, FRAIJI NA, KIESSLICH D, STEFANI MMA. High rate of seromarkers for HIV, HBV and syphilis among blood donors using confidential unit exclusion, before and after HIV-NAT implementation at a major public blood bank in the Brazilian Amazon. **Trasnfusion 59 (2)**: 629-638, 2019.

SPIRA AI, MARX PA, PATTERSON BK, MAHONEY J, KOUP RA, WOLINSKY SM, HO DD. Cellular targets of infection and route of viral dissemination after an intravaginal inoculation of simian immunodeficiency virus into rhesus macaques. **The Journal of Experimental Medicine 183 (1): 215-225**, 1996.

STORRIE MC & SPHAR RL. Seroepidemiological studies of polaris Submarine crews. II. Infectious mononucleosis. **Military Medicine 141 (1)**: 32, 1976.

SUGIMOTO K, UEMA M, SAGARA H, TANAKA M, SATA T, HASHIMOTO Y, KAWAGUCHI Y. Simultaneous tracking of capsid, tegument, and envelope protein localization in living cells infected with triply fluorescent herpes simplex virus 1.**Jornal of Virology 82 (11)**: 5198-5211, 2008.

TANAKA TSO, LEITE TF, FREITAS SZ, CESAR GA, DE REZENDE GR, LINDENBERG ASC, GUIMARÃES ML, MOTTA-CASTRO ARC.HIV-1 Molecular Epidemiology, Transmission Clusters and Transmitted Drug Resistance Mutations in Central Brazil. **Frontiers in Microbiology 10**: 20, 2019.

TANG, J.; SHAO, W.; YOO, Y.J. Human leukocyte antigen class I genotypes in relation to heterosexual HIV type 1 transmission within discordant couples. **Journal of Immunology 181**: 2626-2635, 2008.

TANG J, MALHOTRA R, SONG W, BRILL I, HU L, FARMER PK, MULENGA J, ALLEN S, HUNTER E, KASLOW RA. Human leukocyte antigens and HIV type 1 viral load in early and chronic infection: predominance of evolving relationships. **PLoS One 10 5 (3)**: e9629, 2010.

TANGYE SG, PALENDIRA U, EDWARDS ES. Human immunity against EBV-lessons from the clinic. **The Journal of Experimental Medicine 214 (2)**: 269-283, 2017.

TANNER J, WEIS J, FEARON D, WHANG Y, KIEFF E. Epstein-Barr virus gp350/220 binding to the B lymphocyte C3d receptor mediates adsorption, capping, and endocytosis. **Cell 50 (2)**: 203-213, 1987.

TAKADA K& NANBO A. The role of EBERs in oncogenesis. **Seminars in Cancer Biology 11(6)**:461-467, 2001.

TAYLOR BS, SOBIESZCZYK ME, MCCUTCHAN FE, HAMMER SM. The challenge of HIV-1 subtype diversity. **The New England Journal of Medicine 358 (15)**: 1590-1602, 2008.

TAYLOR GS, LONG HM, BROOKS JM, RICKINSON AB, HISLOP AD. The immunology of Epstein-Barr virus-induced disease. **Annual Review of Immunology 33**: 787-821, 2015.

TEDESCHI V, VITULANO C, CAULI A, PALADINI F, PIGA M, MATHIEU A, SORRENTINO R, FIORILLO MT. The Ankylosing Spondylitis-associated HLA-B*2705 presents a B*0702-restricted EBV epitope and sustains the clonal amplification of cytotoxic T cells in patients. **Molecular Medicine 22**: 215-223, 2016.

THÉ G, GESER A, DAY NE, TUKEI PM, WILLIAMS EH, BERI DP, SMITH PG, DEAN AG, BRONKAMM GW, FEORINO P, HENLE W. Epidemiological evidence for causal relationship between Epstein-Barr virus and Burkitt's lymphoma from Ugandan prospective study. **Nature 274 (5673)**: 756-761, 1978.

THOMAS M, HOPKINS C, DUFFY E, LEE D, LOULERGUE P, RIPAMONTI D, OSTROV DA, PHILLIPS E. Association of the HLA-B*53:01 Allele With Drug Reaction With Eosinophilia and Systemic Symptoms (DRESS) Syndrome During Treatment of HIV Infection With Raltegravir. **Clinical Infection Disease 64 (9)**: 1198-1203, 2017.

THOMPSON MP & KURZROCK R. Epstein-Barr virus and cancer. **Clinical Cancer Research 10 (3)**: 803-821, 2004.

THOMSON MM, SIERRA M, TANURI A, MAY S, CASADO G, MANJÓN N, NÁJERA R. Analysis of near full-length genome sequences of HIV type 1 BF intersubtype recombinant viruses from Brazil reveals their independent origins and their lack of relationship to CRF12_BF.**AIDS Research and Human Retroviruses 20 (10)**: 1126-1133, 2004.

THORLEY-LAWSON DA. Epstein-Barr virus: exploiting the immune system. **Nature Reviews 1 (1)**: 75-82, 2001.

THORLEY-LAWSON DA, HAWKINS JB, TRACY SI, SHAPIRO M. The pathogenesis of Epstein-Barr virus persistent infection. **Current Opinion in Virology 3 (3)**: 227-232, 2013.

TO J, SURYA W, TORRES J. Targeting the Channel Activity of Viroporins. In: **Advances in Protein Chemistry and Structural Biology**. Doney R. 104ª eds. Elsevier. 2016. P. 307-355.

TORRES MA & MORAES MEH. Nomenclature for factors of the HLA system. **Einstein 9 (2)**: 249-251, 2011.

TROTTIER H, BUTEAU C, ROBITAILLE N, DUVAL M, TUCCI M, LACROIX J, ALFIERI C. Transfusion-related Epstein-Barr virus infection among stem cell transplant recipients: a retrospective cohort study in children. **Trasnfusion 52 (12)**: 2653-2663, 2012.

TROMBETTA ES, EBERSOLD M, GARRETT W, PYPAERT M, MELLMAN I. Activation of lysosomal function during dendritic cell maturation. **Science 299 (5611)**: 1400-1403, 2003.

TSHABALALA M, MELLET J, PEPPER MS. Human Leukocyte Antigen Diversity: A Southern African Perspective. **Journal of Immunology Research. (2015)**:746151, 2015.

TSUNETO LT, PROBST CM, HUTZ MH, SALZANO FM, RODRIGUEZ-DELFIN LA, ZAGO MA, HILL K, HURTADO AM, RIBEIRO-DOS-SANTOS AK, PETZL-ERLER ML. HLA class II diversity in seven Amerindian populations. Clues about the origins of the Aché. **Tissue Antigens 62 (6)**: 512-26, 2003.

TUGIZOV SM, BERLINE JW, PALEFSKY JM. Epstein-Barr virus infection of polarized tongue and nasopharyngeal epithelial cells. **Nature Medicine 9 (3)**: 307-314, 2003.

TURNBULL EL, WONG M, WANG S, WEI X, JONES NA, CONROD KE, ALDAM D, TURNER J, PELLEGRINO P, KEELE BF, WILLIAMS I, SHAW GM, BORROW P. Kinetics of expansion of epitope-specific T cell responses during primary HIV-1 infection. **Journal of Immunology 182 (11)**: 7131-7145, 2009.

TZELLOS S & FARRELL PJ. Epstein-barr virus sequence variation-biology and disease. **Pathogens 1 (2)**: 156-174, 2012.

UNAIDS Data 2018. Disponível em: <http://www.unaids.org/sites/default/files/media_asset/unaids-data-2018_en.pdf>. Acessado em: 18/04/2019.

UNAIDS. AIDSinfo. Disponível em: <http://aidsinfo.unaids.org/>. Acessado em: 18/04/2019.

UNAIDS. ESTATÍSTICAS. Disponível em: < https://unaids.org.br/estatisticas/>. Acessado em: 02/02/2022.

URAYAMA, K.Y., JARRETT, R.F., HJALGRIM, H., DIEPSTRA, A., KAMATANI, Y., CHABRIER, A., GABORIEAU, V., BOLAND, A., NIETERS, A., BECKER, N., FORETOVA, L., BENAVENTE, Y., MAYNADIÉ, M., STAINES, A., SHIELD, L., LAKE, A., MONTGOMERY, D., TAYLOR, M., SMEDBY, K.E., AMINI, R.M., ADAMI, H.O., GLIMELIUS, B., FEENSTRA, B., NOLTE, I.M., VISSER, L., VAN IMHOFF, G.W., LIGHTFOOT, T., COCCO, P., KIEMENEY, L., VERMEULEN, S.H., HOLCATOVA, I., VATTEN, L., MACFARLANE, G.J., THOMSON, P., CONWAY. D.I., BENHAMOU, S., AGUDO, A., HEALY, C.M., OVERVAD, K., TJONNELAND, A., MELIN, B., CANZIAN, F., KHAW, K.T., TRAVIS, R.C., PEETERS, P.H., GONZÁLEZ, C.A., QUIRÓS, J.R., SÁNCHEZ, M.J., HUERTA, J.M., ARDANAZ, E., DORRONSORO, M., CLAVEL-CHAPELON, F., BUENO-DE-MESQUITA, H.B., RIBOLI, E., ROMAN, E., BOFFETTA, P., DE SANJOSÉ, S., ZELENIKA, D., MELBYE, M., VAN DEN BERG, A., LATHROP,

M., BRENNAN, P., MCKAY, J.D. Genome-wide association study of classical Hodgkin lymphoma and Epstein-Barr virus status-defined subgroups. **Journal of the National Cancer Institute 104(3)**:240-253, 2012.

VAN BAARLE D, HOVENKAMP E, KERSTEN MJ, KLEIN MR, MIEDEMA F, VAN OERS MH. Direct Epstein-Barr virus (EBV) typing on peripheral blood mononuclear cells: no association between EBV type 2 infection or superinfection and the development of acquired immunodeficiency syndrome-related non-Hodgkin's lymphoma. **Blood 93 (11)**:3949-3955, 1999.

VAN BAARLE D, HOVENKAMP E, DUKERS NH, RENWICK N, KERSTEN MJ, GOUDSMIT J, COUTINHO RA, MIEDEMA F, VAN OERS MH. High prevalence of Epstein-Barr virus type 2 among homosexual men is caused by sexual transmission. **The Journal of Infectious Diseases 181 (6)**:2045-2049, 2000.

VALENZUELA-PONCE H, ALVA-HERNÁNDEZ S, GARRIDO-RODRÍGUEZ D, SOTO-NAVA M, GARCÍA-TÉLLEZ T, ESCAMILLA-GÓMEZ T, GARCÍA-MORALES C, QUIROZ-MORALES VS, TAPIA-TREJO D, DEL ARENAL-SÁNCHEZ S, PRADO-GALBARRO FJ, HERNÁNDEZ-JUAN R, RODRÍGUEZ-AGUIRRE E, MURAKAMI-OGASAWARA A, MEJÍA-VILLATORO C, ESCOBAR-URIAS IY, PINZÓN-MEZA R, PASCALE JM, ZALDIVAR Y, PORRAS-CORTÉS G, QUANT-DURÁN C, LORENZANA I, MEZA RI, PALOU EY, MANZANERO M, CEDILLOS RA, ALÁEZ C, BROCKMAN MA, HARRIGAN PR, BRUMME CJ, BRUMME ZL, ÁVILA-RÍOS S, REYES-TERÁN G; Mesoamerican HIV Project Group. Novel HLA class I associations with HIV-1 control in a unique genetically admixed population. **Scientifc Reports 8 (1): 6111**, 2018.

VICENTE AC, OTSUKI K, SILVA NB, CASTILHO MC, BARROS FS, PIENIAZEK D, HU D, RAYFIELD MA, BRETAS G, TANURI A. The HIV epidemic in the Amazon Basin is driven by prototypic and recombinant HIV-1 subtypes B and F. **Journal of Acquired Immune Deficiency Syndromes 23 (4)**: 327-331, 2000.

VICENZI E & POLI G. Novel factors interfering with human immunodeficiency virus-type 1 replication in vivo and in vitro. **Tissue Antigens 81 (2)**: 61-70, 2013.

VIGA-YURTSEVER S, FRAIJI N, LIRA E, STEFANI MM, KIESSLICH D. High rate of HIV infection in voluntary, first time, young male donors at HEMOAM, a reference blood bank in the Brazilian Amazon: 1992–2012 historical series. **ISBT Science Series 10**: 18-26, 2015.

VIGNEAULT F, WOODS M, BUZON MJ, LI C, PEREYRA F, CROSBY SD, RYCHERT J, CHURCH G, MARTINEZ-PICADO J, ROSENBERG ES, TELENTI A, YU XG, LICHTERFELD M. Transcriptional profiling of CD4 T cells identifies distinct subgroups of HIV-1 elite controllers. **Journal of Virology 85 (6)**: 3015-3019, 2011.

VISSEAUX B, DAMOND F, MATHERON S, DESCAMPS D, CHARPENTIER C. HIV-2 molecular epidemiology. **Infection Genetics and Evolution 46**: 233-240, 2016.

WAINBERG MA, BLAIN N, FITZ-GIBBON L. Differential susceptibility of human lymphocyte cultures to infection by HIV. **Clinical and Experimental Immnuology 70 (1)**: 136-142, 1987.

WANG CY & SUGDEN B. Identifying a property of origins of DNA synthesis required to support plasmids stably in human cells. **Proceedings of the National Academy of Sciences of the United States of America 105 (28)**: 9639-9644, 2008.

WARREN K, WARRILOW D, MEREDITH L, HARRICH D. Reverse Transcriptase and Cellular Factors: Regulators of HIV-1 Reverse Transcription. **Viruses 1 (3)**: 873-894, 2009.

WELZEL TM, GAO X, PFEIFFER RM, MARTIN MP, O'BRIEN SJ, GOEDERT JJ, CARRINGTON M, O'BRIEN TR. HLA-B Bw4 alleles and HIV-1 transmission in heterosexual couples. **AIDS 21 (2)**: 225-9, 2007.

WHELESS SA, GULLEY ML, RAAB-TRAUB N, MCNEILLIE P, NEURINGER IP, FORD HJ, ARIS RM. Post-transplantation lymphoproliferative disease: Epstein-Barr virus DNA levels, HLA-A3, and survival. **American Journal of Respiratory and Critical Care Medicine 178 (10)**: 1060-5, 2008.

WHO. HIV/AIDS. 2020. Disponível em: < https://www.who.int/news-room/fact-sheets/detail/hiv-aids> Acessado em: 02/02/2022.

WILLBERG CB, GARRISON KE, JONES RB, MEIKLEJOHN DA, SPOTTS G, LIEGLER TJ, OSTROWSKI MA, KARLSSON AC, HECHT FM, NIXON DF. Rapid progressing allele HLA-B35 Px restricted anti-HIV-1 CD8+ T cells recognize vestigial CTL epitopes. **PLoS One 5 (4)**: e10249, 2010.

WILLIAMS A, PEH CA, ELLIOTT T. The cell biology of MHC class I antigen presentation. **Tissue Antigens 59 (1)**: 3-17, 2002.

WILLIAMS, H., MCAULAY, K., MACSWEEN, K.F., GALLACHER, N.J., HIGGINS, C.D., HARRISON, N., SWERDLOW, A.J., CRAWFORD, D.H. The immune response to primary EBV infection: a role for natural killer cells. **British Journal of Haematology 129(2)**:266-274, 2005.

WISNER TM & JOHNSON DC. Redistribution of Cellular and Herpes Simplex Virus Proteins from the Trans-Golgi Network to Cell Junctions without Enveloped Capsids. **Journal of Virology 78 (21)**: 11519-11535, 2004.

WIT J, BORGHANS JA, KESMIR C, VAN BAARLE D. Editorial: Role of HLA and KIR in Viral Infections. **Frontiers in Immunology 7**: 286, 2016.

WOLFERS J, LOZIER A, RAPOSO G, REGNAULT A, THÉRY C, MASURIER C, FLAMENT C, POUZIEUX S, FAURE F, TURSZ T, ANGEVIN E, AMIGORENA S, ZITVOGEL L. Tumor-derived exosomes are a source of shared tumor rejection antigens for CTL cross-priming. **Nature Medicine 7 (3)**: 297-303, 2001.

WRIGHT, J.K., BRUMME, Z.L., JULG, B., VAN DER STOK, M., MNCUBE, Z., GAO, X., CARLSON, J.M., GOULDER, P.J., WALKER, B.D., BROCKMAN, M.A., NDUNG'U, T. Lack of association between HLA class II alleles and in vitro replication capacities of recombinant viruses encoding HIV-1 subtype C Gag-protease from chronically infected individuals. **Journal of Virology 86 (2)**:1273-1276, 2012.

WYCISK AI, LIN J, LOCH S, HOBOHM K, FUNKE J, WIENEKE R, KOCH J, SKACH WR, MAYERHOFER PU, TAMPÉ R. Epstein-Barr viral BNLF2a protein hijacks the tail-anchored protein insertion machinery to block antigen processing by the transport complex TAP. **The Journal of Biological Chemistry 286 (48)**: 410402-41412, 2011.

XIAO J, PALEFSKY JM, HERRERA R, TUGIZOV SM. Characterization of the Epstein-Barr virus glycoprotein BMRF-2.**Virology 359 (2)**: 382-396, 2007.

XING Y, SONG HM, WEI M, LIU Y, ZHANG YH, GAO L. Clinical significance of variations in levels of Epstein-Barr Virus (EBV) antigen and adaptive immune response during chronic active EBV infection in children. **Journal of Immunotoxicology 10 (4)**: 387-392, 2013.

YAMAGUCHI J, VALLARI A, MCARTHUR C, STHRESHLEY L, CLOHERTY GA, BERG MG, RODGERS MA. Brief Report: Complete Genome Sequence of CG-0018a-01 Establishes HIV-1 Subtype L. **Journal of Acquired Immune Deficiency Syndromes 83 (3)**: 319-322, 2020.

YANG L, AOZASA K, OSHIMI K, TAKADA K. Epstein-Barr virus (EBV)-encoded RNA promotes growth of EBV-infected T cells through interleukin-9 induction. **Cancer Research 64 (15)**: 5332-5337, 2004.

YAO QY, TIERNEY RJ, CROOM-CARTER D, COOPER GM, ELLIS CJ, ROWE M, RICKINSON AB. Isolation of intertypic recombinants of Epstein-Barr virus from T-cell-immunocompromised individuals. **Journal of Virology 70 (8)**: 4895-4903, 1996.

YATES J, WARREN N, REISMAN D, SUGDEN B.A cis-acting element from the Epstein-Barr viral genome that permits stable replication of recombinant plasmids in latently infected cells. **Proocedings of the National Academy of Sciences of the United States of America 81 (12)**: 3806-3810, 1984.

YATES JL, WARREN N, SUGDEN B. Stable replication of plasmids derived from Epstein-Barr virus in various mammalian cells. **Nature 313 (6005)**: 812-815, 1985.

YATES JL. Epstein–Barr virus DNA replication. In **DNA replication in eukaryotic cells** (ed. ML DePamphilis), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1996. P. 751–774.

YOUNG LS, YAO QY, ROONEY CM, SCULLEY TB, MOSS DJ, RUPANI H, LAUX G, BORNKAMM GW, RICKINSON AB. New type B isolates of Epstein-Barr virus from

Burkitt's lymphoma and from normal individuals in endemic areas. **The Journal of General Virology 68**: 2853-2862, 1987.

YOUNG LS, YAP LF, MURRAY PG. Epstein-Barr virus: more than 50 years old and still providing surprises. **Nature Reviews Cancer 16 (12)**: 789-802, 2016.

YUAN R, CHENG H, CHEN LS, ZHANG X, WANG B. Prevalence of different HIV-1 subtypes in sexual transmission in China: a systematic review and meta-analysis. **Epidemiology and Infection 144 (10)**: 2144-2153, 2016.

ZALANI S, HOLLEY-GUTHRIE E, KENNEY S. Epstein-Barr viral latency is disrupted by the immediate-early BRLF1 protein through a cell-specific mechanism. **Proceedings of the National Academy of Sciences of the United States of America 93 (17)**: 9194-9199, 1996.

ZHANG H, HAN X, ZHAO B, AN M, WANG Z, JIANG F, XU J, ZHANG Z, DONG T, SHANG H. Multilayered HIV-1 gag-specific T-cell responses contribute to slow progression in HLA-A*30-B*13-C*06-positive patients. **AIDS 29 (9)**: 993-1002, 2015.

ZHANG S, KAPLAN AH, TROPSHA A. HIV-1 Protease Function and Structure Studies with the Simplicial Neighborhood Analysis of Protein Packing (SNAPP) Method. **Proteins 73 (3)**: 742-753, 2008.

ZHANG, Y., PENG, Y., YAN, H., XU, K., SAITO, M., WU, H., CHEN, X., RANASINGHE, S., KUSE, N., POWELL, T., ZHAO, Y., LI, W., ZHANG, X., FENG, X., LI, N., LELIGDOWICZ, A., XU, X., JOHN, M., TAKIGUCHI, M., MCMICHAEL, A., ROWLAND-JONES, S., DONG, T. Multilayered defense in HLA-B51-associated HIV viral control. **Journal of Immunology 187(2)**:684-691, 2011.

ZHOU L, LU DP. Immune reconstitution of HLA-A*0201/BMLF1- and HLA-A*1101/LMP2-specific Epstein Barr virus cytotoxic T lymphocytes within 90 days after haploidentical hematopoietic stem cell transplantation. **Virology Journal 16 (1)**: 19, 2019.

ZUR HAUSEN H, SCHULTE-HOLTHAUSEN H, KLEIN G, HENLE W, HENLE G, CLIFFORD P, SANTESSON L.EBV DNA in biopsies of Burkitt tumours and anaplastic carcinomas of the nasopharynx. **Nature 228 (5276)**: 1056-1058, 1970.

ZUR HAUSEN A, BRINK AA, CRAANEN ME, MIDDELDORP JM, MEIJER CJ, VAN DEN BRULE AJ. Unique transcription pattern of Epstein-Barr virus (EBV) in EBV-carrying gastric adenocarcinomas: expression of the transforming BARF1 gene. Cancer Research 60(10):2745-2748, 2000.

ANEXO 1

Preparing your manuscript (Molecular Medicine) (https://molmed.biomedcentral.com/submission-guidelines/preparing-your-manuscript)

Research article

Criteria

Research articles should report on original primary research.

The Discussion section should discuss the application of the results to the understanding of disease and the development of tools for disease diagnosis, treatment and prevention. The main findings should be placed in context to highlight the advance and describe how it moves the field forward. Molecular Medicine strongly encourages that all datasets on which the conclusions of the paper rely should be available to readers. We encourage authors to ensure that their datasets are either deposited in publicly available repositories (where available and appropriate) or presented in the main manuscript or additional supporting files whenever possible. Please see Springer Nature's information on recommended repositories. Where a widely established research community expectation for data archiving in public repositories exists, submission to a community-endorsed, public repository is mandatory. A list of data where deposition is required, with the appropriate repositories, can be found on the Editorial Policies Page.

The Editors of Molecular Medicine welcome authors to send any pre-submission inquiries they might have here. We ask that you please include "Pre-submission inquiry" and the journal name in the subject line of your email and that you include the title and abstract of your proposed submission within the message. Our Editors will do their best to respond to you in a timely manner.

Please note: we ask that you review the sections on this entire page before submitting your work, as omission of any necessary content will delay the processing of your manuscripts. Some information which is often forgotten includes (but is not limited to) the following, but please go through the entire page to make sure you include all necessary sections/information:

- The Declarations section, and its sub-sections
- Corresponding author information (it must stay consistent throughout the process; Molecular Medicine strongly advises having only one Corresponding Author, for ease of communication during the editorial workflow and production of your article)
- Co-author email addresses
- A list of abbreviations

A note to authors from the Feinstein Institutes: please do not submit a manuscript with a membership code, without being invited to do so by our editors. Feinstein authorship does not automatically warrant use of a membership code.

Cover letter

You may suggest up to three potential reviewers in your cover letter. You should not suggest recent collaborators or colleagues who work in the same institution as yourselves. You should provide institutional email addresses where possible, or information which will help the Editor to verify the identity of the reviewer (for example an ORCID or Scopus ID). See our Editorial policies for guidance on suggesting peer reviewers, and Submission Guidelines for further information on what you should include in your cover letter.

Preparing your manuscript

The information below details the section headings that you should include in your manuscript and what information should be within each section.

Please note that your manuscript must include a 'Declarations' section including all of the subheadings (please see below for more information).

Title page

The title page should:

Present a title that includes, if appropriate, the study design e.g.:

"A versus B in the treatment of C: a randomized controlled trial", "X is a risk factor for Y: a case control study", "What is the impact of factor X on subject Y: A systematic review"

Or for non-clinical or non-research studies a description of what the article reports

List the full names and institutional addresses for all authors

If a collaboration group should be listed as an author, please list the group name as an author. If you would like the names of the individual members of the group to be searchable through their individual pubmed records, please include this information in the “acknowledgements” section in accordance with the instructions below

Indicate the corresponding author (please note: molecular medicine strongly advises having only one corresponding author, for ease of communication during the editorial workflow and production of your article)

Abstract

The Abstract should not exceed 350 words. Please minimize the use of abbreviations and do not cite references in the abstract. Reports of randomized controlled trials should follow the CONSORT extension for abstracts. The abstract must include the following separate sections:

Background: the context and purpose of the study

Methods: how the study was performed and statistical tests used

Results: the main findings

Conclusions: brief summary and potential implications

Trial registration: If your article reports the results of a health care intervention on human participants, it must be registered in an appropriate registry and the registration number and date of registration should be in stated in this section. If it was not registered prospectively (before enrollment of the first participant), you should include the words 'retrospectively registered'. See our editorial policies for more information on trial registration

Keywords

Three to ten keywords representing the main content of the article.

Background

The Background section should explain the background to the study, its aims, a summary of the existing literature and why this study was necessary or its contribution to the field.

Methods

The methods section should include:

The aim, design and setting of the study

The characteristics of participants or description of materials

A clear description of all processes, interventions and comparisons. Generic drug names should generally be used. When proprietary brands are used in research, include the brand names in parentheses

The type of statistical analysis used, including a power calculation if appropriate

Results

This should include the findings of the study including, if appropriate, results of statistical analysis which must be included either in the text or as tables and figures.

Discussion

This section should discuss the implications of the findings in context of existing research and highlight limitations of the study.

Conclusions

This should state clearly the main conclusions and provide an explanation of the importance and relevance of the study reported.

List of abbreviations

If abbreviations are used in the text they should be defined in the text at first use, and a list of abbreviations should be provided.

Declarations

All manuscripts must contain the following sections under the heading 'Declarations':

Ethics approval and consent to participate

Consent for publication

Availability of data and materials

Competing interests

Funding

Authors' contributions

Acknowledgements

Authors' information (optional)

Please see below for details on the information to be included in these sections.

If any of the sections are not relevant to your manuscript, please include the heading and write 'Not applicable' for that section.

Ethics approval and consent to participate

Manuscripts reporting studies involving human participants, human data or human tissue must:

Include a statement on ethics approval and consent (even where the need for approval was waived)

Include the name of the ethics committee that approved the study and the committee's reference number if appropriate

Studies involving animals must include a statement on ethics approval and for experimental studies involving client-owned animals, authors must also include a statement on informed consent from the client or owner.

See our editorial policies for more information.

If your manuscript does not report on or involve the use of any animal or human data or tissue, please state "Not applicable" in this section.

Consent for publication

If your manuscript contains any individual person's data in any form (including any individual details, images or videos), consent for publication must be obtained from that person, or in the case of children, their parent or legal guardian. All presentations of case reports must have consent for publication.

You can use your institutional consent form or our consent form if you prefer. You should not send the form to us on submission, but we may request to see a copy at any stage (including after publication).

See our editorial policies for more information on consent for publication.

If your manuscript does not contain data from any individual person, please state "Not applicable" in this section.

Availability of data and materials

All manuscripts must include an ‘Availability of data and materials’ statement. Data availability statements should include information on where data supporting the results reported in the article can be found including, where applicable, hyperlinks to publicly archived datasets analysed or generated during the study. By data we mean the minimal dataset that would be necessary to interpret, replicate and build upon the findings reported in the article. We recognise it is not always possible to share research data publicly, for instance when individual privacy could be compromised, and in such instances data availability should still be stated in the manuscript along with any conditions for access.

Data availability statements can take one of the following forms (or a combination of more than one if required for multiple datasets):

The datasets generated and/or analysed during the current study are available in the [NAME] repository, [PERSISTENT WEB LINK TO DATASETS]

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

All data generated or analysed during this study are included in this published article [and its supplementary information files].

The datasets generated and/or analysed during the current study are not publicly available due [REASON WHY DATA ARE NOT PUBLIC] but are available from the corresponding author on reasonable request.

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

The data that support the findings of this study are available from [third party name] but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of [third party name].

Not applicable. If your manuscript does not contain any data, please state 'Not applicable' in this section.

More examples of template data availability statements, which include examples of openly available and restricted access datasets, are available here.

BioMed Central also requires that authors cite any publicly available data on which the conclusions of the paper rely in the manuscript. Data citations should include a persistent identifier (such as a DOI) and should ideally be included in the reference list. Citations of datasets, when they appear in the reference list, should include the minimum information recommended by DataCite and

follow journal style. Dataset identifiers including DOIs should be expressed as full URLs. For example:

Hao Z, AghaKouchak A, Nakhjiri N, Farahmand A. Global integrated drought monitoring and prediction system (GIDMaPS) data sets. figshare. 2014. http://dx.doi.org/10.6084/m9.figshare.853801

With the corresponding text in the Availability of data and materials statement:

The datasets generated during and/or analysed during the current study are available in the [NAME] repository, [PERSISTENT WEB LINK TO DATASETS].[Reference number]

If you wish to co-submit a data note describing your data to be published in BMC Research Notes, you can do so by visiting our submission portal. Data notes support open data and help authors to comply with funder policies on data sharing. Co-published data notes will be linked to the research article the data support (example).

For more information please email our Research Data Team.

Competing interests

All financial and non-financial competing interests must be declared in this section.

See our editorial policies for a full explanation of competing interests. If you are unsure whether you or any of your co-authors have a competing interest please contact the editorial office.

Please use the authors initials to refer to each authors' competing interests in this section.

If you are a staff member of The Feinstein Institutes for Medical Research and/or Northwell Health, or a board member for Molecular Medicine, it is required to list this in the Competing Interests statement.

If you do not have any competing interests, please state "The authors declare that they have no competing interests" in this section.

Funding

All sources of funding for the research reported should be declared. The role of the funding body in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript should be declared.

Authors' contributions

The individual contributions of authors to the manuscript should be specified in this section. Guidance and criteria for authorship can be found in our editorial policies.

Please use initials to refer to each author's contribution in this section, for example: "FC analyzed and interpreted the patient data regarding the hematological disease and the transplant. RH performed the histological examination of the kidney, and was a major contributor in writing the manuscript. All authors read and approved the final manuscript."

Acknowledgements

Please acknowledge anyone who contributed towards the article who does not meet the criteria for authorship including anyone who provided professional writing services or materials.

Authors should obtain permission to acknowledge from all those mentioned in the Acknowledgements section.

See our editorial policies for a full explanation of acknowledgements and authorship criteria.

If you do not have anyone to acknowledge, please write "Not applicable" in this section.

Group authorship (for manuscripts involving a collaboration group): if you would like the names of the individual members of a collaboration Group to be searchable through their individual PubMed records, please ensure that the title of the collaboration Group is included on the title page and in the submission system and also include collaborating author names as the last paragraph of the “Acknowledgements” section. Please add authors in the format First Name, Middle initial(s) (optional), Last Name. You can add institution or country information for each author if you wish, but this should be consistent across all authors.

Please note that individual names may not be present in the PubMed record at the time a published article is initially included in PubMed as it takes PubMed additional time to code this information.

Authors' information

This section is optional.

You may choose to use this section to include any relevant information about the author(s) that may aid the reader's interpretation of the article, and understand the standpoint of the author(s). This may include details about the authors' qualifications, current positions they hold at institutions or societies, or any other relevant background information. Please refer to authors using their initials. Note this section should not be used to describe any competing interests.

Footnotes

Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.

Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data). Footnotes to the title or the authors of the article are not given reference symbols.

Always use footnotes instead of endnotes.

References

Examples of the Vancouver reference style are shown below.

See our editorial policies for author guidance on good citation practice

Web links and URLs: All web links and URLs, including links to the authors' own websites, should be given a reference number and included in the reference list rather than within the text of the manuscript. They should be provided in full, including both the title of the site and the URL, as well as the date the site was accessed, in the following format: The Mouse Tumor Biology Database. http://tumor.informatics.jax.org/mtbwi/index.do. Accessed 20 May 2013. If an author or group of authors can clearly be associated with a web link, such as for weblogs, then they should be included in the reference.

Example reference style:

ARTICLE WITHIN A JOURNAL

Smith JJ. The world of science. Am J Sci. 1999;36:234-5.

ARTICLE WITHIN A JOURNAL (NO PAGE NUMBERS)

Rohrmann S, Overvad K, Bueno-de-Mesquita HB, Jakobsen MU, Egeberg R, Tjønneland A, et al. Meat consumption and mortality - results from the European Prospective Investigation into Cancer and Nutrition. BMC Medicine. 2013;11:63.

ARTICLE WITHIN A JOURNAL BY DOI

Slifka MK, Whitton JL. Clinical implications of dysregulated cytokine production. Dig J Mol Med. 2000; doi:10.1007/s801090000086.

ARTICLE WITHIN A JOURNAL SUPPLEMENT

Frumin AM, Nussbaum J, Esposito M. Functional asplenia: demonstration of splenic activity by bone marrow scan. Blood 1979;59 Suppl 1:26-32.

BOOK CHAPTER, OR AN ARTICLE WITHIN A BOOK

Wyllie AH, Kerr JFR, Currie AR. Cell death: the significance of apoptosis. In: Bourne GH, Danielli JF, Jeon KW, editors. International review of cytology. London: Academic; 1980. p. 251-306.

ONLINEFIRST CHAPTER IN A SERIES (WITHOUT A VOLUME DESIGNATION BUT WITH A DOI)

Saito Y, Hyuga H. Rate equation approaches to amplification of enantiomeric excess and chiral symmetry breaking. Top Curr Chem. 2007. doi:10.1007/128_2006_108.

COMPLETE BOOK, AUTHORED

Blenkinsopp A, Paxton P. Symptoms in the pharmacy: a guide to the management of common illness. 3rd ed. Oxford: Blackwell Science; 1998.

ONLINE DOCUMENT

Doe J. Title of subordinate document. In: The dictionary of substances and their effects. Royal Society of Chemistry. 1999. http://www.rsc.org/dose/title of subordinate document. Accessed 15 Jan 1999.

ONLINE DATABASE

Healthwise Knowledgebase. US Pharmacopeia, Rockville. 1998. http://www.healthwise.org. Accessed 21 Sept 1998.

SUPPLEMENTARY MATERIAL/PRIVATE HOMEPAGE

Doe J. Title of supplementary material. 2000. http://www.privatehomepage.com. Accessed 22 Feb 2000.

UNIVERSITY SITE

Doe, J: Title of preprint. http://www.uni-heidelberg.de/mydata.html (1999). Accessed 25 Dec 1999.

FTP SITE

Doe, J: Trivial HTTP, RFC2169. ftp://ftp.isi.edu/in-notes/rfc2169.txt (1999). Accessed 12 Nov 1999.

ORGANIZATION SITE

ISSN International Centre: The ISSN register. http://www.issn.org (2006). Accessed 20 Feb 2007.

DATASET WITH PERSISTENT IDENTIFIER

Zheng L-Y, Guo X-S, He B, Sun L-J, Peng Y, Dong S-S, et al. Genome data from sweet and grain sorghum (Sorghum bicolor). GigaScience Database. 2011. http://dx.doi.org/10.5524/100012.

Preparing figures

When preparing figures, please follow the formatting instructions below.

Figures should be numbered in the order they are first mentioned in the text, and uploaded in this order. Multi-panel figures (those with parts a, b, c, d etc.) should be submitted as a single composite file that contains all parts of the figure.

Figures should be uploaded in the correct orientation.

Figure titles (max 15 words) and legends (max 300 words) should be provided in the main manuscript, not in the graphic file.

Figure keys should be incorporated into the graphic, not into the legend of the figure.

Each figure should be closely cropped to minimize the amount of white space surrounding the illustration. Cropping figures improves accuracy when placing the figure in combination with other elements when the accepted manuscript is prepared for publication on our site. For more information on individual figure file formats, see our detailed instructions.

Individual figure files should not exceed 10 MB. If a suitable format is chosen, this file size is adequate for extremely high quality figures.

Please note that it is the responsibility of the author(s) to obtain permission from the copyright holder to reproduce figures (or tables) that have previously been published elsewhere. In order for all figures to be open access, authors must have permission from the rights holder if they wish to include images that have been published elsewhere in non open access journals. Permission should be indicated in the figure legend, and the original source included in the reference list.

Figure file types

We accept the following file formats for figures:

EPS (suitable for diagrams and/or images)

PDF (suitable for diagrams and/or images)

Microsoft Word (suitable for diagrams and/or images, figures must be a single page)

PowerPoint (suitable for diagrams and/or images, figures must be a single page)

TIFF (suitable for images)

JPEG (suitable for photographic images, less suitable for graphical images)

PNG (suitable for images)

BMP (suitable for images)

CDX (ChemDraw - suitable for molecular structures)

For information and suggestions of suitable file formats for specific figure types, please see our author academy.

Figure size and resolution

Figures are resized during publication of the final full text and PDF versions to conform to the BioMed Central standard dimensions, which are detailed below.

Figures on the web:

Width of 600 pixels (standard), 1200 pixels (high resolution).

Figures in the final PDF version:

Width of 85 mm for half page width figure

Width of 170 mm for full page width figure

Maximum height of 225 mm for figure and legend

Image resolution of approximately 300 dpi (dots per inch) at the final size

Figures should be designed such that all information, including text, is legible at these dimensions. All lines should be wider than 0.25 pt when constrained to standard figure widths. All fonts must be embedded.

Figure file compression

Vector figures should if possible be submitted as PDF files, which are usually more compact than EPS files.

TIFF files should be saved with LZW compression, which is lossless (decreases file size without decreasing quality) in order to minimize upload time.

JPEG files should be saved at maximum quality.

Conversion of images between file types (especially lossy formats such as JPEG) should be kept to a minimum to avoid degradation of quality.

If you have any questions or are experiencing a problem with figures, please contact the customer service team at info@biomedcentral.com.

Quick points:

Use double line spacing

Include line and page numbering

Use SI units: Please ensure that all special characters used are embedded in the text, otherwise they will be lost during conversion to PDF

Do not use page breaks in your manuscript

File formats

The following word processor file formats are acceptable for the main manuscript document:

Microsoft word (DOC, DOCX)

Rich text format (RTF)

TeX/LaTeX (use BioMed Central's TeX template)

Please note: editable files are required for processing in production. If your manuscript contains any non-editable files (such as PDFs) you will be required to re-submit an editable file when you submit your revised manuscript, or after editorial acceptance in case no revision is necessary.

Additional information for TeX/LaTeX users

Please use BioMed Central's TeX template and BibTeX stylefile if you use TeX format. Submit your references using either a bib or bbl file. When submitting TeX submissions, please submit both your TeX file and your bib/bbl file as manuscript files. Please also convert your TeX file into a PDF (please do not use a DIV file) and submit this PDF as a supplementary file with the name 'Reference PDF'. This PDF will be used by our production team as a reference point to check the layout of the article as the author intended.

The Editorial Manager system checks for any errors in the Tex files. If an error is present then the system PDF will display LaTex code and highlight and explain the error in a section beginning with an exclamation mark (!).

All relevant editable source files must be uploaded during the submission process. Failing to submit these source files will cause unnecessary delays in the production process.

Style and language

For editors and reviewers to accurately assess the work presented in your manuscript you need to ensure the English language is of sufficient quality to be understood. If you need help with writing in English you should consider:

Getting a fast, free online grammar check.

Visiting the English language tutorial which covers the common mistakes when writing in English.
Asking a colleague who is proficient in English to review your manuscript for clarity.
Using a professional language editing service where editors will improve the English to ensure that your meaning is clear and identify problems that require your review. Two such services are provided by our affiliates Nature Research Editing Service and American Journal Experts. BMC authors are entitled to a 10% discount on their first submission to either of these services. To claim 10% off English editing from Nature Research Editing Service, click here. To claim 10% off American Journal Experts, click here.
Please note that the use of a language editing service is not a requirement for publication in the journal and does not imply or guarantee that the article will be selected for peer review or accepted.

Data and materials

For all journals, BioMed Central strongly encourages all datasets on which the conclusions of the manuscript rely to be either deposited in publicly available repositories (where available and appropriate) or presented in the main paper or additional supporting files, in machine-readable format (such as spread sheets rather than PDFs) whenever possible. Please see the list of recommended repositories in our editorial policies.
For some journals, deposition of the data on which the conclusions of the manuscript rely is an absolute requirement. Please check the Instructions for Authors for the relevant journal and article type for journal specific policies.
For all manuscripts, information about data availability should be detailed in an ‘Availability of data and materials’ section. For more information on the content of this section, please see the Declarations section of the relevant journal’s Instruction for Authors. For more information on BioMed Centrals policies on data availability, please see our [editorial policies].
Formatting the 'Availability of data and materials' section of your manuscript
The following format for the 'Availability of data and materials section of your manuscript should be used:

"The dataset(s) supporting the conclusions of this article is(are) available in the [repository name] repository, [unique persistent identifier and hyperlink to dataset(s) in http:// format]."

The following format is required when data are included as additional files:

"The dataset(s) supporting the conclusions of this article is(are) included within the article (and its additional file(s))."

BioMed Central endorses the Force 11 Data Citation Principles and requires that all publicly available datasets be fully referenced in the reference list with an accession number or unique identifier such as a DOI.

For databases, this section should state the web/ftp address at which the database is available and any restrictions to its use by non-academics.

For software, this section should include:

Project name: e.g. My bioinformatics project

Project home page: e.g. http://sourceforge.net/projects/mged

Archived version: DOI or unique identifier of archived software or code in repository (e.g. enodo)

Operating system(s): e.g. Platform independent

Programming language: e.g. Java

Other requirements: e.g. Java 1.3.1 or higher, Tomcat 4.0 or higher

License: e.g. GNU GPL, FreeBSD etc.

Any restrictions to use by non-academics: e.g. licence needed

Information on available repositories for other types of scientific data, including clinical data, can be found in our editorial policies.

References

See our editorial policies for author guidance on good citation practice.

Please check the submission guidelines for the relevant journal and article type.

What should be cited?

Only articles, clinical trial registration records and abstracts that have been published or are in press, or are available through public e-print/preprint servers, may be cited.

Unpublished abstracts, unpublished data and personal communications should not be included in the reference list, but may be included in the text and referred to as "unpublished observations" or "personal communications" giving the names of the involved researchers. Obtaining permission to quote personal communications and unpublished data from the cited colleagues is the responsibility of the author. Only footnotes are permitted. Journal abbreviations follow Index Medicus/MEDLINE.

Any in press articles cited within the references and necessary for the reviewers' assessment of the manuscript should be made available if requested by the editorial office.

How to format your references

Please check the Instructions for Authors for the relevant journal and article type for examples of the relevant reference style.

WEB LINKS AND URLS: All web links and URLs, including links to the authors' own websites, should be given a reference number and included in the reference list rather than within the text of the manuscript. They should be provided in full, including both the title of the site and the URL, as well as the date the site was accessed, in the following format: The Mouse Tumor Biology Database. http://tumor.informatics.jax.org/mtbwi/index.do. Accessed 20 May 2013. If an author or group of authors can clearly be associated with a web link, such as for weblogs, then they should be included in the reference.

Authors may wish to make use of reference management software to ensure that reference lists are correctly formatted.

Preparing tables

When preparing tables, please follow the formatting instructions below.

Tables should be numbered and cited in the text in sequence using Arabic numerals (i.e. Table 1, Table 2 etc.).

Tables less than one A4 or Letter page in length can be placed in the appropriate location within the manuscript.

Tables larger than one A4 or Letter page in length can be placed at the end of the document text file. Please cite and indicate where the table should appear at the relevant location in the text file so that the table can be added in the correct place during production.

Larger datasets, or tables too wide for A4 or Letter landscape page can be uploaded as additional files. Please see [below] for more information.

Tabular data provided as additional files can be uploaded as an Excel spreadsheet (.xls) or comma separated values (.csv). Please use the standard file extensions.

Table titles (max 15 words) should be included above the table, and legends (max 300 words) should be included underneath the table.

Tables should not be embedded as figures or spreadsheet files, but should be formatted using ‘Table object’ function in your word processing program.

Color and shading may not be used. Parts of the table can be highlighted using superscript, numbering, lettering, symbols or bold text, the meaning of which should be explained in a table legend.

Commas should not be used to indicate numerical values.

If you have any questions or are experiencing a problem with tables, please contact the customer service team at info@biomedcentral.com.

Preparing additional files

As the length and quantity of data is not restricted for many article types, authors can provide datasets, tables, movies, or other information as additional files.

All Additional files will be published along with the accepted article. Do not include files such as patient consent forms, certificates of language editing, or revised versions of the main manuscript document with tracked changes. Such files, if requested, should be sent by email to the journal's editorial email address, quoting the manuscript reference number. Please do not send completed patient consent forms unless requested.

Results that would otherwise be indicated as "data not shown" should be included as additional files. Since many web links and URLs rapidly become broken, BioMed Central requires that supporting data are included as additional files, or deposited in a recognized repository. Please do not link to data on a personal/departmental website. Do not include any individual participant details. The maximum file size for additional files is 20 MB each, and files will be virus-scanned on submission. Each additional file should be cited in sequence within the main body of text.

If additional material is provided, please list the following information in a separate section of the manuscript text:

File name (e.g. Additional file 1)

File format including the correct file extension for example .pdf, .xls, .txt, .pptx (including name and a URL of an appropriate viewer if format is unusual)

Title of data

Description of data

Additional files should be named "Additional file 1" and so on and should be referenced explicitly by file name within the body of the article, e.g. 'An additional movie file shows this in more detail [see Additional file 1]'

ANEXO 2

PARECER CONSUBSTANCIADO DO CEP

DADOS DA EMENDA

Título da Pesquisa: INVESTIGAÇÃO DE MARCADORES IMUNOLÓGICOS E VIROLÓGICOS EM PACIENTES PORTADORES DAS INFECÇÕES PELOS VÍRUS DA IMUNODEFICIÊNCIA HUMANA E VÍRUS EPSTEIN-BARR

Pesquisador: Igor Brasil Costa

Área Temática:

Versão: 5

CAAE: 73927717.3.0000.0019

Instituição Proponente: Instituto Evandro Chagas/SVS/MS

Patrocinador Principal: Instituto Evandro Chagas/SVS/MS

DADOS DO PARECER

Número do Parecer: 3.121.265

Apresentação do Projeto:

Projeto que visa avaliar o perfil imunológico de pacientes co-infectados com HIV e EBV no Estado do Pará,sendo de caráter observacional, analítico e longitudinal, que busca avaliar a relação de polimorfismos em genes codificantes de citocinas anti e pró-inflamatórias com a estimulação da replicação e expressão do HIV e EBV, além de verificar a influência do perfil alélico de HLA na estimulação da resposta imune celular e humoral específicas.

Objetivo da Pesquisa:

Objetivo Primário:

Avaliar o perfil imunológico de pacientes infectados por HIV e EBV no Estado do Pará, Amazônia Brasileira.

Objetivo Secundário:

Avaliar o perfil epidemiológico entre quatro grupos: infectados apenas com HIV, apenas com EBV, coinfectados HIV e EBV e controle; Investigar os níveis séricos das citocinas IL-17A, INF- , TNF, IL-10, IL-6,IL-4 e IL-2 entre os quatro grupos; Determinar as frequências alélicas e genotípicas dos polimorfismos das regiões promotoras dos genes das citocinas entre os quatro grupos estudados; Associar os polimorfismos com os níveis séricos para os quatro grupos; Associar os polimorfismos com a carga viral para HIV e EBV; Avaliar a diversidade genética do HIV e EBV; Associar as cargas virais encontradas para HIV com as cargas virais de EBV; Associar

os níveis séricos de citocinas com carga viral para HIV e EBV; Associar os níveis séricos de citocinas com a contagem de células CD4+/CD8+ para os quatro grupos; Associar os polimorfismos com a contagem de células CD4+/CD8+ para os quatro grupos; Determinar as frequências alélicas e genotípica dos alelos HLA para os loci A, B e DR nos quatro grupos; Associar os alelos HLA com os níveis de CD4+/CD8+, carga viral para HIV e EBV, SNP, dosagem sérica de citocinas nos quatro grupos; Investigar a associação das variáveis clínicas com os polimorfismos, dosagem sérica de citocinas, carga viral para HIV e EBV, alelos HLA, contagem de células CD4+/CD8+ entre os grupos infectados.

Avaliação dos Riscos e Benefícios:

Riscos:

Durante a coleta de sangue, os participantes da pesquisa poderão sentir leve desconforto, que será minimizado pela experiência dos colaboradores que farão as punções. Outro risco oriundo da participação nesta pesquisa é a divulgação de informações pessoais, o qual será minimizado pela codificação dos participantes e de suas amostras, além do profissionalismo da equipe envolvida.

Benefícios:

Compreender melhor de que forma fatores ligados a reposta imune do hospedeiro, como os níveis séricos de citocinas, polimorfismos genéticos nos genes que codificam estas citocinas, assim como diferentes perfis alélicos de HLA, poderiam influenciar ou ser influenciados diretamente ou indiretamente por marcadores de evolução das infecções por HIV e EBV.

Comentários e Considerações sobre a Pesquisa:

Pesquisa bastante relevante que trará informações importantes sobre as características epidemiológicas dos grupos a serem estudados associando aos dados moleculares, tais como a compreensão dos fatores ligados a resposta imune do hospedeiro que podem influenciar no curso da infecção pelos dois vírus.

Considerações sobre os Termos de apresentação obrigatória:

Os termos foram devidamente apresentados.

Recomendações:

Igor Brasil Costa

Recomendamos que a coordenação mantenha atualizados todos os documentos pertinentes ao projeto.

Este CEP se incumbirá dos procedimentos de acompanhamento preconizados pela Resolução 466/12 Publicada no DOU nº 12, 13 de junho de 2013 – Seção 1 – Página 59 e suas complementares, do Conselho Nacional de Saúde/Ministério da Saúde.

PROJETOS COM MAIS DE TRÊS ANOS

1. Deverá ser encaminhado relatório semestral e, ao final, elaborado um relatório consolidado, incluindo os resultados finais da pesquisa, em um prazo máximo de 60 (sessenta) dias, após a finalização da mesma. PROJETOS COM MENOS DE TRÊS ANOS
2. Deverá ser encaminhado relatório incluindo os resultados finais da pesquisa, em um prazo máximo de 60 (sessenta) dias, após a finalização da mesma.

Conclusões ou Pendências e Lista de Inadequações:

Igor Brasil Costa

Diante do exposto, o Comitê de Ética em Pesquisa-CEP, de acordo com as atribuições definidas na Resolução CNS 466/2012 e na Norma Operacional Nº 001/2013 do CNS, manifesta-se pela aprovação do projeto de pesquisa na forma proposta.

Considerações Finais a critério do CEP:

Igor Brasil Costa

Conforme Res. CNS 466/12, a responsabilidade do pesquisador é indelegável e indeclinável e compreende os aspectos éticos e legais da pesquisa. Nesse sentido, ressaltamos as seguintes atribuições do pesquisador:

1. Apresentar o protocolo devidamente instruído ao CEP ou à CONEP, aguardando a decisão de aprovação ética, antes de iniciar a pesquisa;
2. Desenvolver o projeto conforme delineado;
3. Elaborar e apresentar os relatórios parcial (is) e final;
4. Apresentar dados solicitados pelo CEP ou pela CONEP a qualquer momento;
5. Manter os dados da pesquisa em arquivo, físico ou digital, sob sua guarda responsabilidade, por um período de 5 (cinco) anos após o término da pesquisa;
6. Encaminhar os resultados da pesquisa para publicação, com os devidos créditos aos pesquisadores associados e ao pessoal técnico integrante do projeto; e
7. Justificar fundamentadamente, perante o CEP ou a CONEP, interrupção do projeto ou a não publicação dos resultados.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

ANANINDEUA, 24 de Janeiro de 2019

Assinado por:

Arnaldo Jorge Martins Filho (Coordenador(a))

APÊNDICE 1

QUESTIONÁRIO EPIDEMIOLÓGICO

1. Data da coleta de dados: Protocolo:

2. Nome do Paciente:

Dados epidemiológicos:
3. Sexo: Masculino ( ) Feminino ( )

4. Data de nascimento: Idade: anos
5. Estado Civil: ( ) Casado(a) ( ) Solteiro(a) ( ) Separado(a) ( ) Viúvo(a)

6. Ocupação:

7. Endereço:

8. Município:

9. Município de residência anterior (se reside há menos de 05 anos no endereço atual):

10. Naturalidade:

11. Escolaridade:
( ) Não alfabetizado ( ) Alfabetizado ( ) 1° grau incompleto ( ) 1° grau completo ( ) 2° grau incompleto ( ) 2° grau completo ( ) 3º grau incompleto ( ) 3º grau completo

12. Renda familiar (salários): a) < 1 b) 1-3 c) 4-6 d) 7-10 e) > 10 f) Ñ sabe

13. Tabagismo
Já fumou cigarros na vida?
( ) Não (se **não**, ir para a próxima pergunta)
( )Sim. Com que idade iniciou: ( ) Não lembra

14. Etilismo
Já consumiu bebidas alcoólicas na vida?
( ) Não (se **não**, ir para a próxima pergunta)
( ) Sim. Com que idade iniciou: ( ) Não lembra

15. Uso de drogas ilícitas alguma vez:
( ) Sim, mas não quer comentar ( ) Sim, quais:
( ) Não ( ) Não quer comentar

16. Há quanto tempo faz uso de drogas: Anos
Parou? ( ) Sim ( ) Não Ano do último uso:

20. Tem vida sexual ativa: Sim ( ) Não ( )

21. Quanto tempo desde a última relação sexual: anos

22. Início da vida sexual: ( ) 10-15 anos ( ) 16-20 anos ( ) 21-25 anos ( ) >25 anos

23. Opção sexual: ( ) Homossexual ( ) Bissexual ( ) Heterossexual

24. Atualmente tem parceiro fixo: ( ) Sim ( ) Não

25. Sexo com trabalhador (a) comercial do sexo: ( ) Sim ( ) Não ( ) Não sabe

26. Uso de preservativo: ( ) Sempre ( ) Nunca ( ) as vezes

27. Histórico de DST: ( ) Não ( ) Sim Frequência: ( ) 1 ( ) 1 a 5 ( ) >5
Quais:

28. Possui dificuldade para dormir? ( ) Sempre ( ) Nunca ( ) as vezes
Média de horas de sono por dia: horas

29. Possui diagnóstico de câncer?
Qual?
Histórico familiar de câncer?
Qual parentesco?
Qual tipo de câncer?

31. Qual o tempo de diagnóstico do HIV?

Assinatura do responsável pela aplicação do questionário:

APÊNDICE 2

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

O(A) senhor(a) está sendo convidado(a) a participar de uma pesquisa que será desenvolvida no Instituo Evandro Chagas (IEC), em parceria com o Laboratório de Virologia da UFPA, Hospital Universitário João de Barros Barreto (HUJBB), Casa DIA e Centro de Hemoterapia e Hematologia do Estado do Pará (HEMOPA).

Para que decida em participar ou não da pesquisa me foram prestadas as seguintes informações:

- O título do projeto é: INVESTIGAÇÃO DE MARCADORES IMUNOLÓGICOS E VIROLÓGICOS EM PACIENTES PORTADORES DAS INFECÇÕES PELOS VÍRUS DA IMUNODEFICIÊNCIA HUMANA E VÍRUS EPSTEIN-BARR.
- O pesquisador responsável é o Dr. Igor Brasil Costa, Biomédico, pesquisador do Instituto Evandro Chagas, ao qual poderá contatar a qualquer tempo pelos telefones (91)3214-2005 ou (91)3214-2023 ou (91)3214-2086 para esclarecer questões referentes à pesquisa.
- Quaisquer assistências, sejam elas imediatas ou integrais, serão atendidas pela equipe técnica do projeto e dúvidas referentes aos objetivos, metodologia e relevância do estudo poderão ser direcionadas ao pesquisador responsável através dos contatos citados acima.
- O objetivo da pesquisa é avaliar a resposta imunológica contra as infecções pelos vírus da imunodeficiência humana (HIV) e Epstein-Barr (EBV).
- As coletas dos materiais biológicos estarão de acordo com as normas de segurança. Serão coletados dois tubos de sangue para os testes necessários.
- Como riscos da sua participação, por ser um método invasivo, a coleta poderá levar à algum desconforto ou pequenos hematomas na pele no momento de sua realização. Entretanto, a mesma será realizada por equipe técnica treinada utilizando materiais estéreis e descartáveis.
- Os materiais biológicos serão armazenados como biorrepositório até o final desta pesquisa.
- O projeto será submetido à aprovação pelo Comitê de Ética em Pesquisa do Instituto Evandro Chagas, o qual tem como finalidade a minha proteção como sujeito desta pesquisa.
- Sua participação não é obrigatória, assim poderá deixar a pesquisa no momento em que quiser, pois não haverá prejuízo pessoal por esta causa.
- Esta pesquisa não oferece qualquer possibilidade de ajuda financeira aos voluntários que participarem, assim como não irá gerar nenhum ônus aos seus participantes.

- O resultado deste trabalho será convertido em benefício para a ciência básica e saúde pública com o melhor entendimento sobre as infecções por HIV e EBV.
- A participação na pesquisa é sigilosa, isto significa que somente os pesquisadores ficarão sabendo de sua participação e de seus dados pessoais. Não haverá divulgação da identificação individual do participante. Para assegurar o total sigilo dos participantes, serão criados códigos alfa-numéricos utilizados durante todas as etapas da pesquisa.

CONSENTIMENTO LIVRE E ESCLARECIDO

Declaro que li as informações acima sobre a pesquisa, que me sinto perfeitamente esclarecido acerca do conteúdo da mesma, assim como seus riscos e benefícios. Declaro ainda que, por minha livre vontade, aceito participar da pesquisa cooperando com a coleta de materiais biológicos para exame, permitindo que o mesmo seja armazenado para pesquisas futuras.
Belém, ____/____/_____.

Assinatura do participante: _____

Assinatura do pesquisador:

_____