UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE BIOCIÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM GENÉTICA E BIOLOGIA MOLECULAR

Controladores de Elite do HIV e o desenvolvimento de vacinas

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Tese submetida ao Programa de Pós-

Porto Alegre, Março de 2022

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Este trabalho foi realizado no Núcleo de Imunoinformática do Departamento de Genética da Universidade Federal do Rio Grande do Sul (UFRGS).

Dedicatória

Dedico esta tese ao meu filho Mateus e minha esposa Jóice, fontes de amor e estímulo.

AGRADECIMENTOS

Agradeço a minha família constituída, Jóice e Mateus, pelo amor, apoio e compreensão neste período do doutorado.

Aos meus pais Maria e Olides pelo estímulo e apoio na busca do conhecimento. A minha irmã Marta e sua família pelo carinho e incentivo.

Ao meu orientador, Professor Dr. Gustavo F. Vieira, grande mestre que se tornou um amigo. Obrigado por acreditar e ajudar a realizar este sonho.

Aos amigos do Laboratório de Bioinformática, Marcelo e Eduardo, pela ajuda e companheirismo nesta jornada.

As excelentes Alessandra e Ana pela participação no projeto, além do esforço na geração de dados e mapas.

A todos os envolvidos no projeto pelo trabalho em equipe.

SUMÁRIO

Lista de abreviaturas

CD4 – Agrupamento de Diferenciação 4 (do inglês, Cluster of Differentiation 4).

CD8 – Agrupamento de Diferenciação 8 (do inglês, Cluster of Differentiation 8).

CTL – Linfócito T Citotóxico (do inglês, Cytotoxic T Lymphocyte).
EC – elite controllers (controladores de elite)
HLA – Antígeno Leucocitário Humano.
HIV – virus da imunodeficiência humana.
MHC-I – Complexo Principal de Histocompatibilidade de classe I (do inglês, Major Histocompatibility Complex).
PDB – Protein Data Bank.
pMHC – Complexo peptídeo:MHC.
mRNA – RNA mensageiro
TAP – Transportador Associado ao Processamento de Antígenos (do inglês, Transporter associated with Antigen Processing).
TARV – Terapia Antirretroviral
TCR – Receptor de Linfócitos T (do inglês, T Cell Receptor).

RESUMO

O HIV é um vírus altamente mutante, por isso a dificuldade em produzir uma vacina protetora, tanto preventiva como terapêutica. Mesmo com os benefícios dos antirretrovirais, a AIDS persiste como problema de saúde pública, especialmente na África. A resposta imune ao HIV pode ser humoral ou celular. Os pacientes controladores de elite têm carga viral indetectável ou muito baixa sem o uso de medicamentos. Essa condição parece relacionada à resposta T CD8. Alguns alelos HLA são relacionados a essa condição na literatura, especialmente HLA-B*27:05, B*57:01, B*57:03 e B*58:01. A genética do HIV também tem influência, com alguns alelos sendo protetores para um determinado subtipo, mas não para outros. Mutações em determinados epítopos permitem o escape viral mesmo para alelos relacionados à proteção contra a doença. Existe também a possibilidade do mesmo alelo selecionar diferentes vias de escape em grupos étnicos diferentes. Neste trabalho geramos mapas com as frequências dos alelos HLAs relacionados a EC nos países africanos com essa informação disponível e os subtipos do HIV mais frequentes nestes locais. Foram modelados 7 epítopos, avaliando afinidade de ligação, transporte pela TAP, clivagem pelo proteossomo e ranking total de processamento. Foram testadas sequências de HIV depositadas no Uniprot para os subtipos mais frequentes. Algumas mutações afetam a ligação ao MHC, outras têm impacto no ranking de processamento global. O epítopo TW10 foi considerado um bom candidato para abordagens imunoterapêuticas, pois todas as mutações encontradas em sua sequência mantiveram ligação ao MHC e alto ranking de processamento. Com este trabalho desenvolvemos um modo de estimar qual o melhor epítopo a ser usado num país baseado no HLA mais frequente e no subtipo de HIV mais prevalente, com um enfoque de "*geotargeting*", o que apresenta um potencial inovador a ser aplicado no desenvolvimento de novas abordagens vacinais.

ABSTRACT

HIV is a highly mutated virus, that is why it is so difficult to find a vaccine, both preventive and therapeutics. Even with the benefits of HAART, AIDS persists as a public health problem, especially in Africa. The HIV immune response can be humoral or cellular. Elite controllers patients have an undetectable or very low viral load without the use of medication. This condition appears to be related to the T CD8 response. Some HLA alleles are related to this condition in the literature, especially HLA-B*27:05, B*57:01, B*57:03 and B*58:01. HIV genetics is also important, with some alleles being protective for a certain subtype but not for others. Mutations in certain epitopes allow viral escape even for alleles related to disease protection. The same allele can select different escape routes in different ethnic groups. Subsequently, we generated maps with the frequencies of HLA alleles related to EC in African countries where this information was available and with the most frequent HIV subtypes in these locations. Seven epitopes were modeled, evaluating binding affinity, transport by TAP, proteasome cleavage and global processing ranking. HIV sequences deposited in Uniprot for the most frequent subtypes were tested. Some mutations affect MHC binding, others impact global processing ranking. The TW10 epitope was considered a good candidate for immunotherapeutic approaches, as all mutations found maintained MHC binding and high processing ranking. With this work, we developed a way to estimate the best epitope to be used in a country based on the most frequent HLA and the most prevalent HIV subtype, with a “geotargeting” view, which presents an innovative potential to be applied in the development of new vaccine approaches.

Capítulo I-1 Introdução

1.1 Carga da doença pelo HIV

A infecção pelo HIV/AIDS teve seu curso modificado após o surgimento da terapia antirretroviral (TARV) em 1996, com diminuição da mortalidade e melhora na qualidade de vida dos pacientes (Brasil 2018). Entretanto, esta infecção ainda é um problema de saúde pública de importância nacional e mundial. O número de infectados tem crescido em pessoas entre 20 e 39 anos, e foram identificados 32.701 novos casos de HIV em 2020 no Brasil (Secretaria de Vigilância em Saúde. Ministério da Saúde 2021).

Diversos países africanos, particularmente na região subsaariana, enfrentam mortalidade significativa e dificuldade no fornecimento da TARV por questões de custo e logística, bem como legais e sociais. Em 2018 havia 25,7 milhões de infectados pelo HIV na África e houve 1,1 milhões de novas infecções neste continente. A mortalidade relacionada ao HIV na África tem caído principalmente pelo acesso a TARV, mas ainda há muito trabalho a ser feito a este respeito: cerca de 16,3 milhões de pessoas tinham acesso ao tratamento, cerca de 64% da população que deveria estar usando antirretrovirais (WHO 2022).

No Brasil, apesar do tratamento ser gratuito para os pacientes, a mortalidade ainda é relevante. O número de mortes relacionadas à AIDS no Brasil foi estimado em 10.417 em 2020, com quase 920.000 pessoas vivendo com o vírus HIV (Secretaria de Vigilância em Saúde. Ministério da Saúde 2021).

Existe recomendação atual de tratar todos os pacientes vivendo com HIV/AIDS (Brasil 2018). O tratamento inclui diversas classes de antirretrovirais, como os inibidores das proteínas transcriptase reversa, da protease, da integrase e da fusão.

Infelizmente, mesmo com os benefícios da TARV, os pacientes enfrentam os efeitos adversos do tratamento e persiste um quadro inflamatório relacionado ao HIV. Este quadro predispõe à síndrome metabólica, eventos cardiovasculares e neoplasias, mesmo as não diretamente relacionadas ao HIV (Brasil 2018).

Atualmente não é possível eliminar a infecção pelo HIV em uma pessoa apenas com o uso de antirretrovirais (Sobieszczyk 2019). Por isso, a busca de

novos tratamentos e até mesmo uma cura para o HIV é um objetivo a ser alcançado. O uso de anticorpos neutralizantes já foi tentado, mas ainda sem sucesso, o que dificulta a busca por uma vacina baseada apenas na estimulação de resposta humoral ([edited by] John E. Bennett Martin J. Blaser 2015).

1.2 HIV e desenvolvimento de vacinas

Por ser uma infecção viral, o HIV desencadeia as duas linhas principais de defesas do sistema imune adaptativo: a resposta humoral e a celular. Nosso enfoque especial é a resposta adaptativa celular, para alvos virais de célula T, reconhecidos pelos receptores dos linfócitos T citotóxicos no complexo pMHC.

Os mecanismos imunológicos que estão envolvidos nas respostas às infecções por vírus são bastante conhecidos e envolvem basicamente a resposta imune humoral e celular. De maneira simples, a primeira culmina com a geração de anticorpos pelos linfócitos B, os quais têm um papel decisivo na neutralização das partículas virais circulantes nos indivíduos infectados. Entretanto, assim que uma célula é infectada por um vírus e este começa seu ciclo de replicação dentro da mesma, estes organismos não estão mais acessíveis à ação dos anticorpos. Dessa forma, a única resposta capaz de reconhecer que uma célula está infectada e eliminá-la através de mecanismos líticos é a resposta celular, protagonizada pelos linfócitos T citotóxicos. Este reconhecimento é realizado através da interação do receptor de célula T dos linfócitos CD8+ com as moléculas do complexo principal de histocompatibilidade (HLA em humanos), as quais contêm pequenos peptídeos (epítopos) derivados de proteínas próprias, ou dos patógenos em questão. Quando os epítopos forem reconhecidos como exógenos, irão desencadear os mecanismos citotóxicos com a consequente eliminação da célula infectada.

Células apresentadoras de antígenos, como linfócitos T CD4, dirigem a expansão de diferentes populações de células T (Gebre et al. 2021). Linfócitos T CD8 citotóxicos identificam e eliminam células infectadas por patógenos específicos. Linfócitos T CD4 também são ativados e expandem linfócitos B, que produzem anticorpos específicos para um patógeno. Estes anticorpos são críticos

para eliminar uma infecção, tanto se ligando a um micróbio para impedir a entrada nas células, como marcando um patógeno para destruição pelo complemento ou imunidade inata (Gebre et al. 2021).

As abordagens vacinais tradicionais envolvem patógenos atenuados, inativados e com replicação alterada, além de vacinas de subunidades ou conjugadas (Gebre et al. 2021).

Patógenos atenuados geralmente são passados por culturas de células ou embriões até perder capacidade replicativa em células humanas. Em geral estas vacinas produzem uma forte resposta imunológica. Infelizmente, podem ser um risco para pacientes imunodeprimidos (Gebre et al. 2021). Uma alternativa é administrar um patógeno totalmente inativado para diminuir este risco. Ambas as abordagens incluem o crescimento do patógeno em larga escala, o que cria um risco de biossegurança.

Vacinas de subunidades são compostas por um pedaço do patógeno, por isso mais seguras e eliminam a necessidade de culturas. Frequentemente estas vacinas necessitam de "*boosters*" para imunização e adjuvantes (Gebre et al. 2021).

Infelizmente as abordagens tradicionais de vacinas tem se mostrado pouco funcionais contra infecções crônicas ou com infecções recorrentes, como tuberculose e HIV (Xu et al. 2020). A demora na produção de vacinas com essas abordagens dificultam o combate a epidemias emergentes, como a do Ebola. Novas tecnologias incluem vacinas de vetores virais bem como vacinas de ácidos nucléicos, especialmente RNA mensageiro.

Vacinas de mRNA podem ser criadas com sequencias específicas de aminoácidos, entregues em nanopartículas lipídicas (Gebre et al. 2021). Entre as vantagens deste método podem ser citadas a expressão por curtos períodos, o que aumenta a segurança, a facilidade de atualização das sequencias e a produção em larga escala em pouco tempo. Estas vacinas são imunogênicas e seguras, como mostraram as vacinas para o Sars-Cov-2 (Gebre et al. 2021).

Um ponto importante das vacinas de RNA mensageiro é que elas fazem a produção do antígeno dentro do ambiente citoplasmático. Isso faz com que estes entrem na via de apresentação de classe I, elicitando uma resposta citotóxica.

Normalmente essa resposta é muito fraca na vacina de subunidades ou de vírus inativados, ficando a apresentação de antígenos de classe I restrita ao fenômeno de apresentação cruzada. Antígenos externos são apresentados via MHC de classe I.

Vacinas baseadas em vetores por Adenovírus usam a capacidade dos vírus de entrar nas células e introduzir seu material genético (Gebre et al. 2021). Os vírus usados como vetores têm genes retirados para reduzir a capacidade de replicação, gerando maior segurança, e para não aumentar demais o tamanho do genoma com os genes de interesse introduzidos.

Adenovírus são auto-adjuvantes, o que simplifica a composição de vacinas e o processo de conjugação. Também são eficientes em gerar inflamação e resposta imune. A imunidade prévia a Adenovírus pode diminuir a eficácia das vacinas baseadas nestes vetores (Gebre et al. 2021).

Muitas das abordagens vacinais, entretanto, negligenciam ou dão pouca importância a essa importante via de combate às infecções virais e focam seus esforços na correta estimulação da geração de anticorpos. Isso se deve, em boa parte, pelo ainda limitado entendimento de como estes mecanismos de eliminação de patógenos intracelulares obrigatórios são elicitados. É fundamental entender quais proteínas serão imunogênicas na resposta celular (não sendo obrigatoriamente as mesmas da resposta humoral) e quais epítopos dentro dessas proteínas serão considerados imunodominantes no curso da infecção. A busca por estes *fingerprints* ou sinalizadores de infecção pode possibilitar o uso destes epítopos ou antígenos específicos em processos de imunização mais eficazes (Vieira and Chies 2005).

Alguns estudos computacionais possuem este enfoque, mas concentram seus esforços na comparação de sequências e na capacidade dos epítopos de se ligarem às moléculas de MHC. Nosso grupo tem realizado alguns estudos que demonstram que estes padrões imunogênicos não se correlacionam diretamente com a identidade das sequências, mas sim com elementos estruturais formados pela interação da molécula do MHC com os epítopos que ela contém em sua fenda (Rigo et al. 2015).

1.3 Controladores de elite do HIV

No caso da infecção por HIV, este assunto ganha um aspecto interessante. Existe uma classe de indivíduos, os *elite controllers* (controladores de elite) (EC), que são pacientes infectados pelo HIV que não apresentam progressão clínica para AIDS e que mantêm carga viral indetectável (ou em valores muito baixos), com contagem de linfócitos CD4 elevada sem o uso de TARV. Ao que parece, a resposta imune celular CD8 parece desempenhar um papel importante no controle do HIV nestes pacientes, o que poderia representar um campo para novos estudos ([edited by] John E. Bennett Martin J. Blaser 2015).

Este é um grupo bastante heterogêneo de pacientes, conforme (Navarrete-Muñoz et al. 2020): nem todos têm as mesmas características virológicas e imunológicas, nem todos mantêm o controle do HIV pelo mesmo tempo. Uma definição possível seria de pacientes em acompanhamento por pelo menos um ano, sem uso de antirretrovirais e com mais de 90% das medidas de carga viral abaixo de 50 cópias/mL (Navarrete-Muñoz et al. 2020).

Apenas uma porção muito pequena dos pacientes, cerca de 0,15%, consegue manter o controle do vírus por longos períodos (mais do que 10 anos) (Navarrete-Muñoz et al. 2020). O controle imunológico, especialmente a contagem de linfócitos T CD4, depende de fatores do hospedeiro e do vírus (Navarrete-Muñoz et al. 2020).

Um estudo recente encontrou uma frequência de potenciais controladores de elite elevada na bacia do Congo, especialmente na República Democrática do Congo (Berg et al. 2021). Uma possível explicação seria a seleção natural pelo HIV, conferindo uma vantagem de sobrevida destes pacientes, já que nem todos os infectados têm acesso ao diagnóstico e ao tratamento adequados.

A resposta CD8 é importante no controle natural do HIV e isso deve ser levado em conta numa futura vacina. Os epítopos vacinais devem ser desenhados de acordo com os alelos HLA expressos na população imunizada e com os subtipos do HIV circulantes (Collins et al. 2020; Lunardi et al. 2021). Regiões conservadas do HIV, como a proteína p24 Gag, são melhores alvos que regiões com muitas mutações, como as proteínas do envelope (Collins et al. 2020).

Estudos demonstram que esta resposta citotóxica efetiva está relacionada à presença de alelos de MHC específicos. Os mais estudados são HLA-B*5701, HLA-B*5703, e/ou HLA-B*2705, os quais conseguem realizar a apresentação de alvos presentes nas proteínas virais do HIV e, que por sua importância, apresentam uma menor taxa de variabilidade (Payne et al. 2014).

O conhecimento destes elementos que desencadeiam uma correta resposta contra este patógeno fornece informações que podem ser importantes inclusive no estudo de outros alelos. Este tipo de conhecimento é extremamente importante para guiar a escolha de alvos a serem utilizados em novas abordagens terapêuticas.

Vamos estudar nesta tese alelos do HLA relacionados a EC. Posteriormente, veremos os efeitos das mutações de sequências de diferentes subtipos do HIV na ligação ao MHC e outras fases do processamento de epítopos, como transporte pela TAP e clivagem do proteossomo.

2. Objetivos

2.1 Geral

O objetivo deste trabalho é compreender, de um ponto de vista estrutural, aspectos da imunidade celular que estão conferindo proteção diferencial aos indivíduos portadores dos alelos associados ao controle de elite contra a infecção pelo vírus HIV.

2.2 Específicos

- Avaliar efeitos das mutações nas proteínas virais e seu efeito na afinidade de ligação aos MHCs de Classe I associados ao controle de elite (HLA-B*57:01, B*57:03, B*27:05 e B*58:01) na força de ligação ao MHC classe I.
- Determinar o efeito destas mesmas mutações nos outros passos da via de processamento e apresentação de antígenos, como clivagem pelo proteossomo, transporte pela TAP e ranking global de processamento de epítopos.

- Recuperar a frequência dos alelos investigados para todos os países do continente africano, para os quais estes dados estejam disponíveis.
- Gerar mapas com estas informações, permitindo a visualização direta de quais linhagens podem conter mutações que possam estar favorecendo o escape viral nos países africanos estudados, considerando os subtipos mais frequentes nos mesmos.

Capítulo 2 - The influence of HLA/HIV genetics on the occurrence of elite controllers and a need for therapeutics geotargeting view

Artigo publicado na revista The Brazilian Journal of INFECTIOUS DISEASES

BRAZ J INFECT DIS. 2021;25(5):101619

Sociedade Brasileira de Infectologia

The Brazilian Journal of
INFECTIOUS DISEASES

www.elsevier.com/locate/bjid

Review Article

The influence of HLA/HIV genetics on the occurrence of elite controllers and a need for therapeutics geotargeting view

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ARTICLE INFO

Article history:
Received 12 April 2021
Accepted 13 August 2021
Available online 22 September 2021

Keywords:
HIV
HLA class I
Elite controllers
Immunodominant epitopes
Mutation

ABSTRACT

The interaction of HIV-1, human leukocyte antigen (HLA), and elite controllers (EC) compose a still intricate triad. Elite controllers maintain a very low viral load and a normal CD4 count, even without antiretrovirals. There is a lot of diversity in HIV subtypes and HLA alleles. The most common subtype in each country varies depending on its localization and epidemiological history. As we know EC appears to maintain an effective CD8 response against HIV. In this phenomenon, some alleles of HLAs are associated with a slow progression of HIV infection, others with a rapid progression. This relationship also depends on the virus subtype. Epitopes of Gag protein-restricted by HLA-B*57 generated a considerable immune response in EC. However, some mutations allow HIV to escape the CD8 response, while others do not. HLA protective alleles, like HLA-B*27, HLA-B*57 and HLA-B*58:01, that are common in Caucasians infected with HIV-1 Clade B, do not show the same protection in sub-Saharan Africans infected by HIV-1 Clade C. Endogenous pathway of antigen processing and presentation is used to present intracellular synthesized cellular peptides as well as viral protein fragments via the MHC class I molecule to the cytotoxic T-lymphocytes (CTLs). Some epitopes are immunodominant, which means that they drive the immune reaction to some virus. Mutation on an anchor residue of epitope necessary for binding on MHC class I is used by HIV to escape the immune system. Mutations inside or flanking an epitope may lead to T cell lack of recognition and CTL escape. Studying how immunodominance at epitopes drives the EC in a geographically dependent way with genetics and immunological elements orchestrating it may help future research on vaccines or immunotherapy for HIV.

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https://doi.org/10.1016/j.bjid.2021.101619

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Introduction

HIV has led to a pandemic with significant mortality and morbidity. Its detection in the 1980s changed the world. Understanding the virus and its relationship to the immune system is fundamental for preventive or therapeutic vaccines. HIV is highly mutable. This study will investigate HIV type 1 (HIV-1) and the patients who control the virus without antiretrovirals; "elite controllers" (EC)[1] who maintain very low viral loads and normal CD4 counts, besides the interaction of HIV with human leukocyte antigens (HLA) of these patients. HIV-2 was discovered later, occurring mainly in West Africa[2] and accounting for a much lower percentage of infections worldwide.[3]

EC are very rare, approximately 1 in 300 HIV patients or less than 0.5%.[4] This study will focus on immunological aspects presented by this type of patient, but it will not explain all the occurrences.[5]

The geographical distribution of HIV-1 subtypes has been studied based on data collected between 1990 and 2015.[6] While subtype B predominates in North America and Western Europe, most infections in sub-Saharan Africa and India involve subtype C. Subtype A is also common in Africa.

In Brazil, there are several subtypes of HIV-1 and their distribution varies. According to Riedel et al.,[7] B is the most common subtype in Brazil, followed by C, F, and CRF31_BC recombinants, especially in southern Brazil.

Even inside Brazil, there is diversity in HIV subtypes. Subtype B is the most prevalent in the Southeast and Northeast/Midwest regions (90% and 85%, respectively).[7] In the South Region there is a larger prevalence of subtype C when compared to subtype B: Santa Catarina state 48% and 23%, Rio Grande do Sul state 29% and 23%, and Parana state 36% and 38%, respectively.

A study on sequencing and HLA typing in HIV-infected patients who are undergoing antiretroviral treatment in southern Brazil found a more heterogeneous scenario, where the most frequent subtypes of HIV-1 were B (46%) and C (31%), in addition to single BC recombinants (14%), single BF recombinants (7%) and single BCF recombinants (2%).[8]

A recent review[9] studied 2447 HIV-1 genotypes collected between 2008 and 2017 from Brazilians who had never used antiretrovirals. The nationwide prevalence of subtype B was 64.19% and 18.37% of subtype C.

HIV penetrates cells through an interaction between glycoprotein gp120 and cell surface receptor CD4, along with coreceptors CCR5 or CXCR4.[10]

Despite the importance of CD4+ cells in the viral invasion, the first cells of the immune system to have contact with HIV are usually dendritic cells, found in many human tissues, such as the mucosa of genital and anal areas. Once HIV infection becomes established, cytotoxic response and neutralizing antibodies appear, but generally at insufficient levels to eliminate the virus.[10]

A study on the production of cytokines in a cohort of HIV-infected patients found an association between EC and the genetic variant -208A TNF-α (7 of 8 patients).[10] The relationship between TNF-α and natural HIV control requires further studies.

The CCR5Δ32 mutation is also correlated with resistance to HIV infection. The deletion generates a truncated protein that is not carried to the cell surface. Individuals homozygous for this deletion are resistant to infection by HIV-1 with CCR5 tropism, since the mutation prevents the entry of the virus.[11]

After HIV infection, the RNA is undetectable for a few days (the eclipse stage), and then a peak in viremia occurs, with extensive destruction of CD4+ cells, although EC achieve spontaneous control of the virus.[11]

HLA and HIV control

CD8+ T lymphocytes recognize and destroy cells that express non-self peptides in MHC molecules on the surface of nucleated cells. These MHC molecules are also called HLA. In this context, EC also appear to maintain an effective CD8 response against HIV.[11]

HLAs are related to the degree of immune control of HIV, especially among EC. The cellular immune response is linked to the individual's immunogenetic profile, including HLA class I expression.[8]

Some alleles of HLAs are associated with slow progression of HIV infection, such as HLA-B*57, HLA-B*58:01, HLA B*57:03, and HLA-B*27, while others are linked to rapid progression, such as HLA-B*35 and HLA-B*58:02[10,12]

An international consortium studied several HIV EC, finding that alleles such as HLA-B*27:05, and HLA-B*14, were also associated with elite control, while supertypes HLA-B*07 and HLA-B*35 were associated with an increased risk of disease progression.[13] Here is important to highlight that both studies linking HLA-B*35 with rapid progression did not consider its subdivision in Px (B*3502, B*3503, B*3504, and B*5301, e.g.) and Py (HLA-B*3501 and B*3508, e.g.) alleles. Peptides presented by these MHC molecules differ in their F-pocket anchor residue (position 9), where Py alleles bind tyrosine residues, and Px alleles have a more promiscuous pattern in this position. Some studies associate Px alleles with fast progression, while Py seems not to influence disease progression. It denotes the importance of high-resolution HLA sequencing.[14–16]

Nevertheless, some subjects with Py HLA-B*3501 allele more effectively controlled C-clade-infected African cohorts through $NY10_{253\text{-}262}$ Gag-specific epitope, which was not observed in B-clade-infected individuals. It was probably caused by Gag-D260E polymorphism present in ~90% of B-clade sequences, affecting T cell recognition.[17]

HLA-B*35:05, structurally related to Py alleles, also presented a protective effect (the strongest among the analyzed alleles) in HIV-1 CRF01_AE-infected Thai patients, regarding viral load.[18] These studies demonstrate the need to consider both genetics of both host and parasite for each considered population.

In another study, among patients on HIV treatment, the HLA-B*14:02:01 allele was related to CD4 counts below 350 cells/mm^3, indicating an association with a worst clinical outcome.[8] The HLA-B*51:01:01 allele and the HLA-B*07-supertype, associated with an HIV viral load below 100,000 copies/μl of whole blood before treatment, are considered as potential protective alleles, especially in patients infected with HIV Clade C, while the HLA-B*44 supertype is considered a potential allele for faster disease progression.[8] Although the HLA-B*07 supertype was considered related to a faster disease

progression in another study,[13] it is important to remember that, in the first study, patients from Brazil were infected mostly with HIV Clade C,[8] while in the second there were patients from different countries and mainly with HIV subtype B. Some conflicting results indicate a need for a deeper investigation of HLA, recognized cytotoxic epitopes and patients onset relations.

Another important point refers to the pathogen's genetic features. The HLA-B*57:01 and HLA-B*27:05 alleles, for example, show an association with better control of HIV through cellular immune response depending on HIV subtype.[8] The HLA type and virus interaction are major determinants of durable immunological control.[19] The interaction between HLA and HIV subtypes is a crucial element discussed in this article.

The population of Brazil is ethnically diverse, including the descendants of indigenous peoples, Europeans, and Africans. Thus, it is expected a highly diverse frequency composition of HLA alleles. We will discuss HLA diversity in Brazil related to HIV.

In a study of allele frequencies in Brazilian bone marrow donors, the most frequent allele groups were A*01, A*02, B*35, B*44, C*07, DQB1*03, DQB1*06, and DRB1*01.[20] HLA-B*35 frequency in Brazil is 11.8%,[21] which is an important issue that could favor rapid progression of HIV in Brazilian patients. A more recent study updated this information, segregating Px and Py B*35 frequencies. In this study, HLA-B*35:01 and -B*35:08 allele frequencies (Py alleles) summed up to 7%. Px alleles (HLA-B*35:02, -B*35:03 and -B*35:04) accounted for 4.2%.[22] While we already discussed differential disease progression rates for Px and Py alleles, studies from Mexico and Peru did not find differences in this variable among infected individuals. So, once again, the importance of the considered population remains, positioning a country like Brazil, with a high frequency of HLA-B*35 alleles, as an eligible place for a detailed HIV geotargeting investigation.[23,24]

On the other hand, the frequency of protective alleles as HLA-B*57:03 in Brazil is low, with about 0,6% among Rio de Janeiro Caucasians. A study in blood marrow donors in Brazil found an HLA-B*57 national frequency of 2.8%, where the South region presented a similar frequency of 2.8%, compared to 2.4% in the North region.[21]

HLA-B*27 frequency in Brazil was 2.23%, with differences between the South and Southeast regions: 2.66% and 2.16%, respectively.[21] HLA-B*58 frequency in this country was 2.65%, while the frequency in the Southeast region was 2.73 and 1.87% in the South region.

A similar recent study[22] in blood marrow donors from Brazil, using high resolution typing data, updated this information for six ethnic groups by classifying them according to self-reported race group. The frequency of HLA-B*57:03 protective allele ranged from 0.3 to 1.5% in *Amarela* (Asian) and *Preta* (Sub-Saharan African Descent), respectively. In relation to B*27, *Preta* presented 1.25% and *Branca* (European) the higher frequency (2.1%). The frequencies of B*58 subtypes among the analyzed groups ranged from 0.4 to 2.7%, with *Branca* showing the lowest value for B*58:02 and *Preta* presenting the highest frequency of B*58:01. These contrasting values point out that in admixed populations, a personalized approach is even more crucial.

HIV epitopes, HLA, and elite controllers

Some HIV viruses undergo epitope mutations that allow them to escape the CD8 cellular immune response. The CD8+ immune response selectively pressures these mutations, and when they reach essential and more conserved regions of the viral genome, the mutations can result in loss of viral "fitness", resulting in their disappearance from a patient's viral population.[11]

Different HLAs have been associated with relative protection against disease progression and even virus control by the immune system. Goulder et al.[25] studied the relationship between the HIV epitopes and HLA and found that it was generally related to changes in the HIV Gag protein, in which some HLA class I alleles are related to a better prognosis and others to greater susceptibility. It seems to be related to HIV subtype analyzed and population affected.

Mixed populations bring a big challenge. A study in Mesoamerican patients found some HLA alleles to be protective against HIV Clade B, including canonical ones like HLA-B*27:05 and HLA-B*57:01, but also new alleles like HLA-B*39:02, with a Canadian population as control.[26]

The immune response to HIV that leads to control without the use of antiretrovirals seems to be linked to CD8+ lymphocytes. HLA-B*57 is often present in elite controllers. Epitopes of Gag protein restricted to this HLA, such as IW9 (Gag 147−155), KF11 (Gag 162−172), and TW10 (Gag 240−249) generated a considerable immune response measured by interferon and perforin levels in lymphocytes from elite controllers, even with peptide mutations.[27]

As mentioned earlier, human HLA-B*27, HLA-B*57:01, HLA-B*57:03, and HLA-B*58:01 alleles have shown greater correlation with HIV control. Patients with HIV and HLA-B*27 can mount a CD8+ immune response against the KK10 (KRWIILGLNK) epitope of the Gag protein capable of controlling viremia.

According to Ladell et al., the KK10 epitope in the Gag protein appears to be immunodominant in the CD8+ response.[28] Other authors have found HLA-B*14:02 to be protector when interacting with HIV-1 subtype B. In the presence of this HLA and this type of virus, an alternative Env epitope (ERYLKDQQL) becomes immunodominant, highlighting the importance of the interaction between the infectious agent and host genetics.[29] Whether Gag mutations frequently cause loss of viral replicative capacity, Env protein mutations typically are tolerated by the virus without loss of replicative capacity.

In African American patients with HIV subtype B, certain HLAs were related to better viral control, especially HLA-B*14, HLA-B*57:01, and HLA-B*57:03, while other HLAs were related to worse outcomes (HLA-B*15:10, HLA-B*35:01 and HLA-B*53). HLA-B*81 was shown to be protective in Africans with HIV-1 subtype C virus, but HLA-B*13 did not have the same effect in this population.[30]

The geographic ethnic component can influence the response to the virus, explained by genetic diversity of the occurring viruses and the local human population. The relationship of HLAs that confers HIV-1 protection/susceptibility is coordinated by some common alleles around the world,

while specific alleles appear depending on the region and the subtype of the virus investigated.

In two African elite controllers infected with HIV-1 virus subtype C, the following protective HLA alleles were found in patient 1 HLA-B*44:03, HLA-B*81:01, and HLA-DRB1*13, while patient 2 expressed HLA-A*74:01, HLA-B*57:03, and HLA-DRB1*13. For one patient, the HLA-B*81:01 Gag response was immunodominant and likely contributed to viral control. p21, the intrinsic cellular inhibitor of HIV reverse transcription, was also more expressed in these patients than in seronegative donors. The response to HIV seems to be more related to cellular immunity than the presence of neutralizing antibodies.[31] This work emphasizes the importance of a closer look towards the particularities of HIV infection depending on the virus subtype and the population investigated.

Other uncommon alleles were described as protective. A study was conducted on two HIV-infected patients, one characterized as an EC and the other as a progressor. The CD4+ lymphocytes of both were susceptible to HIV with CCR5/CXCR4 tropism, in addition to being heterozygous for the CCR5Δ32 deletion.[1] The HIV subtype of the EC was CRF02_AG, and the subject's partner had the same virus with 100% homology. The EC's HLA was HLA-A*03-A*31, with homozygosis in the HLA-B*07 alleles and HLA-C*07. The elite controller's partner was HLA-B*07-B*52, HLA-C*07 heterozygous and HLA-C*12 heterozygous.[1] The HLA-B*07 is consistently linked to accelerated disease progression in B-clade, but not in C-clade infection.[32] Different expressions of the same virus in two patients show the importance of the interaction between the genetics of the host, the source, and the viral strain in the development of an EC, in addition to being a model for future control of HIV infection.[1]

HLA, mutations, and loss of immunogenicity of epitopes

According to Buggert et al.,[33] some mutations allow HIV to escape the CD8 response while others do not. A study conducted on patients with HLA-B*57:01 who were infected with HIV-1 subtype B and found that some epitopes were related to better cellular response. In this study, KF11 (KAFSPEVIPMF) epitope, a fragment of the HIV-1 Gag protein, is highly conserved in HIV-1 subtype B and maintains its immunogenicity even with mutations. On the other hand, HQ10 and ISW9 epitopes showed a panel of mutations that caused loss of immunogenicity in patients with HLA-B*5701 infected with HIV-1 subtype B.

Caucasians have a 16% frequency of HLA-B*27:05 and/or HLA-B*57:05, and the KK10 Gag epitope seems closely related to HLA-B*27:05 protection.[34] The reason why not everyone with these HLAs become EC is still under study. The T242N mutation, within the HLA-B*57:01 restricted TW10 (TSTLQEQIGW) epitope, was related to loss of HIV control. Kloverpris et al.[35] found a relationship between protective HLA-selected epitopes, like HLA-B*27 and KK10, and viral fitness. Some mutations cause impaired viral replication capacity and are transmitted with benefit to the host. The dominant observed escape mutation in KK10 epitope, R264K, arises at the anchor position-2 (P2) in the epitope that is believed to require Arginine for adequate binding to HLA-B*27.[35] The mutation L268X (where X represents Met or Ile at P6 in the KK10 epitope) occurs prior to R264K, and this is associated with reduced immune control.

Mutations on HLA-B*57 Gag IW9 and TW10 were associated with faster disease progression. This study found that these HLAs selected epitopes in which many mutations occur have an impaired viral replication capacity, demanding compensatory mutations to maintain their virus fitness.

There are differences in HLA protective alleles, like HLA-B*27, HLA-B*57, and HLA-B*58:01, that are common in Caucasians infected with HIV-1 Clade B but cannot show the same protection in sub-Saharan Africans infected by HIV-1 Clade C and presenting a different genetic HLA composition. The KK10 escape mutation R264K in C clade in individuals HLA-B*27-positive is selected when prior escape at L268 is not present. In this situation, the compensatory mutation is typically S165N.[35]

Mutations selected in KK10 epitope include R to K in position 2 in HIV Clade B in 5% of patients.[36] This is a very conserved region in Gag protein. Other African HLAs, like HLA-B*81:01, are also linked to protection to AIDS progression and select the TL9 Gap epitope in the sub-Saharan population.[35]

The HLA-B*57:01 allele selects some protective HIV Gag epitopes, like TW10, IW9, QW9, and KF11, by CD8+ T cells response.[37] One common escape mutation in TW10 epitope is T3N (TSNLQEQIGW) that changes the conformation of the exposed epitope to abolish immune recognition. This mutation enables HIV-1 immune escape in people with HLA-B*57:01.[37]

HIV-1 can increase cellular endocytosis of HLA molecules via nef, limit HLA transcription and peptide processing via tat, and suppress TAP-mediated peptide transport into the endoplasmic reticulum.[37]

Epitope processing and TCR recognition

The endogenous pathway of antigen processing and presentation is used to present intracellular synthesized cellular peptides as well as viral protein fragments via the MHC class I molecule to the cytotoxic-T-lymphocytes (CTLs). In this pathway, the proteins that are destined for the presentation are marked by ubiquitination and subjected to proteolytic cleavage by the immunoproteasome. These fragments of peptides are transported to the lumen of ER by a transporter associated with antigen processing protein (TAP). The TAP proteins also help the loading of the short peptides with appropriate length (nearly nine amino acids) into the cleft of MHC class I molecules. Although proteasome is the main actor in generating the bulk of the CTL epitopes, cytosolic endopeptidases may also be involved in the production of certain CTL epitopes.[38]

The HLA-I (the human MHC-I class of proteins) will present endogenous peptides. Some HLA-I alleles are linked with protection against infectious diseases, like HIV and hepatitis C virus with HLA-B*27. The antigen processing pathway is also important for study of immunological reactions to viruses.[39]

Some epitopes are immunodominant, which means that they drive the immune reaction to some virus. These types of

epitopes share some characteristics, especially structural ones, when complexed to the HLA cleft. Discovering common viral immunodominance can help develop viral vaccines.[40]

CD8+T cells exert immune selective pressure on HCV.[41] In HIV it is observed a preferential expansion of CD8+T "escape specific" cells triggered toward altered epitopes selected during the HIV infection course, especially through HLA-B*27 and HLA-B*57 alleles. These alleles are connected to slower disease progression.[41]

Mutation on an anchor residue of epitope necessary for binding on MHC class I is one way that HIV uses to escape the immune system.[41]

ER aminopeptidase I (ERAPI) is an endoplasmic reticulum resident aminopeptidase involved in antigen presentation.[42] It was studied in a murine model. One study found that CD8+T cells could not recognize HIV-infected cells containing proline at Gag residue 146.[42] Failure of ERAPI to process the mutant peptide resulted in an inability of the optimal epitope to be generated and impaired the immune response.

The Ubiquitin-Proteasome System (UPS) interacts with HIV-1 aiding with degradation and removal of viral proteins.[43] This virus changes UPS to escape from the human immune system.

Eccleston et al.[44] simulated the HIV-1 clade C peptide presentation on TCL surface with bioinformatics tools, which predicts the peptidome of an amino acid sequence, the probability of proteasomal cleavage, TAP affinity, and the affinity (IC50) between the peptide and chosen MHC-I. These pieces of information were combined in a score. Four alleles associated with better control of HIV: HLA-B*58:01, HLA-B*57:01, HLA-B*27:05, and HLA-B*44:03, and four alleles associated with fast progression of the disease: HLA-B*18:01, HLA-B*35:03, HLA-B*07:02, and HLA-B*55:01 were studied. In the first group, the average Gag Total Score was one of the lowest for the controlling alleles, with the highest average scores coming from other proteins: Pol, Env, Nef and Vif. The authors also constructed a combined model of HIV intracellular kinetics and MHC class I peptide presentation. Among nine HIV-1 proteins, Gag peptides predominate at the cell surface, with a Gag:Pol ratio of 18:1, a Gag:Vpr ratio of 23:1, and a Gag:Env ratio of 64:1, and the Env protein was the third most abundant in the cytoplasm, but its epitope is just the sixth most abundant on the cell surface.[44]

Immunodominant epitopes tend to accumulate escape mutations faster than subdominant epitopes. HIV-1 proteins are cleaved by the proteasome, and the fragments are transported to the endoplasmic reticulum by TAP. Virus proteins are processed by endopeptidases into epitopes that go to the cell surface for antigen presentation to TCL. Mutations that affect peptide processing stop the best epitopes formation and impair the availability of these epitopes to loading to MHC-1 molecules in the ER.[45] The Gag epitope ISW9 mutation consists in a change of alanine for proline at position 146 (A146P), blocking recognition of the IW9 epitope by the aminopeptidase I in the ER, which prevents the formation of epitope/MHC-I complex.[42,45]

The preferential processing of immunodominant epitopes can be explained by the presence of specific N-terminal motifs in the precursor protein of the epitope because when these motifs are integrated near to a subdominant epitope it causes a higher production of the epitope.[45]

The reasons that make an epitope immunodominant may include its production efficiency, including kinetics and quantity of peptide produced. Immune escape may happen through mutations within or outside HIV epitopes, which may impair the complete processing of epitopes or induce degradation by intracellular peptidases.[46]

Mutations flanking an epitope may lead to T cell lack of recognition. A mutation of alanine into proline located in front of dominant HLA−B*57 restricted epitopes can be frequently detected in HIV infected persons who are HLA−B*57 positive. Although this mutation occurs outside the epitope, it prevents the recognition of infected cells by epitope−specific CTLs. This mutation blocked the complete processing of the N−extended peptide into the epitope, thus generating a peptide that does not connect efficiently to MHC−I and leads to CTL escape.[46]

Conclusions

We investigated the relationships among HIV EC and the MHC-I system, discussing the viral genetics and geographic elements that could influence cellular responses. The virus characteristics, especially HIV clades, can drive this interaction. Genetic characteristics can contribute or inhibit cellular virus entrance. Human genetic diversity, considering the HLA-I system, has also a direct impact on virus and immune system interaction. Some HLAs are related to a slower disease progression to AIDS, and EC. These relations can be different depending on HIV-1 clades and HLAs most prevalent in a specific geographic region. HIV and HLA can select different epitopes as immunodominant interactions in a population/individual manner. Mutations on HIV-1 epitopes can cause immune escape from CTLs and loss of EC. Other mutations in the flanking regions of the epitope that may affect other stages of antigen processing, such as cleavage by the proteasome and translocation by TAP, can also impact these aspects.

In summary, there are some gaps that should be filled before we understand the complexity of elite controlling and HIV resistance per se. It seems that cellular response, with all cells and molecules involved, is a pivotal player in this process. The HLA presenting protein rules the targets that will be presented to CTLs. But the HLA pool for each population is highly diverse, making the ligandome region-specific. Besides, the circulating HIV viral strains are divergent as well, in a location-dependent way. As we discussed, in this sense, not only mutations inside the epitope regions will interfere in the individual ability to present and generate a response against a specific target, but the flanking amino acid substitutions have also the potential to abolish a cytotoxic event. Only the understanding of the coordinated interplay of these factors will allow the development of more rational HIV immunotherapeutics.

Funding

MAB and GFV received scholarship from Brazilian National Council for Scientific and Technological Development - CNPq.

Conflicts of interest

None.

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Capítulo 3 - The interaction of HLA/HIV genetics on the occurrence of elite controllers and the proposition of a customizable vaccinology strategy

Artigo submetido a revista *AIDS Journal*

O presente manuscrito está organizado conforme a norma de submissão da revista. As Figuras e o Material Suplementar estão após o arquivo principal (*Main document)*.

The interaction of HLA/HIV genetics on the occurrence of elite controllers and the proposition of a customizable vaccinology strategy

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Conflicts of Interest and Source of Funding: no conflicts of interest, no funding source for this article.

Word count: 3482.

Abstract

Objective: HIV vaccine is a difficult goal to achieve. Even the best vaccines based on neutralizing antibodies have around 30% efficacy. Combining cellular and humoral immunities can help to control the disease, also including genetics of the virus and the human host. We studied immunological elements that protect elite controllers from HIV. **Design**: *in silico* prediction study. **Methods**: we recovered HLA alleles related to HIV control and their frequencies in Africa and protein sequences for the most prevalent HIV subtypes in this continent. After this search, we generated maps with this combined data. The impact of mutations on HLA binding affinity, TAP transportation and processing score was assessed in the HIV strains sequences by *in silico* prediction. **Results**: maps with the HIV subtypes impact on T cell responses, for each epitope, were produced. For example, epitope TW10 variants of the main HIV subtypes maintained good MHC binding, proteasome cleavage and global processing ranking across the continent. Epitope TI8 mutations in the main subtypes lose MHC binding affinity and global processing ranking in several countries. **Conclusions**: This is the first attempt to create a landscape linking HIV epitopes, HLA and the effect of mutations on global processing of these targets in a specific population. Some epitopes can be used for specific locations, while others have variants presenting a greater potential to evade immune responses. So, we presented a new way to compile this data making it clearer to prospect customized targets, increasing the expectation to reach the HIV vaccine goal.

Keywords: HIV, HLA class I, Elite Controllers, Immunodominant Epitopes, Mutation, Geotargeting View.

Introduction

HIV is a very mutable virus, and other HIV issues make it difficult to create a vaccine: different virus strains worldwide, immunogens are unable until now to induce reactive neutralizing antibodies against the virus (John E. Bennett, Raphael Dolin 2015), infected latent cells that are not eliminated by host immunity or even long years of antiretroviral therapy (Margolis et al. 2020). The emerging Sars-Cov-2 pandemic brought a fast vaccine development based on the Spike protein as the main antigenic target (Zou et al. 2019).

Some HIV patients have a very low or undetectable viral load and high CD4 count without taking antiretroviral therapy. These patients are called elite controllers (EC) (Bendenoun et al. 2018). This type of patient can be a model for a preventive or therapeutic vaccine (Collins et al. 2020). CD8 T cell responses are important in natural HIV control and their elicitation must be considered in a future vaccine. The vaccine epitopes need to be engineered according both to the expressed HLA alleles in the immunized population and the circulating HIV strains (Collins et al. 2020; Lunardi et al. 2021). Conserved HIV regions, like Gag protein, are better targets than regions with many mutations, like envelope proteins (Collins et al. 2020).

Preventive HIV vaccines showed little protection until now, around 30% of protection in one clinical trial in Thailand (Larijani et al. 2019). The virus is able to escape from cellular immunity and antibodies by the genetic diversity of the envelope structures and variable protein products that change the cell cycle (Larijani et al. 2019).

A cellular immune response is necessary to contain HIV. Humoral immunity has not been sufficient to control the infection. An ideal vaccine should include the stimulation of both immunities (Larijani et al. 2019). T cytotoxic lymphocytes are very important to contain HIV infection, especially against Gag protein epitopes (Zou et al. 2019). Escape mutations from Gag protein frequently result in impaired viral fitness (Zou et al. 2019).

There is also great genetic diversity around the globe. HIV-1 has 4 main groups and many subtypes (Ng'uni et al. 2020), including combined recombinant forms (CRFs). In Africa are also common co-infections by more than one viral variant, increasing the difficulty to create a universal vaccine.

Other difficulties are the lack of knowledge about immune correlates, lack of animal models and low funding on vaccine development (Ng'uni et al. 2020). The traditional vaccines delivery models do not induce appropriate antibodies against HIV and vaccines with attenuated virus cannot be used due to safety issues. New vaccine strategies are needed, especially including the production of intracellular viral antigens to produce T cell responses.

Some HLAs were related to HIV control, like HLA-B*27, HLA-B*51, HLA-B*57, HLA-B*58:01, HLA-A*74:01 (Goulder and Walker 2012) and HLA-B*14 (Gebara et al. 2019). A study with HIV Japanese patients found an 8-mer epitope (TI8, TAFTIPSI) restricted to HLA-B*51:01 to be related to slow disease progression and CTLs specific for this epitope can inhibit HIV replication in vitro (Motozono et al. 2014). Another study related HLA-B*51:01 with disease progression and high HIV viral load (Carlson et al. 2012). This fact highlights the

possibility that the same alleles can select different escape patterns in different ethnic groups (Carlson et al. 2012). Including other HLA-independent mechanisms such as proteasome processing, epitope transport or trimming, is also of primordial importance to study HIV epitopes (Carlson et al. 2012).

In this paper we will study conserved HIV epitopes and their relationship with HLA alleles and different HIV subtypes. We also analyze HLA frequency in some countries, relation with HIV clades and its control, and how epitope mutations can impact the MHC-class I binding affinity and processing.

Methods

We studied HLAs related to HIV control, especially with specific epitopes described in the literature (Goulder and Walker 2012; Motozono et al. 2014). The HLA frequency in Africa was chosen because of high HIV prevalence in this continent. The data were collected at Allele Frequency Net Database (www.allelefrequencies.net). We recovered HIV-1 protein sequences of different subtypes with high frequency in Africa, like group M subtypes B, C, A, D and CRF02_AG (Los Alamos laboratory).

HLA alleles HLA-B*57:01, B*57:03, B*58:01,B*27:05, B*42:01, B*44:03 and A*74:01 were included, also (Goulder and Walker 2012). HLA-B*51:01 was included after some results linking this allele to HIV control (Motozono et al. 2014), but also disease susceptibility (Goulder and Walker 2012).

We build maps with these allele frequencies in Africa and the most prevalent HIV subtypes in the countries with HLA data available using infographics (infogram.com).

The HIV-1 subtypes distribution in Africa was found on the HIV Sequence database (https://www.hiv.lanl.gov/components/sequence/HIV/geo/geo.comp), a platform from Los Alamos National Laboratory.

The strains sequences for HIV-1 subtypes were searched at UniProt (www.uniprot.org) by taxonomy, including HIV-1 clades B, C, A, D and CRF02_AG.

The impact of epitopes mutations in the analyzed sequences was assessed by *in silico* predictions. Binding affinity by NetMHCpan 4.1 (https://services.healthtech.dtu.dk/service.php?NetMHCpan-4.1), MHC-I cleavage by proteasome on NetChop 3.1 (https://services.healthtech.dtu.dk/service.php?NetChop-3.1). The combined prediction, including TAP translocation, was predicted by IEDB web resource Epitope Prediction and Analysis Tools (http://tools.iedb.org/processing), assigning a specific processing ranking score. We considered the ranking position of the wild type described epitope to infer if the mutations impact the worsening of prediction.

Results

Maps and HLA allele frequencies

The maps show that HLA-A*74:01 is common in countries with high prevalence of HIV-1 subtype A and CRF02_AG, like Cameroon (6.2%), Ghana (5.3%), Kenya (6.1%), Uganda (7.3%) and Guinea Bissau (7.1%) (see Map, Supplemental Digital Content 1). HLA-B*58:01 is also common in places where HIV-1 subtypes A and CRF02_AG are prevalent, like Kenya (8.2%), Guinea

Bissau (7.8%), Cameroon (5.7%), Burkina Faso (5.3%), Senegal (6.9%), Uganda (5.0%) and South Africa (4.9%) (see Map, Supplemental Digital Content 2).

HLA-B*27:05 (is uncommon in Africa, with a 2.1% prevalence in South Africa, 0.2% in Kenya and 0.3% in Cameroon (see Map, Supplemental Digital Content 3). HLA-B*57:01 is also rare in that continent and has 3% prevalence in South Africa, 1.8% in Kenya and 0.4% in Zimbabwe (see Map, Supplemental Digital Content 4). South Africa has extreme predominance of HIV-1 subtype C, near to 98.8% (Hemelaar et al. 2019). HLA-B*57:03 is common in Zambia (5.7%) and Zimbabwe (4.4%), where HIV-1 subtype C is also highly prevalent (see Map, Supplemental Digital Content 5).

HLA-B*44:03 is frequent in many countries in Africa, like Morocco (8.1%), Libya (5.1%), Cameroon (8.8%), Central African Republic (7,2%), Ghana (8.7%), South Africa (5.0%) and Zimbabwe (5.8%) (see Map, Supplemental Digital Content 6). Subtypes C and CRF02_AG are common in these countries. HLA-B*42:01 has low prevalence in Morocco (1.9%), Cameroon (3.6%), Central African Republic (1.5%) and high prevalence in Ghana (11.0%), South Africa (5.1%) and Zimbabwe (6.9%), where subtypes C and CRF02_AG predominate (see Map, Supplemental Digital Content 7). The considered epitopes in our analysis are restricted to these above-cited alleles.

Epitope mutations and peptide binding

We investigated the impact of mutations harbored by epitopes related to HIV control described in the literature (Goulder and Walker 2012; Motozono et al.

2014), in the context of population genetics of the African continent and circulating HIV strains in those countries.

TW10 epitope (TSTLQEQIAW), p24 Gag protein was predicted as strong binder on subtypes B, C, A, D and CRF02_AG variants for the 3 HLAs originally related: B*57:01 (HIV Clade B in Caucasians and Hispanics), B*57:03 (Clade A Rwanda and C South Africa; Clade C Zambia, South Africa, Botswana, Zimbabwe) and B*58:01 (Clade C Zambia, South Africa, Botswana, Zimbabwe). Considering their potential to be generated by the antigen processing pathway, all the mutations on Clade B maintain an elevated processing score ranking ranging between 1, 2 or 3 top positions. Mutations present on Clade C sequences were also ranked in first position. Mutations on Clades A, D and CRF02_AG did not have their processing score changed. Regarding their ability to bind to other EC related HLA alleles, all the sequences found were not predicted even as a weak binder for the others HLAs tested. The proteasome cleavage site was maintained for all the variants tested and the three HLAs associated with EC in the literature (Fig. 1).

Epitope TI8 (TAFTIPSI), from reverse transcriptase protein, is related to HLA-B*51:01 (Clade B Japan). The wild type epitope was predicted as processing ranking 10. Clade B sequences were strong binder for this HLA, but mutations I8T and I8R made it lose this condition and were processing ranking 97 and 40, respectively. On Clade C sequences, mutations I8T, I8R and I8K were predicted as non-binders and processing ranking 91, 60 and 32 respectively. Clade A sequences I8T, I8 R and I5L/I8T were not binders for B*51:01 and processing

ranking 93, 32 and 98. For Clade D sequences, I8T mutation was not binder and was processing ranking 98. For CRF02_AG subtype, mutations I8K, I8T and I8R were not binders and processing ranking 63, 96 and 31, respectively. Proteasome cleavage was aborted for sequences from subtypes B (mutation I8T), C (mutation I8T), A (mutation I5L/I8T), D (mutation I8T) and CRF02_AG (mutation I8T) (Fig. 2).

KF11 epitope (KAFSPEVIPMF), p24 Gag protein is described for HLAs B*57:01 (Clade B in Caucasians and Caucasians/Hispanics) and B*57:03 (Clade C in South Africa, Zambia, Botswana, Zimbabwe; and Clade A in Rwanda). Our results showed KF11 peptide variants were also predicted as strong or weak binder for HLA-B*58:01 related allele. Mutation F11V at subtype B sequences makes the epitope loss binding affinity to the eight HLAs tested and also impaired processing ranking for HLA-B*57:01 falling from position 1 to 29. Subtype C A2G/S4N mutation transforms the epitope in a weak binder, but maintains processing ranking 2. Subtype A representatives were described as strong binder for the HLAs B*57:01 and B*57:03 and were processing ranking 1 on both. We have not found KF11 sequences for subtype D in Uniprot. Mutations on the CFR02_AG subtype maintained the epitope as a strong binder (K1R/G2A/N4S/V6I and V6I) with processing ranking 1 or weak binder (A2G/S4N) with processing ranking 2 for HLAs B*57:01 and B*57:03, respectively. Proteasome cleavage was predicted without problems for all sequences tested from subtypes B, C, A and CRF02_AG (see Map, Supplemental Digital Content 8).

Epitope KK10 (KRWIILGLNK), p24 Gag protein is related to HLA-B*27:05 (Clade B in Caucasians). This epitope was a strong binder just for this allele. Mutations on subtype B sequences (R2Q/L6M, R2K/L6M, R2K/L6I, R2G, R2T)

make it loss the binder condition and impaired its ability to be correctly processed, especially R2G and R2T, even for HLA-B*27:05. Clade C mutation R2K/L6M was predicted as a non-binder and processing ranking 8, while I5V remains a strong binder and processing ranking 2 for the same HLA. Clade A mutation K1R maintained the strong binder status and a processing ranking 1. CRF02_AG mutations I6V and K1R were strong binders and processing ranking 2 and 1, respectively. Proteasome cleavage occurrence prediction was affected on sequences tested from the subtypes, including all of the mentioned above (see Map, Supplemental Digital Content 9).

Epitope TL9 (TPQDLNTML), p24 Gag protein is described for HLA-B*42:01 (Goulder and Walker 2012). We predicted that this epitope could also be presented by other HLA alleles: B*51:01, B*07:02, B*08:01 and B*39:01. All the variants keep the binding condition and high proteasome processing ranking. In this case, HLA-B*42:01 is closely related to HLA-B*07:02, so the last was used to predict processing ranking, as the former do not possess prediction for it. Clade B mutation Q3T was predicted as non-binder for B*08:01. The proteasome cleavage was adequate for all the sequences predicted (see Map, Supplemental Digital Content 10).

Epitope AW11 (AEQATQDVKNW), p24 Gag protein is restricted to HLA-B*44:03 (Clade C in South Africa, Botswana, Zimbabwe). Originally this epitope is a strong binder and processing ranking 1 for B*44:03. Subtype B mutations T5S/D7E/W11R and E2G/T5S/D7E make the epitope lose binding affinity and become processing ranking 19 and 26, respectively for B*44:03. Clade C sequences are strong binders and processing ranking 1. The same happens to

subtypes A, D and CRF02_AG. Our analysis showed that the epitope could be also a binder for HLA-B*57:01, B*57:03 and B*58:01. Proteasome cleavage was inadequate for sequences from all subtypes tested (see Map, Supplemental Digital Content 11).

Epitope GR11 (GQMVHQAISPR), p24 Gag protein is the only epitope linked to the locus A (HLA-A*74:01) in this paper (Clade C in Zambia, South Africa, Botswana, Zimbabwe; Clade B in African American; Clades A,C,D in Tanzania). Considering that this allele usually presents nonamers, we selected the fragment with binding affinity predicted as a strong binder (MVHQAISPR) to infer the mutations impact presented by the variants. We predicted MVHQAISPR sequence as strong binder for HLA-A*74:01, but Clade A mutation M1P/I6L was non binder even for this allele. CRF02_AG variant M1P/I6L was predicted as non-binder and V2I/A5S/I6M was weak binder for HLA-A*74:01. Wild type sequence was not predicted as a binder to HLA-A*74:01. Clade B mutations I8L and A7P are weak binders for A*74:01 and processing ranking 22 and 16, but mutations A7P/I8L/S9T and A7S/I8L are not binders and lose processing ranking. Clade C mutation A7P/P10A was weak binder and processing ranking 12. Clades A, D and CRF02_AG sequences were also not binder for HLA-B*74:01 and had low processing ranking. Proteasome cleavage was aborted for sequences from subtypes B (mutations I8L, A7P/I8L/S9T and A7S/I8L), C (mutation A7P/P10A), A (mutations M3P/I8L and V4I), D (mutation 0p17) and CRF02_AG (mutations M3P/I8L and V4I/A7S/I9M) (see Map, Supplemental Digital Content 12).

Discussion

In this paper we did not use just peptide binding to MHC, but included proteasome cleavage, TAP transport and processing ranking to evaluate the epitopes and genetics of HIV variants in each place where HLA alleles were present, that is a fundamental premise to a therapeutics geotargeting view.

This is also a comprehensive review of HIV-1 epitopes for Clades B, C, A, D and CRF02_AG available at UniProt to study the mutation effect on global processing ranking and presentation ability.

We found some epitopes to be strong or weak binders for HLA alleles different from the originally described. This can be explained by the range analysis we did for many HLAs alleles related to HIV control.

Different HLAs have been associated with relative protection against disease progression and even virus control by the immune system. A previous article (Goulder and Walker 2012) studied the relationship between the HIV epitopes and HLA and found that it was generally related to changes in the HIV Gag protein. It seems to be related to HIV subtype analyzed and population affected.

In African American patients with HIV subtype B, certain HLAs were related to better virus control, especially HLA-B*14, HLA-B*57:01, and HLA-B*57:03, while other HLAs were related to worse outcomes (HLA-B*15:10, HLA-B*35:01) (Aleksandr Lazaryan, Wei Song, Elena Lobashevsky, Jianming Tang et al. 2013).

Some HLA alleles belong to the same HLA-B7 superfamily, like HLA-B*07:02, HLA-B*42:01, HLA-B*42:02 and HLA-B*81:01, and have great

prevalence in Sub-Saharan Africa (Kløverpris et al. 2015). The TL9 epitope is presented by this superfamily.

A study found a relationship between HLA protective alleles and viral fitness (Kløverpris et al. 2016). HLA-B*27 restricted KK10 response was one of the first HIV epitopes described (Kløverpris et al. 2016). This HLA allele is related to slow disease progression. The dominant observed escape mutation of KK10 epitope is R264K, witch arises at the anchor position-2 (P2) in the epitope that is believed to require Arginine for adequate binding to HLA-B*27 and is associated with progression to AIDS (Kløverpris et al. 2016).

KK10 mutations in C clade HIV infection, which predominates in Sub-Saharan Africa and in the world, are different. Instead of Ser at Gag-173, the C clade consensus residue at this position is Thr. KK10 escape in C clade HLA-B*27-positive individuals involves the selection of R264K without prior escape at L268 (Kløverpris et al. 2016).

HLA-B*57 is closely related to HLA-B*58:01, which is also protective against HIV progression. TW10 epitope is presented by the two alleles. T242N is the most common mutation and can provide immune escape, but it also impairs viral replicative capacity (Kløverpris et al. 2016).

KF11 epitope is also presented by HLA-B*57/B*58 (Kløverpris et al. 2016). Mutation A163G impairs CD8 immune response and the compensatory mutation S165N restores viral fitness, which is the best solution for the virus.

TL9 epitope is selected by many African HLA alleles, like HLA-B*07:02/B*39:10/B*42:01/B*81:01 and HLA-C*08:02 (Kløverpris et al. 2016).

B*58:01 response restricts the p24 Gag epitope TW10, witch escape impairs viral fitness, while B*58:02 restricts an epitope in Env, which is associated with no selection pressure and high viral load (Kløverpris et al. 2016).

Other HIV studies have shown that one amino acid difference between HLA subtypes, like HLA-B*42:01 and HLA-B*42:02, and HLA-B*57:02 and HLA-B*57:03, is sufficient to have a significant impact on disease outcome (Leitman et al. 2017).

Some HLA alleles are African-specific alleles (eg, HLA-A*74:01, B*15:03, B*58:02) (Koehler et al. 2010). HLA-A*74:01 was described with a protective effect against HIV infection in Africa. In South Africa, A*74:01 is found in linkage disequilibrium with B*57:03 and supplemented the protective effect on viral load of the latter (Koehler et al. 2010). The exact protection mechanism is not known, but this allele shares common peptide-binding properties with HLA-A*32:01, which was related to low HIV viral load in Caucasians (Crux and Elahi 2017).

An article (Angulo et al. 2019) studied HLA alleles and mother to child HIV-1 transmission. Some alleles were related to slower disease progression in children, especially HLA-B*27, B*57, B*58, B*81. However, other allele groups were linked with fast HIV progression, like HLA-B*18, B*35, B*45, B*58. HLA-B*58:02 was associated to faster disease progression, while HLA-B*58:01 was related to slower disease progression (Angulo et al. 2019). We also have discussed this condition in another article (Lunardi et al. 2021).

No single genetic variant yet identified uniformly confers EC condition of HIV-1, and it is likely that different effects of multiple genetic variants are required for HIV-1 control (Crux and Elahi 2017).

A study (Matthews et al. 2012) used a computational approach to find additive effects of HLA alleles on HIV infection. Six pairs of HLA alleles were associated to a favorable impact on HIV infection: A*74 and B*57, A*74 and B*81, B*58:01 and B*81, B*58:01 and Cw*04, A*02 and B*81, B*44 and Cw*04. Four pairs showed association to a detrimental impact: B*58:02 and Cw*16, Cw*06 and Cw*16, B*08 and B*18, B*18 and B*45 (Matthews et al. 2012). An increased breadth of CD8+ T cell targeting as compared to either allele alone can account for the additive effect. This finding demonstrates the potential benefit of combining multiple CD8+ T cell responses in the development of cellular immune vaccines (Matthews et al. 2012).

HLA-B*35 is associated with fast HIV disease progression, but the exact mechanism is unclear (Murakoshi et al. 2018). The Y135F mutation within NefYF9 out of the 4 epitopes, which is selected by HLA-A*24:02-restricted T cell, is a critical factor for the worsening effect of HLA-B*35 on disease outcome (Murakoshi et al. 2018).

All the information discussed above reveals a complex and intertwined scenario, where it is very hard to extract useful elements to be applied in immunotherapeutic. So, we presented a new way to compile this data making it clearer to prospect customized targets.

We predicted that some epitopes even with mutations remain strong binders and high processing ranking, like TW10 for the 3 HLAs related in the literature. This could be a model for future therapeutics.

The TW10 epitope would be a good target for a vaccine or immunotherapy, because even with all the mutations found at UniProt, they were predicted as

strong binder and processing ranking 1 for HLA B*57:01, B*57:03 and B*58:01. Considering the countries with these HLAs, South Africa, Zimbabwe and Rwanda could be good places to test epitopes with this structure.

KF11 epitope maintains good binding strength and processing ranking for Clades B, C, A and CFR02_AG interacting with HLA-B*57:01, B*57:03 and B*58:01. Good places to use this epitope include South Africa, Zimbabwe, Zambia and Kenya.

Mutations on KK10 epitope make it lose binding strength and processing ranking for Clade B, but maintain it on Clades C, A, D and CRF02_AG. Some countries, like South Africa, Zimbabwe and Zambia, would work well with this epitope, while Morocco would not.

TL9 epitope was predicted to HLA-B*42:01, but also with potential for others HLAs, like B*07:02, B*08:01, B*39:01 and B*51:01 for all clades tested, including good processing ranking for these clades. Zambia, Mali and Ghana could be good places to test this epitope.

AW11 epitope was initially predicted to HLA-B*44:03, but our results showed good potential for HLA-B*57:01, B*57:03 and B*58:01 for all clades tested. Countries like Libya, South Africa, Zimbabwe, Uganda, and Kenya would be good places for this kind of epitope.

Epitope MVHQAISPR (nonamer of GQMVHQAISPR) showed potential as binder to A*74:01 in all clades tested sequences, the same HLAs described in the literature. Ghana, Guinea Bissau, Kenya and Uganda could be countries where to use this epitope.

TI8 epitope has mutations on Clade C and A sequences that loose binding condition even with HLA-B*51:01. This epitope could be used in countries like Libya and Morocco, where HIV subtypes B and CRF02_AG are prevalent, as well as HLA-B*51:01, but should not be used in South Africa, where HIV Clade C has 98% prevalent and B*51:01 has a 3% prevalence. This epitope also has problems with proteasome cleavage in some sequences. Because of all these questions, TI8 could also be a negative model for HIV vaccine epitope.

One limitation of this study is the fact we could not evaluate the epitope immunogenicity. We should use another tool to do this analysis. It can be an issue for future research.

An initial analysis showed that we need geographically adjusted HIV vaccines, adapted to the most prevalent HLA and HIV Clade in each place. Antibody based HIV vaccines showed a 30% efficacy until now. Combining different immune responses, like humoral and cellular immunity, and including diverse epitopes, can help to reach the HIV vaccine goal. The EC patients can be a model to identify epitopes for vaccine or immunotherapy purposes.

Acknowledgements
Luciano Werle LUNARDI: wrote the manuscript, performed the MHC processing predictions, recovered the HLA and epitope data.

Alexandra de Azevedo da ROCHA and Ana Carolina Alves de OLIVEIRA performed the MHC processing predictions, generated the maps

Iuri Marques de OLIVEIRA: constructed the database.

Gustavo Fioravanti VIEIRA: elaborated the hypothesis, review the manuscript, general supervision.

We have no funding source for this article.

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Supplemental Digital Content List

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Figure Legends

Figure 1:

African Continent Map showing the main effects associated with the KK10 epitope mutations present in the circulating subtype for each specific location. The colors in the countries indicate the combined frequency of the HLA-B*57:01, B*57:03 and B*58:01 alleles associated with the corresponding epitope.

Figure 2:

African Continent Map showing the main effects associated with the TI8 Epitope mutations present in the circulating subtype for each specific location. The colors in the countries indicate the frequency of the HLA-B*51:01 allele associated with the corresponding epitope.

Supplemental Digital Content 1.pdf

African Continent Map showing the HLA-A*74:01 prevalence.

The colors in the countries indicate the frequency of the allele.

Supplemental Digital Content 2. pdf

African Continent Map showing the HLA-B*58:01 prevalence.

The colors in the countries indicate the frequency of the allele.

Supplemental Digital Content 3. pdf

African Continent Map showing the HLA-B*27:05 prevalence.

The colors in the countries indicate the frequency of the allele.

Supplemental Digital Content 4. pdf

African Continent Map showing the HLA-B*57:01 prevalence.

The colors in the countries indicate the frequency of the allele.

Supplemental Digital Content 5. pdf
African Continent Map showing the HLA-B*57:03 prevalence.

The colors in the countries indicate the frequency of the allele.

Supplemental Digital Content 6. pdf

African Continent Map showing the HLA-B*44:03 prevalence.

The colors in the countries indicate the frequency of the allele.

Supplemental Digital Content 7. pdf

African Continent Map showing the HLA-B*42:01 prevalence.

The colors in the countries indicate the frequency of the allele.

Supplemental Digital Content 8.jpeg

African Continent Map showing the main effects associated with the KF11 Epitope mutations present in the circulating subtype for each specific location. The colors

in the countries indicate the combined frequency of the HLA-B*57:01, B*57:03 and B*58:01 alleles associated with the corresponding epitope.

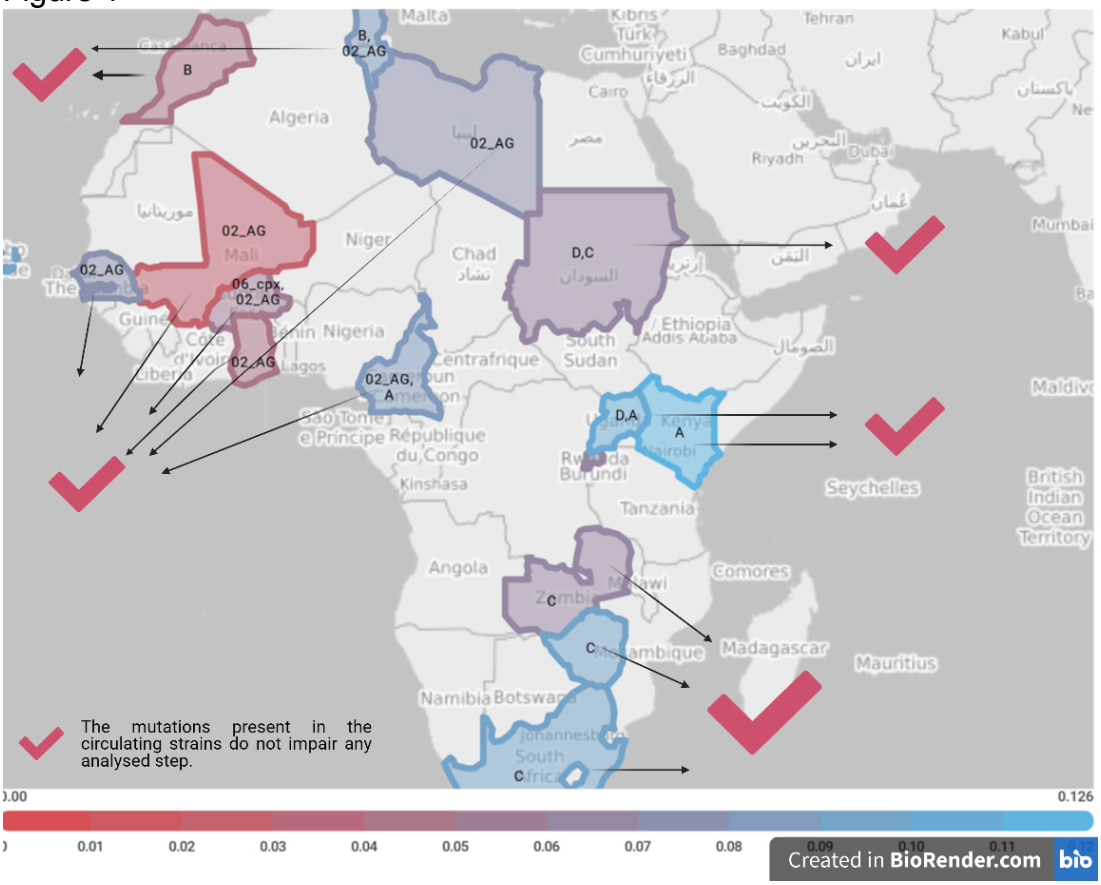
Supplemental Digital Content 9. Jpeg
African Continent Map showing the main effects associated with the KK10 Epitope mutations present in the circulating subtype for each specific location. The colors in the countries indicate the frequency of the HLA-B*27:05 allele associated with the corresponding epitope.

Supplemental Digital Content 10. jpeg
African Continent Map showing the main effects associated with the TL9 Epitope mutations present in the circulating subtype for each specific location. The colors in the countries indicate the combined frequency of the HLA-B*42:01, B*07:02, B*08:01, B*39:01 alleles associated with the corresponding epitope.

Supplemental Digital Content 11. jpeg
African Continent Map showing the main effects associated with the AW11 Epitope mutations present in the circulating subtype for each specific location. The colors in the countries indicate the combined frequency of the HLA-B*44:03, B*57:01, B*:57:03 and B*58:01 alleles associated with the corresponding epitope.

Supplemental Digital Content 12. jpeg
African Continent Map showing the main effects associated with the GR11 Epitope mutations present in the circulating subtype for each specific location. The colors in the countries indicate the frequency of the HLA-A*74:01 allele associated with the corresponding epitope.

Figure 1-



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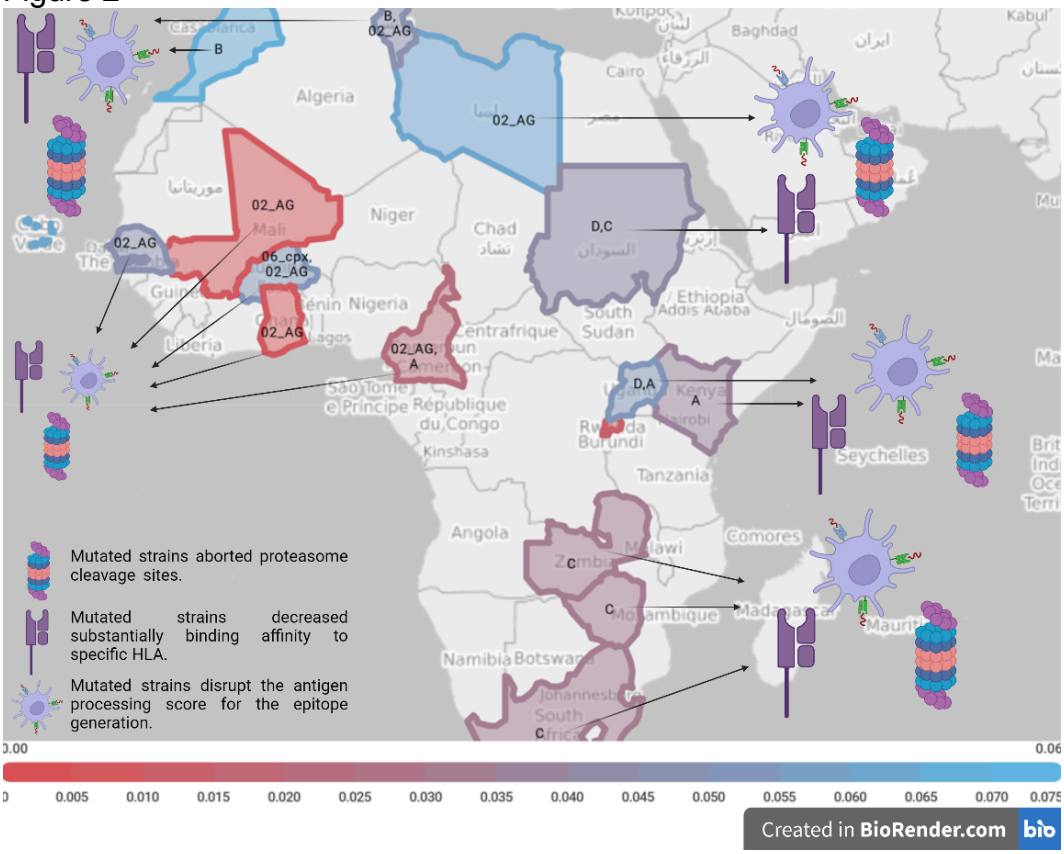

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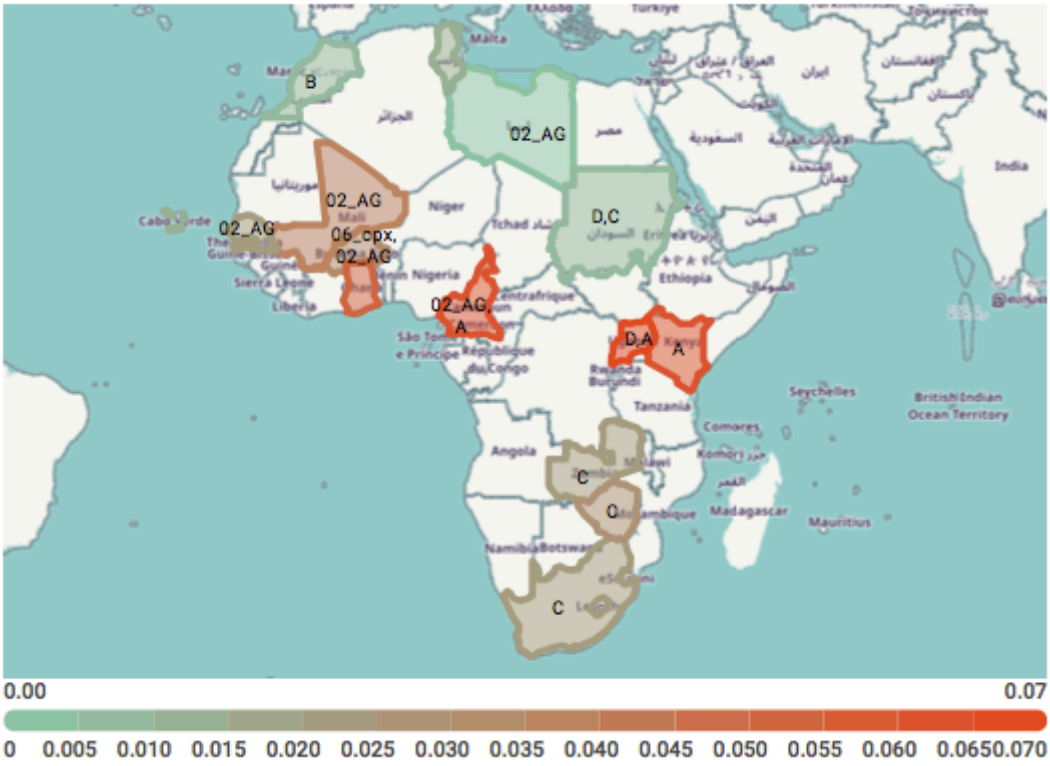

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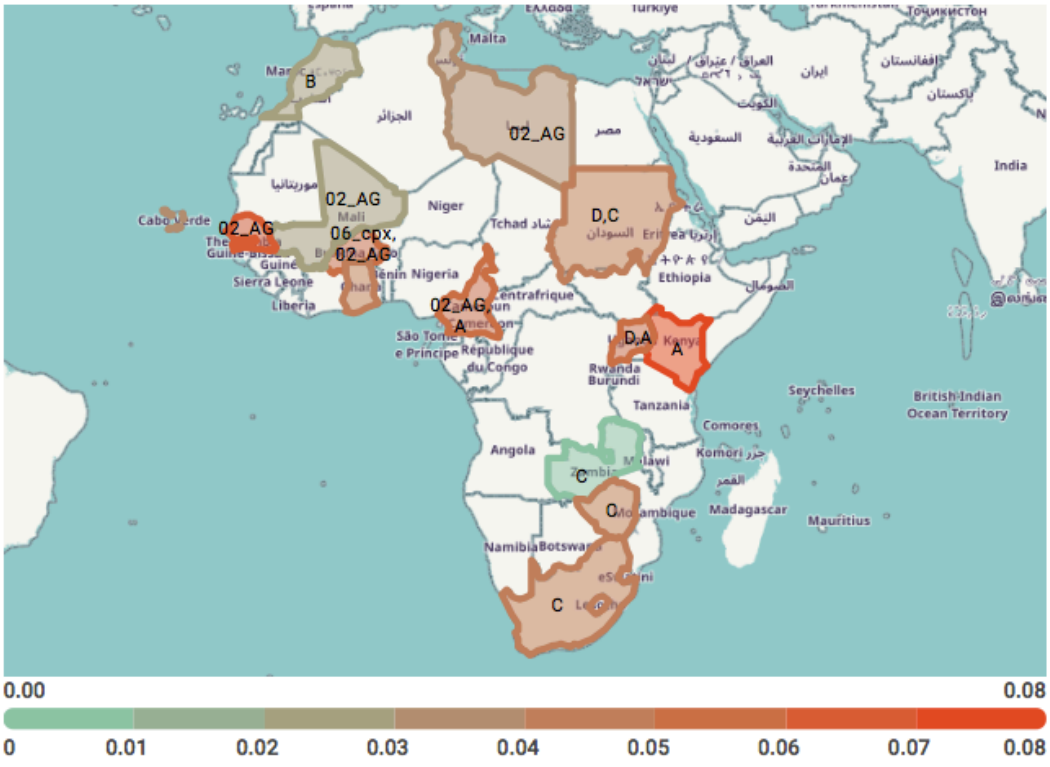

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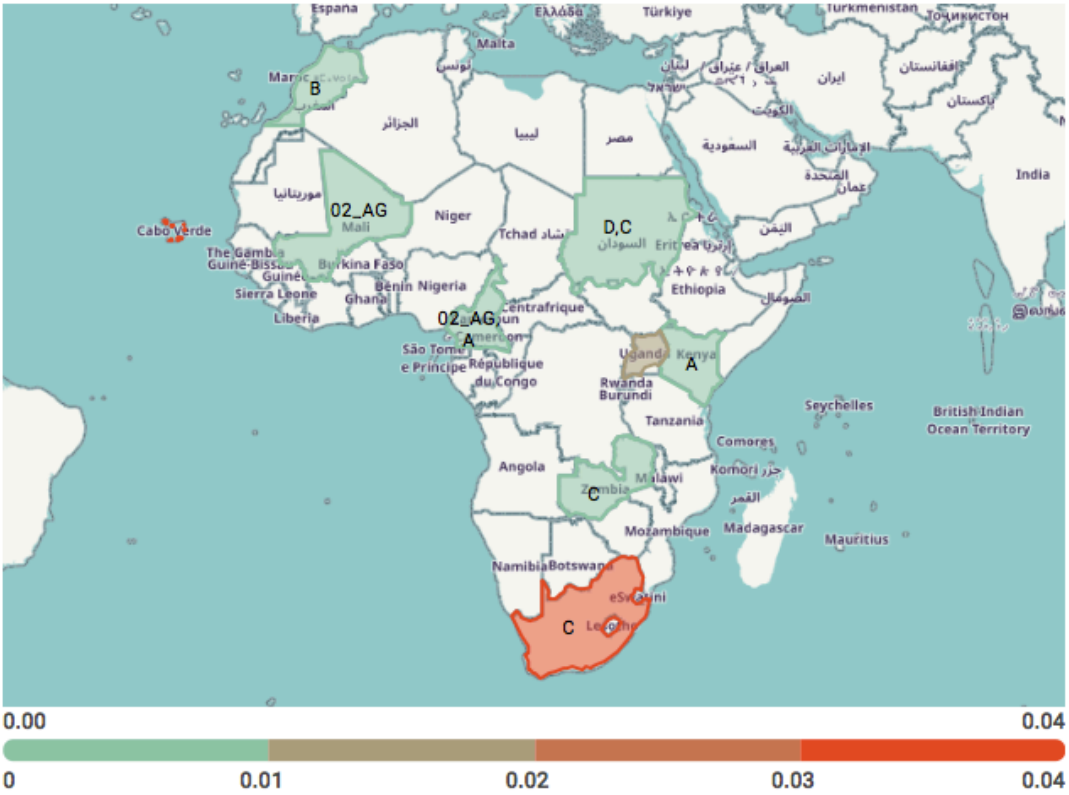

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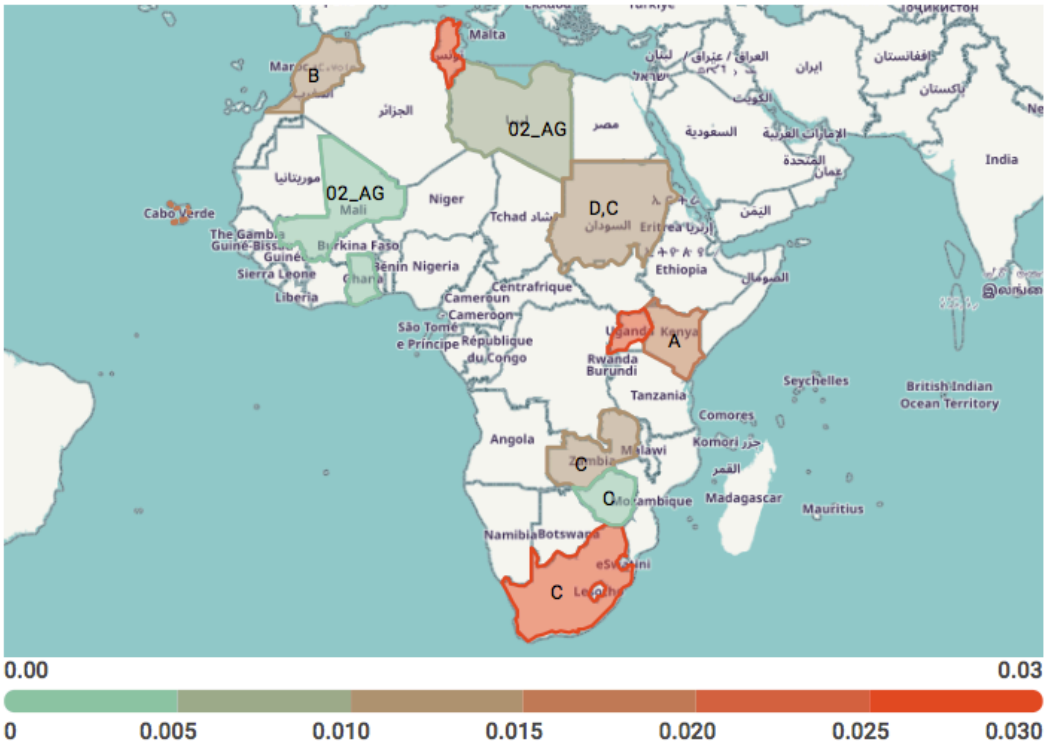

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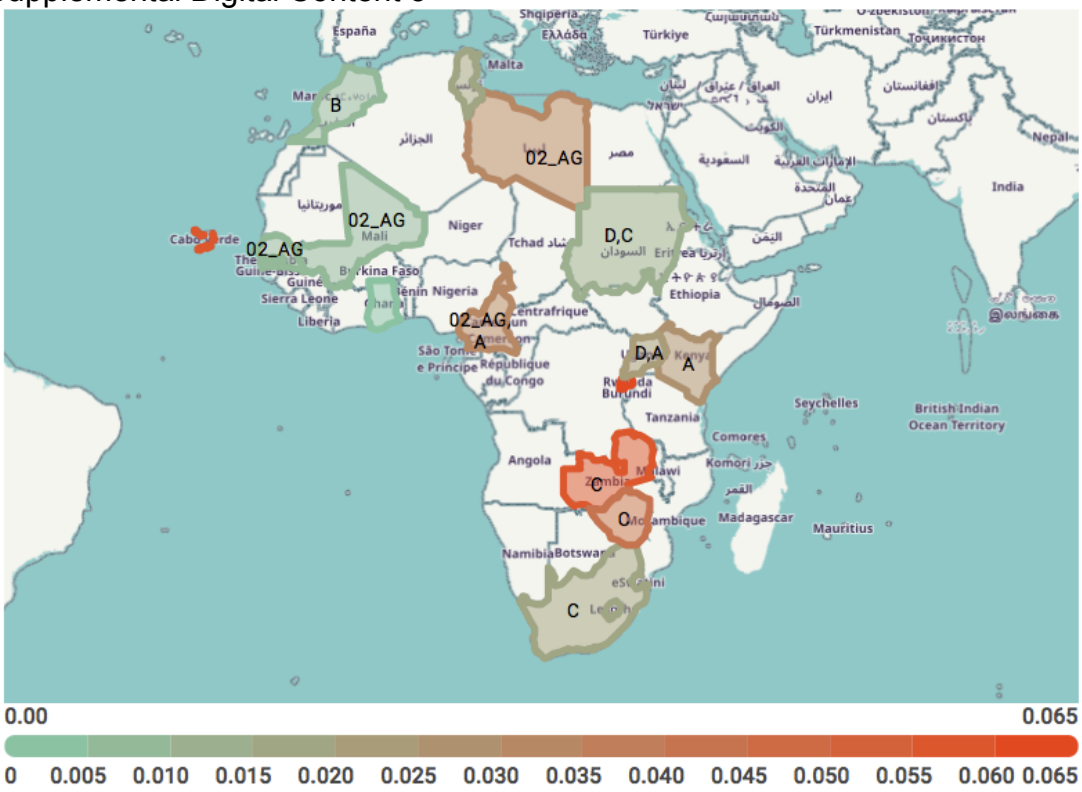

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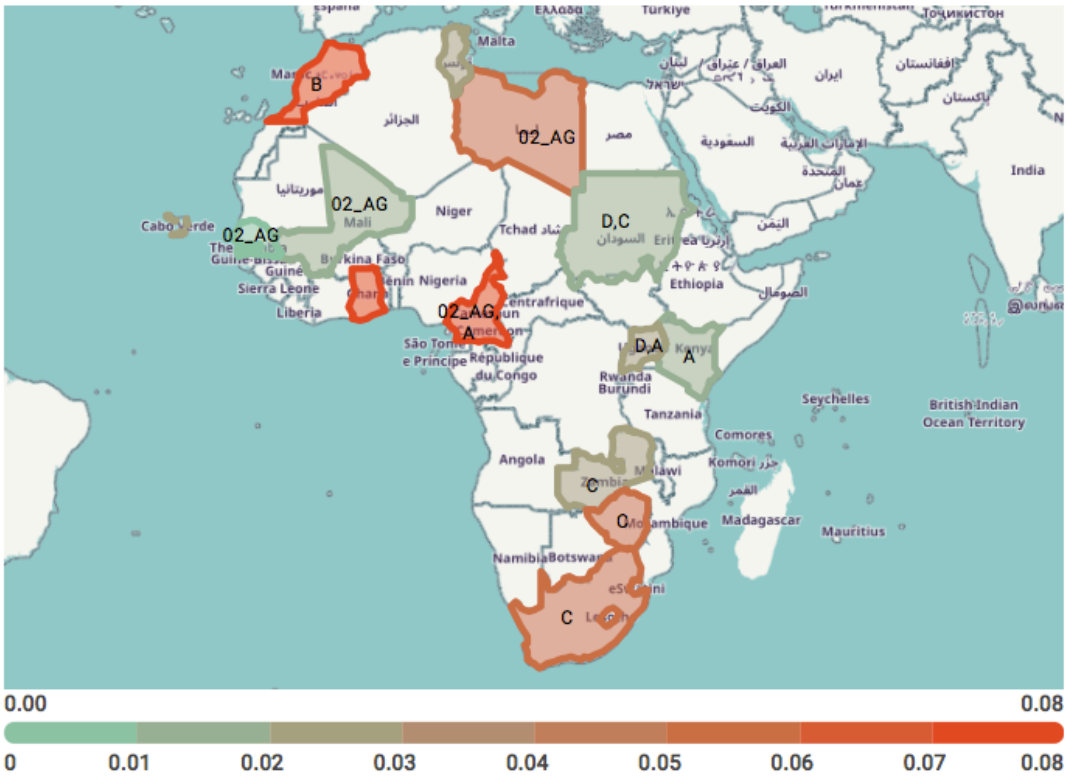

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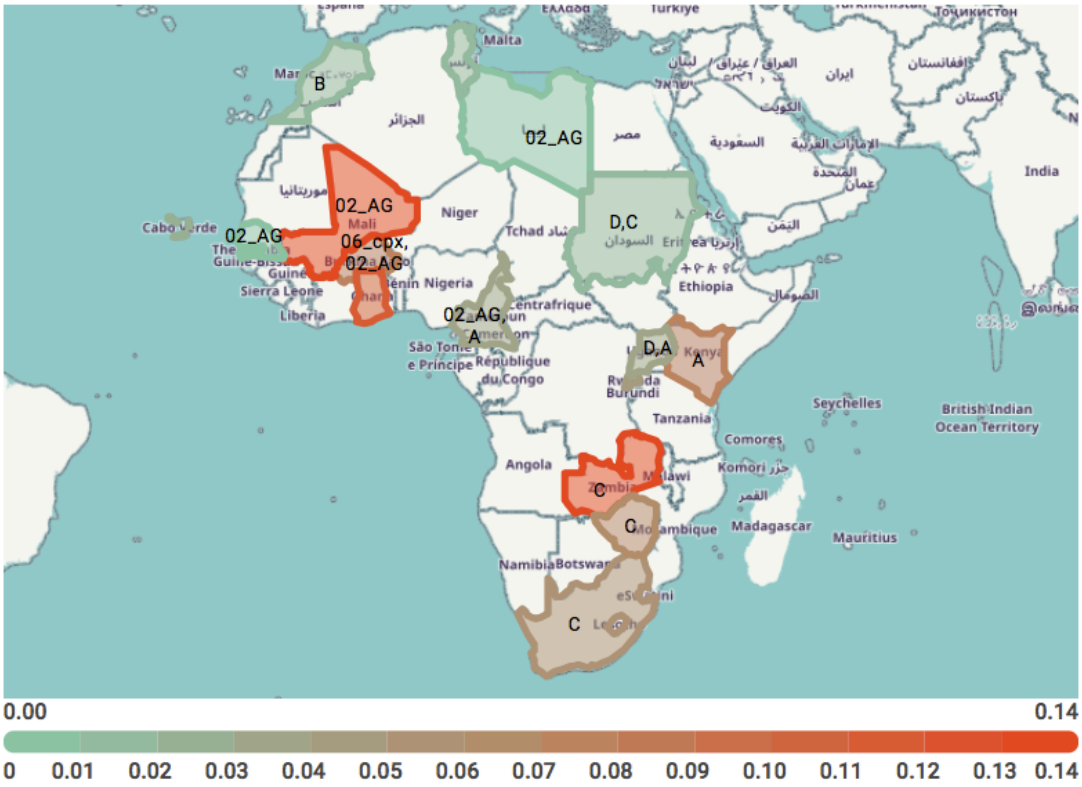

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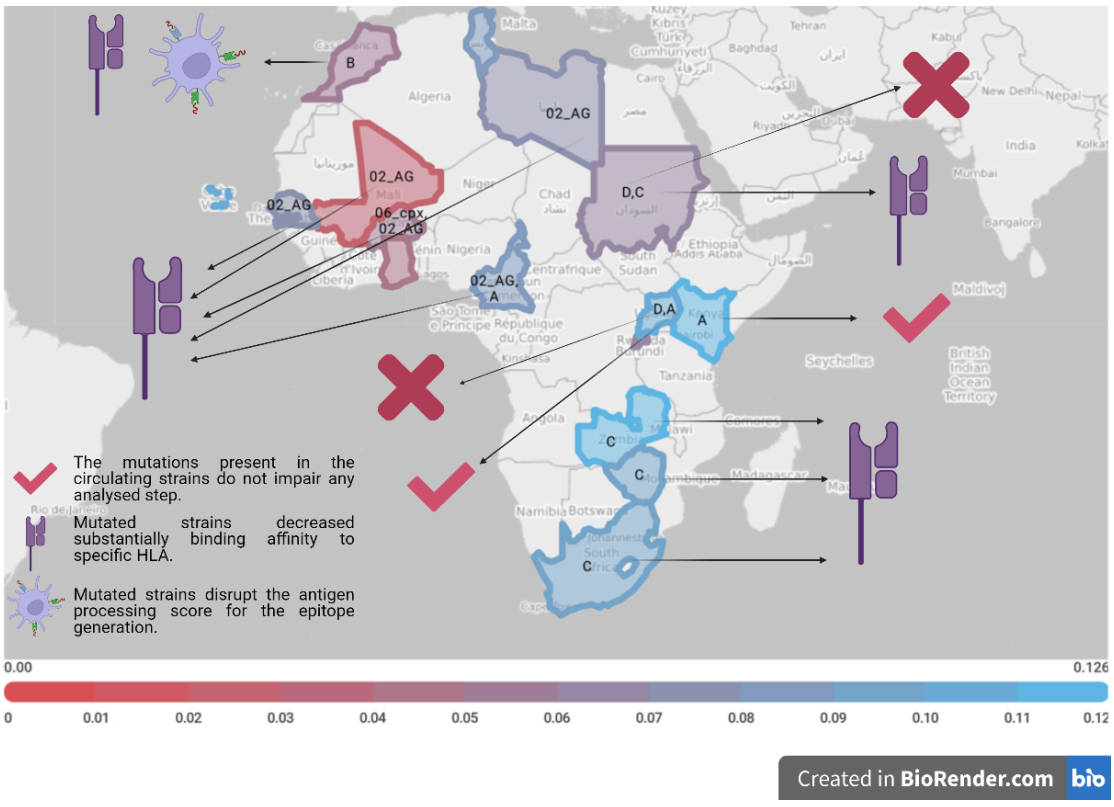

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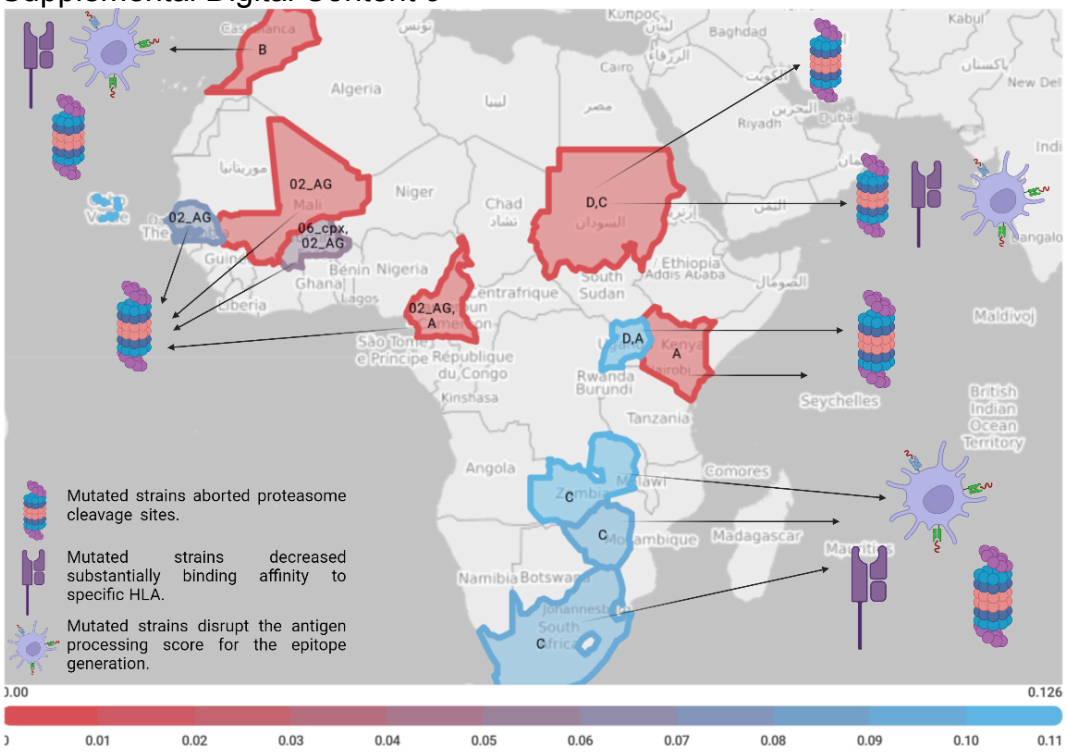

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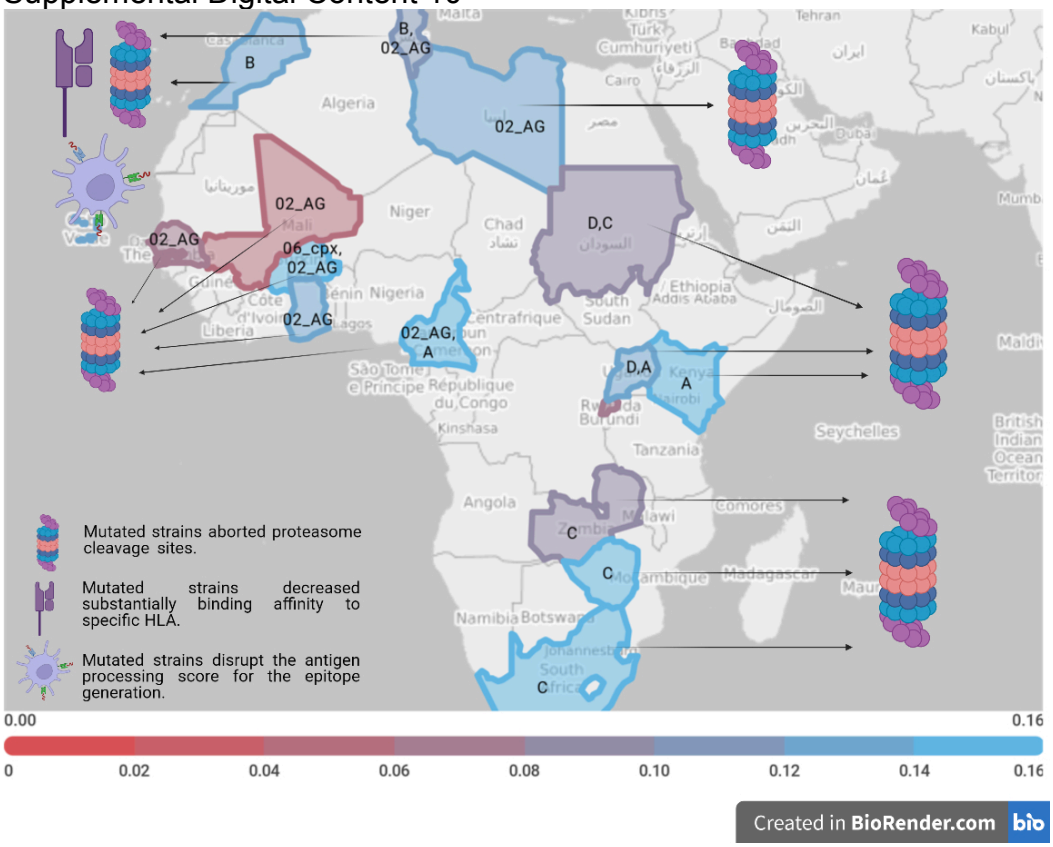

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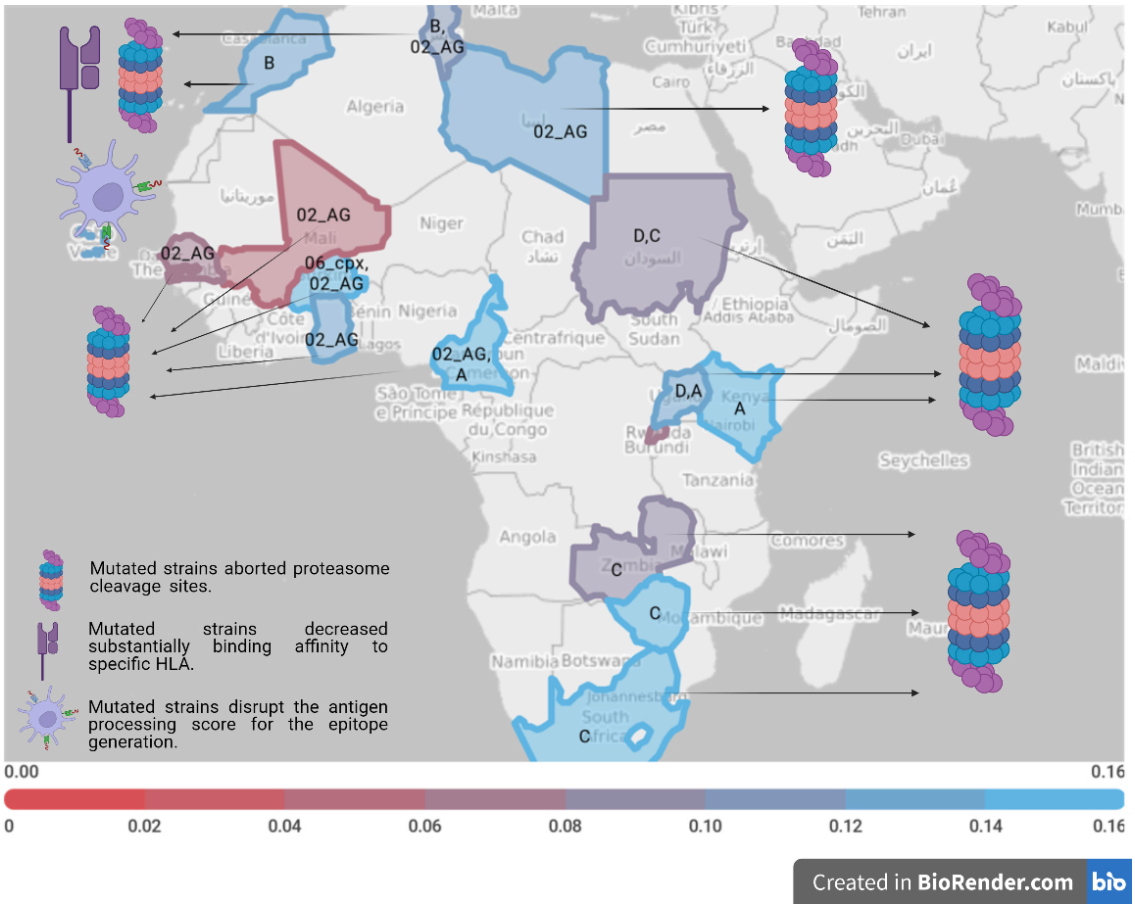

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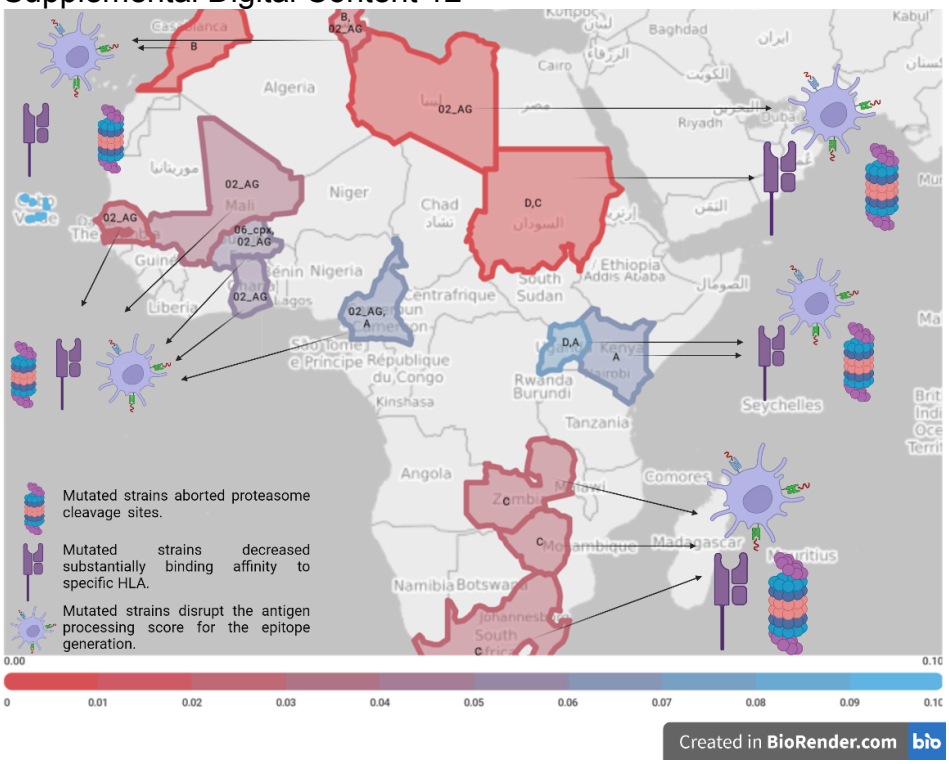

Capítulo 5 – Considerações finais

Nosso objetivo inicial foi estudar do ponto de vista estrutural quais elementos moleculares estão conferindo proteção aos indivíduos portadores dos alelos HLA-B*57:01, HLA-B*57:03, e/ou HLA-B*27:05 contra a progressão da infecção pelo vírus HIV.

Para isso, tentamos implementar a modelagem de complexos pMHC para os alelos HLA-B*57, B*57:01, B*57:02, B*57:03, B*58:01, B*27:05, B*07:02 a partir da ferramenta Docktope na ferramenta de análise do IEDB. Os cristais dos complexos pMHC para estes alelos foram procurados no PDB (Protein Data Bank). Os critérios de busca dos cristais incluíram: resolução superior a 2 angstroms, epítopos com diferentes extensões (9, 10, 11 e 12 aminoácidos). Depois exploramos as estruturas tridimensionais de interesse com o programa UCSF Chimera.

Nós não conseguimos encontrar um número suficiente de cristais com sequências diferentes para cada um dos alelos. Por este motivo, não foi possível criar um sistema de modelagem genérico que permitisse a investigação dos epítopos desejados a partir de um padrão estrutural compartilhado.

O próximo passo foi buscar na literatura epítopos e HLAs relacionados ao controle da infecção pelo HIV, mais especificamente controladores de elite. Foram também recuperados epítopos e HLAs relacionados a uma progressão mais rápida da doença.

Passamos também a estudar o quanto mutações nas sequências dos epítopos afetam a força de ligação ao MHC e outras etapas da apresentação de antígenos. Os diferentes subtipos do HIV e suas mutações também foram incluídos na análise. Percebemos que, conforme os alvos analisados, alguns apresentavam um efeito mais deletério que outros em função do local que ocorriam e das linhagens circulantes presentes nestas regiões.

Por exemplo, os epítopos KF11 e TW10 parecem ser bons alvos para uso imunoterapêutico considerando as características investigadas, pois os mesmos não são afetados pelas mutações ocorridas nas linhagens presentes nas regiões estudadas, pois as mesmas não afetam sua capacidade de serem

gerados como ligantes. Quando comparamos estes dados com informações já presentes na literatura, conseguimos traçar paralelos interessantes. Outro achado interessante foi o HLA-B*07, que é relacionado a progressão da doença no subtipo B do HIV, mas não no subtipo C. O HLA-B*51:01 foi considerado protetor para pacientes japoneses infectados pelo HIV-1 subtipo B, mas relacionado à progressão da doença e carga viral alta em outro estudo. Estes achados apontam para a possibilidade do mesmo alelo selecionar diferentes vias de escape em grupos étnicos diferentes. Percebemos que é importante avaliar em conjunto a genética do vírus, do hospedeiro humano e suas interações.

Este primeiro artigo analisando HLAs relacionados a EC e suscetibilidade ou progressão da infecção pelo HIV foi publicado na revista *Brazilian Journal of Infectious Diseases* em 2021 (Lunardi et al. 2021).

No segundo artigo desta tese avaliamos a frequência dos alelos de HLA relacionados ao controle do HIV, bem como dos subtipos de HIV mais prevalentes em diferentes países da África. Este continente foi escolhido por ter sido a provável origem do HIV e ter a maior participação no número de pessoas vivendo com a doença no mundo.

No total modelamos 7 epítopos, avaliando força de ligação, transporte pela TAP, clivagem pelo proteossomo e ranking global de processamento para HLAs descritos na literatura como relacionados a esses epítopos. Também incluímos sequências de HIV depositadas no Uniprot para os subtipos do HIV mais frequentes nos países da África com frequência alélica HLA conhecida.

A partir dos mapas que construímos, é possível estimar quais epítopos podem ser usados em um determinado país, considerando os HLAs mais frequentes na população e os subtipos de HIV mais prevalentes no território. Colocamos nos mapas dos epítopos os HLAs descritos para o respectivo peptídeo na literatura e incluímos também os que foram preditos em nossos resultados como relacionados.

Foram também levadas em consideração as mutações mais frequentes nos epítopos conforme as sequências dos principais subtipos de HIV prevalentes na África depositadas no Uniprot.

Nossos resultados mostraram que algumas mutações têm impacto na ligação ao MHC, mas a análise global aponta outros impactos na apresentação de antígeno que afetam o ranking de processamento.

A mutação F11V do epítopo KF11 (KAFSPEVIPMF) nas sequências do subtipo B tem impacto importante na força de ligação e ranking de processamento.

Mutações (R2Q/L6M, R2K/L6M, R2K/L6I, R2G, R2T) nas sequências do subtipo B no epítopo KK10 (KRWIILGLNK) causam perda na força de ligação e no ranking de processamento, bem como a mutação do subtipo C R2K/L6M.

Comparando as mutações encontradas e seu impacto predito com as descritas na literatura, o epítopo TL9 foi considerado como relacionado a proteção, incluindo outros HLAs além do HLA-B*42:01, como B*51:01, B*07:02, B*08:01 e B*39:01, indicando que seu uso possa ter uma aplicação ainda mais disseminada na população. As mutações nas sequências depositadas no Uniprot mantiveram ligação ao MHC e alto ranking de processamento, com exceção da mutação Q3T no subtipo B quanto a força de ligação para o HLA-B*08:01.

Quanto ao epítopo KK10, nós encontramos a mesma alteração descrita na literatura (Kløverpris et al. 2016) na posição 2 do epítopo: R2K para as sequências do subtipo B. Para as sequências do subtipo C, a mutação R2K/L6M também afetou a força de ligação e diminuiu o ranking de processamento.

Para o epítopo KF11, as mutações A2G/S4N para os subtipos C, A e CRF02_AG alteraram a força de ligação, mas mantiveram o ranking de processamento elevado para HLA-B*57:01 e HLA-B*57:03. Estas mutações foram consideradas pela literatura como compensatórias e capazes de manter o fitness viral (Kløverpris et al. 2016).

Mesmo pequenas diferenças nos HLAs podem causar respostas imunes bastante diversas. HLA-B*58:01 está associado na literatura como protetor contra a progressão da doença (Kløverpris et al. 2016). Nossos resultados corroboram esta visão, especialmente para os epítopos TW10, KF11 e AW11 em diferentes subtipos do HIV. Já o HLA-B*58:02 está relacionado à progressão da doença. Uma das possíveis explicações é o fato do HLA-B*58:01 restringir um epítopo da proteína p24 Gag, enquanto o HLA-B*58:02 restringe um epítopo da proteína Env,

um alvo que não está associado à pressão de seleção para o HIV e muito mais sujeito a mutações (Kløverpris et al. 2016).

O HLA-B*57:03 também está associado a proteção quanto a doença, enquanto o HLA-B*57:02 não, apesar da diferença entre os dois ser apenas um aminoácido (Leitman et al. 2017). O HLA-A*74:01 também é considerado como de proteção contra a doença. Este alelo é encontrado em desequilíbrio de ligação com o HLA-B*57:03 na África do Sul (Leitman et al. 2017). Nossos resultados mostraram que o epítopo GR11 (GQMVHQAISPR) está relacionado com o HLA-A*74:01, especialmente quanto à ligação ao MHC.

Um estudo (Matthews et al. 2012) encontrou pares de HLAs que teriam um efeito aditivo para proteção ou susceptibilidade à doença, especialmente carga viral e contagem de CD4. Destacamos os com efeito positivo: A*74 e B*57, A*74 e B*81, B*58:01 e B*81, B*58:01 e Cw*04, A*02 e B*81, B*44 e Cw*04, reforçando o achado anterior de (Leitman et al. 2017). Esse efeito aditivo pode ser acreditado a uma resposta CD8 com maior amplitude de alvos quando comparada a apenas um alelo. Este achado demonstra o potencial benefício de múltiplas respostas CD8+ no desenvolvimento de vacinas baseadas em imunidade celular.

Este trabalho demonstra a importância da resposta imune celular para controle do HIV. Essa resposta depende da genética do agente infeccioso, como os subtipos do HIV, e do hospedeiro, como os alelos do HLA Também demonstra a necessidade de ajustar os antígenos de uma vacina para estas duas variáveis. Os EC podem servir como modelo para futuras abordagens tanto de vacinas como de imunoterapia.

Uma perspectiva para o futuro seria, a partir deste conhecimento, gerar um banco de dados capaz de fornecer informações customizáveis considerando tanto a genética dos indivíduos, quanto das linhagens dos patógenos investigados.

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