Review Article

Conceptual Framework and Strategy for Designing Population-specific Epitope-basedHIV-1CTL Vaccine- aProposalfor the Nigerian Population.

Abstract

HIV infection has remained a global health concern for over forty years since its discovery. Although antiretroviral (ARV) drugs have changed the early damning prognosis, the need to prevent new infection is still as pressing as before because individuals who have achieved viral suppression on Antiretroviral therapy are more susceptible than the general population to chronic non-AIDS-defining illnesses due to a progressive immunological dysfunction that current therapeutic options cannot ameliorate. In the case of HIV, vaccination, the go-to intervention for most communicable diseases, is bedeviled by the peculiar molecular bottleneck posed byHIV hypermutability and the diversityof the host HLA genetic profiles. Therefore, the job of vaccine development is challenging. This article reviews the dynamicsof these challenges. It makes a case for a customized vaccine development approach that leverages the lesson learned from the HIV elite controller toward developing a population-based CTL epitope-based HIV-1 vaccine using the Nigerian population as amodel.

Introduction

Human Immunodeficiency Virus (HIV) primarily infects the CD4+ cells (T-lymphocytes, macrophages, and dendritic cells), critical to effective immune responses. The ensuing depletion of the CD4+ T-cell pool leads to progressive and ultimately profound immune suppression (Fields et al.,2007). The advent of effective combination antiretroviral therapy (cART) has significantly slowed down the progression of the disease and improved the quality of life of most affected individuals (Moreno *et al*., 2010).

However, resistance to ART is an ongoingconcern, necessitating identifying new treatment targets and therapeutic options, including the presently unrewardingefforts to design an effective vaccine.Several obstacles stand in the way of developing a protective vaccine against HIV-1 infection. Firstly, because natural immunity fails to prevent or eradicate the infection, there are

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no clear immune correlates of protection in human studies that can provide a template for vaccine development (Pantaleo&Koup, 2004; Plotkin, 2008).Specifically, it is still unclear which type of immune response is more significant for preventing infection: induction of HIV-neutralizing antibodies (systemic vs. mucosal compartments), CD4+ T-helper cells, cytotoxic CD8+ T-cells (Cytotoxic T Lymphocyte [CTL]), innate immunity, or a combination of all forms. Furthermore, immunogens that elicit neutralizing antibodies (NAb) have been elusive due to epitope sequestration in the lipid membrane (Sun *et al.*, 2008) and the fact that epitopes are only transiently exposed during viral entry (Frey *et al.*, 2008). As a result, novel approaches are continuously being employed in HIV-vaccine development.

The exceptional molecular diversity of the virusis the most formidable obstacle to vaccine development. No two HIV isolates are identical. Even isolates from a single individual show significant heterogeneity (Fields *et al*., 2007). Specifically, viral genomic sequences within a single individual can differ by up to 10% (Korber *et al*., 2001). Within a few months of infection,transmission founder (TF) viruses are almost completely replaced by viruses differing at several highly selected genomic loci. This phenomenon is predominantly mediated by CTL-driven immune escape (Goonetelike*et al*., 2009).This extremely high rate of evolution is underpinned by the error-prone reverse transcription step of the virus replication process, the high recombination frequencies in the virus, and the high turnover of progeny virions (Esbjörnsson *et al*., 2010). Together, these produce many mutant viruses with immune-escape potential (Mascola&Montefiori, 2010), serving as moving targets for vaccines.

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Molecular evolution of HIV-1 and Inter-individual variability in HLA genes in the context of vaccine development

A high level of molecular evolution of HIV-1has resulted in the diversification of HIV-1 into genetically distinct subtypes or clades and numerous circulating recombinant forms (CRFs) showing distinct geographic distributions (Esbjörnsson *et al.*, 2010). These strains are classified into four groups: the "major" group M, the "outlier" group O, the "new" or non-M, non-O group N, and the P group (Albert *et al*., 2013).More than 90% of HIV-1 infections belong to HIV-1 group M, within which there are nine genetically distinct subtypes (or clades), namely, A, B, C,

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D, F, G, H, J, and K. Furthermore, the HIV-1 group M is diversified globallyinto clades (A-K), Still, interclade viral recombination has given riseto "circulating recombinant forms" or CRFs, which account for 10% of circulating HIV-1 strains commonly found in geographic areas such as Africa, South America, and Southeast Asia, where multiple subtypes co-exist (Albert*et al.*, 2013).

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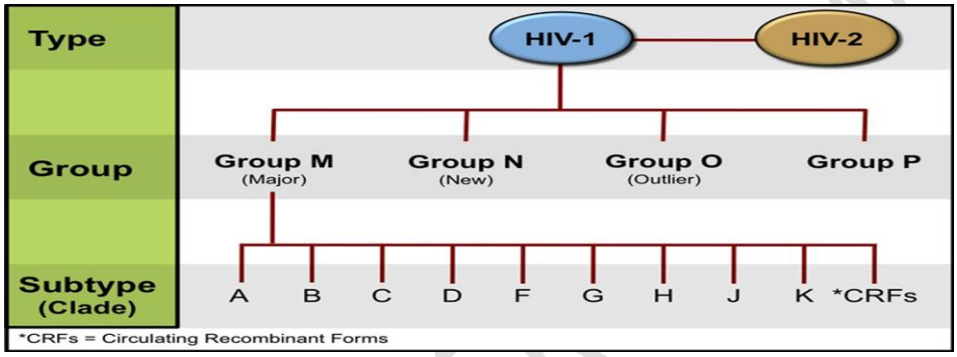

Figure1.HIV-1 groups and subtypes(clades) distributed in different geographical regions

Most HIV-1 recombinants have arisen from Africa, and most contain segments originally derived from clade-A viruses (Tongo *et al.*, 2015). HIV-1 CRF02_AG (recombinant of subtype A and G) and subtype G (HIV-1G) account for most HIV infections in Nigeria(Ajoge*et al.*, 2011).Njoku *et al*. (2016), in astudy in Nigeria involving six sites (Makurdi, Abuja, Enugu, Kaduna, Tafa, and Ojo-Lagos),showed the distribution of the major HIV-1 subtypes across these six sites as: 37.5% CRF02_AG, 27.7% G,and 25.9% G/CRF02_AG, while 8.9% could not be typed. Such massive genetic diversity lays bare the difficultyof developing a vaccine that is immunologically effective across divergent strains (Stephenson & Barouch, 2013)

Mirroring the diversity in HIV-1, numerous studies have shown extensive inter-individual variability in susceptibility to infection and disease progression (Winkler*et al.*, 2004; Fellay*et al.*, 2007; Aziz *et al*., 2019). At one end of the spectrum, individuals develop AIDS within 6-12 months after infection; at the other, they could remain asymptomatic without treatment for over 25 years. Some individuals even remain free of infection despite repeated exposure to the virus (Fields *et al.*, 2007).

Some of the widely studied and well-characterized genetic variants underlying inter-individual variability in the susceptibility to HIV infection and disease outcomes are found in genes encoding HIV entry receptors (CCR5, CCR2) and their ligands (Modi *et al.*, 2006); genes involved in the innate immune response to the initial infection, including **d**endritic **c**ell-**s**pecific **i**ntracellular **a**dhesion molecule-3-grabbing **n**on-integrin [DC-SIGN], killer cell immunoglobulin-like receptors[KIR]; genes encoding viral restriction proteins (ABOBEC3G, tetherin, TRIM5alpha, and SAMHD1) (Kaur & Mehra, 2009); and genes encoding human leukocyte antigen (HLA) class I molecules (Baker *et al.*, 2009). Variability in the latter seems to confer the most robust and most consistent effects on the outcome of the effort of the human immune system to contain the virus, and numerous reports describe the associations of HLA class I polymorphism with clinical, virologic, and immunologic endpoints in the course of HIV-1 infection.

HLA class I and HIV control

Canonically, CD8+ T lymphocytes recognize and destroy cells expressing non-self-peptides in HLA class I molecules on the surface of nucleated cells. The effectiveness of this response is linked to the individual's immunogenetic profile, including HLA class I expression. In HIV, the viral set-point (VSP) (steady stateviral replicationfollowing acute HIV-1 infection) reflects virus-host interplay in which the CTL response (driven by HLA class I interaction with viral antigen) plays a central role (Tang *et al.*, 2002). This is corroborated by the study showing that the first appearance of HIV-1-specific CTL in peripheral blood coincides with the initial decline of VSP and by the failure of the decline of viremia in SIV (simian immune deficiency virus)-infected macaquesthat have been depleted of CD8+ T cells (Altfeld*et al.*, 2006).

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At least three other lines of evidence support this pivotal role of CD8+ T cells in the control of HIV infection: (1) specific HLA class I alleles show a consistent association with either favorable or unfavorable HIV disease outcomes.*HLA- B*27 and -B*57* are the two most dominant alleles associated with favorable disease outcomes (Carrington *et al.*, 1999; Lunardi *et al.*, 2021) and are known to induce CTL responses against conserved HIV-1 epitopes (Tang *et al.*, 2002). These epitopes are derived from the capsid protein, which is essential in viral

assembly. Therefore, its preservation is crucial to the survival of the virus, andan immune response directed at it tends to correlate with a better prognosis (Rihn *et al*., 2013).

The -*B*57* allele, in particular, appears to be the most favourable,regardless ofthe human population or the clade of the virus (Tang *et al.,* 2002), and together with the -*B*27*, constitutes the major contributor to the HLA-mediated HIV-1 specific CTL response. (Table 1). On the other hand, –*B*35* and –*Cw*04* are linked with rapid progression to AIDS (Carrington *et al*., 1999).

HLA CLASS I ALLELE	**EPITPOPE (PEPTIDE IDENTIFICATION)**	**EPITOPE (PEPTIDE SEQUENCE)**
B*57	B57-TW10(**CA**)	TSTLQEQIGW
B*27	B27-KK10(**CA**)	KRWIIGLNK
B*51	B51-LI9(IN)	LPPVVAKEI
B*7	B7-KK10(GP41)	IPRRIRQGL
A*24	A24-RW8(NEF)	RYPLTFGW
CW*8	CW8-AL9(NEF)	AAVDLSHFL
B*35	B35-VY8(NEF)	VPLRPMTY

Table 1: Epitopes presented by various HLA class I alleles. Selected HLA class I alleles and the epitopes they present to CD8+ T cells, the viral origin of the epitopes (in brackets), and their amino acid sequence have been shown. The epitopes presented by the products of *B*27* and *B*57* alleles are derived from the viral C.A. (known to be from a conserved domain) (Altfield*et al.,* 2009)

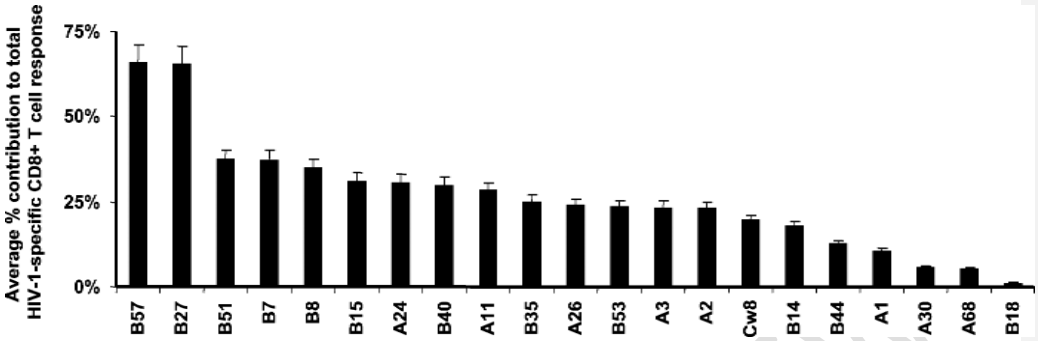

Figure 2: Percentage contribution of various HLA class I alleles to HIV-1-specific CD8+ T-cell(CTL) response- The various HLA class I alleles that present HIV-1 epitopes for CTL recognition. B57 and B27 contribute 66% and 64.5%, respectively to the total HIV-1-specific CTL response in individuals expressing them, making them the two most favourable alleles for the outcome of HIV-1 infection (Adapted from Altfeld*et al*., 2006)

Another piece of evidence comes from studies in non-human primates, where it was shown that the two rhesus macaque MHC class I alleles (Mamu-B*08 and Mamu-B*17) that are associated with control of SIV replication have peptide-binding motifs that resemble those of the two HLA molecules, HLA B*27 and HLA B*57(Gouder & Watkins, 2008; Loffredo *et al*., 2009).

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The HLA genes have extraordinary polymorphism, which allows them to present a vast repertoire of antigenic peptides, and because the alleles are co-dominant, heterozygotes for a given allele present twice as many antigens than homozygotes, the result being a more efficient immune response against pathogens (Carrington *et al*., 1999). There, maximum heterozygosity at HLA class I loci (*HLA-A, HLA-B,* and *HLA-C*) is strongly associated with delayed progression to AIDS. This selective advantage is believed to maintain the extensive allelic diversity in these genes (Carrington *et al*., 1999; Tang *et al*., 1999).

Finally, the appearance of viral mutants escaping CD8+ T-cell recognition is temporarily associated with losing immune control of the infection, suggesting that the main obstacle to viral replication is the HIV-specific CD8+ response (Garcia &Regoes, 2015).

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Virus and host genetic diversity in the context of vaccine development

The dynamic createdby the molecular interaction between HIV-1 genomic diversity and the inter-individual genetic variations of the determinants of host control, particularly HLA-1

genes,seems central to disease outcomes in HIV infection. This is brought to the fore in HIV-1 elite controllers (ECs), a small subset of individuals with a rare ability to spontaneously maintain an undetectable V.L. in the absence of therapeutic intervention. The E.C.s have been shown to have a prevalence of HLA-B alleles, such as *B*27*, *B*57*, and *B*14*, associated with enhanced virological control.The HLA-B alleles are more protective against HIV-1 infection than HLA-A due to their ability to resist Nef-mediated downregulation. This"viral epitope-HLA interaction" forms a reasonable immune correlate of the host's control of HIV-1 and could be fed into a conceptual framework for developing an HIV-1 vaccine.

Nevertheless, the two-prong molecular complexityof the virus and the host still leavesan enduring obstacle in designing a globally effective HIV vaccine capableof elicitingimmune responses againstthe diverse HIV-1subtypes. For one, it is known that certain HIV-1 subtypes are associated with specificdemographics,suggesting compartmentalized epidemicscharacterized by subtype/clade specificities (Abecasis*et al*., 2013), table 1. The implication is obvious: Fordesigning and developing an effective HIV-1 vaccine, one size will most likelynot fit all. Regions with subtype specificities and specific HLA profileswould needcustomized vaccine design and development.

Table 2: HIV-1 CRF02_AG (recombinant of subtype A and G) and HIV-1 G account for most (approx. 80%) HIV-1 infections in Nigeria (Ajoge*et al.*, 2011; Bhosa*et al.*, 2019); other strains predominate elsewhere)

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HIV-1 Subtypes	Percentage contribution to HIV-1 infections worldwide
CRF 01_AE	5.8
CRF02_AG	2.7
CRF07_BC	1.1
A	5.8
A6	1.0
B	**55.9**
C	**16.8**
D	3.2
F	0.9
G	01
Other	5.8
Total	100

HIV-1 Subtypes	Percentage contribution to HIV-1 infections in the African population
CRF02_AG	7.6
A	**19.2**
A1C	1.09
A1D	1.6
B	0.9
C	**49.2**
D	11.4
G	1.7
Total	100

HIV-1 Subtypes	Percentage contribution to HIV-1 infections in the Nigerian population
CRF 02_AG	**38.3**
CRF06_cpx	2.7
02G	6.0
A	1.6
A3	1.3
C	1.3
G	**39.4**
Other	8.6
Total	100

In considering the Nigerian situation, theHIV-1subtypes driving the Nigerian epidemic have been characterized. The next step is identifying immunogenicT-cell epitopes among these HIV-1 subtypescapable ofinducing long-lasting and potent CTL response against HIV-1. Relatedly, these epitopes must be recognized by the HLA class 1prevalent in the Nigerian population, to which the vaccine would be targeted (a promising HIV-1 vaccine design for a given populationmust consider the HLA class I alleles prevalent in that geographic region and the CTL epitopes restricted by the prevalent HLA class I alleles in that population).

Unfortunately, there is a paucity of data on HLA diversity, especially in the Nigerian population.Therefore, large-scale HLA-1 genotyping data from the diverse ethnic groups in Nigeria would be a necessary piece of the vaccine design puzzle. Epitope selection could be approached computationally, using computational vaccinology tools, which make epitope selection basedon the essential requirements for a suitable vaccine: immunogenicity, effective proteasomal cleavage, epitope processing, and epitope conservation.The derived epitopes can then be concatenated to produce the vaccines (Moise et al., 2015).

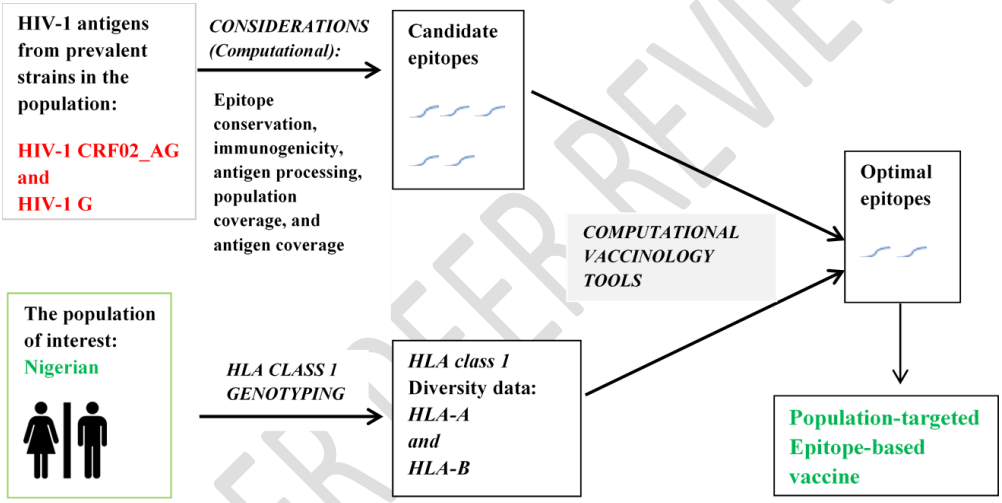

Figure 3: Conceptual framework: The target antigens derived from the most dominant HIV-1 strains in Nigeria are screened with respect to theconsiderations for a good vaccine. These antigens areparsed against the HLA-I data fromthe target population with the aid of computational vaccinology tools to determine an optimal set of immunogenic epitopes.The latter is then utilized in the designof a CTL epitope-based vaccine specific to the Nigerian population (Toussaint et al., 2008; Moise et al., 2015)

Conclusions

The slow progress and insignificant success in finding an effective HIV vaccine necessitate novel approaches to this quest. One such is trying to leverage the understanding of the regional diversity ofHIV-1 strains and HLA profiles- the two determinants of the effectiveness of a CTL vaccine- to customize such vaccines for different population groups. With improvements in computing power and the availability of more genotyping data for both virus and host, a lot promise exists in this area.

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