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HIV-1 Polymorphism: a Challenge for Vaccine Development - A Review

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The perspective for the development of anti-HIV/AIDS vaccines became a target sought by several research groups and pharmaceutical companies. However, the complex virus biology in addition to a striking genetic variability and the limited understanding of the immunological correlates of protection have made this an enormous scientific challenge not overcome so far. In this review we presented an updating of HIV-1 subtypes and recombinant viruses circulating in South American countries, focusing mainly on Brazil, as one of the challenges for HIV vaccine development. Moreover, we discussed the importance of stimulating developing countries to participate in the process of vaccine evaluation, not only testing vaccines according to already defined protocols, but also working together with them, in order to take into consideration their local information on virus diversity and host genetic background relevant for the vaccine development and testing, as well as including local virus based reagents to evaluate the immunogenicity of the candidate vaccines.





Key words: HIV-1 polymorphism - HIV/AIDS - vaccine

Since the identification of the viral etiology of the Acquired Immunodeficiency Syndrome (AIDS) in 1983, with the isolation of the Immunodeficiency Virus Type 1 (HIV-1) from patients with persistent lymphadenopathy (Barre-Sinoussi et al. 1983), the perspective for the development of anti-HIV/AIDS vaccines became a target sought by several research groups and pharmaceutical companies. However, the complex virus biology in addition to a striking genetic variability and the limited understanding of the immunological correlates of protection have made this an enormous scientific challenge not overcome so far.

Indeed, HIV is one of the most important emergent pathogens of this century. Based on phylogenetic analyses, it was estimated to have emerged in the human population around 1931 (Korber et al. 2000), becoming a worldwide public health challenge fifty years later.

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As recently reviewed by the Joint United Nations Programme on HIV/AIDS (UNAIDS 2001), since the description of the first cases at the early beginning of the eighties in USA and Europe, more than 21,8 million deaths have occurred due to HIV/AIDS. Forty million individuals, adults and children, were estimated to be living with HIV/AIDS worldwide by the end of 2001 and Latin America contributes with almost 4% of these cases. HIV/AIDS was the cause of 4.2% of deaths in the world in 1999 being the fifth cause of death worldwide. Only in 2001, 5.3 million adults and children were estimated to be infected worldwide, with 14,000 new infections a day. Around 95% of these new cases occurred in developing countries. Roughly 14% were in children under 15 and 86% in people aged 15-49, of whom almost 50% are women and about 50% are 15-24 years old.

Although the development of combined antiretroviral therapy has had a great impact on the reduction of AIDS morbidity and mortality, its application is quite limited worldwide due to the high cost and complex medical approach for the more deprived areas, complicated by the emergence of drug resistant strains. Thus, the development of HIV/AIDS vaccine is of high priority for public health.

There is a newly growing optimism in the international scientific community concerning the possibility of developing safe and effective preventive vaccines. However, despite the progress in recent years, one of the major points is still to be clarified concerning the importance of the intriguing virus diversity on vaccine efficacy. Indeed, viruses isolated from different patients, and especially from different geographic regions, show considerable genetic and thus antigenic variations, which may be a limiting factor to the development of a universal vaccine.

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MOLECULAR EPIDEMIOLOGY OF HIV

The molecular epidemiology of HIV and its potential implications for vaccine development and efficacy, as well as for other aspects of HIV infection such as local epidemiological features, virus transmission and pathogenesis, has been an issue of great concern (reviewed by Workshop Report 1997a,b). Indeed, HIV is a highly polymorphic virus and two types, HIV-1 and HIV-2, are participating in the AIDS epidemic. While HIV-2 has been basically restricted to the African continent, HIV-1 is associated with the worldwide pandemic. Phylogenetic analyses of virus samples from different geographic regions have revealed that HIV-1 can be divided into three groups: O (outlier), N (new, non-N, non-O) and the M group (Major). While samples from groups O and N are limited to the African continent, with some isolated cases of group O in Europe and in the United States, group M variants are responsible for the AIDS pandemic and were found to be subdivided into major genetic forms, including distinct subtypes and sub-subtypes (A1, A2, B, C, D, F1, F2, G, H, J and K). Moreover, 14 circulating recombinant forms (CRF 1 to 14), in addition to unique recombinant viruses and several other HIV-1 unclassified genomes have already been identified (reviewed in McCutchan 2000, CRF homepage <http://hiv-web.lanl.gov> for continuous updating), as well as mixed infections (Janini et al. 1996, 1998, Ramos et al. 1999, Flores et al. 1999). This striking worldwide genetic variability is depicted in the [Figure](#).

The HIV-1 subtypes are geographically distributed, with multiple subtypes and recombinant viruses co-circulating in many areas of the world. All of them are similarly transmitted through sexual contact, blood contamination, and vertically from mother to child.

Pairwise nucleotide distance among the HIV-1 subtypes showed 20% for the envelope, 16% for the gag and 13% for the polymerase genomic regions (Robertson et al. 1999). Such a high genetic variability has direct influence on the antigenic constitution of these viruses. Indeed, comparative analyses of relevant B

cell, CD4⁺ T helper cell and cytotoxic CD8⁺ T-lymphocytes (CTL) epitopes located in these regions have shown an important level of variability in a greater or lesser extent among the HIV-1 subtypes (HIV Molecular Immunology 2000).

It is therefore important to know whether the HIV-1 strains circulating in the countries where the vaccine will tentatively be used, are genetically and antigenically related to the vaccine prototype being tested. In order to obtain this type of information, it is necessary to keep HIV molecular surveillance programs, checking systematically the distribution of the HIV-1 subtypes, as well as the introduction of new variants.

With the perspective of HIV/AIDS preventive vaccine trials at the beginning of the nineties to be conducted in different world regions, in both developed and developing countries, the knowledge of the molecular epidemiology of HIV rapidly increased worldwide (Figure) and Brazil was one of the countries participating in this international effort.

Several studies on the molecular epidemiology of HIV-1 in Brazil showed the predominance of subtype B, with the presence of additional subtypes like F (Morgado et al. 1994, 1996, 1998a, Galvão-Castro et al. 1996, Tanuri et al. 1999, Brazilian Network for HIV Isolation and Characterization 2000), C (WHO 1994, Loureiro et al. 1998, Antunes 1998), D (Morgado et al. 1998a, Tanuri et al. 1999) and, more recently, the presence of subtype A (Caride et al. 2001, Guimarães et al. unpublished). Recombinant B/F and B/C viruses have also been described (Sabino et al. 1994, Gao et al. 1996, Cornelissen et al. 1996, Tanuri et al. 1999, Vicente et al. 2000, Guimarães et al. 2001), in addition to dual subtype infections (Janini et al. 1996, 1998, Ramos et al. 1999).

Due to the interest for vaccine purposes, the majority of these studies were originally performed based on the analysis of the envelope gene, and, more recently, the evaluation of gag and polymerase genomic polymorphisms have already been assessed. In addition to the inter-subtype diversity, genetic and antigenic differences have also been described among the subtype B viruses circulating in Brazil, with the identification of a subtype B variant called B", which differs from the classical subtype B viruses by the presence of the GWGR motif at the top of the envelope gp120 V3 loop, instead of the GPGR molecular signature (Potts et al. 1993, Morgado et al. 1994, Brazilian Network for HIV Isolation and Characterization 2000). These two subtype B variants can be distinguished by both genetic (Morgado et al. 1998b, Covas et al. 1998) and antigenic (Bongertz et al. 1994, Hendry et al. 1996, Casseb et al. 1998) approaches, although consistent cross-neutralization has been observed between them (Bongertz et al. 1997, 1998). Such observations are of important consideration for vaccine evaluation in our country as the B" subtype B variant was found to be highly prevalent in some Brazilian areas, corresponding to 57% of the subtype B samples detected in Ribeirão Preto, SP (Covas et al. 1998) and 37% of those from Rio de Janeiro, RJ (Morgado et al. 1998a,b).

Brazil is a huge country and differences in the pattern of subtype distribution have been identified among the geographic regions. Indeed, whereas similar proportions of B and F subtypes were observed in a study conducted in Manaus (northern Brazil) (Vicente et al. 2000), in the southeast, several studies have shown the predominance of subtype B (~85%) followed by subtype F (~10-15%) (Morgado et al. 1994, 1998a, Tanuri et al. 1999, Brazilian Network for HIV Isolation and Characterization 2000). Moreover, a clear predominance of subtype B (> 90%), with very limited cases of F and C subtypes were, respectively, observed in HIV-1 samples from the northeast (Couto-Fernandez et al. 1999) and central-west (Stefani et al. 2000). The presence of subtype C in south Brazil was firstly detected in 1 out of 5 HIV-1 samples collected in Porto Alegre, RS, in a setting of a WHO international study (WHO 1994). In a recent analysis conducted by our group (unpublished data), we observed in 34% of the HIV-1 subtype C infected samples collected in Porto Alegre, RS, in 1998, strongly contrasting with the pattern of HIV-1 subtype distribution already verified for other Brazilian geographic regions. This subtype seems to be spreading all over the world and is presently the most prevalent strain in the AIDS pandemic (Esparza & Bhamarapavati 2000).

Following the Brazilian pattern, studies on the molecular epidemiology of HIV-1 in other South American countries also showed the predominance of subtype B, with the presence of additional subtypes like F in Argentina, Bolivia, Uruguay, Peru, Paraguay, and Venezuela (Campodonico et al. 1996, Marquina et al. 1996, Velarde-Dunois et al. 2000, Masciotra et al. 2000, Russel et al. 2000, Castro et al. unpublished) and isolated cases of A in Chile (Desgranges et al. 1998), Peru (Russel et al. 2000) and French Guyana

(Kazanji et al. 2001) and E in Uruguay (Artenstein et al. 1995). Moreover, unique recombinant B/F viruses and/or CRF_012 B/F have also been described in Argentina (Marquina et al. 1996, Fernandez-Medina et al. 1999, Carr et al. 2001), Uruguay (Carr et al. 2001) and Venezuela (Delgado et al. 2001, Castro et al. 2001). Data from Colombia and Ecuador have only shown the presence of subtype B so far (Navas et al. 1999, Russel et al. 2000).

The general picture of the HIV-1 subtypes and recombinant viruses circulating in South American countries is summarized in the [Figure](#). In general, a quite similar virus pattern distribution is observed among the countries, which certainly will be reflected in similar HIV/AIDS vaccine strategies to be applied for South America.

■ [HIV VACCINE CANDIDATES](#)

The first generation of HIV vaccine concepts favored the protective humoral immune response, characterized by the presence of neutralizing antibodies mainly to the V3 loop of the viral envelope. The first Phase I protocol, corresponding to a gp160 recombinant protein started in 1987, less than 5 years after the virus identification (Dolin et al. 1991). Several other protocols were followed also focusing on the envelope gp120 or the full-length gp160, produced in different cell systems by distinct vaccine developer initiatives (reviewed by Bojak et al. 2002). Indeed, the first HIV-1 vaccine presently in a Phase III efficacy trial corresponds to this vaccine concept (Francis et al. 1998). This efficacy trial is now being conducted in the USA and Thailand using, respectively, bivalent HIV-1 gp120 B/B and B/E subtype vaccines (Berman et al. 1999), and the first results are expected soon. However, the difficulty to neutralize primary isolates of the virus was always a limiting factor of these vaccine concepts (Mascola et al. 1996), which are not able to induce antibodies against broadly conserved neutralizing epitopes (reviewed by Moore et al. 2001). Although there is no correlation between HIV-1 subtypes and virus neutralization, cross-clade neutralization has been described in a greater or lesser extent by several authors (reviewed by Moore et al. 2001), thus reflecting the presence of common antibody epitopes, at least among groupings of HIV-1 subtypes and variant viruses.

A highly relevant factor described in recent years by many research groups was the association between the presence of HIV-1 specific cytotoxic CD8⁺ T-lymphocytes (CTL) and the decrease of the plasma viral load in HIV infection (reviewed by Brander & Walker 1999). Indeed, a negative correlation was demonstrated between the frequency of CTL and progression to disease in HIV infected individuals (Klein et al. 1995, Musey et al. 1997). Also, dramatic increases in viral load were observed in chronically SIV-infected macaques after CD8⁺ T cell depletion treatment, which coincidentally decreases with the reappearance of CD8⁺ cells (Jin et al. 1999, Schmitz et al. 1999). These findings have certainly strengthened the importance of the cell-mediated immunity in the control of the HIV infection and disease progression, leading to a redirection of the HIV vaccine protocols for the utilization of live vectors able to induce MHC class I presentation of processed antigenic peptides to CD8⁺ T-lymphocytes. Furthermore, broad cross-reactive CTL recognition has been demonstrated in relation to the CTL epitopes located in very conserved regions of HIV-1 genome among the HIV-1 subtypes (Ferrari et al. 1997, Cao et al. 1997, McAdam et al. 1998), although reactions of higher magnitude have been described for subtype-specific CTL responses (Rowland-Jones et al. 1998, Cao et al. 2000, Novitsky et al. 2001).

In this sense, recombinant pox viruses and DNA vaccine concepts have already been tested in Phase I clinical trials. Promising alternative vector-based approaches, like recombinant poliovirus, Venezuelan equine encephalitis virus (VEE), BCG, salmonella and shigella, among others, are under development for HIV vaccines (reviewed by Bojak et al. 2002).

If on one hand, no specific CTL responses were observed in the volunteers vaccinated with HIV-1 recombinant protein vaccine protocols, on the other hand, limited neutralizing antibody production was also observed when recombinant pox virus vaccines were administered alone in human HIV-1 vaccine trials (Mulligan & Weber 1999). Considering the relevance of both humoral immune response and cell mediated immunity on HIV control, more promising vaccine approaches were further proposed, denominated as prime-boost regimen including vaccine concepts with complementary immunogenic profiles, associated with the inclusion of multi-antigen components consisting of selected HIV-1 structural as well as regulatory

antigen-encoding genes. Together, these types of vaccine approaches are attempting to amplify the immune response to the virus, inducing a multi-specific, long lasting humoral and cell mediated immunity able to prevent HIV infection and/or progression to disease.

Since the first human trial of HIV-vaccine at the end of the eighties, around 30 different HIV-1 candidate vaccines were tested in more than 60 phase I/II clinical trials (see Bojak et al. 2002 for updating). The majority of them have been conducted in USA and Europe, but a few others have already been conducted in developing countries like Thailand, China, Cuba, Uganda and Brazil (Esparza & Bhamarapravati 2000, WHO-UNAIDS Report 2001).

Indeed, the first Brazilian Phase I vaccine trial was conducted in Brazil in 1994, testing an HIV-1 subtype B gp120 V3 loop peptide-based vaccine concept (MN-V3) in 30 healthy volunteers from two cities (Rio de Janeiro and Belo Horizonte). As already described, this vaccine candidate was shown to be safe, but induced a limited antibody and T CD4+ lymphocyte proliferative response to the MN strain vaccine peptide (Sutmoller et al. 1998). More recently, a new HIV-1 subtype B multi-antigen recombinant canarypox/recombinant gp120 Phase I prime-boost protocol was implemented and is presently being conducted in our country.

■FINAL REMARKS

The results of the first Phase III human trial of HIV-1 vaccines will be available soon and other vaccine concepts like recombinant canarypox/recombinant gp120 prime-boost strategies are planned to enter in the Phase III efficacy evaluation in a near future (Cohen 2001) in some world regions, including South America (WHO-UNAIDS Report 2001).

Although the immunological relevance of the genetic subtypes for HIV-1 vaccine efficacy is still a matter of extensive investigation and discussion, some international efforts were made for the inclusion of local prevalent subtypes and variant viruses in the vaccine protocols to be tested. For instance, the recombinant gp 120 Phase III trial in USA includes two subtype B variants, MN and a subtype B primary isolate, whereas the protocol being conducted in Thailand employs subtype B MN and a region-specific gp120 subtype E primary isolate (Mulligan & Weber 1999). In the African continent, where several HIV-1 subtypes and recombinant viruses predominate, a subtype A based Phase I protocol is being tested in Kenya (Mwau et al. 2001). Considering the spreading of subtype C worldwide, comparative analysis of subtype C samples from different countries, including Brazil, are being performed with the objective of future vaccine development (Rodenburg et al. 2001).

In conclusion, the development of safe HIV-1 vaccines able to induce broad and long-lasting immune responses affording protection against the array of prevalent variants already described worldwide (including subtypes, recombinants and variant viruses) is presently a distant target to be achieved. However, even considering the difficult challenges to be overcome, urgent vaccine measures are of high priority for public health in order to control the devastating spread of HIV mainly in the poorer world areas. Based on the knowledge already accumulated over time, it is important to stimulate Phase I/II trials for the scientifically defined promising vaccine concepts as well as to move on to Phase III with the more immunogenic and feasible ones. It is of paramount importance to stimulate developing countries to participate in the process of vaccine evaluation, not only testing vaccines according to already defined protocols. But, working together with them, in order to take into consideration their local information on virus diversity and host genetic background relevant for the vaccine development and testing, as well as including local virus based reagents to evaluate the immunogenicity of the candidate vaccines is of utmost importance as well.

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[Figure](#)

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