Review

A review of vaccine research and development: The human immunodeficiency virus (HIV)∗

Marc P. Girard a,∗, Saladin K. Osmanov b,1, Marie Paule Kieny b,1

a University Paris 7, 39 rue Seignemartin, FR 69008 Lyon, France
b Initiative for Vaccine Research, World Health Organization, 20 Avenue Appia, CH-1211Geneva 27, Switzerland

Received 19 November 2005; received in revised form 10 February 2006; accepted 13 February 2006
Available online 28 February 2006

Abstract

Since the discovery of AIDS in 1981, the global spread of HIV has reached pandemic proportions, representing a global developmental and public health threat. The development of a safe, globally effective and affordable HIV vaccine offers the best hope for the future control of the pandemic. Significant progress has been made over the past years in the areas of basic virology, immunology, pathogenesis of HIV/AIDS and the development of antiretroviral drugs. However, the development of an HIV vaccine faces formidable scientific challenges related to the high genetic variability of the virus, the lack of immune correlates of protection, limitations with the existing animal models and logistical problems associated with the conduct of multiple clinical trials. More than 35 vaccine candidates have been tested in Phase III clinical trials, involving more than 10,000 volunteers, and two Phase III trials have been completed, themselves involving more than 7500 volunteers. Multiple vaccine concepts and vaccination strategies have been tested, including DNA vaccines, subunit vaccines, live vectored recombinant vaccines and various prime-boost vaccine combinations. This article reviews the state of the art in HIV vaccine development, summarizes the results obtained so far and discusses the challenges to be met in the development of the various vaccine candidates.

© 2006 World Health Organization. Published by Elsevier Ltd. All rights reserved.

Keywords: Acquired immunodeficiency syndrome; AIDS; Human immunodeficiency virus; HIV-1; Simian immunodeficiency virus; SIV; Vaccines

Contents

1. Introduction....................................................................................................... 4063
2. Disease burden.................................................................................................... 4063
3. Virology.......................................................................................................... 4064
4. HIV vaccine research: challenges and difficulties.................................................. 4065
4.1. Why is it so difficult to develop an HIV vaccine?............................................................... 4065
4.2. Animal models.............................................................................................. 4066
5. Vaccines.......................................................................................................... 4066
5.1. Live attenuated vaccines..................................................................................... 4066
5.2. Inactivated vaccines........................................................................................ 4066
5.3. Virus-like particles (VLP).................................................................................... 4067
5.4. Subunit vaccines............................................................................................ 4067

The authors alone are responsible for the views expressed in this publication, which does not necessarily reflect the views of the World Health Organization.

∗ Corresponding author. Tel.: +33 478 748 531.
E-mail addresses: marc.girard36@wanadoo.fr (M.P. Girard), osmanovs@who.int (S.K. Osmanov), kienym@who.int (M.P. Kieny).

0264-410X/$ – see front matter © 2006 World Health Organization. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2006.02.031
1. Introduction

The Acquired Immunodeficiency Syndrome (AIDS) emerged in the human population in the summer of 1981. There is now convincing evidence that AIDS is a zoonosis and that its etiological agent, the human immunodeficiency virus (HIV), crossed the simian-human species barrier before the middle of the 19th century [1,2] and probably arose and spread among humans as a result of increased unsafe injection and transfusion practices in postcolonial Africa, enabling the virus to adapt to humans through serial passages. Today, HIV/AIDS is the leading cause of death in sub-Saharan Africa and the fourth biggest killer in the world. An estimated 14,000 people/day (5 million persons/year, including 600,000 children less than 15 years of age) become infected with HIV, with more than 95% of them living in underdeveloped regions of the world [3]. The number of HIV infections is equally distributed between men and women, but infection rates in young women in today’s Africa are close to three times higher than those among young men, reflecting the degree to which gender inequities are driving the epidemic, as many women in developing countries lack socio-economic independence, education and access to health information and services, and have difficulty avoiding exposure to the virus.

The development of a safe, effective, easy to administer and affordable AIDS vaccine is urgently needed. The first Phase I trial of an HIV vaccine was conducted in the USA in 1987. Since then, more than 35 candidate vaccines have been tested in over 65 Phase III clinical trials, involving more than 10,000 healthy human volunteers in more than 10 countries [4,270]. Two Phase III trials have been carried to completion [5] and a third one is in progress [6]. However, we still are years away from an effective HIV vaccine, due to multiple hurdles and challenges. The development of a safe and effective vaccine is hampered by the high genetic variability of the virus [7,8], the lack of knowledge of immune correlates of protection [9,10], the difficulty of generating broadly neutralizing antibodies [11], the absence of relevant and predictive animal models and the complexities related to the preparation and conduct of multiple large-scale clinical trials, especially in developing countries [12,13].

This article reviews the current progress in HIV/AIDS vaccine research and development and the many challenges that still need to be met in the field [14-17].

2. Disease burden

At the end of 2005, the global number of adults and children living with HIV/AIDS was estimated by WHO/UNAIDS to have reached 40.3 million with an estimated 2.9–3.5 million HIV-infected persons dying every year from the disease [3].

Sub-Saharan Africa remains the hardest-hit region in the world, with at least 25 million infected people, accounting for 60% of the people living with HIV/AIDS and 77% of AIDS deaths in the world. The overwhelming majority of HIV infections in the region stems from heterosexual transmission [18]. In many African countries, the overall HIV prevalence in the adult population can be greater than 10%, with figures reaching up to 38.8% in some areas. Among the most severely hit countries are South Africa, with more than 5.6 million infected people and a prevalence of 30% among 21–29-year-old adults, especially women, together with Botswana, Mozambique, Zimbabwe, and Tanzania. The highest infection rates are found among commercial sex workers, truck drivers and seasonal migrant workers. Sub-Saharan Africa also is home to an estimated 500,000 HIV-infected infants who became infected prior to the introduction of mother-to-child prevention programmes based on the use of antiretroviral drugs. In addition, sub-Saharan Africa faces numerous wars and civil conflicts, producing large numbers of refugees who are at heightened risk of contracting HIV. Life expectancy in South African countries like Botswana, Swaziland or Zimbabwe, where a quarter to more than a third of the general population is infected with HIV, has decreased from 65 years in 1985–1990 to 37 years in 2000–2005.

A remarkable success story in the fight against AIDS was achieved in Uganda, which faced the onset of a severe HIV epidemic in the mid 1980s. Through voluntary HIV counselling, expanded treatment of STDs, awareness campaigns and community mobilization encouraging delayed initiation of sexual activity, monogamy and use of condoms, the level of infection has declined significantly since 1992—from nearly 30 to 11.2% in prenatal clinic settings in Kampala and from 13 to 5.9% in clinics outside major urban areas. Another important HIV prevention strategy could be adult male circumcision, which was found in a recent study in South Africa to offer close to 65% protection rate from HIV infection. Two other randomized controlled trials are underway in Kenya and
Uganda to obtain more data on the potential protective effect of circumcision on female-to-male transmission.

The estimated number of people living today with HIV in Asia and the Pacific region is more than 8.3 million, but the accuracy of the figure is questionable in view of the fast pace at which the epidemic is expanding. It has been projected that if current trends continue, the region will contribute 40% of all new infections worldwide by the end of the decade. Increasing sex trade, use of illicit drugs, and high rates of sexually transmitted infections contribute to an increased vulnerability of the populations in the region. Gender inequities also play an important role in the epidemic, as young girls are frequently steered towards commercial sex work by their families. Substantial transmission also occurs among men who have sex with men, with prevalence rates of 14–20% reported in certain male homosexual communities.

The estimated number of adults and children living with HIV in Latin America and the Caribbean at the end of 2005 was 2.1 million. While in some countries HIV infections remain concentrated mainly among men who have sex with men and injecting drug users, other countries are experiencing increasing rates of heterosexual transmission.

The Eastern European countries continue to experience one of the sharpest increases in the number of new HIV infections, most of which occur among injecting drug users. The number of people living with HIV/AIDS in the region is estimated to be above 1.6 million, 70–80% of whom are less than 30 years of age. More than 300,000 HIV seropositive persons are living in Russia alone, where commercial sex workers, injection drug users and prisoners are major victims of the tuberculosis and HIV epidemics [19].

The introduction of the Highly Active Antiretroviral Treatment (HAART) in industrialized countries has considerably reduced disease progression to AIDS and transformed HIV infection from a lethal disease to an effectively manageable chronic disease. AIDS among infants, which a decade ago took the lives of thousands of babies per year may be on the verge of elimination. Thus, in New York city, the number of babies born with HIV fell from 321 in 1990 to 5 in 2003, mostly due to education, counselling, testing and antiviral treatment of the mother during pregnancy. However, successes achieved in treatment and care are not being matched by progress in prevention, as some 75,000 individuals become infected with HIV every year in industrialized countries, where an estimated 1.6 million people are living with HIV/AIDS, and where a new wave of rising HIV infection rates is emerging, particularly in the young and marginalized communities.

3. Virology

The HIV, together with the simian, the feline, and the bovine immunodeficiency viruses (SIV, FIV, and BIV, respectively), the Visna virus of sheep, the caprine arthritis-encephalitis virus (CAEV) and the equine infectious anemia virus (EIAV), belongs to the genus Lentivirus in the family Retroviridae. These enveloped RNA viruses produce characteristically slow, progressive infections. Lentivirus replication depends on the presence of an active reverse transcriptase responsible for the transformation of the viral RNA genome into a proviral DNA copy that integrates into the host cell chromosome. The provirus is eventually transcribed into a set of mRNAs that encode the viral proteins and progeny genomic RNA.

Two types of HIV have been described: HIV-1 and HIV-2, the latter being less virulent, less transmissible and geographically limited to West Africa. HIV-1 is phylogenetically close to SIVcpz, a commensal virus in chimpanzees, and probably arose as the result of a single transmission event from chimpanzees to humans [1], whereas HIV-2 is closely related to SIVmac, the etiological agent of simian AIDS, and to SIVsm, a commensal virus in sooty mangabey monkeys. HIV-1 is further subdivided into three groups, M ("major"), O ("outlier"), and N ("non M/non O"). The vast majority of the HIV-1 strains responsible for the global AIDS pandemic belong to group M, which has evolved in humans to form at least 10 genetic subtypes, also known as clades, designated by letters from A to K [20]. HIV-1 subtype B predominates in industrialized countries as well as in Latin America and the Caribbean. Subtypes A and D are more common in Central Africa. Subtype C accounts for the majority of infections in southern Africa, eastern Africa and India. Genetic subtypes, in turn, have diversified further. Even within a given subtype, antibodies that are specific for isolates from one patient typically do not recognize isolates from other patients. Over the course of the epidemic and as a result of frequent dual and superinfections, HIV strains can recombine and form mosaic viruses, some of which, called "Circulating Recombinant Forms" (CRF), can gain epidemiological dominance [20–23]. The major CRFs are CRF02_AG, prevalent in western Africa, CRF01_AE, which predominates in south-eastern Asia, and CRF07_BC and CRF08_BC, which are prevalent in China. Amino acid sequence of the viral envelope glycoprotein shows up to 25–35% divergence between different subtypes and up to 20% divergence within any given subtype, which constitutes a formidable challenge to vaccine development.

The genome of HIV is a single-stranded positive sense RNA molecule, about 9.5 kb in length, which encodes the typical retrovirus proteins Gag, further cleaved into M (matrix), C (capsid) and N (nucleocapsid), Pol, cleaved into protease, reverse transcriptase and integrase, and Env, a 160 kD glycoprotein eventually cleaved into an external gp120 subunit and a transmembrane gp41 subunit that together form trimeric spikes on the surface of the virion. In addition, the HIV genome encodes a variety of non-structural proteins, such as the transactivator protein Tat [24], the splice regulator protein Rev [25] and accessory proteins such as Nef [26,27], Vif [28], Vpr [29] and Vpu.

The mature virion contains a cone-shaped protein capsid, which encapsidates two strands of the genomic RNA, the replication enzymes, tRNAs and cellular proteins such as...
between different SIV strains in SIV-infected monkeys [59].

Similarly, recombination was shown to readily occur between different SHIV variants and generating increased virus diversity in HIV-infected persons, leading to the emergence of recombinant virus and host cell membranes through its hydrophobic N-terminal fusion peptide and a fusion active hairpin structure involving two heptad repeats that can fold into a six-helix coiled-coil bundle [33–36]. Neutralizing human monoclonal antibodies, such as IgGb12 and 2G12, have allowed the identification of several neutralization epitopes on gp120 [37], some of which are carbohydrate-specific [38,39] and others overlap the CD4 receptor- or coreceptor-binding sites [40–43], but all of them appear to be little accessible to the cognate antibodies due to hindrance by the many glycosylation motifs on the molecule [44] as well as by the hypervariable loops [45,46], which act as antigenic decoys [47]. Two monoclonal fusion-blocking antibodies (2F5, 4E10) also have been described, with corresponding epitopes located at the base of the gp41 ectodomain [48–53]. A note of caution has been raised by the results of one study, which showed that these monoclonal antibodies were reactive with the human phospholipid cardiolipin [54,55].

Neutralizing antibodies induced in response to gp120 are primarily targeted to the hypervariable loops of the molecule and only rarely do they recognize the receptor binding sites, which makes it hard to generate broadly cross-reactive neutralizing antibodies against primary virus isolates from patients [11,15,72–75]. The complete lack of efficacy of antibody responses raised by monomeric gp120 vaccines in protection against HIV infection has been proven beyond any doubt in the world’s first two Phase III clinical trials of AIDS vaccines [5,76].

Although neutralizing antibodies administered passively to non-human primates can provide protection against experimental SHIV infection [77–85], proof of their protective role in infected humans remains circumstantial [86]. The best evidence for a role of HIV-1 neutralizing antibodies in vivo is the rapid and constant selection of neutralizing antibody-escape variants during the course of infection [69,70]. However, contrary to laboratory-adapted virus strains, which use CXCR-4 as a coreceptor (“X4” strains), and against which protection in chimpanzees could readily be achieved by inducing neutralizing antibodies targeted to the hypervariable V3 loop [87,88], primary virus isolates, which use CCR-5 as a coreceptor (“R5” strains), are difficult to neutralize, which casts doubt on the possibility for a vaccine to elicit protection against infection by the induction of neutralizing antibodies alone. In view of all these problems, recent vaccine approaches have focused on the induction of cellular immune responses [14,15,23,89–91]. Evidence for the role of CD8+ T cells in the control of virus replication includes temporal correlation between the appearance of HIV-specific CD8+ T cells and the decline of primary viremia [92,93], the fact that several HLA class I alleles (HLA-B57, HLA-B27, HLA-B63) are associated with slow disease progression [94], the early selection of CTL escape viral mutants during primary infection [95–98] and the rapid increase of viral loads in macaques infected with SIV after experimental depletion of CD8+ T cells [99–101]. The induction of a cellular immune response against HIV, especially a CD8+ CTL response, although not being able to provide sterilizing immunity and protection from infection, should hopefully enable vaccinees to control virus replication following infection, reduce their virus load, slow down...
their progression towards disease, and reduce the probability of secondary transmission of the virus.

4.2. Animal models

A significant obstacle in HIV vaccine research has been the difficulty in developing an appropriate animal model. The only animals susceptible to experimental infection with HIV are chimpanzees, Pan troglodytes, and pigtail macaques, Macaca nemestrina. Both maintain low levels of persistent virus load and do not develop clinical manifestations of AIDS. African monkeys are the natural hosts to a variety of SIVs (SIVagm, SIVsm, SIVsyk, SIVcol, etc.), but do not appear to develop a clinical disease following infection with these viruses [102]. In contrast, Asian monkeys, especially rhesus monkeys from India, are highly susceptible to SIVmac infection and progressively develop an immunodeficiency syndrome, which fully mimics human AIDS [103]. Plasma virus levels during primary and chronic SIVmac251 infection in macaques parallel those observed in humans, some animals containing viremia spontaneously and progressing to disease slowly, as in HIV-1 infected human long-term non-progressors, and others maintaining high virus loads and behaving as fast progressors [104,105]. As in HIV-1-infected humans, the cellular immune responses to SIVmac during primary and chronic infection differ significantly [106], and evidence of immune escape is readily documented [96–98]. Perhaps most importantly, the mucosal immune system, especially the memory CD4+CCR5+ T cells in the gut-associated lymphoid tissue (GALT), is the major site of viral replication and of CD4+ T cell depletion in SIV-infected macaques [107–110] as in HIV-1-infected individuals [111–114]. The SIV/macaque model is currently, therefore, considered to be the most appropriate animal model for studies on potential protective immune responses against HIV [115,116].

Another animal model used in HIV vaccine research is based on the use of SIV/HIV hybrid viruses (SHIVs) that were engineered to carry the env gene from an HIV-1 isolate in the context of an SIV genome. These viruses can replicate in thesus macaques, and after serial passages in the animal eventually lead to the emergence of highly pathogenic SHIV variants that are capable of wiping out the circulating CD4+ T-cell population of the animal within a few weeks after infection and generating a lethal immunodeficiency syndrome within a year. However, the relevance to HIV of these pathogenic SHIVs, such as SHIV 89.6P [117–119], is being questioned [120,121]. They exhibit a “X4” cellular tropism and, paradoxically, turn out to be much easier to contain by vaccine-induced immune responses than “R5” viruses such as SIV [122,123]. SHIV strains with an “R5” tropism [124,125] probably are a more appropriate and predictive model than “X4” SHIVs, but they have not yet been widely used in vaccine protection experiments.

The monkey models suffer from the fact that experiments to evaluate vaccine efficacy require challenging the vaccinated animals with a huge dose of virus, usually in the range of $10^5$–$10^7$ TCID50, equivalent to up to 5 x $10^5$ SIV RNA copies/ml. Such a high dose of virus is required to achieve 100% infection of control animals in the placebo group after a single exposure. However, high doses of virus do not correspond to the natural exposures to HIV in humans, where concentrations of less than $10^3$ to a few $10^5$ HIV RNA copies/ml of seminal plasma have been reported [126–128]. It has recently been observed that repeated low dose (10–30 TCID50) mucosal challenge of monkeys with SIV results in the same viral and immunological kinetics of infection as high dose challenges [129]. Such a new type of mucosal challenge might change the results of preclinical vaccine efficacy studies in the future (see as an example, [271]).

Of note is the fact that the challenge virus used in animal model experiments is nearly always homologous to the vaccine, giving good chances of success that are unrealistic in humans.

5. Vaccines

5.1. Live attenuated vaccines

The observation that nef-deleted mutants of SIV could confer protection against challenge with pathogenic SIV in thesus macaques [130–132] served as a model in favor of a live attenuated HIV vaccine approach. However, the SIV Δnef mutant establishes a life-long, persistent low-grade viral infection. It does not protect the vaccinated monkeys against superinfection with wild-type virus, although it does protect the animals from progression to AIDS. SIV Δnef still may cause AIDS, especially when administered orally to infant monkeys [133,134]. Additional deletions or mutations of the virus can result in further attenuation but at the expense of its protective efficacy [135–137]. In view of such safety concerns, this approach has not been actively pursued [138,139], but efforts are currently being made by IAVI, among others, to try and explore systematically the nature of the protective immune responses generated with live attenuated SIV vaccines in macaques [140].

5.2. Inactivated vaccines

The difficulty of inactivating HIV-1 with formalin without losing or destroying the antigenicity of the viral envelope has been a deterrent to the development of whole inactivated HIV vaccines. The obstacle was circumvented by using sublethal doses of formalin followed by heat inactivation at 62 °C [141]. The resulting killed virus preparation was shown to induce in mice and non-human primates modest but significant titers of antibodies capable of neutralizing heterologous primary isolates of HIV in a variety of infectivity assays [142]. Another way to inactivate HIV or SIV infectivity has been to target the two nucleocapsid protein zinc finger domains, which bind Zn ions in tetrahedral coordination com-
plexes that are essential for virus infectivity. These complexes can be disrupted by mild oxidation with 2,2'-dithiodipyridine (dithiothreitol-2) [143] or by alkylation with N-ethylmaleimine (NEM). Both compounds have been found to completely inactivate the infectivity of HIV-1 and SIV, while keeping the envelope glycoprotein spikes intact and functional [144]. The immunogenicity of the resulting preparations was studied in the SIV/macaque model [145]: vaccinated monkeys experienced decreased viremia after challenge and showed no significant depletion of CD4+ T cells.

5.3. Virus-like particles (VLP)

When coexpressed in cells, for example using either a baculovirus or a vaccinia virus expression system, the Gag and Env proteins of HIV or SIV spontaneously assemble to form pseudo-virions, i.e. virus-like particles (VLPs) that only contain envelope and core proteins. SIV VLPs have been tested as immunogens in non-human primate models after priming with vaccinia virus recombinants or by the nasal route using the cholera toxin B subunit (CTB) as an adjuvant [146,147]. The administration of VLP vaccines via mucosal surfaces might be a promising strategy to elicit mucosal and systemic anti-HIV immune responses [148]. Gag-Env pseudovirions were also tested in guinea pigs and found to elicit low but significant titers of neutralizing antibodies against both homologous and heterologous primary HIV-1 isolates when administered in the presence of a block copolymer adjuvant or of alum combined with Cpg oligonucleotides [149].

Heterologous VLPs have also been used as a delivery vehicle for HIV genes. Expression of the structural proteins of Kunjin virus, an arbovirus, was used to prepare VLPs that encapsidate recombinant Kunjin RNA replicons encoding the HIV-1 gag gene [150]. The resulting VLPs were found to elicit strong CTL and antibody responses to HIV Gag.

Still another approach has been to induce systemic and mucosal neutralizing antibodies against HIV by immunization with bovine papillomavirus (BPV)-HIV-1 gp41 chimeric VLPs. The ELDKWA amino acid sequence (2F5 epitope) from HIV-1 gp41 was fused to the N-terminus of the BPV L1 capsid protein and the resulting chimeric protein was used to generate chimeric VLPs. The latter elicited gp41-specific IgG after intra-muscular immunization and both systemic IgG and intestinal secretory IgA after oral immunization in mice. HIV neutralization activity could be detected in some of the mouse sera [151].

5.4. Subunit vaccines

5.4.1. Envelope-based subunit vaccines

Initial trials of HIV-1 Env-based subunit vaccines showed that soluble recombinant envelope glycoproteins gp120 or gp140 (gp120 prolonged with the ectodomain of gp41) were well-tolerated and elicited neutralizing antibodies to the homologous vaccine strain, but not to heterologous primary virus isolates [76,152,153]. These vaccines elicited sterilizing immunity against homologous virus challenge in animal models [87,88,154]. Two gp120 subunit vaccines were further evaluated in a Phase II trial in the USA [155], and one such vaccine, based on monomeric gp120 added with alum (VaxGen), was tested in a double-blind, placebo-controlled Phase III efficacy trials. The first trial involved 5000 volunteers at risk (mostly men who had sex with men) in the USA, Canada and the Netherlands, who were immunized every 6 months with a 300μg mixture of two subtype B gp120s. The second trial involved 2500 volunteers in Thailand (mostly injection drug users), who were immunized with a mixture of a subtype E (CRF_EAE) and a subtype B gp120s. None of these trials showed a statistically significant reduction of HIV infection in the vaccinees in spite of continuous booster immunizations during the 36 months of the study. A reduction of the number of HIV infections was observed in certain ethnic subgroups in the first trial, correlating with a higher level of anti-gp120 antibody, but the numbers were too small to provide statistical confidence [5].

The subtype E/B gp120 vaccine from the trial in Thailand is presently being used for booster immunizations in a prime-boost Phase III trial, which was launched in late 2003 in Thailand in collaboration between the Ministry of Health of Thailand, WRAIR, Sanofi-Pasteur and VaxGen. This community-based trial, which includes 16,000 volunteers and is meant to last for 4 years, involves a priming immunization with a recombinant canarypox virus (ALVAC) vaccine that expresses CRF_EAE gp120 and subtype B Gag, Pol and Nef antigens followed by boosts with the gp120 subunit vaccine [6].

Other envelope-based subunit vaccine approaches aimed at eliciting HIV neutralizing antibodies are at an early clinical stage of evaluation. These include trimeric gp140 molecules stabilized by the addition of heterologous trimerization domains at the C-terminus of the gp41 ectodomain [156,157], and similar trimers in which the gp120 moiety was deleted of the variable V2 loop, in order to expose neutralization epitopes overlapping the CD4-binding site. This approach is developed by Chiron [158–160]. Immunization of rabbits and macaques with a DNA vaccine encoding a V2-deleted gp140 from a South African subtype C HIV-1 strain, followed by boosting with oligomeric V2-deleted gp140 in MF59 adjuvant elicited high titers of Env-binding antibodies and low level heterologous neutralizing antibodies [161]. Gp140-GCN4 trimeric immunogens were found to induce a modest but significant heterologous neutralization activity, which could be efficiently increased by emulsification in adjuvants AS01B, AS02A or AS03 from GSK [162].

Gp140 trimers internally stabilized by an intermolecular disulfide bond between gp120 and gp41 (SOS gp140), have been developed with an expectation to induce both neutralizing and fusion-blocking antibodies [163,164]. Priming with DNA encoding a membrane-bound form of the SOS gp140 protein followed by repeated immunization with
the soluble trimers resulted in high titer antibodies active against neutralization-sensitive HIV-1 strains, but even the most potent of the sera had a quite limited ability to cross-neutralize primary heterologous HIV-1 strains [165].

Still another approach has been to covalently couple monomeric gp120 or oligomeric gp140 molecules to soluble CD4 or to synthetic mimetics of the CD4 receptor in order to induce the conformational changes that are supposed to take place in the glycoprotein spike at the time of virus entry, resulting in the exposure of neutralization epitopes that overlap the coreceptor-binding site. This approach has been developed by Merck and by the University of Maryland [166], which also engineered single chain derivatives of gp120 covalently linked to the first two domains of the human CD4 molecule [167]. The single chain fusion proteins were shown to elicit a broadly neutralizing antibody response in guinea pigs, but this property may have primarily been due to the induction of anti-CD4 antibodies. In contrast, covalently linked gp120 CD4 complexes were reported to elicit broadly neutralizing antibodies in macaques that were not related to anti-CD4 antibodies [168]. However, this observation was not confirmed in later experiments (Sadoff J, personal communication). Replacing the CD4 binding sequences by the appropriate synthetic mimic might be key to this problem but higher affinity mimetics need to be developed [64].

Engineering hyperglycosylated derivatives of gp120 was attempted in order to target the immune response to conserved epitopes on the molecule that form part of the CD4-binding site [169]. This approach is under development.

Induction of fusion-blocking antibodies by immunization with recombinant oligomeric gp41 molecules is another promising new approach that still is at an early preclinical stage of development [170,171].

Finally, IAVI's Neutralizing Antibody Consortium has undertaken to solve the crystal structure of the complexes formed between a broadly HIV-neutralizing human monoclonal antibody and the viral envelope glycoprotein spikes to try to unravel new clues for vaccine design [172]. It has been generally recognized that the extremely high level of genetic variability of HIV and its continuous evolution over time within one single individual, as well as between different population groups and diverse geographical regions represents a major challenge for the development of HIV vaccines capable of eliciting broadly reactive neutralizing antibodies against globally prevalent HIV strains [173]. To address this challenge, multiple parallel vaccine strategies are being explored, such as the use of cocktails of envelope immunogens derived from globally prevalent HIV strains [55,174], that of multivalent DNA or live vectored vaccines incorporating envelope genes from various clades, as well as combinations of live vectored vaccines as a prime followed by vaccination with a subunit recombinant vaccine as a boost [175,176]. In addition, novel avenues to circumvent the problem of the broad genetic diversity of HIV have been opened by the development of synthetic genes derived from theoretically defined consensus or ancestral HIV envelope sequences, which have been expressed in an immunogenic form [177–179]. The problem still, however, remains basically unsolved [11].

5.4.2. Non-structural protein subunit vaccines

The hope that immune responses directed against antigens that are expressed very early in the virus life cycle might lead to rapid elimination of infected cells has prompted the development of subunit vaccines based on the viral trans-activator Tat [180–182]. Immunization with Tat protected macaques against SHIV infection [183–185] or resulted in attenuated virus replication in the animals [186]. However, immunization with different forms of the Tat protein did not demonstrate any efficacy against virus challenge [187,188] and a recent study using an adenovirus type 5 (Ad5)-HIV Tat recombinant vaccine elicited no protection in rhesus monkeys against a SHIV challenge, in spite of demonstrable Tat-specific T cell and antibody responses [189]. A detoxified Tat subunit vaccine developed by Neovax in collaboration with SanoFil Pasteur, has been evaluated in a Phase I trial and should eventually enter Phase II trials. A Phase I clinical trial of another subunit Tat vaccine was carried out in Italy on HIV seropositive and HIV negative volunteers [190], showing that the vaccine was well-tolerated and immunogenic. Phase II trials are planned to begin presently in seronegative volunteers at risk as well as in HIV-infected volunteers. At the same time, the AIDS Vaccines Integrated Project (AVIP), a European consortium, is developing a novel vaccine approach based on the use of a trimeric gp140 molecule deleted of the V2 loop (gp140 ΔV2 SF162) that will be mixed with either Tat or Nef [191].

Different vaccine approaches using the Tat antigen have also been developed such as a Tat-adenyl cyclase fusion protein [192] or a Tat-Nef fusion protein that was combined together with a recombinant gp120 subunit vaccine in the AS02A adjuvant (an oil-in-water emulsion with monophosphoryl lipid A and the saponin derivative QS21). This combined vaccine was able to prevent the development of AIDS in rhesus monkeys following challenge with a pathogenic SHIV strain [193]. The Tat-Nef fusion protein, which is developed by GSK, was recently tested in human volunteers in a Phase I clinical trial using MVA as a vector and was found to induce strong CD4+ T cell and antibody responses.

5.5. Naked DNA and live recombinant vaccines

The majority of recent HIV vaccine studies have aimed to develop T-cell-stimulating vaccines that induce a HIV-specific CD8+ CTL response, whose role in control of virus load and evolution of disease has been well-documented in the monkey model (see above). Although vaccines that stimulate the cellular arm of the immune response are not expected to provide protection against infection, they should allow vaccines that get infected to control virus replication and reduce viral loads, thus resulting in lower probability of virus transmission to seronegative partners. A variety of vaccines were
Results from preclinical studies on plasmid DNA vaccines in non-human primates were rather disappointing, even though successful protection against a SHIV challenge could be observed in macaques [195,196] as well as in the HIV-1/chimpanzee model [197]. Immunogenicity of the vaccines could be improved by modification of the promoter, the use of synthetic genes with optimized codons, insertion of the transgene into an alphavirus replicon, or coexpression of cytokines IL-2 or GM-CSF [198,199]. Naked DNA vaccines expressing the HIV-1 gag gene and either IL-12 or IL-15, which were developed by Wyeth, are being tested in Phase I trials in the USA, Brazil and Thailand. DNA vaccines were actually found to be most useful as priming vaccines in prime-boost strategies, using live recombinant vaccines for booster immunization (see below). These strategies were tested in monkeys against pathogenic SHIV challenges and shown to result in reduction in virus load and protection against disease and death in the vaccinated animals [200]. The same approach was, however, definitely less successful in protection against SIV challenge [122,201,202].

The first live recombinant HIV vaccines that were developed were based on the use of vaccinia virus as a vector. Safety considerations prompted the search for other, non-replicative poxvirus vectors. A canarypox virus vector (ALVAC) was developed by Sanofi-Pasteur and intensively evaluated in multiple Phase III trials [203–205]. It is now being tested in a Phase III trial in Thailand (see above). The modified vaccinia virus Ankara (MVA) vector was tested in multiple prime-boost vaccine protection studies in macaques [206] and is currently still undergoing evaluation in Phase III trials in human volunteers in the USA and in several African countries, most often as a boost in combination with a DNA vaccine priming [23,207]. A subtype C multigenic env, gag, tat–rev, nef MVA recombinant developed by Therion in collaboration with IAVI is thus undergoing trial in India, whereas another MVA recombinant that expresses subtype A env, gag, pol and was developed by the NIH is in Phase I trial in the USA, soon to be followed by Thailand. The fowlpox virus (FPV) also has been used as a vector for HIV vaccines and has been tested in Phase I trials in Australia [208]. It is currently being evaluated in combination with MVA in the USA. These three vectors were used to express a variety of HIV antigens, such as Gag, Env, Pol and Nef. However, the immunogenicity of the poxvirus-based HIV vaccines in humans has usually been relatively modest, with less than 35% of the vaccinees scoring positive for T-cell responses at any point in time, as determined by IFN-γ ELISPOT assays. This was emphasized by the disappointing results of recent IAVI-sponsored DNA prime-MVA boost studies in the UK and Africa.

Replication-defective adenovirus type 5 (Ad5) represents one of the most promising live viral vectors for HIV vaccines [209–211]. It was first used by Merck for the development of a pilot, monovalent Ad5–gag recombinant that was shown to be highly immunogenic in mice, nonhuman primates and human volunteers. More than 50% of the volunteers showed significant, long-lasting HIV-1-specific CD8+ T-cell responses to HIV-1 peptides, including secretions of IFN-γ, IL-2, and TNF-α. A trivalent recombinant Ad5–gag/pol/nef vaccine has now been engineered and tested in human volunteers. A multi-center Phase II trial of this trivalent candidate vaccine has been launched, involving some 1200 men and 400 women at high-risk who will be followed for 3 years after three immunizations at 0, 4 and 26 weeks (NIAID and Merck). The trial is taking place in several centers in North America, Peru, Brazil, the Caribbean Islands and Australia, with final results expected in 2008.

Another non-replicative adenovirus vector is being developed by the NIH Vaccine Research Center (VRC), together with a DNA-based vaccine. These are multicomponent vaccines, which express the Env glycoprotein from clades A, B and C and the Gag, Pol and Nef proteins from clade B, and are designed for use in a prime-boost regimen strategy [175]. The DNA vaccine was initially tested in Phase I trials in the USA, where it showed good immunogenicity, and is undergoing a Phase I trial in Uganda in collaboration with the Makerere University and WRAIR. The VRC Ad5 recombinant candidate vaccine also has undergone testing in a Phase I study in the USA and is now being tested on those volunteers who were earlier primed with the DNA vaccine. A Phase I DNA-Ad5 prime-boost trial was recently started in the USA, Brazil and South Africa and will presently be extended to East Africa in collaboration with IAVI.

There is little doubt that the best results so far, in terms of percent human responders and levels and duration of T-cell responses to HIV-1 Gag in human volunteers, have been obtained with the Ad5 recombinant vaccines, either delivered alone or as boosting immunogens after plasmid DNA priming [212]. However, these vaccines are confronted with the problem of frequently pre-existing anti-vector immunity in the human population, especially in developing countries, which may dampen the immune response to the HIV transgenes [213]. This has prompted the development by Merck, Crucell and Transgene, in collaboration with IAVI, of candidate vaccines based on less prevalent human adenovirus serotypes (Ad6, Ad35, Ad11, or Ad24) to replace the Ad5 vector in future HIV vaccine trials [214,215]. Like Ad5, these vectors readily multiply to large yields in PRC-6 cells. Nonreplicative chimpanzee adenoviruses (AdC58, AdC6 and AdC7), against which humans do not have neutralizing antibodies, also are being explored as novel vectors by GSK and IAVI. In addition, as the major adenovirus neutralization epitopes are carried by the penton spike on the adenovirus virion, the development of adenovirus chimeras that escape anti-Ad5 pre-existing immunity has been achieved by replacing the fiber gene of an Ad5 vector by that from a rare Ad subtype. Adenovirus chimeras such as Ad5/Ad1 or Ad5/Ad35 have been constructed and successfully tested in animal models [216].
A different vaccine strategy using adenovirus vectors also has been developed by the NIH, employing replication-competent Ad recombinants in which the Ad vector is deleted only in the E3 region [217]. AdΔhr-, AdΔhr-, or AdΔhr-HIV recombinants together with boosts of subunit envelope glycoprotein were found to protect chimpanzees against challenge with a primary, heterologous HIV-1 isolate [218,219]. Protective efficacy was also demonstrated in the SIV/macaque model, using a replication-competent AdΔsr-SIV env/rev and AdΔsr-SIV gag priming followed by SIV gp120 boosting [220].

The list of other virus vectors used to construct live recombinant vector-based HIV vaccines is long [17,194]. Some of the most promising vector candidates include the measles virus (MV), vesicular stomatitis virus (VSV), Sendai virus (SeV) and Venezuelan equine encephalitis virus (VEEV). The Schwarz attenuated MV strain, which has a long standing safety and efficacy record as a live attenuated measles vaccine, was engineered to express the HIV gp160 molecule deleted of the V3 loop. The recombinant was found to elicit neutralizing antibodies with broad specificity after a single immunization in mice [221]. Recombinant MV-HIV vaccines were also successfully tested in a SHIV/macaque model. Their development is currently planned in collaboration between the Pasteur Institute and GSK.

AIDS vaccines based on an attenuated VSV vector expressing the Gag and Env proteins were found to provide complete protection against T-cell loss and disease progression in the SIV/macaque model, with all the vaccinated animals having low or undetectable virus load levels for up to 14 months after challenge [222]. VSV, which is developed as a vector for HIV by Wyeth, is a particularly attractive vector for the construction of live recombinant HIV vaccines, including, among others:

- Adenovirus-associated vector [229,230], which is developed by Targeted Genetics and IAVI and was tested in Phase I trials in Germany, Belgium and India. This candidate vaccine recently entered Phase II evaluation in South Africa, later to be followed by trials in Uganda and Zambia.
- BCG, which was developed as a vector for HIV by the Japanese NIH and already underwent a Phase I trial in Thailand with an early construct [231]. Recombinant BCG-based vectors may have potential as an HIV vaccine when administered in combination with a recombinant poxvirus vector-based vaccine in a “prime-boost” vaccine strategy [232].
- Attenuated Salmonella, that are used as vectors for the development of oral vaccines at the University of Maryland [233].

Poliovirus attenuated Sabin strains from the oral polio vaccine (OPV) also have been engineered to express short sequences of the HIV genome and could be used as an oral vaccine [234], but the future of this approach remains uncertain in view of the probable adverse impact of pre-existing immunity in the human population and the fact that OPV is progressively abandoned, whenever possible, in favor of the inactivated polio vaccine (IPV) in the poliomyelitis eradication program.

Another picornavirus, rhinovirus, recently has been engineered to express the 2F5 epitope on its surface. Intranasal administration of the recombinant virus induced broadly neutralizing antibodies, which, if confirmed, would represent a remarkable achievement [273].

It is too early at this time to evaluate the chances of success of these various vector-based vaccine approaches.
5.6. The development of prime-boost combinations

The experience gained so far with the first generation of candidate HIV vaccines has been that many were modestly immunogenic and only induced short-lived immune responses. One of the strategies experienced over the last decade to increase their immunogenicity was to combine these vaccines in “prime-boost” vaccination regimens, initially using DNA vaccine candidates for priming followed by live viral vectored vaccines for boosting [23,175,235]. Another type of prime-boost vaccine regimen was developed using two different live recombinant vectors expressing the same antigens, such as an Ad5 vector followed by a poxvirus vector [236], or two successive adenovirus vectors, such as Ad11 and Ad35, or two successive poxviruses, such as MVA and FPV, whose combination has been tested in Phase I trials in the USA and Brazil by Therion in collaboration with the NIAID. Heterologous adenovirus prime-boost vaccine regimens using successively Ad11 and Ad35 vectors, or the same vectors in reverse order, were found to elicit in mice higher frequency immune responses than homologous regimens [237], emphasizing the advantage of multiple vector-based vaccines. In these monkeys, responses arising from an Ad5-poxvirus (MVA or ALVAC) prime-boost regimen were significantly greater than those elicited by homologous regimens with the individual vectors. However, the increased immunogenicity observed in monkeys was not confirmed in human volunteers. Combinations of different vectors in heterologous prime-boost regimens are likely to be developed in the future to circumvent the problem of anti-vector immunity, which follows immunization with any live recombinant vaccine.

Another prime-boost strategy has been developed in the hope to strengthen both the humoral and cellular immune responses to vaccination, by combining a T-cell stimulating vaccine such as recombinant plasmid DNA or a live recombinant vectored vaccine with a subunit vaccine, especially one based on envelope proteins (gp120, gp140 or gp41) [203]. There is no strong evidence, however, that such a dual vaccination regimen resulted in a significantly higher antibody or T-cell response.

For any prime-boost strategy to be commercially feasible, the combined regimen needs to demonstrate significantly greater efficacy over single modality vaccines in order to balance the increased costs and complexities associated with developing two vaccines, including potential regulatory and licensing problems, as well as logistical hurdles with the delivery of the vaccines in the field.

5.7. Fusion proteins and peptides

Multiepitopic combinations of peptides, fusion proteins and long lipopeptides are at an early stage of clinical development, either alone or in prime-boost combinations with live vector-based recombinant vaccines. Vaccine constructs that express a series of minimal epitopes arranged in a string-like fashion have been explored as a potential strategy to generate diverse CTL responses and bypass the natural hierarchy of epitope bias [238,239]. Multi-epitope DNA immunogens can efficiently prime for broadly reactive CTL responses [89,240,241], but do not necessarily overcome hierarchies of epitope dominance [242]. A multiepitope DNA vaccine developed by Epimmune is currently undergoing a Phase I trial in the USA and will be followed by booster immunization with a multiepitope recombinant fusion protein.

Induction of persistent HIV Gag-specific CD8+ CTL responses was evaluated in a Phase I trial involving immunization with a fusion protein comprising the HIV p24Gag protein and detoxified Bacillus anthracis lethal factor to target antigen-presenting cells (Avant Therapeutics and WRAIR) [243].

Synthetic lipopeptides containing MHC class I-restricted T-cell epitopes were found to induce strong CD8+ T-cell responses against HIV in mice, non-human primates and humans without additional adjuvant [244–246]. Lipopeptides with sequences corresponding to that of CTL epitope-rich regions in the HIV-1 Gag and Nef proteins were tested in Phase I trials and were shown to induce strong, multiepitopic CD4+ and CD8+ T-cell responses. Parallel Phase II trials were started in 2004 in the USA and in France under the sponsorship of NIAID and ANRS, respectively, to study the efficiency of lipopeptides as priming or boosting immunogens, but the trials had to be interrupted due to the occurrence of a severe neurological side effect in one of the US volunteers. The trial now has resumed in France.

6. Concluding remarks

The history of the HIV pandemic is now well into its third decade. Tremendous progress has been made in our understanding of the complex interaction between HIV and the host immune system that has laid ground for the development of new, potent antiviral drugs able to control virus replication. However, many basic questions that bear on the feasibility of developing an HIV vaccine still remain unanswered, including identification of protective immune mechanisms, addressing the high variability of the virus and its ability to evade immune responses and eliciting potent virus-neutralizing antibodies with broad specificity against primary HIV-1 isolates [10,11,14–16,247,248].

The experience with current viral vaccines suggests that an effective vaccine or vaccine regimen against HIV infection might need to induce both neutralizing antibodies and cell-mediated immunity. One of the challenges for HIV vaccine research, therefore, is to understand how to design envelope immunogens to effectively elicit long-lasting high titer broadly neutralizing antibodies. Meanwhile, the attention of the AIDs vaccine field has focused on the induction of HIV-specific cellular immune responses, including CTL, based on the hypothesis that such a response would
progressors. The CD4+ T-cell response also was implicated as a correlate of protection in long-term protection provided by the live attenuated SIV. Another player in the immune response, which probably has not received enough attention so far, is the CD4+ cell-stimulating vaccines, but not in the SIV/macaque model, where virus control following vaccination and challenge often appears to be short-lived, ultimately leading to vaccine failure [122,123,201,202,274].

These vaccines, therefore, urgently need to be tested in clinical trials on human volunteers at risk [15]. Of note is the technical challenge associated with the design and follow-up of large Phase III trials of vaccines that are not meant to induce protection against infection. Indeed, although the correlation between viral loads at early times after infection and speed of evolution of the disease are well documented in natural infection, the translation of this correlation in vaccinated populations remains to be established. In addition, a number of questions remains unanswered at present, like the nature of the best end points for long-term follow-up of volunteers in Phase III clinical trials, and the time during which volunteers will need to be monitored. These technical questions are accompanied by a number of ethical questions relating to standard of care and duration of the sponsors’ responsibility for providing this care. The population-wide effects of vaccines that do not prevent infection but only reduce viral load levels in vaccinees are largely unknown, but a few studies based upon mathematical models suggest that even a 1-log reduction in viral load, which is a reasonable expectation with the currently available candidate vaccines, would have a major impact and significantly reduce HIV mortality within 20 years after introduction of the vaccine [249].

Among the great many hurdles that are met in the development of HIV vaccines is the lack of scientific knowledge on the nature and level of the immune responses that would be required to achieve protection against HIV infection and/or development of the disease. The most sensible strategies adopted to date in HIV vaccine research therefore aim at eliciting an effective response of both arms of the immune system. One way to increase the immune response to HIV would be to help target the antigens to the antigen-presenting cells, especially dendritic cells (DC). Coupling the antigen of choice to a DC-specific monoclonal antibody [250] has yielded impressive results in mice, enhancing the efficiency and kinetics of the immune response as compared with soluble antigen. This approach is now being studied in nonhuman primates.

Another player in the immune response, which probably has not received enough attention so far, is the CD4+ effector T cells, which seem to play a critical role in the protection provided by the live attenuated SIV/AvNef vaccine in macaques (Johnson P, personal communication), and which also were implicated as a correlate of protection in long-term non-progressors [251–254]. The CD4+ T-cell response to vaccines might be an important correlate of potential vaccine efficacy that should more systematically be taken into account.

Last, but not least, it would seem of paramount importance to develop HIV vaccines that stimulate the mucosal immune system so as to block the major virus transmission route. The first cellular targets of HIV-1 are CD4+ CCR-5+ memory T cells that are mostly found in the mucosal lymphoid tissue of the GALT, where the early phase of HIV infection and replication take place [110–114,255]. The need, therefore, is to develop vaccines capable of raising an immune barrier at the site of the genital, rectal and intestinal mucosa to efficiently prevent HIV-1 infection. At this time, however, the design of such vaccines remains a major challenge [256]. HIV-1-specific mucosal CTLs could be efficiently induced in the intestinal mucosa by intrarectal administration of a synthetic peptide vaccine incorporating a detoxified E. coli heat-labile toxin, LT(R192G). Following a SHIV challenge, the mucosally immunized monkeys readily cleared the virus to undetectable levels both in blood and in the intestine and did not experience any CD4+ T-cell depletion [257]. Mucosal HIV vaccine delivery should be considered among the most effective immunization strategies for the induction of mucosal CTL that could provide early protection against HIV replication and amplification [258,259]. Mucosal HIV vaccine delivery should be considered among the most effective immunization strategies for the induction of mucosal CTL that could provide early protection against HIV replication and amplification [258,259]. Immuno- nization of macaque monkeys by the nasal or oral route using a VSV vectored vaccine expressing SIV antigens Gag and Env resulted in significant protection against SIV infection [222]. Alphavirus replicons represent another type of recombinant vaccine that can be administered efficiently by the mucosal route [260]. The administration of a HIV-1 VLP vaccine by the mucosal route is under study [147,148]. The tonsillar route of immunization has been explored in macaque monkeys using tonsillar sprays of SIV vaccines [261,262]. Generally speaking, the nasal route of immunization is probably the most advantageous one, both from the point of view of public acceptance and for its potential efficacy, but the use of nonliving vaccines by the nasal route still raises a few problems including that of adjuvants [263–265]. The production of mucosally targeted immunogens also has been attempted in plants. A chimeric protein was expressed in plants that combi- nes the GM1 ganglioside-binding B subunit of the cholera toxin (CTB) fused to a peptide (P1) which spans the 2F5 and 4E10 epitopes and the galactosylceramide-binding site at the C-terminus of the ectodomain of HIV-1 gp41 [266]. The CTB-P1 fusion protein was found to induce vaginal and intestinal antibody responses after intranasal immunization in mice. This might pave the way to a new generation of mucosally administered HIV vaccines.

Most of the efforts to develop and evaluate HIV vaccines are borne by the National Institute of Allergy and Infectious Diseases (NIAID), the Centers for Disease Control (CDC) and the Walter Reed Army Institute for Research (WRAIR) in the USA, as well as by the French National AIDS Research Agency (ANRS) in France, the International AIDS Vaccine Initiative (IAVI) in New York, the European Union, initia- tives at WHO and UNAIDS, and the African AIDS Vaccine Programme (AAVP). The recent commitment of the Bill and Melinda Gates Foundation has resulted in the foundation of
a Global HIV Vaccine Enterprise [267]. The creation of a Center for HIV/AIDS Immunology (CHAVI), which could receive more than US$300 million from the NIAID over the next 6 years, is part of this novel effort to speed up and coordinate the search for an HIV vaccine [268]. The HIV Vaccine Trial Network (HVTN) established by NIAID in 2000, with 25 clinical sites on four continents, represents a major resource for clinical HIV vaccine research. The European Union has similarly created the European and Developing Countries Clinical Trials Partnership (EDCTP) with the aim to help developing countries strengthen their capacity in testing the efficacy of new drugs, microbicides, and vaccines. Strong efforts are indeed needed to harmonize vaccine trials and help to define the next clinical step in a ‘learning by doing’ procedure based on continuous vaccine improvement in iterative clinical trial processes [269]. Developing countries are essential partners in this international effort.

There is little doubt that the development of a safe, effective, and affordable HIV vaccine constitutes a top priority for HIV/AIDS research and a formidable scientific and public health challenge at the dawn of this century.

Acknowledgement

The efficient editorial help of Olga Assossou is gratefully acknowledged.

References


Hel Z, Vennin D, Pouilly M, Tsai WP, Giuliani L, Woodward R, et al. Vienna control following antiretroviral treatment and thera-

[105] Parker RA, Rogan MM, Reimann KA. Variability of viral load in plasma of rhesus monkeys inoculated with simian immunodefi-


[119] Wharton AM, Cook N, Hall GA, Sharp S, Bud EW, Crane MP. Repair and evolution of nef in vitro modulates simian immunodefi-


[121] Babu TW, Mao Z, Trynierzewska E, Tsai WP, Parks RW, Montefiori DC, et al. Containment of simian immunodeficiency virus infection in vaccinated macaques: correlation with the magnitude of virus-


[125] Johnson RP, Lifson JD, Czajak SC, Cole KS, Manson KH, Glick-Goldstein AM, et al. Highly attenuated vaccine strains of simian immunodefi-
cency virus protect against vaginal challenge: inverse rela-

[126] Bahn TW, Liska V, Khimani AH, Ray NB, Dailley PJ, Prinimick D, et al. Live attenuated, multiply deleted simian immunodefi-


[128] Vlahos R, L. Klimis AI, Ray NB, Dailley PJ, Prinimick D, et al. Live attenuated, multiply deleted simian immunodefi-


References


