Vaccines & Vaccine Technologies

Chapter: HIV Vaccine Development: Tools and Knowledge
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Introduction

One of the greatest ideal aspirations of science and health organizations is the discovery of a safe and effective HIV vaccine. The hope in developing HIV vaccines increased after results obtained with the RV144 human clinical trial using recombinant poxvirus vector carrying HIV-1 envelope (env), gag and protease genes as priming and recombinant Env proteins as booster [1]. The RV144 trial provided modest but unprecedented 31% of protection against HIV acquisition in a low-risk population and the immune correlates hypothesized that binding antibodies against the variable (V1 and V2) region of HIV-1 envelope contributed to protection against HIV-1 infection [1,2]. Binding antibodies were associated with antibody dependent cellular cytotoxicity mechanism and brought to light immune functions not previously associated with HIV prevention. Since the identification of HIV as the pathogenic agent of acquired immunodeficiency syndrome (AIDS) for more than 30 years, no vaccine achieved licensure. The epidemic caused by HIV/AIDS is far from being controlled and is continuously growing in some parts of the world. The infection that was initially associated with homosexual male behavior in 1980’s today affects both genders equally, with children being 1% of total infected people. An effective preventive vaccine should induce protective immune response in the mucosa and in other organs to prevent sexual, horizontal as well as vertical transmission however the achievement of these objectives face several challenges related to the still unknown ideal immune response that would confer protection and/or disease control. Despite the success of antiretroviral therapy (highly active antiretroviral therapy – HAART) in improving the quality of life of HIV infected individuals, its toxicity committed the adherence of patient to treatment and there is clear evidence of the necessity for an HIV vaccine. The scientific findings in HIV vaccine studies and HIV vaccine trials have so far improved the knowledge in biology and immunopathology of HIV-1. The studies are now focused on the better understanding of the initial steps of transmission and to gather discoveries in human anti-HIV innate and adaptive immune response, prerequisites for the correct design and development of an immunogen able to reach the ideal protection against HIV acquisition and control towards
AIDS’s progression. Animal models such as mice and non-human primates are generally used to test the immunogenicity of vaccine candidates; however they do not represent the real context of HIV infection in humans. Human clinical trials are under evaluation using different immunogens and immunization schemes. Neutralizing and binding antibodies induction is currently the most pursued outcome [3] on HIV vaccine field considering the rapid spread of HIV and reservoirs establishment after immune cells infection. Helper and cytotoxic CD4+ T lymphocytes became as relevant as cytotoxic CD8+ T lymphocyte (CTL) in the interruption towards AIDS progression and CD4+ T cell has their defined role in antibody maturation and affinity [4]. Historically successful vaccines achieved disease eradication using whole inactivated or attenuated microorganisms and induced neutralizing antibodies. For HIV several types of immunogens were tested from inactivated HIV to viral vectors expressing HIV genes as well as proteins and subunits. HIV vaccine concept is moving between preventive and/or therapeutic immunogens aiming to induce neutralizing and binding antibodies together with CD4 and CD8 T cell immune responses. Even with the discoveries about structure of broadly neutralizing antibodies and strategies to circumvent the diversity of HIV, questions are still rising concerning how to effectively elicit these antibodies and answers are far away from being completely elucidated. The last completed efficacy trials are targeting new fields of investigation aiming to improve the design of new HIV immunogens. The development of a unique vaccine that induces neutralizing, binding antibodies and also T cell responses able to avoid the infection and to block virus replication remains elusive and perhaps new vaccine approaches should combine two different immunogens to reach the two arms of immune response. The pieces of the HIV vaccine intricate puzzle still have to be placed in order even though scientists have been working hard on diverse fields of HIV science to stop its transmission.

Why Knowledge about Epidemiology is Important to the Development of an HIV Vaccine?

Since its discovery, HIV affected more than 70 million people, killed almost 36 million and nowadays more than 35 million are infected and living with HIV/AIDS. The infection has a worldwide distribution and the most affected region is the Sub-Saharan Africa, with more than 69% of world’s infected people [5]. Although current overall trend reflects stabilization or even decrease in HIV incidence, in nine countries from Eastern Europe and Central Asia, there were an increase of more than 25% between 2001 and 2009 [6]. HIV infection remains the fourth largest cause of death in the world.

Among the total HIV infected individuals, three million are children (under 15 years old) and more than a half are women. It is estimated that over 7,000 new HIV infections occur daily, 97% of them taking place in low- and middle-income countries. Among these new daily infections, approximately 900 affects children and nearly 6,000 affects adults (of each 47% are women and 39% young men). In 2013 UNAIDS reported that 10% of total HIV infected people are reaching 50 years old [7].

Considering the broad range of age groups burdened with HIV infection and the different profile of maturity and quality of immune cells, the design of HIV immunogens construction should be taken into account these features. The new generation immunogens should generate effective responses in immature, mature and senescent immune systems. It is also important to know and understand the geographical distribution of HIV circulating forms, its epidemiology and the predominant mode of transmission in the location where clinical trials
will be performed. The pre-existing immunity against the vector in case it makes part of the vaccine formulation should also be considered.

**Understanding the HIV Structure and HIV Components that should be Considered as Part of a Vaccine**

The HIV as a retrovirus has two single-stranded ribonucleic acids (ssRNA). Once released into infected cell’s cytoplasm, ssRNAs are transcribed by the viral reverse transcriptase (RT) into a double-stranded deoxyribonucleic acid (dsDNA), which is integrated into host genome by the viral integrase activity [8]. HIV viral capsid is composed of proteins derived from post-translational processing of the Gag protein, including matrix protein (p17), capsid protein (p24) and nucleocapsid protein (p7). The capsid stabilizes viral genome and contains essential viral enzymes, such as protease (which cleavage and helps in maturation of viral peptides sequences), reverse transcriptase (Reverse transcription into cDNA) and integrase (insertion of viral DNA into the host genome). Surrounding the capsid there is a lipid bilayer derived from the host cell membrane where are anchored trimers of glycoproteins (gp120) and transmembrane proteins (gp41). The gp120 and gp41 are responsible for viral entry into host cell. Viral particles also comprise the regulatory proteins Nef (negative factor, which enhances viral replication and down modulates CD4 and MHC class I molecules expression), Vif (viral infectivity factor) and Vpr (targets the integration complex to the nucleus). Concerning the non-structural proteins there are Rev (cytoplasm transportation of RNA from the nucleus), Tat (inhibits premature termination of gene expression) and Vpu (CD4 degradation and new viral particles liberation) are non-structural proteins. So the genome of HIV is encoded by two RNA strands with nine genes, three of them encode structural proteins: gag (comprises capside and nucleocapsid proteins), pol (comprises enzymes) and env (comprises envelope glycoproteins). The other six genes encode regulatory and non-structural proteins: nef, vif, vpr, rev, tat and vpu.

Preventive vaccines usually induce neutralizing antibodies and in the case of HIV must be made up of inactivated virus or subunits containing envelope glycoproteins. On the other hand, therapeutic vaccines are effector T cell inducers and must contain internal proteins such as Gag, generally being made up of live attenuated virus or vectored formulations [9]. So far HIV immunogens used in phase II or III clinical trials aimed the induction of protective immunity against the envelope glycoproteins and T effector cells to the inner structural proteins (Gag, Pol) or non-structural (Nef). The choice of a promising protective HIV immunogen must consider the viral variability. The selected immunogen must protect against existing virus variants and the immune response generated is able to fight with a pathogen with an extraordinarily high mutation rate, which drive to an unbroken increasing of its antigenic diversity. Several different circulating subtypes and recombinant strains of HIV are causing the epidemic around the world, and then knowledge of molecular HIV epidemiology of each region is an important factor for vaccines design. Since the ideal scenario is to obtain a single and universal vaccine against all strains of HIV, there is a trend to construct immunogens containing conserved HIV regions [10]. This approach hypothesizes that the immune response against highly conserved regions of HIV proteins, common to major variants and escape mutants, could circumvent the immune scape of immunodominant variable HIV epitopes by targeting the response to the conserved epitopes between subtypes and recombinant forms of the virus.
What about the Correlation between HIV Life Cycle, Treatment and Vaccine Development?

Once in the body, HIV envelope (gp 120) engages the CD4 protein and subsequently the chemokine receptors (CCR5 and/or CXCR4) in host expressing cells to promote viral entry [11]. This binding triggers structural modification of the envelope glyprotein and the insertion of gp41 into the host cell membrane [12] resulting in fusion of the viral envelope and cell membrane. Fusion inhibitors and chemokine receptor antagonists prevent the HIV attachment to CD4+ molecules or the CCR5 co-receptor interaction [13], blocking the entrance of viruses into target cells. Accordingly, therapeutic vaccination with CD4+ T cell with disrupted CCR5 expression leads to sustained and significant increase in CD4 T cell counts [14]. Also, antibodies against certain gp120 regions are able to block HIV attachment and infection, however none of the vaccines used so far was able to induce broad and sustained neutralizing antibodies covering all the circulating forms of HIV. The transference of anti-HIV highly broadly neutralizing antibodies is being tested to prevent mother-to-child-transmission [15]. The impairment of HIV binding to host cells has been considered as a curative therapy strategy. The functional cure of HIV achieved by transplantation of homozygous Δ32 deletion of CCR5 gene, with no expression of CCR5 on cell surface, in an HIV infected patient with acute myeloid leukemia opened opportunities to new approaches for treatment and vaccine development [16]. The lack of CCR5 expression in the cells of the transplanted patient interrupted disease progression since the viral entry was impaired. Unfortunately the stem cell transplantation in HIV patients as a curative strategy is hampered by the high mortality rate after allogeneic transplantation, the difficulties in HLA matching and the low frequency of Δ32 homozygous individuals in the population [17].

After the step of fusion to the cell membrane, HIV capsid is disassembled in the cytoplasm releasing viral RNA to be reverse transcribed into a cDNA copy by the reverse transcriptase enzyme. In this process, the viral RNA is used as a template for DNA synthesis and this RNA is degraded by the reverse transcriptase afterwards. Regarding the antiretroviral therapy, nucleoside reverse transcriptase inhibitors (NRTIs) are applied to interrupt the HIV replication via competitive inhibition of HIV reverse transcriptase and the termination of viral DNA chain [18]. Next step on the viral life cycle is transportation of the duplicated cDNA to the nucleus and its insertion into the host cell chromosome by the viral integrase. The integrated viral DNA is then referred to as proviral DNA. During integration, host DNA repair enzymes circularize a small proportion of the HIV cDNA into episomes with one or two copies of the viral long terminal repeats (LTR). The integrase inhibitors block the integration enabling the formation of a greater proportion of LTRs episomes.

The proviral DNA may remain inactive or, with cell activation, transcribed into messenger RNA (mRNA) and genome RNA. The HIV mRNA may be translated to produce viral enzymes and structural proteins. The production of some functional proteins depends on the cleavage of long polyproteins by the viral protease. This enzyme is also responsible for the maturation of virus particles in late viral life cycle. Currently there are several drugs able to inhibit protease activity, preventing the cleavage of HIV polyproteins [18].

Generally, patients under HAART receive a combination of drugs acting at different steps of the HIV life cycle, however a great number of patients do not succeed to keep undetectable plasma viral load after prolonged periods of treatment due to poor adherence or to the development of drug resistance. The combination of HAART and therapeutic vaccines must be
considered, since the vaccine would stimulate the immune response to contain infection and thus prevent the failure in case of development of drug resistance. Most recent therapeutic vaccine strategies suggest the use of drugs to activate viral replication in latent cells from viral reservoirs to purge the latent virus followed by their elimination using antiretroviral combination that will act in association with the effective and broad HIV immune response elicited by a HIV vaccine [19].

How does HIV Infection Establish and Progress in Humans and How We can Apply this Knowledge to Vaccine Design?

After exposure to HIV, the transmission occurs through the mucosal barrier by cell–free virions, infected cells or virions are attached to dendritic cells [20]. Anal sex is the major cause of HIV transmission between men having sex with men and on the other hand, among heterosexual couples, vaginal intercourse also strongly contributes to infection. The dynamics of HIV spreading in gut and vaginal mucosa differs due to the initial number of cell layers that the virus must surpass to reach the target cells. Recent data showed the rapid spread (less than four hours) of Simian Immunodeficiency Virus (SIV) in the rectum of macaques after high dose of SIV’s challenge [21]. Potent and numerous specific CD8+ T cells elicited by experimental immunization did not protect non-human primates from SIV intrarectal challenge [22]. This recent finding suggests that broad CD4+ and CD8+ T cell response generated by conventional vaccine vectors may not be sufficient to contain HIV replication soon after infection.

The initial phase of infection consists of HIV propagation in partially activated CD4+ T cells followed by greater propagation in activated CD4+CCR5+ T cells in the gut associated lymphoid tissue (GALT) [23]. The clinical acute phase is characterized by an intense depletion of CD4+ T cells in peripheral blood associated with a depletion of CD4+CCR5+ T cells in GALT. A robust HIV replication occurs during two to four weeks after infection, reaching typically 10 million copies per milliliter [24]. Propagation and dissemination of the virus to other lymphoid tissues occurs and the viral reservoir consisting of the resting CD4+ T lymphocytes or latently infected cells is established contributing to the failure of viral eradication, even with effective ART [20]. Another major obstacle to HIV vaccine development is the inability of current HIV vaccine candidates to hamper the establishment of HIV infection as well as the viral reservoirs, which occurs soon after infection.

The immune response takes place during the acute phase of infection and can be evaluated by the presence of HIV specific binding antibodies and specific T cells two to four weeks after infection [20]. The adaptative cellular and humoral anti-HIV immune responses are detected in the majority of infected individuals and the infection can be partially controlled until the viral escape. As the viral evolution occurs the selection of immune escape variants takes place and this happens during all stages of infection contributing to viral persistence [25]. Some researchers suggest that a vaccine candidate containing conserved HIV epitopes shared by several variants represent the best way to subvert the escape mutants [10].

The acute infection without antiretroviral therapy (ART) is characterized by the high viral load and low CD4 T cell counts [20]. While the chronic infection is characterized by viral replication in lymphoid tissues resulting in generalized immune activation, prolonged production of viral particles, increased cell turnover and finally, without treatment or failure to respond to ART, destruction of the CD4+ T cell compartment and rapid disease progression. The median-time between the HIV seroconversion is six months and AIDS symptoms used to
be 8-10 years before the ART introduction, infected individuals clinically progress towards AIDS at different rates [26,27]. Usually, without treatment, when CD4 T cell counts drop under 350-200 cells/mL opportunistic infections appear.

Defined cohorts of HIV infected patients based on their clinical status are the best approach to find ideal immune responses to guide the prophylactic or therapeutic HIV vaccine development [28]. While some HIV long-term non-progressors (LTNP) or slow progressors keep stable CD4 counts and low viremia for several years, the elite controllers even better handle the infection maintaining high counts of CD4+ T cells and viremia under 75 copies/ml. Defining immune responses and host’s mechanisms in LTNP and elite controller would provide clues to vaccine development as these patients naturally have mechanisms to control HIV-1 replication. Also, HIV exposed-uninfected individuals might provide information about natural resistance to infection that can be incorporated in the immune responses to be achieved by a HIV-1 vaccine. LTNP and elite controllers manifest higher frequency of heterozygote to CCR5-∆32 gene deletion and certain class I HLA alleles (HLA-B57, HLA-B27, HLA-B14, BLA-B51) associated to protection against disease progression [29]. As we cannot control or change the immunogenetics of HIV vaccine recipients it is better to try to mimic the generation of similar phenotypically CD8+ T-cell immune response as are reported in LTNP and elite controllers [30,31].

Recently it has been proposed the use of antiretroviral treatment as a strategy to keep viremia below detection levels (functional cure) or to eliminate virus from the host (sterilizing cure). The first evidence of functional cure was the treatment of an early infected subject, known as Berlin Patient I, (Berlin Patient II was the receiver of the CCR5-∆32 transplant) who spontaneously controlled the HIV replication during acute infection after treatment interruption. Also, Boston patients were considered functional cured from HIV after transplantation of bone marrow stem cells for cancer treatment. These two patients underwent intermittent HAART and were considered HIV free during 2.6 and 4.3 years, respectively. Unfortunately, their viral load rebounded after experimental therapy interruption [32]. The exploration of their immune responses can clarify how to control disease progression. Another case of functional cure associated to antiretroviral treatment was the Mississipi child an HIV-positive newborn who was started on ART within 30 hours of age and have no signs of HIV after discontinuation of treatment at 18 months, until 41 months old the child still functionally cured after being off treatment [33].

The association of immunological factors that lead to functional cure associated with the knowledge of immune response that hamper the AIDS development in LTNP and elite controllers may help to define mechanisms for viral replication control and maintenance of immune equilibrium as goals to be reached in an ideal vaccine [29].

**Why has it been so Hard to Find a Vaccine for HIV Prevention and/or Contention?**

Several obstacles challenge the HIV vaccine development. First, the extreme viral diversity impairs the design of a global vaccine covering the nine subtypes and more than 35 circulating recombinant forms [34]. The immune pressure and the error-prone replication by HIV reverse transcriptase gives rise to a huge number of mutant genomes derived from a single founder HIV, these diverse virions are highly susceptible to immune escape 31. Second, it is still necessary to define the immune correlates of protection in HIV infection and disease
progression, even though it is already clear the need for both neutralizing/binding antibodies and cellular immune response stimulation to properly fight against the virus [35]. The third challenge is the inexistence of an ideal validated animal model. Rhesus macaques have widely been used, however there still are certain obstacles as the follows: simian immunodeficiency virus is not HIV, simian’s immunogenetics differs totally from human’s, there is not enough number of SIV variants to mimic HIV variability [32]. Another important issue is that vaccine development cannot mimic HIV infection. Since natural immunity against HIV does not prevent infection, and neither control disease progression, nor prevents subsequent infection by different HIV isolates [31,32]. Also, the ability to rapidly integrate into the host genome makes the HIV faster than immune response development [31,32]. Lastly, HIV sanctuaries as central nervous system, retina, testes and blood-tissue barriers limit penetration of ART agents and contribute to hidden viral reservoirs to the immune host [36].

Considering the HIV vaccine development there are many obstacles to surpass, however the new findings related to protection generated in RV144 trial, the identification of the correlate of protection in the RV144 trial and new knowledge about neutralizing antibodies encourage scientific community to search for a preventive and therapeutic vaccine. Of course there is a hard way to reach this goal nevertheless new technologies are raising and guiding the construction of new immunogens, which will try to circumvent all the complicated issues imposed, firstly by HIV characteristics and secondly by the complexity of human immune responses that can or not block HIV infection.

Which type of Immunogens has already been and should be Used as HIV Immunogens?

As definition, immunogen is any substance capable of eliciting humoral and cellular immune responses without stimulating immunological tolerance. In respect to HIV, several potential immunogens were already described considering structural and non-structural proteins. Despite the wealth of knowledge about peptides/epitopes able to generate different patterns of immune responses against the virus, the difficulty in developing an efficient antiviral vaccine lies on the tremendous sequence diversity due to HIV-1 high mutational rates. A recent large-scale analysis concerning the diversity of approximately 58,000 HIV-1 clade B sequences reported over 26 years demonstrated that only 9% of the viral proteome presents highly conserved positions relevant for cellular immune response, mostly in pol, gag and env genes. Only two Pol positions have shown complete conservation. The plasticity of the virus was highlighted by the high diversity of Rev, Vpu, Env and Nef proteins, which presented 80% or more of variance among analyzed sequences [37]. In addition to this remarkable variability, the immunogenic conserved domains are in their majority hidden by tertiary or quaternary protein structure, becoming inaccessible to immune system during viral life cycle.

The currently knowledge in HIV immune response guides the vaccine development towards two types of approaches: (i) induction of antibody response and/or (ii) induction of potent and effective T cell effector immune response. In an attempt to achieve such immune responses, different types of immunogens have been developed or used in human HIV vaccine trials: proteins or peptides, DNA plasmids and recombinant viral vectors.

Whole inactivated virus

The first vaccine strategies tried to emulate other successful antiviral vaccines using whole inactivated or mutant attenuated forms of SIV as immunogens [38-40], but safety concerns
based on incomplete inactivation and reversion of attenuation impaired their development to be used in humans.

**Proteins and peptides**

First human clinical trials, that used as immunogens a combination of proteins derived from different HIV-1 strains comprised of entire envelope glycoprotein subunits (AIDSVAX and VaxGen), resulted in absence of protection and without effect on disease progression in infected individuals [41-43]. Recently, phase I trials with a vaccine composed only by Tat recombinant protein, which is well conserved among HIV clades, was highly immunogenic, inducing functional antibodies and cellular immune responses in HIV infected and non-infected individuals [44-46]. The Tat early regulatory protein plays a major role in HIV replication and AIDS pathogenesis. Phase II study is ongoing to evaluate the capacity of Tat protein to induce protection against infection.

Recombinant proteins have also been frequently used as immunogens. For instance, the oligomeric gp160 from ANRS VAC14 trial consists of a hybrid soluble gp160 molecule in which the fused gp120 and gp41 portions derived from different HIV-1 isolates. This strategy did not induce anti-gp160 specific antibodies in HIV- negative volunteers [47]. In contrast, anti-HIV-1 neutralizing antibodies were elicited in healthy individuals immunized with the trimeric gp140 immunogen, which comprises the gp120 subunit fused only with the external domain of gp41 [48,49].

Protein-based vaccines are generally limited in their ability to induce strong Th1 immunity. This can be greatly improved by enhancing protein uptake by dendritic cells, the professional antigen-presenting cell. One approach already successfully tested in mice [50] and non-human primates [51] is to introduce the protein Gag p24 into the monoclonal antibody that efficiently target the DEC205 receptor on dendritic cells. Another strategy relies on lipopeptides-based vaccines [52,53], such as HIV-LIPO-5 vaccine, which apply a mix of synthetic HIV-1 peptides derived from Gag, Pol and Nef proteins, each coupled to a palmytoil tail in an attempt to facilitate peptide’s entry into dendritic cells [54]. Vaccinated healthy individuals tolerated the vaccine and developed HIV-specific sustained CD8 and CD4 T cell responses [49]. No trial was performed so far to analyze its prophylactic or therapeutic capacity. The peptides may even be lapidated and anchored on the immunopotentiating reconstituted influenza virosome (IRIV), which serves as a lipid carrier for antigen delivery, mimicking the native viral membrane, and as a safe human adjuvant [55]. A peptide of the HIV-1 gp41 anchored to the virosome (MYM-V101 vaccine) was able to elicit systemic and mucosal antibodies in a Phase I trial [56].

**DNA plasmids**

After the first failure of the human trial that tried to elicit protective immune response using envelope glycoproteins, vaccines efforts were then redirected towards naked DNA and recombinant viral vectors carrying HIV protein gene sequences [57]. As a unique feature, DNA plasmid vaccines do not contain antigens themselves, since protein antigen is codified by a gene cloned into a bacterial plasmid under the control of a eukaryotic promoter. DNA vaccines are expression vectors using transcription proteins and organelles from transfected eukaryotic cells to express the antigen in situ [58].

The plasmid structure contains adjuvant elements not yet well established which stimulates innate immune response directing the adaptive immune response. Naked DNA vaccine is a quite versatile strategy able to elicit cytotoxic T lymphocyte response (CTL), T-helper and humoral
responses in experimental models [59,60], but human trials with only DNAs codifying different HIV genes have evaluated this strategy with no high protection rates nor long-lasting response in humans [61-63]. Electroporation of DNA vaccines has improved its immunogenicity and is under evaluation to several infectious and chronic diseases (HIV, HCV, Malaria and cancers) [64]. HIV DNA vaccine administered by electroporation is safe and increase immune response [65,66]. Also, current strategies use DNA vaccines as “prime” with heterologous boost to achieve a stronger and broader anti-HIV immune response [67-69].

In order to improve DNA priming activity, different approaches have been used to induce stronger T cell response and overcome the large antigenic diversity. This way, the selection of conserved viral regions, which contain the lowest variability between viruses at each amino acid position, points to the use of integrase, core-capsid, reverse transcriptase and protease sequences as preferential targets to stimulate a cross-clade CTL response [70,71]. Other strategies aiming to cope with viral sequence diversity are the use of antigens classified as subtype-matched, consensus, single sequences or multiple antigens, used alone or in combination [72,73].

In parallel to proper antigen choice, a well-designed DNA vaccine must also provide high levels of antigen expression by transfected cells, since protein yield is a limiting factor for immune response development [74]. Viral and human genomes differ greatly regarding to codon usage, GC content, mRNA secondary structures and many other features, impairing efficient pathogen gene expression in human cells [75,76]. To overcome this issue, sequence optimization through genetic algorithms seeks to change such parameters in transgenic sequence to better meet human cells requirements without changing the resulting amino acid sequence. This way, codon usage optimization of HIV-1 gp120 genes has already proved to increase antigen expression in human cell lines transfected with the DNA vaccine, resulting in consistently higher anti-Env antibody responses in immunized Balb/C mice over their counterpart using wild type env insert [77]. Codon usage-optimized of other HIV-1 genes such as gag, pol, env and nef had also their expression increased in a range from 20- to 250-fold in transfected human cells, when compared to non-optimized constructs. Mice immunized with DNA optimized gag gene developed stronger antibody and cellular immune responses than its non-optimized counterpart [78]. Alterations in antigen sequence are also necessary to eliminate identified viral RNA inhibitory/instability sequences [79-81].

In some cases in order to produce specific immune responses it may be necessary to target DNA codified proteins to specific tissues, cell types or even subcellular compartments [82]. DNA vaccines targeting HIV antigens to the Major Histocompatibility Complex Type II (MHC II) processing compartment (MIIC) through its fusion with the Lysosome-Associated Membrane Protein 1 (LAMP-1) or dendritic-cell-LAMP are targeting approaches that have already proven to induce stronger systemic and mucosal cellular and humoral immune responses in diverse experimental models (non-human primates, adult and neonate mice), with more efficient stimulation of T helper cells and prolonged immunological memory to HIV antigens [83-85]. Targeting DNA vaccines to specific cells with broader antigen-presenting capacity may also be achieved through fusing antigens to cell-specific soluble ligands. One example of this strategy is the antigen targeted to dendritic cells through fusion to the programmed death-1 protein (PD-1). Mice immunized with DNA vaccines codifying HIV-1 Gag p24 fused to PD1 elicited robust CD8 T cell response as well as high titers of anti-Gag antibodies [86]. Other improvements in DNA vaccines focus on delivery [87-91], plasmid design [92-94], adjuvants [90,95,96], and dosage [78], all of which have been extensively tested.
It is well known the property of immune system to respond robustly and vigorously after boosting the system with the same antigen. Prime-boost immunizations are frequently used to strengthen and broaden HIV-specific immune responses [97,98]. Heterologous prime-boost is even better to elicit a strong immune response [99]. The human trial DP6-001 used a heterologous prime-boost regimen combining a polyvalent DNA prime vaccine with a protein subunit boost. Although immunizations resulted in high titer serum antibody against diverse HIV-1 viruses and cross-subtype HIV-specific T cell responses [100], the high frequency of severe side effects [101] interrupted trial progression. The HVTN-049 phase I human trial, in contrast, was safe and well tolerated by healthy individuals immunized with a prime of DNA plasmid encoding the codon-optimized HIV-1 Gag and gp140 env genes and a boost with the recombinant trimeric Env protein, but the trial resulted in a weak neutralizing antibodies induction [102]. A safe, well-tolerated and more immunogenic heterologous prime-boost strategy was the HVTN-064 trial. It applied a reverse immunization scheme, priming with a recombinant protein consisting in a string of 18 HIV-1 epitopes, and boosting with a plasmid vaccine encoding 21 conserved epitopes of HIV-1 [103].

Other promising prime-boost strategies combine DNA prime vaccine with recombinant viral vectors boost, which have been massively used in recent human clinical trials.

**Viral vectors**

Recombinant viral vector vaccines consist of live attenuated viruses that are genetically engineered to carry DNA encoding protein antigens from the pathogen and represent the best manner to present the antigens to the immune system as they simulate natural infection. Viral vectors carry DNA effectively into a host cell and also acts as adjuvant due to its capacity to replicate in transduced cells and activate innate immune response through the recognition of viral structures by the pattern recognition receptors and activation of intracellular signals leading the inflammatory genes transcription and initiating the inflammatory response [104-110], the activation of innate immune system by the vector itself may also interfere with the efficient HIV antigen expression, since transduced cells produces IFN that can block viral replication and release several factors that attract other immune cells to kill the infected cell. As an alternative, genetically modified vectors that lack specific transcriptionally active regions to decrease immune activation have been used in vaccine formulations [111-113]. Strategies used in DNA vaccines such as conserved antigens, targeting and sequence optimization are also suitable for improving viral vectors immunogenicity. These vectors are generally used as boosts in immunization protocols due to the development of immunity against the vector itself that can lead to a reduction in vaccine efficiency [114]. Adenovirus and poxvirus-based vectors are the most commonly used in prime-boost regimens against HIV [115].

Human replication-defective adenovirus Ad5 vector have shown the ability to induce potent CTL responses in macaques [116-119] and humans [120], but its pre-existing immunity in human population raises concerns about the reduction of magnitude and quality of immune responses [121], [122]. Despite that, when used as DNA prime Ad5 boost strategy, the pre-existing immunity against Ad5 did not impair the generation of high IFN-γ ELISPOT responses against HIV-1 antigens [117]. Therefore, DNA and adenovirus combinations have been tested in phase I and phase II clinical trials, generally with both DNA and Ad5 codifying the same combination of different genes (gag, pol, nef) derived from different HIV-1 clades [123-125].

The poxvirus vectors such as modified vaccinia virus Ankara (MVA) and canarypox virus (CPV), on the other hand, present less pre-existing immunity prevalence in human population.
[96], as well as a large capacity for heterologous DNA insertion into vector backbone and cytoplasmic gene expression [31]. Both viruses do not replicate in human cells what make them safer and attractive for vaccine studies. DNA-MVA prime boost immunizations are also constant in phase I/II human clinical trials [126-128], using combinations of several plasmids and vectors codifying conserved HIV-1 proteins or CTL-epitopes strings from different viral strains. These regimens were able to induce both cellular and humoral immune responses against HIV-1[129], and most importantly, polyfunctional T helper responses towards Env proteins [31].

DNA-CPV prime boost regimens have been tested in clinical trials [130,31], but virus vector prime with protein subunits boost is the most common immunization regimen utilizing CPV vectors [31], including the phase III RV144 study, the most successful immunization protocol against HIV-1 tested so far [1]. RV144 was a phase III clinical trial performed in healthy heterosexual adult individuals who were immunized with ALVAC vCP1521 prime AIDSVAX B/E recombinant gp120 boost strategy. ALVAC vCP1521 is a CPV-based vector that expresses four genes products from HIV-1: Gag p55, p15, a portion of protease gene, a portion of gp120 and the anchoring transmembrane region of gp41.

Other viral vectors have also been used in human clinical trials, but in a lesser extent. The alpha virus Venezuelan Equine Encephalitis virus (VEE) have been used as virus-like replicon particles (VRP), which encode the viral replicases and the HIV-1 antigen, but have their structural genes deleted [131]. VRPs are morphologically identical to live alpha virus particles and retain similar cell tropisms but are safe, single-cycle, propagation-defective vectors restricted to a single round of infection and expression [132]. These vectors are especially attractive as vaccine vectors because they express antigens to high levels, target expression to dendritic cells, and are capable of both humoral and cellular immune responses to the vectored gene products [133,134].

The Adeno-Associated Viruses (AAV) are dependoviruses that commonly infect humans but is not associated with any disease. Even though 80% of humans are seropositive for AAV [135], there is a very low incidence of neutralizing antibodies against it in human populations, allowing its use as vaccine vector in clinical trials [136-138]. Immunizations only with the AAV vector was found to be weakly immunogenic. Recombinant Tiantan vaccinia virus (rTV) is considered as one of the most promising viral vectors because of its capacity to induce long-lasting responses against foreign antigen [139]. In China, TV had most widely been utilized to eradicate smallpox and showed good safety records [140]. Its safety and immunogenicity as anti-HIV vaccine has been evaluated in an ongoing phase II clinical trial, using a DNA plasmid as prime and rTV as boost.

**Next generation immunogens**

Beyond being the first trial vaccine against HIV-1 to show any degree of protection, the RV144 trial also unearthed valuable correlates of protection in humans, revealing promising branches to be pursued by vaccine researches. One of the most highlighted branches is the antibody-based HIV vaccine, development of which was invigorated by the clear correlation between plasma concentration of immunoglobulins and protection from infection demonstrated by the trial–[1]. In combination with the discovery of naturally occurring potent broadly neutralizing antibodies (bnAbs) [141,142], the trial data boosted the search for immunogens capable of stimulating bnAbs through immunization. Broadly neutralizing antibodies are a special group of antibodies characterized by the ability to neutralize a broad range of HIV,
prevent [131] and suppress [132] infection, but are found only in serum from 5% [143,144]
or more [145,146] of HIV infected individuals, several years after infection. They were shown
to interact with highly conserved regions from Env protein [147-154] in virus escape variants
through conformational contortions in antibody structure generated by a complex maturation
process that undergoes unusual high levels of mutations [155-157]. Due to this intriguing and
not completely understood maturation process, there is no heterologous Env protein capable
of eliciting this kind of immune response so far, but a lot of discoveries have been done in this
field [158-161].

Based on advances in the understanding of the bnAbs epitopes structure, two major
approaches have been used for the development of immunogens. One of them is the
reverse vaccinology, which grafts one epitope to a selected protein scaffold that allows the
stabilization and maintenance of its structure, optimizing immune presentation [162-164].
The other approach is an attempt to mimic the native Env spike, creating a soluble gp140
protein containing all bnAb epitopes in the right quaternary context [165,166]. Despite these
advanced technologies, until the moment there is no immunogen demonstration of anti-HIV
bnAbs generation.

The struggle to achieve bnAbs stimulation pointed to the necessity of understanding the
antibody maturation as well as the cells that drive this process. More recent researches are
focusing on follicular helper cells, CD4+ T cells allocated in germinal centers (sites of B cell
selection and maturation) [167] which stimulate proliferation and differentiation of B cells
[166,168,169] The hope is that the proper activation of these cells, together with specific
B cells, would allow appropriate stimulus for bnAbs production. On the other hand, it was
demonstrated that the induction of those bnAbs might be easier than currently thought by the
discovery of a bnAb (CH103) that undergoes less somatic mutations and neutralizes 55% of
HIV-1 isolates. The lower amount of mutations may represent an easier pathway to induce the
production of bnAbs by immunogens.

Overall, the wealth of data provided by the remarkable progress in HIV vaccine research in
the last 3 years raised optimism in an effective vaccine development. The expectation is that
the RV144 and the next post-RV144 trials results analysis, combined with new technologies for
BnAbs production and strategies to overcome HIV diversity will shed lights on the development
of new immunogens capable of eliciting a protective immune response against HIV-1.

**HIV Vaccine Human Trials**

From the HIV identification more than 218 clinical trials has been performed with several
HIV immunogens since 1987, 76 of which with different products in prime-boost regimens,
from those only six moved to phase II/III clinical trials [170,171]. The majority of vaccines
candidates used in human trials were proteins or peptides, poxvirus vectors, DNA plasmids,
adenvirus vectors [170]. As already been highlighted, the development of HIV vaccines was
target first to induction of neutralizing antibodies, after to induction of CTL response and now
to immunogens combinations to induce of effective neutralizing and binding antibodies and
effector cellular immune responses [170].

The two firsts HIV vaccine human trials tested the envelope proteins (VAX 003 and VAX 004).
The first one was the VAX004 that used recombinant gp120 HIV-1 envelope proteins from two
subtype B strains; it was a double blind, placebo-controlled trial given seven injection of the
vaccine or placebo, to 5403 volunteers, with the primary endpoint the HIV-1 seroconversion
in 36 months [37]. The volunteers were men who have sex with men (MSM) or women at high risk for heterosexual transmission of HIV-1. The estimated vaccine efficacy was 6% without prevention of HIV-1 infection in this trial, moreover no significant differences in viral loads of vaccinated and placebo groups were found [37]. Neutralizing antibody generated in VAX004 was against a virus easy-to-neutralize suggesting that level and breadth were insufficient for HIV-1 infection protection [172].

The second efficacy vaccine trial, the VAX003, was performed in Asia and used recombinant gp120 from HIV-1 from B and E subtype (AIDSVAX B/E) [38]. It was a double blind, randomized, placebo-controlled efficacy trial conducted among injection drug users (n=2546) in Thailand, the primary end point was the HIV-1 infection. There were no differences between incidence of HIV-1 infection between the vaccine and placebo arms, the vaccine did not affect the viremia after HIV-1 acquisition.

At the time, with the first failures of HIV vaccine human trials the field moved to immunogens that elicit CD8 T cell responses. In non-human primates, the depletion of CD8 T cell increased the viremia [173], while immunization of these animals with adenovirus vectors induced sustained and effective immunity by CD8 T cell controlling viremia [174] that time viral vectors carrying HIV immunogens became the stars on HIV vaccine development.

Two phase IIb clinical trials were conducted in the early T-cell based HIV immunogens era: the Step (HVTN502) and the Phambili (HVTN503) trials. The Step trial was a multicenter (North and South America, Caribbean and Australia), randomized, placebo-controlled, double-blind in which high HIV risk homosexual men and heterosexual from both genders were immunized with non-replicating human adenoviral subtype 5 vector expressing Gag/Pol/Nef from HIV-1 subtype B [118]. Step trial was a proof-of-concept of protection against HIV-1 elicited by T cell or reduction of early plasma HIV-1 levels. In 2007 the trial was interrupted as vaccinated group presented higher HIV incidence than placebo group, the mean of plasma viral load were comparable in both groups. Among vaccine recipients 75% presented T-cell response. The hazard risk to HIV-1 infection was higher in Ad5 seropositive and uncircumcised men than in Ad5 seronegative or circumcised men [117]. Despite Step trial did not reach the protection against HIV infection, subsequent evaluations showed the induction of immune pressure against the virus [175].

At the same time, the Phambili study (HVTN 503) was conducted using the Step trial immunogen [176]. During the enrolment of volunteers in HVTN 503 the vaccinations were halted as no vaccine efficacy were found in HVTN502 trial. The Phambili study was also a double blind, randomized, placebo-controlled, which recruited HIV-1 uninfected heterosexual individuals from both genders. In South Africa the predominant HIV-1 circulating subtypes is clade C, while de vaccine was composed by HIV-1 genes from B clade, the unprotected heterosexual behavior is the main cause of transmission. From 3000 recruited participants only 801 received the immunization regimen resulting in 34 HIV-1 infections in the vaccine and 28 on the placebo group, at time HVTN 503 confirmed the lack of vaccine efficacy already seen in Step trial. Long term analysis of the HVTN502, 42 months after vaccination, it was shown a higher rate of HIV-1 infection in the vaccine than placebo recipients [177].

Up to now the unique HIV vaccine efficacy trial that presented modest efficacy was the RV144 [1]. In 2009, the RV144 vaccine efficacy gave back hope to scientific community to still search for a preventive HIV vaccine. The RV144 was a multicenter, randomized, double-blind, placebo-controlled efficacy trial enrolling 16,402 subjects with high heterosexual risk.
The volunteers were immunized with four priming injections of a recombinant canarypox vector vaccine (ALVAC-HIV) followed by booster with recombinant glycoprotein 120 subunit vaccine (AIDSVAX B/E). In the modified intention-to-treat analysis involving 16,395 subjects (exclusion of 7 subjects who were HIV-1 infected at baseline) the vaccine efficacy was 31.2%. After all, the vaccination had no effect on HIV-1 post-infection viremia or CD4+ T cell count. The use of multiple statistical methods guided the better understanding of RV144 data. The vaccine included genetic fragments of HIV subtype B and E. Subtype E is the predominant strain in Thailand and Southeast Asia. Two immune correlates of risk were evidenced: IgG antibodies against variable region 1 and 2 (V1V2) from HIV-1 envelope proteins (Env) that correlated inversely with HIV-1 infection acquisition and the IgA binding antibodies to Env which correlated directly with acquisition of HIV-1 infection [2]. The importance of anti-HIV non-neutralizing IgG and IgA highlighted the need to better understand the different types of immunoglobulins and their protective functions in the HIV entry sites. RV144 trial brought back the importance of the viral Env immunogens to prevent HIV-1 acquisition [64].

The next trial was the HVTN 505 [178] that immunized men or transgender women who have sex with men (n=2504) with three injections of DNA (expressing clade B Gag, Pol, Nef and Env proteins from A, B and C clades) with 1 month interval followed by one boost at 6 month after beginning of the immunization with rAd5 (expressing clade B Gag-Pol fusion protein and Env glycoproteins from A, B and C clades). In April 2013 after discussions and decisions made by data and safety monitoring board, HVTN 505 trial was ended; the regimen was immunogenic but without vaccine efficacy with augmented HIV acquisition in the vaccinated group [176].

The lessons learned until today with human clinical trials showed the importance of clinical efficacy studies during the vaccine discovery process. The strong and sustained partnerships with diverse communities are critical. Preventive technologies are intersecting and creating opportunities to evaluate immunogens combination strategies. After all, the HVTN 505 added essential information to the adenovirus vector debate in the HIV vaccine field and its results supported efforts to induce cross-reactive broadly neutralizing antibodies, optimizing protein boosts and new generation of viral vectors.

Understanding Animal Models Used for HIV Vaccine Studies

One of limitations to find a preventive vaccine against HIV is the lack of an ideal animal model which explain the complex and full range of aspects of HIV infection and pathogenesis as to any other human disease [179-181].

Animal models must support HIV infection and virus replication and the animals cells must express several similar or identical human key proteins related to: HIV cell entrance (CD4 molecule, CCR5 or CXCR4 chemokine receptors), transcription factors (cyclin T1), nuclear export factors (CRM1), factors associated to virus budding and innate restriction factors [178]. The species-specific feature of HIV infection, replication and budding is associated with its adaptation to overcome restriction factors and exploit cellular cofactors in humans rather than in others species. There are several animal models and viruses available that can be used, this way the researchers should be aware of the question to be addressed in order to select the right option. The animal models used for HIV/AIDS research included: chimpanzees (HIV infection), several monkey’s species (SIV infection) and humanized mice.
Mice

Despite the fact that mice are widely used in scientific research and have a well-defined genetic background added to laboratories facilities as wide range of reagents for studying immune response, they are not permissive to HIV [182]. While the immunogenicity of HIV vaccines can be evaluated in mice or other small animals, the viral replication, dissemination and control cannot [181]. The huge divergence in immune response between vaccinated mice and humans are related to the host-range restrictions as anatomical, physiological, genetic and immunological differences between animals [183]. After all, the homozygous major histocompatibility complex (MHC) genes in inbred mice strains give an unnatural immune response which is not comparable to humans, which carry highly polymorphic MHC alleles [180]. Inbred wild type and knockout mice have been commonly used to understand the mechanism of immunogenicity of immunogens new compounds (DNA, viral and other vectors, adjuvants etc). The distinct functional innate and adaptative cell responses against the virus in mice do not mimic those in humans, impairing the comparison of immune response to HIV immunogens between the given species [184].

In any way, the best and cheapest model to study HIV immunogens is the humanized mice [178]. These immunodeficient mice engrafted with humanized tissues or immune cells can provide the target cells to be infected by HIV and can also the factors necessary for HIV replication.

Humanized mice are generated in animals with severe combined immunodeficiency (SCID) carrying a recessive mutation in the gene encoding the DNA-dependent protein kinase catalytic polypeptide (PRKDC), which impairs the recombination of T cell receptor genes and disrupts the differentiation of T and B lymphocyte progenitor cells, resulting in the absence of T and B cells. The murine innate immune system is not affected; however the absence of murine adaptive immunity abrogates de rejection of human transplanted cells [185]. Human peripheral blood mononuclear cell, fetal tissues (or part of it) and even bone marrow can be transplanted in these animals to develop the mice-human chimera [180].

The most useful humanized mice models used in HIV vaccine development are:

(a) SCID-hu-PBL-mice: engraftment of peripheral blood lymphocytes (PBL) which circulate and populate the lymph nodes, bone marrow, spleen and genital mucosa [186]. They produce antibodies and have the CD4+ T cell loss after HIV infection [187,188]. They are commonly used to investigate HIV pathogenesis and vaccinology. The use of PBL model improved knowledge in the field of: passive immunization with anti-HIV envelope monoclonal antibodies; testing Env-based vaccines and the use of inactivated-HIV pulsed dendritic cells to induce immune response [178] PBL-mice do not support mucosal transmission.

(b) BLT mice: Bone-marrow-liver-thymus mice are NOD SCID animals engrafted with fetal human liver cells and thymus tissue irradiated and then transplanted with human stem cell from the same donor. Human cells are found in peripheral blood, lung, liver, gut-associated lymphoid tissue and vagina. Here human T cells are developed in the human engrafted thymus. In this BLT model after HIV infection they develop several AIDS related signs: CD4+ T cell depletion, sustained high viral load, HIV humoral and cellular immune response. They can be infected by mucosal route.

The most important limitation of humanized mice model is the artificial immune environment created by human immune cells in a mouse tissue. However as they are the nearest murine model to human, the evaluation of new immunogens against HIV have been moving forward
using BLT humanized mice. Recently several evidences concerning BLT mice developing anti-HIV immune responses similar to human HIV infection were published. BLT humanized mice expressing protective HLA haplotypes suppressed virus as humans carrying the identical HLA genotype. After all, humanized mice generated responses against HIV conserved regions associated with control of viral replication as it has already been shown in humans [189]. The migration of HIV infected T cells to the lymph nodes and the formation of multinucleated syncytia in the same tissue were confirmed in the HIV infected BLT mice, experimentally implying the role of HIV infected cells on the local and rapid HIV spread to lymph nodes as hypothesized in humans [190]. Considering the B cell compartment, humanized BLT mice transplanted with human hematopoietic stem/progenitor cell, which was transduced with viral vector construct encoding anti-HIV IgA genes, was protected against CD4 T cell depletion after vaginal HIV-1 challenge [191]. The protective role of passive antibody transfer was confirmed in BLT model by reproducing early experiments using a combination of broadly neutralizing antibodies that controlled HIV-1 infection, suppressing viral load. After all, the longer half-life of antibodies compared to short life of antiretroviral therapy reinforces the importance of this therapeutic modality in HIV disease [192].

The future of humanized mice in HIV research is promising even considering them an artificial model. The chimeric human-mice immune system is remarkably disorganized, maybe due to the genetic engineering required. Removing mice immune system to be replaced by a human system could unknowingly break up the necessary genes and their expression to organize properly the lymphatic system in humanized mice [193]. Hampering their use, the maintenance of immunodeficient humanized mice in a sterile environment is difficult; humanized mice are not easy to be generated and should be de novo generated for each new experiments, the cost to keep this mice is high [178]. Otherwise, humanized or wild type mice can be used in HIV vaccine field to delineate the immune response, to analyze the inflammatory and signal transduction pathways and should be considered depending upon the question to be addressed.

**Non-human primate models**

Several species of non-human primate (NHP) are used in anti-HIV vaccine development studies [177-179]. Research in NHP was targeted to simian immunodeficiency virus (SIV) or recombinant simian-human immunodeficiency virus (SHIV) in natural and non-natural simian species. It is important to understand the features of the SIV or SHIV interactions in the selected simian specie to better interpret the data obtained in vaccine testing in the NHP model. NHP are the most similar model to humans to study HIV immunogens based on the well-developed immune system and its associated tissues [194].

Historically, chimpanzees (*Pan troglodytes*) were the first model used to study HIV/AIDS disease and were abandoned for ethical and logistical reasons [195,196,192]. Infrequently chimpanzees develop disease and usually only 10 years after the SIV or SHIV infection [197]. SIV is a natural host of African monkeys and apes (non-pathogenic model) and they normally do not develop disease despite high levels of virus replication [198,199]. The Asian macaques (*Macaca* species) are the SIV artificial or unnatural model. This model is ideal to compare to human HIV-1 infection, as they develop AIDS related disease, presenting high viremia, chronic CD4+ T cell depletion and opportunistic infections [177-179,200].

Natural SIV hosts are useful to study pathogenesis and immune correlates of protection against SIV / SHIV. Among them, the mostly studied are the Sooty mangabeys (smm, *Cercocebus atys*) and the African green monkeys (agm, *Chlorocebus aethiops*), and their SIV are referred as SIVsmm and SIVagm [177-179]. After natural SIV hosts infection by their own SIV,
they develop high-sustained viremia, severe depletion and turnover of CD4+ T cell, however they maintain healthy CD4+ T counts and their mucosal immunity, there is no microbial translocation, lack of chronic infection, their architecture of lymph nodes are normal and they preferentially infect the central memory T cells \[196\]. Several similarities and differences of pathogenic (non-natural host) and non-pathogenic (natural host) simian models were defined \[196\]. Regarding the similarities between African and Asian macaques and humans infection there is: high viremia in both models: short in vivo life span of productively infected cells and during acute infection they have significant loss of mucosal CD4+ T cells and high levels of innate and adaptive immune activation \[196\].

Unnatural SIV hosts are the preferential and widely used model in HIV vaccines studies \[177,201\]. The most studied macaques are: rhesus (Macaca mulatta) and the cynomolgus (Macaca fascicularis) \[196\]. These three species of macaques develop similar disease as human AIDS, characterized by acute and chronic progressive loss of CD4+ T cell followed by clinical immunodeficiency, opportunistic infections and development of neoplasia \[202,203\]. The differential outcome after SIV / SHIV infection is related to each monkey specie evolutionary characteristics \[177\]. SIV/NHP studies resulted in several advances in anti-HIV vaccine development based on discoveries of viral pathogenesis and HIV immune responses, guiding to design new vaccines and strategies to reach effective protective immune responses \[204\]. For experimental efficacy studies it is important to challenge monkeys with virus containing envelope and others essential proteins from HIV.

Why HIV does not infect some NHP cells? The inability of HIV to infect NHP is related to differences in restriction cellular factors such as TRIM5a and APOBEC3G among others \[205,206\]. Restriction factors are cellular proteins linked to innate immunity that can interfere with crucial and specific steps of virus replication, acting as post-entry blockers. These restriction factors accumulate amino acid differences between species, as virus evolved mechanisms to overcome these restriction factors in their own host, once in a different and new hosts they become susceptible to these innate blockers, resulting in an inability of virus from a species grow in a new host specie as demonstrated with HIV in several NHP species \[204\].

To understand NHP studies in which HIV immunogens are been tested it is important to know the most used challenge virus. The SIVmac and related viruses originated from cross-species transmission of the SIVsm that naturally infects sooty mangabeys (SM) \[207\]. SIVmac strains differ from HIV in several features as presence of vpx gene in SIV, only 53% nucleotide similarity and distinct organization of SIV overlapping reading frames compared to HIV \[178\].

Among the studied uncloned SIV there do SIVmac251 and SIVsmE660 which are viral isolates owe genetic heterogeneity desirable for analysis of diversity typical of early-chronic HIV-1 infection \[208\]. The limitation of uncloned viruses in efficacy and immunogenicity studies of new HIV vaccines are the differences in stock virus preparations by distinct laboratories, which complicate the interpretation and comparison of the results by different groups \[206\]. Other pathogenic viral clones are SIVmac239 and SIVsmE543-3, derived from SIVmac251 and SIVsmE660, they are CCR5 tropic and replicate better in memory CD4+ T cells, however their Env surface glycoproteins were not neutralized by antibodies \[209,210\]. These SIV strains are useful to evaluate heterologous protection after vaccination considering the genetic distance between SIVmac251 and SIVmac239 from SIVsmE660 and SIVsmE543-3, this genetic distance is comparable as the described between HIV-1 isolates from the same clade \[211-213\]. Immunization of NHP with diverse MHC genotypes with SIV mutants deleted of multiple gp120 N-glycans, used as potent live attenuated vaccine, induced near-sterile immunity.
against SIVsmE54 [211]. Another vaccine scheme using cloned SIV elicited protective immune responses against heterologous virus in highly diverse MHC NHP [178]. There are other strains derived from the first isolated and generated in rhesus SIVmm (Macaca mulata, mm), pig-tailed SIVnm (Macaca nemestrina, mn), however their use in research is not as established as the African monkeys.

SIV challenge is not the ideal approach to be used to test HIV based immunogens in NHP. For example, antibodies against Env SIV is not neutralizing to Env HIV-1 [214]. One of the genetic difference among these viruses are the lack of vpu in SIV that impairs the discoveries about the role of immunity to this protein in HIV immunogens tested in NHP and then challenge with SIV [215], besides that SIV has vpx which is not present in HIV-1 among other differences between HIV and SIV enzymes. Moreover, structural differences among envelope from HIV-1 and SIV lead to qualitative differences in the antibody responses [216].

To overcome these limitations several chimeric simian-human immunodeficiency viruses were engineered [178,213]. The first generation of chimeric SHIV express HIV Env, and were produced by replacing env, tat and rev from SIVmac239 by their HIV-1 corresponding genes [217]. The first generation SHIV, expressing Env, was highly pathogenic, marked by high level of replication and rapid depletion of CD4+ T cell besides their inability to induce antibodies [214,218]. The potential of SHIV replication in macaques improved after extensive passage of this chimeric virus in monkeys and its application in HIV vaccinology is aiming to induce neutralizing antibodies [217]. In vaccine studies SHIV 89.6P is the most used strain, as a chimeric virus expressing HIV Env, its limitation is the easy protection developed by macaques’ vaccination, which represents a paradox in the context of human infection as in humans is really hard to induce neutralizing antibodies [178]. Genetic engineering makes it possible to generate SHIV tropic to CCR5, the original SHIV 89.6P is CXCR4 tropic, and these new viruses are being improved to originate virus representing the most relevant targets for Env-based vaccines (SHIVSF162P3, SHIV1157ip3N4) [219,220]. Advances in construction and improvement of simian-tropic HIV-1 (stHIV-1) and its application in cynomolgus macaque [221] represent the innovative virus construction to verify vaccine efficacy.

Even with several concerns against the utility of SIV natural or unnatural hosts concerning the evidences of protection, NHPs are the most appropriate animals to study HIV vaccine. Attenuated HIV vaccines are prohibited in humans for ethical and safety concerns, however they represented the most efficient immunogens already been evaluated [35,179]. Recently it was found that the frequency of T cell specific response in lymph node was found associated with the efficacy of live attenuated SIV vaccine [222]. The maintenance of protective T cell response associated to persistent attenuated vaccine replication in follicular helper T cell seems to elicit immune response that contain and suppress early SIV replication [221]. As already showed in RV144 trial, ADCC also contributed to protection after challenge of NHP vaccinated with attenuated SIV [223]. These evidences point to the need of continuous antigenic stimulation in the maturation of protective immunity in SIV model perhaps by keeping activated effector memory T cells or driving the affinity maturation of neutralizing antibodies in lymph nodes [179].

Several NHP studies were used in proof-of-concept as protection against SHIV by mediated passive transfer of neutralizing antibodies [224,225]. These evidences indicate that a HIV immunogens designed to generate and maintain neutralizing antibodies would protect against HIV-1 [179]. Other proof-of-concept in NHP was to elicit sustained neutralizing activity of antibodies and long-lasting protection against high pathogenic SIV in rhesus macaques [226] using delivery integrative vector to muscle, adeno-associated virus, expressing SIV Env-specific antibody that is synthesized in myofibers and passively distributed to the body.

Until now, none of T CD8+ cell based vaccine has been proved to protect against HIV-1
infection in human trials, the proof of concept of T cell vaccine in NHP model also did not reach prevention of SIV or SHIV infection, on the other hand, T CD8 cell based vaccines resulted in CD8 T cell response and block of virus replication [227,228]. Also, optimized HIV-1 vaccine candidates as Ad5 and canarypox virus vectors blocked acquisition of heterologous, neutralization-resistant virus challenge in rhesus monkeys [229].

In a proof-of-concept study the proper selection of the virus being challenged guides the evaluation of proper vaccine. The research on HIV vaccine development in NHP progressed from challenging immunized macaques with high amounts of cloned virus to lower dose swarm-based mucosal infection with SIV mac251 or SIVsmE660, and is moving toward use of SHIV and simian-tropic HIV-1. As majority of vaccines were able to induce broadly neutralizing antibodies, the focus in NHP model moved to passive immunization as well as vectored delivery methods, also scientists tried to mimic RV144 in monkeys to understand the protection mechanisms and develop viral vectors (rAd26 and MVA) to elicit protective cellular immune responses [228].

Concluding HIV-1 vaccine candidates must continue to be tested in humanized mice, generating knowledge about immunogenicity of these products rapidly and with low costs where there is no NHP center established. Concerning NHP model advances in proof-of-concepts studies provide evidences that reaching a preventive and therapeutic HIV-1 vaccine could be feasible. However NHP as an animal model has its limitations and while the correlates of immunity were not completely defined in humans the ideal approach is to continue the parallel studies in humans and NHP to clarify the mechanisms of infection protection.

Conclusion

The design and development of an effective prophylactic anti-HIV vaccine is far from becoming a reality; however the advances in the field improved during the last two decades. Even with failures of HIV-1 vaccine clinical trials, the modest protection revealed in RV144 trial shed light into the understanding of HIV immune correlates of protection and created a hope in HIV vaccine field. New approaches are being conducted from now onward, guided by translational research and long-term follow-up analysis of HIV vaccine human trials, and bringing knowledge and advancement in the field. Currently the focus relies on the complete activation of immune system with the attempt to develop immunogens able to simulate the HIV infection and to elicit both anti-HIV cellular and humoral responses, since clinical studies have pointed out these aspects to hamper the infection and/or progression towards AIDS. The scientific evidences, reached in search for an HIV vaccine research, contributed to improve knowledge in all fields of basic and applied sciences. Experimental animal models (like humanized mice) as well as the simian-human model were improved to elicit an anti-HIV response that would be more similar to the human HIV infection and more suitable for the understanding of immunogens mechanisms that would reach the ideal anti-HIV immune response.

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