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REVIEW

Current progress in the development of a prophylactic vaccine for HIV-1

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Lena J Gamble[1]
Qiana L Matthews[1,2]

[1]Division of Human Gene Therapy, Departments of Medicine, Pathology, Surgery, Obstetrics and Gynecology, The Gene Therapy Center, [2]Center for AIDS Research, University of Alabama at Birmingham, Birmingham, AL, USA

Abstract: Since its discovery and characterization in the early 1980s as a virus that attacks the immune system, there has been some success for the treatment of human immunodeficiency virus-1 (HIV-1) infection. However, due to the overwhelming public health impact of this virus, a vaccine is needed urgently. Despite the tireless efforts of scientist and clinicians, there is still no safe and effective vaccine that provides sterilizing immunity. A vaccine that provides sterilizing immunity against HIV infection remains elusive in part due to the following reasons: 1) degree of diversity of the virus, 2) ability of the virus to evade the hosts' immunity, and 3) lack of appropriate animal models in which to test vaccine candidates. There have been several attempts to stimulate the immune system to provide protection against HIV-infection. Here, we will discuss attempts that have been made to induce sterilizing immunity, including traditional vaccination attempts, induction of broadly neutralizing antibody production, DNA vaccines, and use of viral vectors. Some of these attempts show promise pending continued research efforts.

Keywords: HIV, prophylactic vaccine, AIDS, viral vectors, sterilizing immunity

Introduction

Since its discovery and characterization in the early 1980s as a virus that attacks the immune system, leaving patients unable to fight off opportunistic infections, there has been an ebb and flow of effective treatments and hope as scientists continue to search for ways to eradicate human immunodeficiency virus-1 (HIV-1) from the human population similar to what has been accomplished in the case of smallpox. The majority of the effort and nearly all of the success has come in the area of patient treatment rather than inhibition of contraction or spread of the virus. A class of treatments, antiretroviral therapies (ARTs) and later highly active antiretroviral therapies (HAARTs), has been the mainstay of disease control during the last 15 years. Notwithstanding the increased life span of patients, increased time to full-blown AIDS, and decreased contraction of opportunistic infections and AIDS-related diseases (ie, non-Hodgkin's lymphoma, Kaposi's sarcoma, etc) by patients treated with HAART, there are several reasons why development of an HIV-1 vaccine is still warranted. Five of these reasons are as follows: 1) nearly two-thirds of the patients who contract HIV-1 live in underdeveloped countries and cannot afford the expensive HAART regimen,[1] 2) both the ART and HAART regimen are complex and are disruptive to patients' lives and diets, making long-term compliance an issue,[2] 3) the potential side effects of ART/HAART treatments negatively affect the long-term health of patients and include diabetes, cardiovascular disease, fractures, etc,[3–5] 4) development of HAART drug resistance,

Correspondence: Qiana L Matthews
Division of Human Gene Therapy, Department of Medicine, University of Alabama at Birmingham, 901 19th Street South, BMR2-R534, Birmingham, AL 35294, USA
Tel +1 205 934 0573
Fax +1 205 975 7476
Email qlm@uab.edu

and 5) the presence of latent HIV-1 reservoirs harboring viral strains that were produced through mutation throughout the duration of the infection of the host also play a role in the failure of HAART.[6] These reasons, as well as many others, underscore the need for a prophylactic HIV-1 vaccine.

Possibly, the strongest argument for development of a prophylactic vaccine may be the need for control of the virus spread worldwide. Every day, 7500 patients worldwide are infected with HIV-1.[1] Production of a vaccine that could inhibit infection, reduce spread, or both would aid in the reduction of the burden of AIDS and AIDS-related diseases. The expenses incurred by the AIDS epidemic can hardly be calculated. They range from tens of thousands of dollars per patient for the HAART regimen, to millions of dollars required for building of orphanages by governments for children whose parents have succumbed to the disease, to the unknown cost of educational materials and condoms in the effort to prevent further spread of the disease. This public health challenge has not gone unnoticed and has been addressed by scientists' ongoing efforts to develop a safe and effective HIV-1 vaccine.

Prophylactic vs therapeutic vaccines

A prophylactic HIV-1 vaccine would offer sterilizing immunity to patients, preventing infection upon presentation of the virus. A prophylactic vaccine must also be effective at all possible portals of HIV-1 entry, especially the mucosa.[7] For this to occur, the vaccine must offer broad and durable immunity. Several consortia have worked diligently to produce a vaccine that will induce broadly reactive neutralizing antibodies (Nabs). These consortia include major international efforts as well as efforts of individual countries, regions, and institutions including, but not limited to: the International AIDS Vaccine Initiative Neutralizing Antibody Consortium,[8] the Center for HIV-AIDS Vaccine Immunology, the HIV Vaccine Trials Network, US Military HIV Research Program, the Collaboration for AIDS Vaccine Discovery, and the Vaccine Research Center at the National Institutes of Allergy and Infectious Diseases of the National Institutes of Health. To date, however, no HIV-1 candidate vaccine has induced broadly reactive Nabs.[8]

In the absence of a vaccine that can prevent infection of HIV-1, there are still many benefits to be realized from production of a therapeutic vaccine. A therapeutic vaccine would be supremely valuable if it were able to increase the titer of virus necessary for infection, increase the time to clinical manifestation of virus, control viral load after infection, and reduce secondary transmission.[9–13] A vaccine that could induce this type of response would invariably decrease contagiousness, decrease the need for costly and potentially dangerous ART/HAART, and decrease the number of opportunistic infections of patients.

While the effect of controlling the normal HIV-1 pathology with therapeutic vaccines will be favorable for the individual patient as well as society, the effect of preventing HIV-1 infections in humans with a prophylactic vaccine is also broadly appealing. This potential for eradicating the HIV-1 virus from human hosts drives scientists to continue to find ways to circumvent the challenges presented by this unique virus in order to induce production of the Nabs that are critical for sterilizing immunity. This review, therefore, will focus on the specific challenges presented by HIV-1 and strides that have been made toward creating a prophylactic vaccine, including past efforts that have failed and lessons that have been learned from those failures. We will also discuss novel vaccine options and some of the promising trials that are currently underway.

Current challenges to creating an HIV-1 vaccine

While several problems face scientists who are attempting to create an HIV-1 vaccine, three problems in particular have posed extremely daunting challenges. These three problems are 1) degree of diversity of the virus, 2) ability of the virus to evade the hosts' immunity, and 3) lack of appropriate animal models in which to test vaccine candidates. These three major problems will be discussed in more detail below.

Degree of diversity

Traditionally, prophylactic vaccines have been made by exposing some part of a pathogen's structure as an antigen to the host's immune system, and eliciting an immune response, resulting in the production of long-term memory lymphocytes that are capable of mounting a strong immune response upon later infection with the pathogen. The premise upon which this manipulation of the immune system is based is the ability of the immune system to make long-lasting antibodies to conserved structures on exposed proteins that are native to the pathogen. Ideally, both humoral and cell-mediated immunity would be induced creating long-lasting immunity. Traditional attempts to recreate this process using live attenuated simian immunodeficiency virus-1 (SIV-1) viruses in an effort to vaccinate macaques against SIV-1 have been proven safe and effective in macaques that were subsequently challenged with SIV-1.[14,15] However, an incidental study of the effect of live-attenuated HIV-1 (containing deletions of the *nef* gene and

the long terminal repeat) was proven pathogenic in humans when three out of six treated patients developed late-onset immunosuppression.[16–18] Killed viruses have also been tested as a potential vaccine approach, but safety concerns have halted their use. These safety concerns include incomplete inactivation of the virus leading to potential residual infectivity during the vaccine preparation.[19] Due to the ineffectiveness of traditional vaccine approaches to date, scientists have attempted to use recombinant HIV-1 proteins to stimulate the production of Nabs. These attempts failed due to their inability to induce a lasting, broad range of Nabs that would inhibit infection in humans.[20–23] Perhaps these failures are a result of the inherent diversity of HIV-1. This diversity has presented a major roadblock to development of a prophylactic vaccine. There are three main groups of HIV-1 (M, O, and N)[24] as well as a recently discovered group, P.[25] Each group consists of several subtypes, clades. The various clades display biological differences with respect to transmission,[26] replication,[27] and disease progression.[28,29] These differences result in an inability to produce a generalizable vaccine that would induce the breadth of Nabs necessary to counter an infection by a wide range of HIV-1 clades that may be encountered in a natural setting.[30] The degree of diversity seen in HIV-1 is greater than that of any other virus observed.[31,32] This problem is being addressed by development of multiclade (multiple *env* and/or subtype B *gag, pol, nef*)[33,34] and mosaic vaccines which incorporate sets of 10 immunogenic proteins from 4 different clades or bivalent proteins from clades B and C.[35–37] There are proof of principle studies that illustrate immunological protection against HIV-1 in nonhuman primates that were passively treated with broadly reactive Nabs.[38–40] These studies show that protection against infection with HIV-1 can be conferred by the presence of broadly reactive Nabs. The next step toward production of a prophylactic vaccine would involve induction of production of these or similar broadly reactive Nabs by the host's immune system.

Immune evasion

The rate at which the HIV-1 virus mutates, due to the nature of the reverse transcriptase enzyme responsible for transcribing its RNA, ensures that nearly every daughter virion will have a different genome than its parent.[41] When these changes occur in the HIV-1 Env protein that is needed for antibody recognition, they inhibit the immune system's ability to mount a sufficient response. One attempt to circumvent this problem has been to induce the production of Nabs to the conserved regions of HIV-1 proteins. A major problem with this approach is that the conserved regions of HIV-1 proteins are often shielded from exposure to Nabs within the HIV-1 envelope. The native structure of the envelope protein, reportedly the only HIV-1 protein susceptible to Nabs,[31] shields it from the immune system as a glycosylated trimer of heterodimers. The glycosylation of the envelope protein allows for the carbohydrates to masquerade as 'self' thereby forming an immunologically silent face and protects neighboring epitopes via an 'evolving glycan shield'.[42–44] Additionally, the gp41 coreceptor binding site, another conserved site, is not presented until primary binding to $CD4^+$ has occurred.[45] An attempt to create antibodies to the CD4-binding region of the gp120 protein was made in rhesus macaques in 2007 and results indicated that vaccinated hosts were able to withstand challenge with SHIV.[46] Other attempts to create an HIV-1 vaccine have focused on overcoming the ability of HIV-1 to escape immune surveillance through use of antibodies that are able to neutralize diverse isolates of HIV-1. These antibodies include PG9, PG16,[47] 2F5, 2G12, 4E10, b12,[48–51] and most recently sCD4-17b[52] and others.[53] Identification of these antibodies gives hope that their induction or the induction of other such broadly reactive Nabs may provide the basis for a prophylactic vaccine in the future.

Lack of appropriate animal models

The use of animal models for development of therapeutics offers the benefit of thorough testing and validation prior to introduction of a vaccine in humans. In the past, vaccines were made by observing and then mimicking the immune response mounted by individuals who had recovered from a particular disease. To date, however, there are no known cases of individuals who have recovered from HIV-1 infection. However, data can be gathered from long-term nonprogressors – patients who have been infected with HIV-1 for at least 7 years and do not display any HIV-1-related symptoms.[54,55] Another option that may be critical to the development of a prophylactic vaccine is the use of relevant animal models. Such models will allow for analysis of the effect of a potential vaccine on an intact host prior to use in humans.

One particular challenge with the use of animal models for development of a prophylactic HIV-1 vaccine is that there are very few naturally occurring disease models of HIV-1. Only a few nonhuman primates are susceptible to infection with HIV-1 and infected animals do not progress to AIDS.[56] Therefore, it is important to use other disease models that mimic the HIV-AIDS pathologic progression.[57] One such potential model is feline immunodeficiency virus (FIV). FIV was discovered in 1986 and is known to cause an AIDS-like disease in domestic cats and mimics HIV-related dementia

in humans.[58] A vaccine for FIV was approved by the FDA in 2002.[59] While the FIV model is potentially informative, its use is not sufficient as a basis for development of a prophylactic HIV-1 vaccine.

An ideal animal model would display a pathological response to infection with HIV-1 that is very similar to the one that occurs in humans. Unfortunately, HIV-1 does not cause pathology leading to the development of AIDS in any host other than humans.[60–63] However, animal models have been developed and used that allow partial understanding of the pathology of HIV-1, the natural immunological response to infection, and the response of the host to novel therapeutics. One of these models involves the simian immunodeficiency virus$_{MAC}$ (SIV$_{MAC}$) that replicates and causes an AIDS-like disease in baboons, cynomolgus, and pigtailed macaques. While the similarities of SIV$_{MAC}$ to HIV-1 have allowed for insight into pathology, transmission, and immunological response of the infected host to the virus, the differences between SIV$_{MAC}$ and HIV-1 are still too great to be able to draw conclusions regarding potential human responses to an HIV-1 prophylactic vaccine.[63] Therefore, to broaden the scope of animal model usage, a chimeric SHIV virus was engineered to incorporate both SIV and HIV-1 proteins or genes.[64] While macaques infected with SHIV do go on to develop AIDS, the time to progression is much different from the time to progression to AIDS of HIV-1-infected humans. Infection of macaques with SIV$_{mac}$251 strain mimics HIV-1 infection in humans by leading to chronic, slow disease progression. Route and dose required for infection, viral tropism, replicative capacity of the viruses, and pathology of SIV/SHIV-infected monkeys are all very different than these parameters in humans.[65,66] This distinction has been well characterized by the recent Phase IIb STEP trial, which involved 3000 healthy, uninfected volunteers. The result of this trial was termination at its first scheduled efficacy assessment due to its failure to suppress viral load in subsequently infected individuals and then-suspected increased HIV-1 infection due to interaction of the immune system with vaccine components.[67] The vaccine, a recombinant adenovirus serotype 5 (Ad5) virus incorporating the *gag*, *pol*, and *nef* genes from HIV-1, had been previously tested in an SHIV model in macaques and the results of that experiment were not suggestive of the results of the human trial.[68]

This disparity underscores the need for animal models that more closely reflect the pathology seen in human infection with HIV-1 as well as identification of immunological correlates of protection that reflect control of HIV-1 viral load in human subjects. Therefore, the search for an appropriate animal model or the appropriate use of current animal models in the search for a prophylactic HIV-1 vaccine continues. Until a model can be derived that will allow for observation of each stage of infection, progression of disease, and response of the immune system in a way that is comparable to this process in humans, we will not be able to logically predict which vaccine candidates should be moved forward to clinical trials.

Several attempts to stimulate the immune system to provide protection against HIV infection have been attempted so far (Table 1). Hope for creating a prophylactic vaccine lies in the ability of the scientific community to identify and induce a broad neutralizing antibody response that would offer sterilizing immunity to vaccinated patients. To this end, several novel approaches are being studied.

Novel vaccine options

As mentioned in the previous section, there are several daunting problems facing scientists who are attempting to create an HIV-1 vaccine. In hopes of creating a vaccine which elicits sterilizing immunity to HIV-1, researchers have focused their efforts on (1) the use of plasmid DNA vaccines, (2) live recombinant vectors for vaccine development (expressing or presenting HIV antigens), and (3) mucosal immunity. These critical topics will be discussed in more detail below.

Plasmid DNA vaccines

Vaccines should elicit a robust immune response that is long lasting and is able to provide protection against various strains of a pathogen. Plasmid DNA vaccinations can induce a strong humoral and T-cell response. DNA-based vaccination has been used as a powerful tool to fight against parasitic, fungal, bacterial, and viral infections.[115–119] There are multiple advantages for using plasmid DNA for vaccination: they are generally safe, nontoxic, and through the delivery of a gene encoding important immunogenic epitopes, the DNA-based vaccine exploits biosynthetic machinery of the host cell. One such example was in 1990, whereby Wolff and colleagues illustrated protein expression after intramuscular (IM) injection of plasmid DNA into myocytes.[120] Despite these promising results, there had been speculation regarding DNA vaccination strategies. For example, it was shown that protein production in response to DNA plasmids that contained HIV inserts elicited substantial cellular response in mice and nonhuman primates. However, these products were poorly immunogenic in humans.

One strategy to improve immune response of the plasmid DNA vaccine strategy is by coadministration of DNA

Table 1 Historical vaccine attempts to inhibit HIV-1 infection

Vaccine attempt	Mechanism of action	Outcome	References
Live attenuated			
Nef-deleted viruses	Deletion of *nef* gene in SIV; deletion of *nef* gene in HIV	Excellent short-term protection in nonhuman primates. Disease-causing mutants generated from vaccine	14,69–72
SIV1A11	Deletion of *vpr* and a portion of gp41	Animals never developed immunodeficiency but were not protected against challenge with wild-type virus	73
SIV_{MAC}-M4	Multiple mutations in the transmembrane protein intracytoplasmic domain	Animals developed immunodeficiency 1 year after infection with this virus but showed low or undetectable viremia levels 1 year post-challenge with SIV_{251}	74
Whole inactivated HIV-1[a]			
Simian model	Formaldehyde-inactivated or subunit SIV vaccines	Animals resistant to infection with HIV produced in human cells but not HIV produced in macaque cells	75–77
Feline model	Dual inactivated strains used for vaccination	Protection against heterologous strains conferred	78–81
Protein subunits and synthetic peptides			
Recombinant env glycoproteins	Stimulate humoral immune response	Some antibody production and lymphocyte proliferation but no clinical benefit to date	82–90
Recombinant Gag subunits	Stimulate humoral response	Induced production of anti-p24 antibodies but no clinical benefit	91–94
DNA vaccines			
Direct injection	Encoding HIV-1 *env* and *rev*	Produced a robust humoral and cellular response	95
Viral vectors expressing HIV-1 genes			
Retroviral vectors	CD4-specific transduction of HIV-1 genes: *env, vpu, tat,* and *rev*	Induction of humoral and cellular anti-HIV-1 responses in vivo	96,97
Rabies virus	Attenuated RV-expressing SIV-1 proteins	SIV_{MAC}-challenged macaques expressed higher antibody and CTL responses than nonvaccinated controls	98,99
Alphavirus	HIV-1 strain R2 env expression followed by administration of soluble oligomeric gp120	Induction of humoral and cell-mediated responses that were protective against heterologous HIV challenges in rhesus macaques	100
Canarypox	Prime with ALVAC-HIV (vCP1521) boost with AIDSVAX B/E	Inhibition of infection noted in vaccinees, although viremia was not reduced in those vaccinees that did become infected	101
Adeno-associated viruses	Gene transfer in muscle of antibodies or antibody-like immunoadhesins	Long-lasting neutralizing activity in serum of monkeys against SIV	102
Ads	Vaccination with a recombinant Ad5 construct	Induction of humoral and cellular responses in mice, dogs, chimpanzees, or nonhuman primates	103–106
Other			
Virus cocktails	HIV-1 vaccination with successive immunizations containing recombinant DNA, recombinant vaccinia virus, and recombinant *env* proteins	Following challenge with SHIV strains that were not used in vaccination cocktail, four of six vaccinated macaques lived through the 44-week observation period as compared to one of six control macaques	107
Fibroblasts	Immunization with retroviral vector-transduced fibroblasts expressing human immunodeficiency virus type-1 IIIB ENV/REV proteins	Induction of CTL and antibody responses in rhesus monkeys	108
Dendritic cell-based vaccines	Immunization with retroviral-vector transduced dendritic cells	Induction of CTL and antibody responses in cynomolgus monkeys	109
Virus-like particles	Goal – present artificially produced partial HIV-1 proteins in order to stimulate Nabs	Humoral and cellular response achieved in mice or rabbits	110–114

Note: [a]Proof of principle studies using SIV or FIV.
Abbreviation: CTL, cytotoxic T-lymphocyte.

plasmids coding for cytokines (eg, INF-g, IL-2, IL-12, IL-18, and IL-15).[121–124] A second strategy which has been utilized to improve plasmid DNA vaccination has been the administration of plasmid DNA with adjuvants (eg, CpG oligodeoxynucleotides), or the use of DNA-delivery systems (eg, microparticles, cochleates, and linear polyenimines).[125–128] A third strategy to improve vaccine efficacy involves the coadministration of plasmid DNA in combination with viral vectors. For instance, research performed by Harari and colleagues in 2008 demonstrated that vaccination by means of an HIV-1 clade C DNA prime in combination with a pox vector (NYVAC) boost induces a reliable polyfunctional and long-lasting anti-HIV T-cell response in human participants.[129] Along these same lines, work recently published by Jaoko and group demonstrated safety and immunogenicity of a multiclade HIV-1 Ad-based vaccine alone or in combination with a multiclade HIV-1 DNA vaccine in Africa. These results also demonstrated that DNA priming increased the frequency and magnitude of cellular and humoral responses; however, there was no effect of recombinant Ad5 dosage on immunogenicity endpoints.[130]

The previously mentioned DNA-delivery strategies have been used in combination with viral vectors or alone by means of a variety of immunization routes (eg, IM, intravenous [IV], intradermal [ID], intranasal [IN], oral, rectal, or vaginal). In the majority of reported studies, DNA vaccines have been administered by the IM and/or ID routes. However, as it relates to HIV vaccination, mucosal immunity could potentially be an important factor to consider, with mucosal immunity being achieved optimally by IN or oral routes of administration. The topic of mucosal immunity will be discussed in more detail in a later section within this review.

After immunization, it is assumed that the DNA vaccination immunogen is produced in the skeletal muscles, dendritic cells, and macrophages at the site of immunization. However, in adults, the skeletal muscles are not involved in a high level of protein synthesis as compared to the liver. Therefore, the delivery of DNA to cells, which are capable of high protein synthesis, such as hepatocytes, epithelia cells of the intestines, or salivary pancreas, may result in high levels of protein expression. The hepatocytes express enzymes involved in the formation of intrachain and interchain disulfide bonds required for proper folding and assembly of proteins. In addition, the liver expresses glycosyltranferases, which are essential for synthesis of both *N*- and *O*-linked glycan side chains; this may not be the case for other cell types,[131,132] the significance of this point being the fact that broadly cross-clade Nabs such as 2G12 recognize glycan moieties on the heavily glycosylated HIV-1 envelope antigens.[44,133,134] Another advantage of protein expression within the liver is that significantly lower amounts of DNA are needed for protein expression of a particular antigen in the hepatocytes vs another cell type. For the immunization of humans, milligram quantities of DNA are necessary to achieve adequate levels of immune response.[119] Any method whereby there would be a reduction in DNA quantity needed to vaccinate humans would provide significant economic advantages. Based on the previously mentioned reasons, it is not a surprise that the liver has been exploited extensively as a site for gene delivery due to its ability to produce proteins and glycoproteins.[135–138]

Hydrodynamic delivery is the application of controlled hydrodynamic pressure in capillaries to enhance endothelial and parenchymal cell permeability; this methodology had its inception in the late 1990s with investigations into intravascular injection of plasmid DNA solution for gene delivery in whole animals.[139–142] Hydrodynamic plasmid DNA delivery is well tolerated in mice. In 2008, Raska and colleagues demonstrated in mice that IV hydrodynamic vaccination with HIV-1 envelope DNA injections resulted in high levels of expression of HIV antigen in the liver. In mice, immunological data illustrated that hydrodynamic administration of HIV-1 plasmid DNA was superior to vaccination with DNA by IN, ID, IM, and intrasplenic routes. Further results illustrated that after boosting, hydrodynamic vaccination yielded levels of HIV-1-specific antibodies that were 40-fold higher than those elicited by other routes tested.[132]

However, this delivery scheme is not feasible in large animals and humans. As an alternative, receptor-mediated DNA binding to hepatocytes could be a viable approach. Molecules with terminal galactose residues covalently linked to DNA are recognized by the hepatocyte-expressed galactose-specific asialoglycoprotein[143] receptor for internalization.[144] This alternative would avoid delivery through the hepatic system and the need for expansion of the blood volume. In addition, galactose-linked DNA packaged in delivery vehicles such as liposomes, choleates, or microspheres can be given by oral administration, which would be absorbed by the intestine and ultimately delivered to the hepatic vein. As an additional alternative to hydrodynamic delivery in humans, it might be possible to express HIV antigens in the liver by means of plasmid DNA delivery via viral vectors such as the Ad. Ads have been shown to transduce the liver efficiently in vivo by means of the hexon proteins.[145,146] In this regard, production of translation of HIV-1 proteins primarily in the liver might allow for the production of heavily glycosylated HIV-1 envelope antigens and thus the production of Nabs.

Live recombinant vectors for vaccine development

Viral vectors are potent inducers of cellular and humoral response. Viral vectors can express proteins from bacteria or viral pathogens to vaccinate against infectious diseases. There are several viral vaccine vectors that have been used successfully in models for vaccination. These vectors include alphaviruses, human rhinoviruses (HRVs), Ads, picornaviruses, poxviruses, measles viruses, influenza, and vaccinia viruses.[30,129,147–156] Each of these vectors has its respective disadvantages and advantages with respect to vaccine development. Some advantages of a few of these vectors include their ability to naturally infect a wide variety of cell types and tissues of interest.[157–162] Each respective vector has its own set of disadvantages. For instance, one disadvantage of using the poliovirus or the HRV as a vaccine vector is the insert size limit restriction of these vectors as compared to the large insert size (~8 kb) accommodation of Ad vectors.

The most common disadvantage of the majority of viral vaccine vectors is reduced vaccine efficacy due to vector pre-existing immunity (PEI).[163–167] Various strategies have been employed to circumvent the problems associated with vector PEI. Specifically, as it relates to Ad vectors, PEI is a tremendous problem. Of the identified serotypes of Ad vectors, human serotypes 5 (Ad5) and 2 (Ad2) have been the most extensively used for gene therapy protocols. Ad5 has been used for HIV-1 vaccination protocols, most recently in the STEP study. As it relates to Ad2 and Ad5, PEI to these vectors may be found in up to 50% of the American population and up to 95% of the population of other countries. This Ad PEI can limit the effectiveness of Ad-based vaccinations.[168–170] To circumvent Ad2 or Ad5 PEI, researchers have employed the use of vector chimeras,[166,171] use of alternative serotypes,[172–178] and the use of nonhuman Ads,[151] such as chimpanzee Ad. The chimpanzee Ad virus was demonstrated to not be significantly neutralized by human sera, which gives chimpanzee Ad an advantage for human vaccine development.[179–181]

Other strategies have been used to reduce the immune response against Ad vectors such as the use of helper-dependent Ad (HD-Ads) vectors,[182–187] the use of Ad delivery in combination with biochemical modifications such as PEGylation,[188–194] and the use of vector delivery by means of cell vehicles.[195,196] With respect to the HD-Ads, these vectors were produced to further increase the safety and cloning capacity of first-generation Ad vectors. HD-Ads lack Ad genes and contain only the packaging signals and end terminal repeats. These vectors were designed to avoid cellular immunity and diminish liver toxicity, thus promoting long-term transgene expression.[197–200] The reduced immune response against HD-Ads has allowed for transgene expression in mice and baboons for years.[182,183,185,200] This long-term transgene expression could be helpful for antigen production for an HIV vaccine, thus producing an opportunity to have increased protection against HIV, with reduced frequency of vaccinations.

Although Nabs to Ad5 may reduce the immunogenicity of Ad5-based vectors in animal model systems, their effect on the immunity in subjects with previous Ad5 exposure is still largely unknown. As previously mentioned, the STEP trial, which tested a Merck recombinant Ad5 (rAd5) vaccine (encoding HIV-1 *gag*, *pol*, and *ne1* genes), failed to yield protection, either by lowering viral load or by decreasing acquisition of infection.[13] Analysis of data from this study aroused speculation that subjects with pre-existing Nabs from wild-type Ad5 infection had an increased risk of HIV infection after vaccination. One recent study has shown that there was no causative role for Ad5-specific $CD4^+$ T cells in increasing HIV-1 susceptibility in the Merck trial.[201] In this regard, there are multiple studies ongoing to elucidate a concrete finding with respect to the role of Ad5 PEI and increased activation of $CD4^+$ T cells in the mucosal milieu.[202,203]

Recently, there was a report by Cheng and colleagues that attempted to characterize the specificity of rAd5 Nabs in Ad5-immune subjects and determine the impact of Ad exposure on immune responses elicited by Ad5-based vaccinations. Cheng and colleagues reported that rAd5 Nabs were directed toward different components of the Ad virion, depending on whether the Ad5 infection was natural or from Ad-based HIV vaccine trials. For example, Ad Nabs generated by natural infection are directed primarily to fiber components, while vector exposure elicits responses primarily to capsid proteins other than fiber. Nabs elicited by natural infection significantly reduced the $CD8^+$ and $CD4^+$ cell responses to HIV Gag after DNA/rAd5 vaccination. This report concluded that Ad5 Nabs differ based on the route of exposure and that previous Ad5 exposure compromises Ad5 vaccine-induced immunity to weak immunogens, such as HIV-1 Gag.[204] These results have a tremendous impact on HIV-1 vaccine trials and the design of next generation viral vaccine vectors.

Viral vectors such as Ad, influenza, and polio have been used as vaccine vectors for many reasons. One important advantage of these vectors, which makes them attractive, is that they can provide mucosal immunity because they can easily infect the mucosal surfaces as well as act to induce cytokine and chemokine production at the mucosal entry sites. Ad, influenza, and polio also have the advantage of

being able to be delivered orally, without the use of needles. This is an important fact in developing countries where needle cost is prohibitive to vaccine administration. As it relates to HIV vaccine development, mucosal immunity is a debatable factor to consider.

Mucosal HIV immunity

When deciding upon a vaccine agent, the importance of considering if the ultimate goal is to induce systemic immunity, mucosal immunity, or both is worth careful consideration.[205–207] It is believed that 80% of HIV-1 infection will occur from heterosexual viral transmission and most of the rest will occur from homosexual or perinatal transmission.[152] Although the biology of sexual transmission is poorly understood, it is clear that the essential first step in the infection pathway is the transfer of infectious virus or HIV-infected cells through the mucosal surfaces. After HIV has entered a new host, the HIV or HIV-infected cells will soon encounter susceptible host target cells at the mucosal point of entry where the virus replicates and then invades local lymphatic tissues, initiating systemic HIV infection. On this basis, strong immunity is required to provide a protective immunological barrier at the most common point of entry, the mucosal surfaces of the reproductive tract. Due to the compartmentalization of the secretory and systemic immune systems, parenterally administered antigens do not consistently stimulate mucosal immunity.[152] Therefore, it is important to consider a vaccine regime that induces mucosal immunity.

Since $CD4^+$ $CCR5^+$ memory T cells are the primary target of HIV infection in the gut and mucosa and rapid depletion of this subset occurs early after infection,[208,209] several studies have investigated the role of HIV mucosal immunity. Previous studies have demonstrated the importance of a mucosal SIV/HIV vaccine producing both strong mucosal antibody and $CD8^+$ response capable of blocking the escape of virus from the intestinal mucosa into systemic lymphoid organs.[207,210–214] However, in other instances, the necessity of exclusive mucosal HIV immunity will be further debated based on the promising results found in a heterologous prime/boost regimen using DNA/89.6-expressing SIV and HIV-1 transcripts[215,216] and modified vaccinia virus Ankara (MVA/89.6)-expressing SIV and HIV-1 transcripts under the control of vaccinia virus early/late promoter. In this case, either ID or IM DNA/MVA vaccination was able to provide protection against a intrarectal SHIV-89.6 challenge.[153] Along these same lines, recently, promising results were found by Hessell and colleagues in 2010. Hessell and colleagues demonstrated that after an IV administration of monoclonal antibodies 2F5 or 4E10 to six monkeys followed by a $SHIV_{ba\text{-}L}$ challenge, five out of six monkeys from either group showed complete protection and sterilizing immunity. A low level of viral replication could not be ruled out for the six monkeys in either group.[217]

Replicative Ad yields a robust immune response at the mucosal sites partly because Ad is known to infect and replicate in epithelial cells.[218–221] Various strategies have been used to achieve mucosal immunity via the oral route. One such strategy embodies the development of replication-defective recombinant Ad serotype 41 (Ad41) vector.[222] Serotype 41 vectors are being currently used because Ad41 has a natural tropism for the gut and causes no pathological disease outside of the gastrointestinal tract.[223] Ad41 vectors are likely to have a preferential tropism for the gut because Ad41 appears to have a resistance to acidic pH[224] and the capsid configuration of long and short fibers allows the Ad41 virus to preferentially infect the gut.[177,225]

Live recombinant vectors for vaccine development engineered to express/present HIV-1 antigens

As previously mentioned, viral vectors are potent inducers of cellular and humoral responses. Of note, viral vectors have been practically used for human applications and have progressed treating a variety of disease contexts such as cancer and infectious diseases.[226–229] Traditional viral vector immunization embodies the concept that the vector uses the host cell machinery to express antigens, which are encoded as transgenes within the viral vector. Cellular and humoral immune responses are generated against these antigens. Over the last 20 years, several viral vectors have been derived to express HIV-1 antigens for vaccine purposes.

Some researchers have taken an alternative approach to conventional transgene expression of antigens by means of viral vectors; this alternative approach embodies the capsid incorporation of antigens. This innovative paradigm is based upon the vector presenting the antigen as a component of the capsid rather than an encoded transgene. Incorporation of immunogenic peptides into the vector capsid offers potential advantages. In this regard, the processing of the capsid-incorporated antigen via the exogenous pathway should result in a strong humoral response similar to the response provoked by native Ad capsid proteins. In this arrangement, potentially, HIV peptide antigens accrue the potent immunostimulatory effects of the native Ad vector capsid proteins, which effectively perform an adjuvant function. On this basis, the immune response directed against vector

capsid proteins with repetitive vector administration should achieve a booster effect against the incorporated antigen.[230] Most importantly, as it relates to HIV infection, this strategy yields the potential of generating antibodies to HIV proteins. Recent crystallographic, cryo-electron tomography, and molecular modeling studies have provided valuable insight to molecular surfaces recognized by antibodies as well as assisted in rationale vaccine design of immunogens.[231–235] These structural technologies can also potentially improve the abilities of scientists to advance the antigen capsid-incorporation strategy. If the antigen capsid incorporation is effective, it can provide a way forward with respect to inducing sterilizing immunity.[68,236,237]

The antigen capsid-incorporation strategy has been used for Ad-based vaccines in the context of many diseases.[230,238–242] One of the first examples where the antigen capsid-incorporation strategy was used was with research performed by Crompton in 1994.[242] Crompton and colleagues inserted an eight-amino acid sequence of the VP1 capsid protein of poliovirus type 3 into two regions of the Ad2 serotype hexon. One of the chimeric vectors produced grew well in tissue culture. In addition, antiserum raised against the Ad with the polio insert specifically recognized the VP1 capsid of polio type 3. As it relates to Ad5 serotype, Wu and group demonstrated that His_6 epitopes could be incorporated into Ad hexon hypervariable regions (HVRs) 1–7 (now reclassified as 1–9) without perturbing viral viability and any major biological characteristics such as replication, thermostability, or native infectivity. This study by Wu and colleagues demonstrated that His_6 appeared to be surface exposed at these regions.[243] With respect to peptide incorporation within Ad5 hexon, HVR2 and HVR5 appear to be the most promising locales for peptide/antigen incorporation based on X-ray and peptide analyses along with molecular studies.[244] Our laboratory and others have focused on incorporations at HVR5 or single-site incorporations (such as fiber and pIX).[230,238–241,243,245,246] However, we recognized that the ability to place antigen within multiple sites of the Ad capsid protein would hold important potential for presenting multiple epitopes/antigens or several copies of the same epitope within a single Ad vector-based vaccine.

In an effort to create multivalent HIV vaccine vectors, our 2008 study explored the use of Ad5 HVR2 and HVR5 in hopes of creating vectors which contained HIV antigenic epitopes at both locales. To compare the flexibility and capacities of Ad5 HVR2 and HVR5, we genetically incorporated identical epitopes of incrementally increasing size within HVR2 or HVR5 of Ad5 hexon. We incorporated identical epitopes ranging from 33 to 83 amino acids within the Ad5 hexon HVR2 or HVR5 region. Viable viruses were produced with incorporations of 33 amino acids plus a 12-amino acid linker at HVR2 or HVR5. In addition, viable viruses were produced with incorporations of up to 53 amino acids plus a 12-amino acid linker at HVR5. With respect to identical antigen incorporations at Ad5 HVR2 or HVR5, HVR5 was more permissive allowing an epitope incorporation of 65 amino acids in total. These model antigens were surface exposed via ELISA analysis. In vivo immunization with these vectors illustrated an antigen-specific immune response.[240]

Along these same lines, Abe and colleagues evaluated the ability of Ad5-based vectors expressing an HIV transgene to induce antigen-specific immune responses under Ad5 preimmune conditions. To overcome limitations that are generally experienced as a result of PEI to Ad5, they constructed vectors that have a modification in the HVR5. Their study characterized various immunological parameters generated by these vectors such as vector neutralization, acquisition of adaptive immune response, and comparison of protective immunity. First, in order to evaluate the utility of the modified Ad vector, they measured the neutralizing activity of sera by a modified Ad vector. They administered Ad-Luc (luciferase protein expressed as a transgene in the Ad E1 region), Ad-HisLuc (His_6 epitope presented in HVR5 region and luciferase protein expressed as a transgene), or Ad-END/AAALuc vector (containing three amino acid mutations in HVR5 and expressing luciferase protein) to mice IM. After administration of these vectors, neutralizing activity against Ad5 was observed for 0–8 weeks. The hexon-modified vector (Ad-HisLuc) generated the lowest Ad5-specific neutralizing activity, which was significantly lower than what was generated by Ad-Luc at weeks 6 and 8, and by Ad-End/AAALuc vector at week 8. The individual neutralizing activity of Ad-HisLuc immunization was significantly lower than that of Ad-Luc immunization. Additional studies performed by Abe and colleagues support the concept that modified hexon thwarts Ad5 Nabs and promotes cellular immune responses.[247] Studies performed by this research group indicate that a change in the immunogenic epitope is necessary to avoid neutralization by pre-existing Nabs.

Our recently published work exploits the antigen capsid-incorporation strategy for HIV vaccination. Our novel vectors were constructed in hopes of moving toward the goal of creating vectors that will provide cellular and humoral HIV immunity. Our study is the first of its kind to genetically incorporate an HIV antigen within the Ad5 hexon HVR2 alone or in combination with genomic incorporation of a

Gag transgene (Ad5/HVR2-MPER-L15(Gag)). In this study, we successfully incorporated a 24-amino acid epitope of HIV-1 within HVR2. The HIV-1 region selected for HVR2 incorporation was the membrane proximal ectodomain region (MPER) derived from HIV-1 glycoprotein 41 (gp41). Our rationale for choosing a portion of the MPER (EKNEKELLELDKWASLWNWFDITN) derived from gp41 was based on the fact that the gp41 envelope protein ectodomain is a target of three broadly neutralizing anti-HIV-1 antibodies.[248] When the MPER was incorporated into HVR2 in combination with transgene expression, we observed growth kinetics and thermostability changes similar to those of other capsid-incorporated vectors generated in other studies,[249,250] indicating that incorporation of the MPER epitope within HVR2 was not dramatically detrimental to virological characteristics.[250,251] In addition, we demonstrated that the MPER epitope is surface exposed within HVR2. Most importantly, we observed a humoral anti-HIV response in mice vaccinated with the hexon-modified vector. The MPER-modified vector allows boosting compared to AdCMVGag, possibly because the Ad5/HVR2-MPER-L15(Gag) Ad elicits less anti-Ad5 immune response. It is possible that the MPER epitope reduced the immunogenicity of the Ad5 vector. This finding is noteworthy because HVR2 has not been fully explored for antigen capsid-incorporation strategies.[252] These vectors are currently being analyzed by cryo-electron microscopic analysis to determine the critical correlates related to antigen placement/configuration and immune response.

In addition, with respect to HIV-1 vaccination, the antigen capsid-incorporation strategy has been evaluated within the context of HRV. Research groups have constructed human rhinovirus:HIV-1 chimeras in an effort to stimulate immunity against HIV-1.[148,253] In an effort to develop HIV-1 vaccines, researchers within this same group generated combinatorial libraries of HRV capsid-incorporated HIV-1 gp41 epitope. Their results indicated that they were successful in eliciting antibodies whose activity can mimic the Nab effect.[149]

Commercial and clinical Ad development of HIV-1 vaccines have progressed preferentially more than vector systems such as HRV because the flexibility of Ad generally exceeds current rhinovirus systems. For example, because HRV is a relatively small RNA virus, the HRV platform can display an array limited to 60 copies of a single HIV-1 epitope.[148,253] In contrast, the Ad vector platform allows incorporation of the HIV-1 MPER epitope into three structurally distinct locales, including HVR2, HVR5,[247] and protein IX (our unpublished data). In comparison, the Ad MPER antigen capsid-incorporation display platform could present an array of 720 HIV-1 epitope copies within Ad hexon and 240 HIV-1 epitope copies within pIX. If a multivalent Ad vector is generated with HIV-1 epitopes within the hexon and the pIX locales, this would represent 960 HIV epitopes within one Ad vector. Another significant difference between the Ad and HRV platforms is in the number of locales that have been successfully used for heterologous epitope insertion. Finally, in contrast to the rhinovirus that lacks this capacity, the Ad platform has sufficient coding capacity allowing for HIV-1 transgene expression in combination with presenting the same or a different antigen on the viral capsid surface. This latter finding is important because it provides the basis for constructing vectors that will provide cellular and humoral HIV-1 immunity. Vectors which provide both cellular and humoral immunity may be the way forward with respect to prophylactic HIV vaccine development.

Promising results in an effort to produce an HIV vaccine

Recently, there have been encouraging developments regarding HIV vaccination. In the 1980s, in Thailand, there was a substantial increase in the prevalence of infection with HIV-1.[254–256] By first observation, these groups consisted of intravenous-drug users and commercial sex workers; this infected group then expanded to the general population.[101] By the mid 1990s, the overall seroprevalence of HIV-1 reached a peak of 3.7% among members of the Royal Thai Army and of 12.5% among people from Northern Thailand.[255,257] The Thai Ministry of Public Health acted by starting an effective HIV-prevention campaign. With this effort, the number of new HIV-1 infections per year decreased from an estimated 143,000 in 1990 to 14,000 in 2007.[255,258–260] Although this decrease was promising, there was still a desire to do more to prevent HIV infection. To achieve this goal, an HIV Phase III study was begun.

The Thai Phase III HIV vaccine study, also known as RV144, opened in the fall of 2003. The placebo-controlled trial tested the safety and effectiveness of a prime-boost regimen of two vaccines: ALVAC-HIV vaccine (the prime), a modified canarypox vaccine, and AIDSVAX B/E vaccine (booster), a gp 120 vaccine. The vaccines were based on the subtype E and B HIV-1 strains that commonly circulate in Thailand. The subtype B HIV-1 strain is the most commonly found strain in the United States. The trial, conducted in the Chonburi and Rayong provinces of Thailand, enrolled 16,402 women and men aged 18–30 years at various levels of risk for HIV infection. Study participants received the placebo or ALVAC HIV vaccine at enrollment and again after 1, 3,

and 6 months. The placebo or AIDSVAX B/E vaccine was given to participants at 3 and 6 months. Participants were tested for HIV-1 infection every 6 months for 3 years. During each clinic visit, study participants were counseled on how to prevent HIV-1 infection.

The results showed that 74 of 8198 placebo recipients became infected with HIV-1 compared with 51 of 8197 participants who received the vaccine. This level of effectiveness in preventing HIV-1 infection was found to be statistically significant. The vaccine strategy had no effect, however, on the amount of virus in the blood of volunteers who acquired HIV-1 infection during the study. Based on the final analysis of the study, the surgeon general of the US Army, the trial sponsor, announced that the prime-boost investigational vaccine regimen was safe and 31% effective in preventing HIV-1 infection. With respect to an HIV-1 vaccine that can provide sterilizing HIV immunity, this is the best result in humans to date. However, the modest protection effect appeared limited to low-risk individuals, and there were data which suggest that this effect was confined to the first year following administration of the vaccine. Efforts must continue to focus on evaluating the immune response induced by the vaccine to establish potential correlates of protection.

Conclusion

Over the last three decades, the world has been faced with the emergence and subsequent epidemic of HIV/AIDS. There has been much progress with respect to diagnosis and prevention. On the treatment front, there have been several significant advances with respect to drug development (ie, ART/HAART). However, there is a desperate need for an effective and safe vaccine. There has been tremendous difficulty with regard to developing a vaccine that provides sterilizing immunity. This has been the case due to some of the factors mentioned in this review such as HIV diversity, immune evasion, and lack of appropriate animal models. Due to these obstacles, many researchers assumed that the control of HIV-1 viremia by vaccination would be a more realistic goal than the development of sterilizing immunity.

The road to a safe and effective HIV-1 vaccine received a serious setback in the fall of 2007 with the premature termination of the Merck-HIV-1 Vaccine STEP trial due to the lack of efficacy and early speculation that the vaccine might have increased the risk of HIV infection in some populations of vaccinees. In late 2009, promising results came in from Thailand in response to their efforts to create a safe and effective vaccine against HIV-1. A community-based, randomized, multicenter, double-blinded, placebo-controlled efficacy trial using a prime-boost combination showed 31% effectiveness in preventing HIV-1 infection. These results lend promise to the hope of producing an HIV-1 vaccine vector that yields sterilizing HIV-1 immunity.

In the future, research scientists must work together to increase HIV-1 vaccine effectiveness beyond 31%. Realization of this goal may be accomplished by some of the techniques mentioned in this review, such as acquisition of HIV mucosal immunity, development of effective prime-boost strategies, development of better animal models, better molecular antigen modeling and presentation, avoidance of PEI (by the means of using novel vector serotypes in combination with PEGylation), and/or induction of Nabs (by means of capsid incorporation of HIV antigens within viral vectors). These are just a few considerations that scientists and clinicians must consider with respect to the development of an effective and safe HIV-1 vaccine. Scientists and clinicians must also consider that one vector or scheme may not be sufficient with respect to providing effective HIV-1 immunity and some combination of the above-mentioned potential strategies may offer the most promising method of producing an effective HIV-1 prophylactic vaccine.

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Disclosure

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