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BioDrugs. Author manuscript; available in PMC 2013 September 06.

Published in final edited form as:
BioDrugs. 2009 ; 23(3): 137–153. doi:10.2165/00063030-200923030-00001.

Progress on the induction of neutralizing antibodies against HIV-1

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Abstract

The Human Immunodeficiency Virus Type -1 (HIV-1), the causative agent of AIDS in humans, is one of the most catastrophic pandemics to affect human health care in the latter 20th century. The best hope of controlling this pandemic is the development of a successful prophylactic vaccine. However, to date, this goal has proven to be exceptionally elusive. The recent failure of an experimental AIDS vaccine in a phase IIb study named the STEP trial, intended to solely elicit cell mediated immune responses against HIV-1, has highlighted the need for a balanced immune response consisting of not only cellular immunity but also a broad and potent antibody response which can prevent the infection of HIV-1. This article will review the efforts being made up to this point to elicit such antibody responses, especially with regards to the use of a DNA prime-protein boost regimen which has been proven to be a highly effective platform for the induction of neutralizing antibodies in both animal and early phase human studies.

Since its discovery in the early 1980's, HIV-1 has been implicated in the deaths of more than 20 million individuals. It is estimated that more than 33 million people are currently harboring an active infection, many even without knowledge until later development of Acquired Immunodeficiency Syndrome (AIDS). With an estimated 2.5 million people infected in 2007 alone, spread of HIV-1 shows little signs of slowing [1]. The best hope of controlling this pandemic is an effective prophylactic vaccine. While it is generally believed that the development of both effective humoral and cellular immunity is required to provide protection against HIV-1 infection, there has never been a clear roadmap on how to achieve such a goal.

In the last two decades, a great deal of information and knowledge has been accumulated regarding the properties of various immune responses as observed in HIV-1 infection and studies of prophylactic vaccine development. Unfortunately, several late phase clinical trials of HIV-1 vaccine candidates have failed to provide any efficacy. At the same time, we also witnessed enormous progress in the induction of humoral and cellular immunities against HIV-1 that resulted from novel strategies of antigen design and vaccination approaches. These allow us to further investigate potential protective mechanisms and develop more effective vaccines to prevent the infection. The most recent phase IIb trial, the STEP trial, was a novel attempt to deliver an HIV-1 antigen using a non-replicating adenoviral vector, intended to prevent disease through the induction of a potent cellular immune response. While people are still debating whether the inadequate levels of cellular immunity may be responsible for the failure of this candidate vaccine, this outcome has highlighted the need

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for a balanced immune response consisting of not just cellular immunity, but also the inclusion of a broad and potent neutralizing antibody response.

Limitation of the T Cell-based HIV-1 Vaccines

In recent years, the focus of the HIV vaccine field has largely been on the induction of strong cell mediated immune responses against the virus. This is especially true for the large effort put forth in inducing strong cytotoxic T lymphocytes (CTL) responses directed against the virus. Focus on the induction of CTL responses was driven by a number of discoveries implicating $CD8^+$ T cells as vitally important in prevention and control of viral infection. Early work on the role of CTL responses in viral infection determined that the induction of CTLs is the primary correlate for the control of viremia in early infection [2, 3]. These findings were corroborated with the discovery that $CD8^+$ T cells were absolutely required to control SIV infection [4]. Additional evidence in human patients capable of controlling viral replication without therapy, so called "elite controllers", supported this notion further when strong and effective CTL responses correlated with viremic control in these individuals [5, 6].

The theory behind the design of a T cell vaccine is that the presence of a strong and immediate CTL response present at the time of viral exposure would, at a minimum, reduce viral loads in infected individuals by reducing acute viremia. This theory was supported by data indicating that strong CTL responses were shown to be capable of protecting against viral infection in SHIV protection models [7–9]. Because of the success in raising strong T cell responses and the protection seen in SHIV challenge models, the T cell vaccine appeared to be an attractive platform for vaccine development. However, despite this success in raising strong T cell responses and the protective capabilities of the vaccines when facing SHIV challenges, the effect of these vaccines on more highly pathogenic viral challenges was much less substantial [10, 11]. Furthermore, the entire T cell vaccine theory has been built on the "post-infection" protection model because it considers the induction of "sterilizing immunity" against HIV-1 as an impossible mission. Therefore, it is unfortunate, but not entirely surprising, that T cell-based vaccines, as shown in the STEP trial, despite being well tolerated and immunogenic in humans, ultimately proved ineffective in the best of cases and possibly detrimental in the worst. [12–15].

Challenges of Raising Antibody Based Vaccines against HIV-1

While there will be continued effort to improve the magnitude and breadth of T cell responses in future HIV-1 vaccine development, there has been a renewed focus on the induction of functional antibody responses to HIV-1 as a means to provide an early and even possibly sterilizing immune response. The induction of a strong functional antibody response, such as in the form of broadly neutralizing antibodies (NAb), is currently one of the most sought after goals in the field of HIV-1 vaccine development.

Unfortunately, HIV-1 contains an array of protective mechanisms which makes the elicitation of a broad and potent neutralizing antibody response an exceptionally difficult task. Much of the difficulty in raising functional antibody responses can be attributed to the high degree of diversity found in the envelope (Env) glycoprotein, the major target of neutralizing antibodies (NAb) to the virus [16]. In addition to the difficulty in overcoming high levels of sequence diversity, functional Nabs must also be able to overcome a series of intrinsic defenses present in the HIV-1 Env. This includes high levels of glycosylation, epitope masking by variable loops, cryptic binding domains, the high degree of entropy present in the Env protein, and masking of functionally important domains by quaternary interactions resulting from trimerization of the Env complex [17]. Additionally, because

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HIV, as a retrovirus, integrates into the host cell's genome, there exists only a very narrow window for neutralizing antibodies to act before the establishment of an infection

Despite all of the protective mechanisms the virus utilizes, a number of monoclonal antibodies (mAb) have been identified that are capable of neutralizing a relatively wide number of primary isolates [18]. To date, the only means of providing sterilizing immunity has been the passive transfusion of these mAbs before or shortly after viral challenge [19–24]. While relatively high levels of these antibodies were required to prevent infection, the success of these studies demonstrates that sterilizing immunity based on an antibody mediated mechanism is indeed feasible.

Raising Antibody Responses to HIV-1 antigens

A significant amount of work has been invested in raising high quality, functional antibody responses targeted to the HIV-1 virus. Much of this work has focused on two areas: 1) modulation of vaccine antigens and 2) the immunization regimens used to deliver these antigens. Early attempts to raise antibody responses to the virus primarily used HIV-1 envelope glycoproteins (Env) from T cell line adapted (TCLA) viruses. This was before the realization that there is a significant difference in the antigenicity of envelope derived from TCLA and primary HIV-1 isolates. One of the first such studies utilized the TCLA isolate IIIB in a chimpanzee challenge model to study the protective effects of subunit based immunizations. In this study chimpanzees received three immunizations of gp120 or transmembrane and cytoplasmic tail truncated "gp160" (gp140) formulated in aluminum hydroxide (alum). Based upon the presence of homologous neutralizing antibody responses exceeding 1:160 in their immunized animals, a challenge of homologous HIV-1 isolate IIIB was administered. Both of the gp120 immunized chimpanzees in this study remain free from viral infection six months after challenge [25].

The apparent success of this study and others [26, 27], led to the testing of TCLA based recombinant proteins in human clinical trials. Many of these trials, based on immunization with subunit Env derived from the TCLA isolates IIIB or MN, proved to be both safe and immunogenic [28–31]. These trials often succeeded in raising binding and neutralizing antibody responses against TCLA viruses similar to those seen in protected chimpanzees [30, 32, 33]. Based upon this information, two phase III efficacy trials were conducted, one in North America and the Netherlands [34] and a second in Thailand [35]. In the North American and Netherland locations more than 5400 individuals were enrolled to receive a bivalent vaccine consisting of two clade B rgp120s derived from the isolates MN and GNE8 adjuvanted in alum. All of the vaccinees studied generated positive binding antibodies to homologous Env and many generated a homologous neutralizing antibody response to the vaccine strain MN [36]. Despite this, there was no observed reduction in the rates of infection between the placebo and vaccine groups, indicating that the vaccine was not efficacious.

Similar results were also observed in the Thailand location of this trial. The Thailand arm of the study enrolled more than 2500 intravenous drug users and differed from its North American counterpart only in the chosen antigens. In an attempt to better represent circulating virus at the Thailand location, a bivalent formulation consisting of a clade B gp120, MN, and a clade E derived gp120, A244, was chosen. Again, gp120 binding antibodies and MN neutralizing antibodies were generated as a result of immunization, but no difference in the rates of infection existed between the placebo and vaccine arms of the trial [35]. The failure of these trials was actually predicted because in vitro neutralization assays performed before the start of these two trials already demonstrated that antibodies elicited by TCLA Env antigens, such as MN, were not able to neutralize primary HIV-1

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isolates. The failure of these vaccine trials may be in part due to the selection of antigens, specifically those derived from TCLA isolates, chosen for the formulation. However, it still remains to be seen whether a selection of antigens better representative of those in circulating populations may provide a protective response against viral challenge.

Raising the quality of the antibody response against HIV

Because of the proven ability of Nabs to provide sterilizing immunity in non human primate challenge models [19–24], an enormous amount of effort has been put into designing immunogens which are capable of eliciting a functional neutralizing antibody response (Table 1). These efforts have focused on a number of different techniques including, but are not limited to the manipulation of the envelope sequence, making structural modifications to the envelope, and increasing the immunogenicity of potentially important but poorly immunogenic epitopes.

Centralized Antigens

One of the most highly criticized elements of the two failed phase III trials was the antigen selection used in the vaccine, in particular the inclusion of the TCLA derived envelope MN. The MN isolate is not representative of the majority of isolates seen circulating in the global population. Because of this, it should be of little surprise that this particular isolate, when used as an immunogen, did not elicit a broadly cross reactive immune response. In an effort to design an immunogen more representative of the isolates more likely to be observed in an *in vivo* setting, the use of consensus or centralized envelope sequences has garnered some attention in recent years. One such study generated an artificial envelope antigen based on the 5 constant regions of gp120 (C1 to C5) and the V3 loop of the group M consensus sequence [37]. Both gp120 and gp140 forms of this consensus M protein were investigated as immunogens in a guinea pig model. After five immunizations of either the gp120 or gp140 proteins administered in Ribi-CWS adjuvant the resulting sera were capable of neutralizing the sensitive isolates SF162 and Bx08 to high titer. Additionally, positive neutralizing antibodies against more difficult to neutralize isolates, SS1196 and QH0692 were also generated. V3 peptide adsorption performed in an attempt to identify the specificity of neutralizing antibodies elicited with this construct demonstrated that while sensitive isolates were primarily being neutralized by the antibodies to the V3 loop, other unknown Ab specificities were elicited that were largely responsible for neutralization of the more resistant primary isolates.

Based upon the first consensus M immunogen’s ability to raise a functional, but limited, antibody response, a second generation group M consensus envelope was also tested [38]. As opposed to the first generation construct, this envelope encoded the consensus sequence for the entire envelope, as opposed to primarily the constant regions. Some modifications to the variable loops of the envelope were made as a result of the interpretation of the consensus sequences by the authors. These modifications resulted in the variable loops being slightly shorter than the average wild type envelope. Protein from different gp140 constructs of this consensus M gene when administered in Ribi adjuvant, successfully broadened the neutralizing antibody response when compared to immunization with wild type sequences derived from the primary isolates JR-FL (clade B), 92RW020 (clade A), or 97ZA012 (clade C). However, the overall breadth was still very limited, with the sera still unable to neutralize the majority of prototypic HIV isolates. When this serum was tested for neutralizing specificity, a large proportion of neutralizing activity could be adsorbed with V3 peptides. Because the V3 loop may not be accessible on a large fraction of primary isolates, the large percentage of V3 directed antibodies potentially explains the limited breadth of neutralization observed when immunization with this antigen.

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In attempts to focus the antibody response more on a single subtype, subtype C ancestral and consensus genes have also been generated [39]. When these immunogens were administered as three DNA immunizations in guinea pigs, the resulting sera was capable of recognizing a greater breadth of contemporary clade C antigens than sera generated by immunization with a wild type clade C immunogen. Despite the increase breadth of cross reactivity, however, very little homologous or heterologous neutralization was observed. Only one of the immunized animals generated antibodies capable of neutralizing its homologous isolate, while none of the animals generated antibodies capable of neutralizing heterologous primary isolates. Similar studies have also been completed with subtype B ancestral [40] and consensus immunogens [41]. The use of the subtype B consensus immunogen was evaluated in guinea pigs after three DNA immunizations encoding the different forms of the consensus B envelope gene. Elicited humoral responses were then compared to that of guinea pigs immunized with the wildtype isolates CAAN5342.A2 and WITO4160.27. In this study, the consensus B immunogens elicited neutralizing antibodies to isolates with a range of sensitivities including, SF162, SS1196, and a subset of viruses representative of those found in acute infection, so-called tier 2 viruses [42, 43], a phenomenon not observed with immunization of the wild type immunogens. Based upon the successful neutralization of a number of isolates, the authors continued in an attempt to identify the mechanism of neutralization they were observing. An HIV-2 virus either pre-exposed to CD4 or containing a graft of the MPER region of gp41 was used as a means to identify the presence of co-receptor or MPER targeted neutralizing antibodies. However, none of the immunized animals generated antibodies with these specificities.

Variable Loop Deletions

Generation of centralized immunogens among a large group of viral isolates is only one approach to enhancing the immunogenicity of HIV constructs. Others strategies have focused on structural modification of existing HIV-1 Env antigens. It has been well established that variable loops can protect functionally important domains [44–47]. Theoretically, deletion of variable loops should expose these functionally important domains allowing antibodies to be elicited to the previously obscured regions. Operating under this theory, a number of studies have modified the wild type envelope by deleting variable loops. One of the first such studies to investigate the role of variable loop deletion in altering the immunogenicity of a parental envelope evaluated the effects of V1/V2 and V3 deletions on the immunogenicities of several forms of the HXB2 envelope [48]. This study evaluated the wild type and variable loop deleted forms of gp120, gp140, and gp160 immunogens delivered by DNA immunizations for their ability to raise binding and neutralizing antibodies in rabbits. Results from this study indicated that variable loop deletion of the gp140 and gp160 constructs increased the amount of binding antibodies elicited to the gp120 subunit of the envelope. Despite the increase in binding titers however, the immunization with the wild type gp120 subunit was still the most effective at eliciting binding antibodies. Neutralization of the sensitive isolate IIIB was also evaluated in this study. Immunization with the strictly wild type gp120 elicited the highest neutralizing antibody titers against this isolate. The elimination of the variable loops from this construct had a detrimental effect however, eliminating all observed neutralizing activity. This is likely due to the extreme sensitivity of this isolate to variable loop mediated neutralization. In addition, although the variable loop deletions increased binding titers in the gp140 and gp160 immunized animals, they still did not manage to neutralize the IIIB isolate in any cases.

Another of these studies looked at the effect of elimination of only the V2 loop of SF162 on its ability to raise a humoral immune response [49]. In this study, rabbits were given DNA immunizations encoding a full length SF162 gp140 immunogen, or one with a partial deletion of the V2 loop. Raised antibody responses were then tested for neutralization

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breadth and potency against a panel of homologous and heterologous isolates. When tested against the homologous SF162 isolate, more potently neutralizing sera was raised by immunization with the V2 deleted construct. Additionally, neutralization was also seen more frequently and with higher titers against six other heterologous clade B isolates. This pattern was also observed in rhesus macaques immunized with the same constructs followed by a protein boost of homologous V2 deleted gp140. Again, more broad and potent neutralizing antibody responses were raised when the V2 deleted construct was used as an immunogen compared to the wild type SF162 immunogen.

Further study of V2 deletions characterized the changes in antibody specificities elicited compared to immunization with the wild type envelope [50]. Interestingly, immunization with the V2 deleted construct resulted in an increase in targeting to the V3 loop of SF162 as well as a modulated ratio of serum antibodies capable of being outcompeted by sCD4 binding to gp120. The utility of V2 deletions in subtype C immunogens has also been evaluated [51]. Using the viral envelope TV1 as a model subtype C immunogen, a comparison of the immunogenicity of unmodified and V2 deleted immunogens were made. Similar to the results seen with SF162 immunogen, an increase in the potency of homologous neutralization was observed when the V2 deleted constructs were used. Additionally, an increase, albeit very limited one, in the breadth of neutralization against heterologous clade B and C viruses was also observed with the use of this V2 deleted gp140.

Characterization of humoral responses raised by wild type and variable loop deleted gp140 constructs was also performed in comparison to the humoral responses seen in chronic SHIV infections in macaques and heterologous HIV infection in humans [52]. Interestingly, the quantity, quality, and specificity of antibody responses differed greatly between the groups. In gp140 immunized animals, the gp120 subunit of the immunogen appeared to be more immunogenic than the gp41 subunit. This trend was not observed in SHIV infected macaques or HIV infected humans where gp41 was equally if not more immunogenic than gp120. Additionally, overall binding titers were also significantly lower in immunized animals compared to infected ones. Another significant difference between immunized and infected animals involves the neutralizing specificity of the serum. While the variable loop modified constructs were very capable of neutralizing homologous SF162 virus, it was discovered that this is largely due to recognition of the V1 loop, a phenomenon not observed in infected animals. While this area is accessible on most HIV viruses, it is highly polymorphic, potentially explaining the limited neutralization breadth that is observed with these gp140 immunizations.

More drastic modifications to the HIV envelope have also been made. The removal of the V1/V2, V3, and V4 loops alone and in combination on an HXBc2 background have also been investigated [53]. While immunization of all of these constructs resulted in high binding titers to recombinant gp120, the best NAb titers resulted from immunization with the wild type Env protein. Immunization with the V1/V2 and V3 deleted constructs elicited an antibody response with little to no neutralizing activity. Epitope mapping analysis revealed that, expectedly, deletion of the variable loops can shift the targeting of elicited antibodies. However, oftentimes the shift in recognition is to areas that are not exposed on the surface of the protein. Because of results such as this it is likely that simple deletion of variable loops will not provide the necessary increase in potency and breadth of neutralization to effectively combat an infection.

Glycosylation Mutants

While immunizing with variable loop deletions have resulted in some increases in the quality of the antibody response, other less drastic alterations to the viral envelope have also

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been evaluated for their effect on immunogenicity. One such modification is the alteration of the glycosylation pattern on the surface of the HIV-1 Env. The surface of the Env is very highly glycosylated, with carbohydrates encompassing up to 50% of the total molecular weight of the protein. It has been well documented that changes in the glycosylation pattern of the envelope protein can have significant effects on the antigenicity of the envelope and neutralization sensitivity of the parental virus [54–59]. Because changes in the glycosylation pattern of these envelopes can have a drastic effect on their phenotype, it may also be possible that altering the glycosylation pattern could be used to modulate the immunogenicity of the protein. One potential use of changing the glycosylation patterns on the envelope is to dampen immune responses to undesirable epitopes. Because it is relatively difficult to raise antibodies to sugars that should rightfully be identified as “self” by the immune system, the addition of glycans in unwanted areas should have the effect of focusing the humoral response to desirable areas of the envelope. Efforts have been made to this extent in attempts to focus antibodies to the CD4 binding site [60]. In this study, the addition of seven extra glycans eliminated binding of the undesirable nonneutralizing antibodies, 15e, b6, b3, F91, and F105 while preserving the binding site of the broadly neutralizing CD4 binding site antibody IgG1 b12. Use of this immunogen in rabbits however ended with mixed results. Rabbits immunized with this construct in Ribi adjuvant generated positive binding antibodies to wild type Env protein, but raised a highly limited neutralizing antibody response [61]. Analysis of the immune serum also revealed very limited amounts of antibody with a specificity similar to that of mAb b12, an antibody specificity which this construct was intended to enhance. The neutralization results mirror this with sera generated from this construct often incapable of neutralizing even the highly sensitive isolates SF162 and HXBc2. As intended, immunization with the hyperglycosylated mutant did have the effect of dampening the elicitation of most of the weakly neutralizing antibodies similar to b6 and F105. Unfortunately, it did not succeed in eliciting b12 like antibodies. This in combination with lower levels of V3 crown directed antibodies may potentially explain the disappearance in neutralizing activity in this type of sera.

The concept of dampening immune responses to unwanted areas through hyperglycosylation was continued with the use of a hyperglycosylated trimeric gp140 constructs [62]. Trimerization of a hyperglycosylated gp140 construct through the use of a heterologous trimerization domain resulted in significantly reduced availability of the V2 and V3 loops as measured by monoclonal antibody binding. This was reflected in the immune sera resulting from protein based immunizations with this construct. Neutralization of the sensitive isolate SF162 was dramatically reduced as a result of fewer antibodies being elicited to the variable loops of the virus. In the context of dampening the immune response to unwanted areas this strategy succeeded, however within the context of focusing the antibody response to more desirable areas, such as the CD4 binding site, this strategy still needs to undergo further development.

Hyperglycosylation of the envelope to dampen immune responses to a particular region is only one strategy involving the manipulation of glycosylation sites on the envelope protein. Another strategy involves the elimination of particular glycosylation sites in order to enhance the immunogenicity of the envelope. One such attempt at this eliminated N linked glycosylation sites in the first and second variable loop of an infectious SIV isolate. This glycosylation mutant isolate was then used to infect rhesus monkeys. The resulting humoral responses were then compared to those elicited by infection with the parental wild type virus [63]. Relative to humoral responses raised by the wild type virus, the mutant glycosylated virus demonstrated a shift in specificity to the degycosylated region of the V1/V2 loop as well as an increase in the neutralizing activity of the sera. A second study determined that the elimination of a single N linked glycan at the stem of the V2 loop in a 89.6 background could have dramatic effects of the phenotype, antigenicity, and immunogenicity of a model

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immunogen [64]. Immunization of macaques with a vaccinia vector encoding the glycan deleted construct, followed by boosting with recombinant protein dramatically increased the potency of neutralization to homologous mutant and parental virus, as well as increased the breadth of neutralization against a panel of heterologous clade B derived primary isolates. However, whether these results will hold true in other envelopes, or if this specific to the 89.6 background remains to be seen. A number of other studies have attempted similar mutational studies, eliminating N linked glycosylation sites, however in each of them it was shown that the mutants were no better immunogens than their parental virus [65, 66].

Envelope Trimerization

The native envelope spike on an HIV virus is a structure consisting of three subunits each of gp120 and gp41. It is possible that an effective immunogen may need to mimic this trimeric structure in order to elicit an effective neutralizing antibody response. The creation of a trimeric mimic has proven to be a difficult task, however, owing mostly to the fact that gp120-gp41 and gp41-gp41 interactions on the surface of a virion are governed only by weak noncovalent interactions. Because this limitation makes the production and evaluation of trimeric immunogens a difficult task, a number of strategies have been employed in attempts to overcome this hurdle. One such strategy that has been employed is to eliminate the cleavage site that would normally result in the processing of the precursor gp160 into its mature gp120 and gp41 components. Further modification of this construct by elimination of the transmembrane and intracellular tail of gp41 results in a relatively stable trimeric construct that can be used for immunogenicity studies. One such study that used this strategy immunized rabbits with a monomeric gp120 or trimeric gp140 construct derived from the HIV-1 IIIB envelope in Ribi MPL-SE adjuvant [67]. The resulting antibody response was capable of binding gp120 and gp160 constructs from homologous and heterologous isolates. An increase in the potency of the neutralizing antibody response in animals immunized with the trimeric gp140 construct was also observed against the TCLA viruses NL4–3 and MN. When this serum was tested against more prototypical primary isolates however, no neutralizing activity was observed. Despite this, the trimeric gp140 constructs were also tested in macaques. The resulting sera were again capable of neutralizing TCLA strains of HIV. Epitope mapping analysis of the sera revealed that usually more than 50%, and as high as 77%, of the neutralizing activity could be adsorbed using V3 peptides. The predominance of V3 directed antibodies, and the limited exposure of this loop in primary isolates could potentially explain why so little neutralization of primary isolates was observed.

Other studies have also used the strategy of eliminating the cleavage site between gp120 and gp41 in an attempt to increase the yield of oligmers produced. One of these compared the immunogenicity of monomeric gp120 to oligomeric gp140 derived from the CD4 independent isolate R2 [68]. After four protein based immunizations in the very powerful AS02A adjuvant, antibody responses were tested for neutralizing activity against a large panel of primary isolates from clades B and C. In groups of three rabbits, at least two of three rabbits that received gp140 based immunizations were capable of neutralizing all but one of the viral isolates tested. In comparison, two of three rabbits that received monomeric gp120 based immunogens were only capable of neutralizing nine of the forty six isolates tested.

A second means used to stabilize the trimeric interaction and study its immunogenicity is through the addition of heterologous trimerization domains to the HIV envelope. One example of this introduced the GCN4 or foldon trimerization domain into the Yu2 gp140 background. Immunization with this trimerized construct was initially shown to be effective at raising a more potent neutralizing antibody response in mice [69]. Vaccination of these trimeric constructs using a wide variety of adjuvants in guinea pigs generally increased the

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potency of neutralization against selected homologous and heterologous clade B isolates [70]. However, it did little in expanding the breadth of neutralization of isolates when compared to immunization with monomeric gp120. Analysis of this neutralizing activity revealed that gp120 immunized animals had neutralizing activity directed primarily towards the V1 loop of the Yu2 virus with additional neutralizing activity directed towards the V3 loop. Interestingly, immunization with the gp140 constructs redirected neutralizing activity away from the V3 and V1 loops to other areas of the virus. Trimerized Yu2 gp120 constructs were also generated using the GCN4 motif and tested in rabbits [71, 72]. The general pattern of an increase in potency of the neutralizing activity of the sera was again observed in this study. Interestingly, this study also looked at the effects of stabilizing the Yu2 gp120 in a CD4 bound state in its trimeric form through site directed mutagenesis. The additional modifications of stabilizing the core in its trimeric form increased the potency of the elicited neutralizing antibody response even further. However, the overall breadth of neutralization was not increased appreciably against the more difficult to neutralize isolates JR-FL and TRJO.58. Also interesting to note was that the specificity of this neutralizing activity differed between the two studies. In guinea pigs immunized with the trimeric structures most neutralizing activity could be attributed to V1 recognition, while in rabbits immunized with a similar construct almost no neutralizing activity could be attributed to V1 reactivity.

Other attempts to induce trimerization of the envelope have also been successfully made. Instead of the introduction of heterologous trimerization motifs, the stability of a normal envelope trimer has been enhanced through the introduction of disulfide bonds to stabilize the gp120-gp41 interaction and mutations in the gp41 region have been made to stabilize gp41-gp41 interactions [73, 74]. These modifications allowed the trimer to be cleaved normally, while still maintaining a stable trimeric interaction. In a study evaluating the immunogenicity of this disulfide bond stabilized construct, high titer binding antibody but only weak neutralizing activity was observed [75]. Specifically, the homologous JR-FL isolate, from which the immunogen was generated, was neutralized only sporadically upon immunization with this construct. Neutralization of the TCLA isolate MN was also evaluated in this study. Sera generated through immunization with this construct frequently neutralized the virus with high titer, however there was no trend for higher neutralizing antibody titers being raised in the animals immunized with the trimer. A second study also evaluated the effects of trimerization by immunizing rabbits with a trimerized construct resulting from the deletion of the gp120-gp41 cleavage site, a trimerized construct based on an intermolecular disulfide bonds, or immunization with monomeric gp120. Again in this study there was little improvement in the functional antibody response elicited by the trimeric proteins. Sporadic neutralization of the homologous isolate JR-FL was seen in trimer immunized animals, as well as sporadic neutralization of the sensitive isolate BaL. However, almost no neutralization of resistant primary isolates was observed. Analysis of neutralizing sera in both of the above studies indicated that neutralizing activity was not directed towards the V1V2 loops of the virus or the MPER region. Only limited amounts of neutralizing activity could be assigned to the V3 loop as well, leaving the exact specificity of any neutralizing activity that was observed largely undefined.

Gp41 targetting (epitope grafts)

A series of neutralizable epitopes are found in the membrane proximal external region (MPER) of gp41. However, this region of gp41 has been shown to be poorly immunogenic. In efforts to increase the immunogenicity of this region, the linear epitopes in the MPER region of gp41 recognized by broadly neutralizing antibodies, specifically 2F5 and 4E10, have been grafted into other areas of the envelope. In one such study, transplantation of the 2F5 epitope into either the V1, V2, V3, or V4 loop was tested [76]. DNA based

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immunizations of mice with these grafted constructs, generated positive binding antibodies against this MPER epitope when it was placed in either the V1 or V3 loop. However, immunization of guinea pigs failed to raise positive antibody titers to the intact MPER when positioned in these same loops. Interestingly, repeated immunizations of the MPER graft in the V2 loop raised positive antibody responses targeted to the grafted epitope in guinea pigs. Despite the positive recognition of this epitope however, no MPER based neutralization was observed against the virus IIIB.

The immunogenicity of the MPER epitope grafted in to the V1/V2 loop was also assessed in mice and rabbits [77]. This grafted region was further manipulated by the addition or deletion of residues flanking the epitope in order to manipulate the exposure of the helix. These grafts were proven capable of binding the MPER directed antibody 4E10, and their immunogenicity in rabbits was assessed. Again, while many gp120 binding antibodies were generated in this study, no neutralizing antibodies targeted to the MPER region were detected.

Anti-Idiotypic Immunogens

Yet another interesting approach to the focusing and generation of an effective antibody response is to use anti-idiotypic antibodies as immunogens to focus the antibody response on a desirable domain. Anti-idiotypic antibodies capable of binding a CD4 binding site directed fraction of human sera from HIV infected individuals has also been evaluated as immunogens [78]. This study enriched a fraction of CD4 binding site directed antibodies from four long term non progressors and used this fraction to generate anti-idiotypic monoclonal antibodies in mice. Two monoclonals were generated that were capable of binding b12 and were subsequently used in their Fab form adjuvanted in IFA as immunogens in rabbits. The best of the two Fab immunogens, P1, was capable of neutralizing the sensitive isolate HxB2 in three out of five rabbits. However, no data on the neutralizing activity of this sera against more representative primary isolates is given.

Vaccination Approaches for Delivery of HIV-1 Env antigens

The ultimate goal of raising a strong antibody response against HIV-1 is to prevent HIV infection. The design of an effective antigen to be used in a vaccine is only one part of the overall effort to generating a protective antibody response. A second, equally important task is to optimize the delivery method that an optimal Env antigen can be administered to humans so that a successful antibody response can be raised. To pursue this goal a number of different strategies have been implemented (Table 2). The use of traditional subunit protein vaccine was attempted first, the details of which have been discussed above. However, the sheer difficulty in raising effective antibody responses against the virus has necessitated the use of novel immunization approaches.

Viral vector based vaccines

One of these novel approaches is to use viral vectors to deliver HIV-1 antigens. One such application of this approach has been evaluated in a phase I human trial using an adenovirus vector to deliver HIV-1 Env antigens [79]. This study used an adenovirus delivery system made replication incompetent through the deletion of the E1 and E4 region as well as the part of the E3 region of the viral genome. Inserted into the virus were genes encoding a Gag-Pol fusion protein, intended for elicitation of T cell responses, as well as three gp140 Envelope genes each derived from a single Clade, A, Clade B, and Clade C isolate. Four weeks after immunization, 93% of individuals were capable of recognizing the homologous clade B antigen by IP-Western blot. When antibody responses were measured by ELISA however, only 50% of the individuals were capable of recognizing one of the three antigens

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used in vaccine formulation. Despite the positive binding titers induced in some individuals, no neutralizing activity was detected against the highly sensitive isolate SF162, or the TCLA isolate HXB2. Therefore, while this vaccine proved to be relatively safe, it failed to generate a highly immunogenic humoral response against even very sensitive HIV-1 viruses.

The use of a canarypox virus to deliver HIV antigens has also been evaluated [80]. In this phase II human trial, uninfected individuals were immunized with the canarypox vector vCP1452, encoding the gp120 protein of MN fused to the gp41 region of the HIV isolate LAI, plus the entire *gag* gene and CTL epitopes derived from the nef and pol proteins. The canarypox immunization was either administered alone, or boosted with a subunit protein boost of a bivalent formulation of gp120 derived from HIV-1 isolates MN and GNE8 for a total of four immunizations. Positive binding antibodies were raised against the Gag protein in 23–36% percent of individuals depending on the immunization group. More relevantly however, between 70% and 83% of individuals raised neutralizing antibody responses against the homologous isolate MN. Notably, individuals that received only canarypox based immunizations elicited lower titers of neutralizing antibody against MN. Neutralization of the heterologous isolate IIIB was also evaluated in a limited number of samples. When heterologous neutralization was taken into account, those individuals who received only the canarypox based immunization fared significantly worse than those who received a subunit protein booster. Specifically, the individuals assayed who received only canarypox immunizations never successfully neutralized IIIB, while individuals who received a subunit protein boost were able to neutralize the virus in 70% of cases. The generation of antibody responses capable of neutralizing TCLA strains of virus using a similar canarypox prime-protein boost immunization regimen has also been mirrored in a number of other studies, demonstrating the utility of a heterologous prime-boost regimen [81–84]. Despite this ability, the overall quality of antibody responses in this trial does not appear to be better than those reported in trials conducted with strictly subunit protein based immunizations. Notably, the ability of individuals to neutralize a significant number of neutralization resistant primary isolates has yet to be demonstrated in these trials.

DNA vaccines

The sole use of DNA based immunizations to raise HIV-1 specific antibody responses has also been tested in human trials. In one trial, three DNA immunizations encoding three envelope antigens, one each derived from clades A, B, and C, as well as the T cell antigens, Gag, Pol, and Nef were given to healthy human volunteers using a needle free injection system[85]. Antibody responses from this trial were then evaluated by ELISA and neutralizing antibody assay. Humoral responses recognizing the clades A and C envelopes were generated in 71% of individuals with the clade B being recognized in 64% of individuals in the trial. Despite the presence of binding antibodies, functional, neutralizing antibodies were entirely lacking. None of the vaccinated individuals generated neutralizing antibodies against the sensitive HIV-1 isolate MN, indicating the overall lack of immunogenicity of this approach in generating a strong antibody response.

These results are mirrored by second human DNA only vaccine trial [86]. This trial delivered DNA encoding a Gag-Pol-Nef fusion protein plus modified envelope constructs derived from clades A, B and C via a needle free injection system. Similar to other DNA only trials, binding antibodies as determined by ELISA were raised in 60% of individuals. However again, a total lack of neutralizing antibody responses were raised against the sensitive HIV-1 strain MN. A third trial utilizing only DNA based immunizations encoding Gag, Pol, Env, Rev, Tat and Vpu delivered by a tradition needle based intramuscular injection also failed to produce any detectable NAb titers against the HIV-1 isolates ADA or MN [87]. The sole use of DNA based immunizations in humans has highlighted the fact that

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as a whole, the DNA vaccine is not very immunogenic by itself. However, it is puzzling that the immune sera can not neutralize even highly sensitive HIV-1 isolates when positive binding antibody responses were clearly identified. One possible explanation is the use of a modified Env antigen (gp145) [88] in these DNA vaccine alone studies [86]. It has multiple deletions in gp120 and gp41 domains [88], resulting in most of the construct remaining cell associated. The combination of these mutations may have adversely affected critical envelope conformation that is required for eliciting neutralizing antibody responses.

DNA vaccine prime – viral vector boost

Based upon the limited ability of vaccines utilizing a single modality to raise an effective antibody response against HIV-1, combinations of heterologous immunization approaches have also been attempted. One such study used a DNA prime and adenovirus boost to elicit cellular and humoral immunity in rhesus macaques [89]. This study used DNA and rAd5 viruses expressing three HIV envelopes from clades A, B, and C, either alone or in combination as well as a fused Gag-Pol-Nef construct intended to raise cell mediate immunity. While this study generated strong cell mediated immune responses to the virus, functional antibody responses were still somewhat lacking. Positive binding antibody titers were raised in immunized animals as well as positive neutralization of the sensitive isolates HxB2 and SF162. Inhibition of three clade A isolates, UG29, UG031, and 44951 by 50% was observed in animals receiving a combination of all three envelope immunogens. However, 50% inhibition of the majority of primary isolates from clades B and C was entirely lacking. Despite the low level of neutralizing antibody responses, immunization did demonstrate positive effects after an 89.6P challenge. Immunized animals demonstrated better control of viral infection as well as better preservation of the CD4+ T cell compartment.

Other studies evaluating the elicitation of antibody responses using combinations of DNA and adenoviruses have also been performed [90]. In this study immunization of rhesus macaques with a chimeric HxBc2/BaL gp145 construct delivered either by a DNA prime-adenovirus boost or strictly repeated immunizations with rAd was evaluated. Immunization with a single Ad5 vector generated higher binding titers against the gp140 protein compared to immunization with only DNA vaccine. However, repeated boosting of the rAd5 immunized animals with addition rAd5 virus did not enhance the antibody response. In contrast, the DNA primed animals when administered a rAd5 boost demonstrated a rapid rise in envelope binding titers. Neutralizing activity raised by the two immunization approaches was also evaluated. Neutralization of the 89.6 isolate was found to be significantly greater in animals that first received a DNA prime, indicating the superiority of this combination immunization approach relative to immunization with only rAd5. However, the breadth of neutralization using the DNA prime rAd5 boost format was still somewhat limited, with only about a third of tested clade B isolates being neutralized by sera generated from immunization with either an 89.6 or chimeric HxBc2/BaL construct.

DNA Prime – Protein Boost

The use of DNA vaccines to raise humoral responses against HIV-1 was first seen in the early nineties where it was shown that a DNA plasmid encoding HIV-1 Env derived from TCLA was capable of raising HIV-1 specific antibody responses in small animals [91, 92]. The antibodies raised by this approach were capable of both binding recombinant Env protein as well as neutralizing the HIV-1 isolate IIIB. The utility of this approach was further demonstrated in a SHIV challenge model in cynolomogous macaques [93]. In this study, animals that received DNA immunizations generated a strong immune response that resulted in a lowered viral load compared to unimmunized animals. Additionally in this

study, one of four immunized animals was protected from viral challenge upon completion of the DNA immunization regimen.

Other than its obvious ability to generate an immune response, there are a number of positive aspects of DNA immunizations which make them an attractive option for use as a platform for an HIV-1 vaccine. The first of these is the endogenous production and processing of a chosen antigen. When a DNA immunization is given, antigen encoding plasmids are taken up directly by cells at the injection site of the host, thereby making antigen production similar to that of a live attenuated vaccine. This allows the protein to undergo well regulated translation processes allowing for native folding, as well as normal post translational modifications, such as glycosylation, of the antigen of interest. Additionally, because of the endogenous production of the antigen, the produced protein can be efficiently presented to the immune system through class I and class II MHC complexes, allowing for an efficient T cell response to the antigen. Furthermore, the DNA vaccine has also proven to be a very safe alternative to subunit and live attenuated vaccines [94–98]. Because DNA vaccines are normally non-replicative, non integrative, and can only encoded the protein(s) of interest, DNA vaccines allow the researcher to elicit an antibody response with the specificity of a subunit vaccine and the native antigen processing of a live attenuated vaccine, all without the safety risk of reversion of an attenuated viral strain into a more pathogenic one.

In addition to its relative safety, DNA base immunizations provide an excellent platform for studying different properties of a particular antigen , screening of different immunogens [99, 100], identifying immunogenic and neutralizing domains of a target [101], as well as identifying effective immunization regimens [102].

Despite the DNA vaccine's ability to generate an immune response against model antigens, a number of caveats still existed. This includes relatively low in vivo transfection efficiency leading to low levels of antigen production. Because of this, a significant effort has been applied in order to increase the potency of DNA vaccines. This includes studying different delivery mechanism for the DNA itself. These methods include electroporation [103, 104], needle free jet systems [105–107], gene gun [104, 107], and microneedle injections [108], all of which are intended to increase the efficiency of DNA delivery over a traditional intramuscular injection [104].

Increasing the efficiency of DNA delivery is only one aspect in the effort to increase the potency of DNA immunizations. Work has also focused on the design of the DNA constructs themselves in order to enhance antigen production on the level of the individual transfected cell. One of the primary improvements that has been made was the advent and implementation of codon optimization to maximize the efficiency of tRNA usage in the cell [109–111]. Optimizing each codon to utilize the most prevalent tRNA present in the cell allows for more efficient protein translation, resulting in a higher quantity of antigen being produced. Other work to increase the amount of antigen produced has focused on manipulating the leader sequences and promoters of these constructs [110]. The simultaneous manipulation of all of these factors in a potential construct was shown to improve the immunogenicity of a gp120 protein in a mouse model.

Despite improvements in the design of the DNA construct and the increase in efficiency in the delivery, a DNA immunization is still only capable of producing limited quantities of antigen at levels much lower than that delivered by inactivated or subunit vaccines. Because of this, as witnessed in DNA vaccine alone human HIV-1 trials, it is not immunogenic enough on its own to generate an effective antibody response against the virus. Despite this, DNA immunizations are highly effective in priming the body's immune system and works

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best when used in combination with another immunization approach, usually with DNA administered as a priming immunization followed by a boost of other modality. One of the simplest and most effective of these combination approaches for the elicitation of humoral immunity is a DNA prime followed by a traditional subunit protein boost [112].

Early studies using the DNA prime-protein boost approach utilized TCLA derived Env proteins in the vaccine formulation [113]. Rabbits in this study were immunized with DNA based immunizations encoding gp120, gp140, or a replication incompetent form of HXB-2 and subsequently boosted with rgp160 derived from the HIV-1 isolate IIIB in incomplete Freund’s adjuvant (IFA). While only limited binding antibody was generated after the DNA immunizations, boosting with recombinant protein greatly increased binding titers in immunized animals. Analysis of serum avidity elicited by each immunization regimen indicated that use of a combination approach elicited a higher avidity antibody response than with the use of DNA immunizations alone. The combination DNA prime-protein boost approach generated homologous neutralizing antibody titers significantly greater than that observed with immunization of naïve animals with only recombinant protein. A heterologous neutralizing antibody response against MN and SF2 were also generated using this prime boost immunization regimen. Titers in immunized animals varied from 1:148 to exceeding 1:3000 against MN and 1:37 to 1:269 against SF2. However, in agreement with later studies, the use of a TCLA derived immunogen, was shown incapable of generating a heterologous neutralizing antibody response against more difficult to neutralizing primary isolates.

Extensive ground work has been done demonstrating that a DNA prime-protein boost strategy is an effective means by which to raise antibody responses in both small animals and non-human primates [114, 115]. However, many of these studies suffered from the inability to neutralize the more relevant primary isolates of HIV-1. One breakthrough, in an attempt to overcome the limitations of TCLA derived immunogens, used the gp120 derived from the primary isolate JR-FL as a model immunogen [116]. In this study rabbits were immunized in either a DNA prime-protein boost format, or with only recombinant gp120 protein derived from the primary isolate JR-FL. Sera generated by both immunization approaches contained high levels of binding antibody to homologous envelope, but the neutralizing antibody response generated by each immunization regimen differed dramatically. One example of this was observed with the neutralization of the sensitive isolate SF162. Both immunization regimens were capable of generating a neutralizing antibody response against this sensitive isolate, however the DNA primed animals did so with much higher titer. Additionally, animals that received a DNA prime were capable of neutralizing the homologous strain of HIV-1, JR-FL, in a PBMC based neutralization assay. Inhibition of this neutralization resistant primary isolate was not observed in animals that were immunized with only protein. Additionally, sera raised by the DNA prime-protein boost approach were also frequently capable of neutralizing other heterologous clade B isolates including 1196 and 0692.

The superiority of the DNA prime-protein boost approach was proven again concurrently in a separate study looking at the effect of oligomerization state on the elicitation of neutralizing antibodies [75]. Here, the use of a DNA prime-protein boost approach elicited a 12 fold higher binding antibody response when compared to immunization with only protein, clearly demonstrating the potential of this platform for eliciting a functional antibody response to the virus. A third example of the effectiveness of the DNA prime-protein boost vaccination approach using monomeric gp120 immunogens generated a consistent NAb response against neutralization resistant primary isolates [75]. While the use of the DNA prime-protein boost approach was able to enhance the binding and neutralizing antibody response elicited when compared to immunization with subunit protein, the overall

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breadth of neutralizing activity was still somewhat limited. The most likely explanation for this phenomenon was the use of only a single, subtype B envelope in the vaccine formulation. One attempt that was made to increase the breadth of the neutralizing activity was to include multiple, genetically distinct envelopes into a single polyvalent formulation [117]. In this study, rabbits were immunized in a DNA prime-protein boost format consisting of either monovalent or polyvalent formulations of gp120 derived from clades A, B, C, D, E, F, and G. Sera generated by immunization with these constructs were then tested in a pseudovirus based neutralization assay against a panel of 14 viruses from clades A-E.. Data from this study revealed that immunization with a polyvalent formulation significantly increased the breadth of neutralization against this multiclade panel, nearly doubling the number of isolates neutralized compared to the monovalent immunization groups [117].

The success of the DNA prime-protein boost immunization format further showed its promise in non human primate studies. To this extent, a modification of the polyvalent formulation above, consisting of two clade B gp120s, one clade C gp120, and one clade E gp120, plus Gag derived from NL4–3 was tested for its protective efficacy in rhesus macaques[118]. Animals received a combination of DNA and protein based immunizations and were subsequently rectally challenged with the R5 SHIV BaL. At the time of challenge, animals had generated an antibody response capable of neutralizing the sensitive isolates of HIV, MN and SF162, as well as the challenge strain of BaL. Immunization with the above regimen provided sterilizing immunity to four out of six macaques from the SHIV challenge, based upon detection of viral RNA in the blood. Relative to the control group of seven naïve animals who all became infected and demonstrated high viral loads, the remaining two immunized macaques that became infected demonstrated lower levels of viral RNA in the blood. Because Gag antigen is from an HIV-1 isolate while the virus received a SHIV challenge, the protection is clearly mediated by Env-induced immunity, most likely due to anti-Env antibodies.

Other studies have also confirmed the utility of the DNA prime-protein boost approach in nonhuman primates. One of these studies used this prime-boost approach in neonatal macaques [119]. Immunization of animals in this study with DNA encoding vpu and the IIIB envelope, followed by boosting with recombinant IIIB derived gp160 protected 4 of 15 animals from a homologous IIIB intravenous SHIV challenge. Another study utilizing the DNA prime-protein boost approach immunized rhesus macaques with gp120 or gp160 forms of envelope derived from HXBc2 [115]. Homologous neutralizing antibody titers exceeding 1:1000 were generated as a result of immunization with these constructs. Following an intravenous challenge of SHIV HXBc2 none of the immunized monkeys became infected based upon negative re-isolation of HXBc2 at every bleed after challenge.

Based on the successful protection of nonhuman primates in a SHIV protection model, the DNA prime-protein boost approach was tested in a Phase I clinical trial [120]. Again, a polyvalent envelope formulation was used, this time consisting of five envelopes from clades A, B, C, and E. After three DNA immunizations and two protein boosts, humoral responses were evaluated by solid phase antibody binding and neutralizing antibody assays. Immunization with this polyvalent Env vaccine formulation and regimen elicited broad and high titer binding antibody responses in all individuals enrolled in the trial against gp120 antigens from clades A to H of HIV-1 as evaluated by ELISA and Western blots. Additionally, neutralizing antibody responses were detected in 100% of individuals against the sensitive HIV-1 isolates MN, NL4–3 and SF162 at titers as high as 1:2000. Neutralization of the homologous primary isolates included in the vaccine was also frequently observed. Specifically, neutralizing activity was detected in more than 60% of individuals against the subtype C vaccine strain 96ZM652. In addition to eliciting neutralizing antibodies against sensitive and homologous isolates, which has not always

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been demonstrated by some other trials, the new polyvalent DNA prime-protein boost regimen also generated neutralizing activity against difficult to neutralize heterologous primary isolates from clades A, B, C, D and E in a high throughput pseudotyped virus based neutralization system [120]. Positive NAb titers were identified in each of the 22 vaccinees included in the analysis with about 60% vaccinees having positive NAbs against 80–100% of pseudotyped viruses included in the assay. This represents a significant improvement over the neutralizing antibody responses reported with immunization via protein, DNA, or viral vectors alone as well as combinations of viral vector prime-protein boost and DNA prime-viral vector boost studies. Additionally, while this vaccine was successful in generating a cross reactive antibody response, it also proved to be safe and well tolerated [121].

The underlying mechanism as to why the DNA prime-protein boost immunization regimen is more effective at raising a functional antibody response is still not fully understood. One potential explanation for this, is that the avidity of the antibody response generated against the HIV envelope by a prime-boost immunization is higher than that generated by immunization with either modality alone [113, 116]. These studies indicated that boosting with recombinant protein vastly increased the avidity of the antibody response elicited by DNA priming. The generation of a higher avidity antibody response may be one of the defining features necessary to elicit effective neutralization of HIV-1.

A second possibility as to why the DNA prime-protein boost approach elicits a higher quality antibody response has also been recently investigated. Comparison of antibody specificities generated by immunization with only recombinant protein or with the DNA prime-protein boost approach has revealed that the incorporation of a DNA prime alters the specificity of elicited antibodies [122]. Based upon an enhanced breadth of neutralizing antibody response observed in the DNA primed animals, the specificities of the antibodies elicited by each immunization approach was studied. Recognition of linear peptides derived from the group M consensus sequence revealed that immunization with either approach generated binding antibodies to the C1 and V3 regions of the envelope. However, immunization with the DNA prime-protein boost approach also elicited antibodies with specificities for the flanking regions of the V1/V2 loop, the C2 region, and the junction of the V5 and C5 regions. Mapping of these regions onto the crystal structure of liganded gp120 revealed that these uniquely recognized regions all mapped very closely to known contact residues for CD4. Through the use of a monoclonal antibody competition assay it was confirmed that increased titers of CD4 binding site directed antibodies were present in animals that received a DNA prime. Interestingly however, V3 directed antibodies were also observed to be elicited in higher titers in the DNA primed animals. Further investigation to this phenomenon revealed that while the V3 directed antibodies were more prevalent in the DNA primed animals, they were not playing a significant role in the neutralization of primary isolates. This left the increased levels of CD4 binding site antibodies as the most likely mechanism for the observed enhanced neutralizing antibody response. With recent studies implicating the CD4 binding site as a primary target for individuals with broadly neutralizing activity [123], the ability of a DNA prime-protein boost approach to elicit antibodies with this specificity is a remarkably important attribute of this platform.

Summary

The development of an effective HIV-1 vaccine still faces numerous challenges before it may become a reality. This includes enhancing the antibody response to neutralize a wide array of viral isolates and to do so with a high degree of potency. However, with current work underway in using a polyvalent Env formulation delivered by the DNA prime-protein boost approach in eliciting broadly cross reactive antibody responses, this challenge may prove not to be insurmountable.

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Acknowledgments

This work is supported in part by NIH research grants R29AI40337 and R01AI65250 to S.L. M.V was supported an NIH training grant 5 T32 AIO7349–18.

References

1. UNAIDS/WHO. Global AIDS epidemic continues to grow. 2006. [cited 2007 26 March]; Available from: http://www.who.int/hiv/mediacentre/news62/en/index.html
2. Borrow P, Lewicki H, Hahn BH, Shaw GM, Oldstone MB. Virus-specific CD8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection. Journal of virology. 1994 Sep; 68(9):6103–10. [PubMed: 8057491]
3. Koup RA, Safrit JT, Cao Y, Andrews CA, McLeod G, Borkowsky W, et al. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. Journal of virology. 1994 Jul; 68(7):4650–5. [PubMed: 8207839]
4. Schmitz JE, Kuroda MJ, Santra S, Sasseville VG, Simon MA, Lifton MA, et al. Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. Science. 1999 Feb 5; 283(5403):857–60. New York, NY. [PubMed: 9933172]
5. Bernard NF, Pederson K, Chung F, Ouellet L, Wainberg MA, Tsoukas CM. HIV-specific cytotoxic T-lymphocyte activity in immunologically normal HIV-infected persons. AIDS (London, England). 1998 Nov 12; 12(16):2125–39.
6. Pontesilli O, Klein MR, Kerkhof-Garde SR, Pakker NG, de Wolf F, Schuitemaker H, et al. Longitudinal analysis of human immunodeficiency virus type 1-specific cytotoxic T lymphocyte responses: a predominant gag-specific response is associated with nonprogressive infection. The Journal of infectious diseases. 1998 Oct; 178(4):1008–18. [PubMed: 9806028]
7. Amara RR, Villinger F, Altman JD, Lydy SL, O'Neil SP, Staprans SI, et al. Control of a mucosal challenge and prevention of AIDS by a multiprotein DNA/MVA vaccine. Science. 2001 Apr 6; 292(5514):69–74. New York, NY. [PubMed: 11393868]
8. Shiver JW, Fu TM, Chen L, Casimiro DR, Davies ME, Evans RK, et al. Replication-incompetent adenoviral vaccine vector elicits effective anti-immunodeficiency-virus immunity. Nature. 2002 Jan 17; 415(6869):331–5. [PubMed: 11797011]
9. Barouch DH, Santra S, Schmitz JE, Kuroda MJ, Fu TM, Wagner W, et al. Control of Viremia and Prevention of Clinical AIDS in Rhesus Monkeys by Cytokine-Augumented DNA Vaccination. Science. 2000; 290:486–92. New York, NY. 2000 20 October. [PubMed: 11039923]
10. Casimiro D, Wang F, Schleif W, Liang X, Zhang Z-Q, Tobery T, et al. Attenuation of simian immunodeficiency virus SIVmac239 infection by prophylactic immunization with dna and recombinant adenoviral vaccine vectors expressing Gag. Journal of virology. 2005; 79(24):15547–55. [PubMed: 16306625]
11. Liang X, Casimiro DR, Schleif WA, Wang F, Davies ME, Zhang ZQ, et al. Vectored Gag and Env but not Tat show efficacy against simian-human immunodeficiency virus 89.6P challenge in Mamu-A*01-negative rhesus monkeys. Journal of virology. 2005 Oct; 79(19):12321–31. [PubMed: 16160159]
12. HIV vaccine failure prompts Merck to halt trial. Nature. 2007 Sep 27.449(7161):390. [PubMed: 17898737]
13. Steinbrook R. One step forward, two steps back--will there ever be an AIDS vaccine? N Engl J Med. 2007 Dec 27; 357(26):2653–5. [PubMed: 18160684]
14. Pantaleo G. HIV-1 T-cell vaccines: evaluating the next step. The Lancet infectious diseases. 2008 Feb; 8(2):82–3. [PubMed: 18060842]
15. Ledford H. HIV vaccine may raise risk. Nature. 2007 Nov 15.450(7168):325. [PubMed: 18004331]
16. Korber B, Gaschen B, Yusim K, Thakallapally R, Kesmir C, Detours V. Evolutionary and immunological implications of contemporary HIV-1 variation. British medical bulletin. 2001; 58:19–42. [PubMed: 11714622]

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17. Pantophlet R, Burton DR. GP120: target for neutralizing HIV-1 antibodies. Annu Rev Immunol. 2006; 24:739–69. [PubMed: 16551265]
18. Burton DR, Stanfield RL, Wilson IA. Antibody vs. HIV in a clash of evolutionary titans. Proceedings of the National Academy of Sciences of the United States of America. 2005 Oct 18; 102(42):14943–8. [PubMed: 16219699]
19. Conley AJ, Kessler JA II, Boots LJ, McKenna PM, Schleif WA, Emini EA, et al. The consequence of passive administration of an anti-human immunodeficiency virus type 1 neutralizing monoclonal antibody before challenge of chimpanzees with a primary virus isolate. Journal of virology. 1996 Oct; 70(10):6751–8. [PubMed: 8794312]
20. Emini EA, Schleif WA, Nunberg JH, Conley AJ, Eda Y, Tokiyoshi S, et al. Prevention of HIV-1 infection in chimpanzees by gp120 V3 domain-specific monoclonal antibody. Nature. 1992 Feb 20; 355(6362):728–30. [PubMed: 1741059]
21. Hofmann-Lehmann R, Vlasak J, Rasmussen RA, Jiang S, Li PL, Baba TW, et al. Postnatal pre- and postexposure passive immunization strategies: protection of neonatal macaques against oral simian-human immunodeficiency virus challenge. Journal of medical primatology. 2002 Jun; 31(3):109–19. [PubMed: 12190851]
22. Hofmann-Lehmann R, Vlasak J, Rasmussen RA, Smith BA, Baba TW, Liska V, et al. Postnatal Passive Immunization of Neonatal Macaques with a Triple Combination of Human Monoclonal Antibodies against Oral Simian-Human Immunodeficiency Virus Challenge. Journal of virology. 2001 Aug; 75(16):7470–80. [PubMed: 11462019]
23. Mascola JR, Lewis MG, Stiegler G, Harris D, VanCott TC, Hayes D, et al. Protection of Macaques against pathogenic simian/human immunodeficiency virus 89.6PD by passive transfer of neutralizing antibodies. Journal of virology. 1999 May; 73(5):4009–18. [PubMed: 10196297]
24. Mascola JR, Stiegler G, VanCott TC, Katinger H, Carpenter CB, Hanson CE, et al. Protection of macaques against vaginal transmission of a pathogenic HIV-1/SIV chimeric virus by passive infusion of neutralizing antibodies. Nat Med. 2000 Feb; 6(2):207–10. [PubMed: 10655111]
25. Berman PW, Gregory TJ, Riddle L, Nakamura GR, Champe MA, Porter JP, et al. Protection of chimpanzees from infection by HIV-1 after vaccination with recombinant glycoprotein gp120 but not gp160. Nature. 1990 Jun 14; 345(6276):622–5. [PubMed: 2190095]
26. el-Amad Z, Murthy KK, Higgins K, Cobb EK, Haigwood NL, Levy JA, et al. Resistance of chimpanzees immunized with recombinant gp120SF2 to challenge by HIV-1SF2. AIDS (London, England). 1995 Dec; 9(12):1313–22.
27. Berman PW, Murthy KK, Wrin T, Vennari JC, Cobb EK, Eastman DJ, et al. Protection of MN-rgp120-immunized chimpanzees from heterologous infection with a primary isolate of human immunodeficiency virus type 1. The Journal of infectious diseases. 1996 Jan; 173(1):52–9. [PubMed: 8537682]
28. Schwartz DH, Gorse G, Clements ML, Belshe R, Izu A, Duliege AM, et al. Induction of HIV-1-neutralising and syncytium-inhibiting antibodies in uninfected recipients of HIV-1IIIB rgp120 subunit vaccine. Lancet. 1993 Jul 10; 342(8863):69–73. [PubMed: 8100910]
29. Belshe RB, Clements ML, Dolin R, Graham BS, McElrath J, Gorse GJ, et al. Safety and immunogenicity of a fully glycosylated recombinant gp160 human immunodeficiency virus type 1 vaccine in subjects at low risk of infection. National Institute of Allergy and Infectious Diseases AIDS Vaccine Evaluation Group Network. The Journal of infectious diseases. 1993 Dec; 168(6): 1387–95. [PubMed: 8245523]
30. Belshe RB, Graham BS, Keefer MC, Gorse GJ, Wright P, Dolin R, et al. Neutralizing antibodies to HIV-1 in seronegative volunteers immunized with recombinant gp120 from the MN strain of HIV-1. NIAID AIDS Vaccine Clinical Trials Network. Jama. 1994 Aug 10; 272(6):475–80. [PubMed: 7913731]
31. Graham BS, Keefer MC, McElrath MJ, Gorse GJ, Schwartz DH, Weinhold K, et al. Safety and immunogenicity of a candidate HIV-1 vaccine in healthy adults: recombinant glycoprotein (rgp) 120. A randomized, double-blind trial. NIAID AIDS Vaccine Evaluation Group. Ann Intern Med. 1996 Aug 15; 125(4):270–9. [PubMed: 8678389]
32. Pitisuttithum P, Berman PW, Phonrat B, Suntharasamai P, Raktham S, Srisuwanvilai LO, et al. Phase I/II study of a candidate vaccine designed against the B and E subtypes of HIV-1. Journal of acquired immune deficiency syndromes (1999). 2004 Sep 1; 37(1):1160–5. [PubMed: 15319676]

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33. Francis DP, Gregory T, McElrath MJ, Belshe RB, Gorse GJ, Migasena S, et al. Advancing AIDSVAX to phase 3. Safety, immunogenicity, and plans for phase 3. AIDS research and human retroviruses. 1998 Oct; 14(Suppl 3):S325–31. [PubMed: 9814961]
34. Flynn NM, Forthal DN, Harro CD, Judson FN, Mayer KH, Para MF. Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. The Journal of infectious diseases. 2005 Mar 1; 191(5):654–65. [PubMed: 15688278]
35. Pitisuttithum P, Gilbert P, Gurwith M, Heyward W, Martin M, van Griensven F, et al. Randomized, double-blind, placebo-controlled efficacy trial of a bivalent recombinant glycoprotein 120 HIV-1 vaccine among injection drug users in Bangkok, Thailand. The Journal of infectious diseases. 2006 Dec 15; 194(12):1661–71. [PubMed: 17109337]
36. Gilbert PB, Peterson ML, Follmann D, Hudgens MG, Francis DP, Gurwith M, et al. Correlation between immunologic responses to a recombinant glycoprotein 120 vaccine and incidence of HIV-1 infection in a phase 3 HIV-1 preventive vaccine trial. The Journal of infectious diseases. 2005 Mar 1; 191(5):666–77. [PubMed: 15688279]
37. Gao F, Weaver EA, Lu Z, Li Y, Liao HX, Ma B, et al. Antigenicity and Immunogenicity of a Synthetic Human Immunodeficiency Virus Type 1 Group M Consensus Envelope Glycoprotein. Journal of virology. 2005; 79(2):1154–63. January 2005. [PubMed: 15613343]
38. Liao HX, Sutherland LL, Xia SM, Brock ME, Scearce RM, Vanleeuwen S, et al. A group M consensus envelope glycoprotein induces antibodies that neutralize subsets of subtype B and C HIV-1 primary viruses. Virology. 2006 Sep 30; 353(2):268–82. [PubMed: 17039602]
39. Kothe DL, Li Y, Decker JM, Bibollet-Ruche F, Zammit KP, Salazar MG, et al. Ancestral and consensus envelope immunogens for HIV-1 subtype C. Virology. 2006 Sep 1; 352(2):438–49. [PubMed: 16780913]
40. Doria-Rose NA, Learn GH, Rodrigo AG, Nickle DC, Li F, Mahalanabis M, et al. Human immunodeficiency virus type 1 subtype B ancestral envelope protein is functional and elicits neutralizing antibodies in rabbits similar to those elicited by a circulating subtype B envelope. Journal of virology. 2005 Sep; 79(17):11214–24. [PubMed: 16103173]
41. Kothe DL, Decker JM, Li Y, Weng Z, Bibollet-Ruche F, Zammit KP, et al. Antigenicity and immunogenicity of HIV-1 consensus subtype B envelope glycoproteins. Virology. 2007 Mar 30; 360(1):218–34. [PubMed: 17097711]
42. Mascola JR, D'Souza P, Gilbert P, Hahn BH, Haigwood NL, Morris L, et al. Recommendations for the Design and Use of Standard Virus Panels to Assess Neutralizing Antibody Responses Elicited by Candidate Human Immunodeficiency Virus Type 1 Vaccines. Journal of virology. 2005 Aug; 79(16):10103–7. 2005. [PubMed: 16051803]
43. Li M, Gao F, Mascola JR, Stamatatos L, Polonis VR, Koutsoukos M, et al. Human immunodeficiency virus type 1 env clones from acute and early subtype B infections for standardized assessments of vaccine-elicited neutralizing antibodies. Journal of virology. 2005 Aug; 79(16):10108–25. [PubMed: 16051804]
44. Stamatatos L, Cheng-Mayer C. An envelope modification that renders a primary, neutralization-resistant clade B human immunodeficiency virus type 1 isolate highly susceptible to neutralization by sera from other clades. Journal of virology. 1998 Oct; 72(10):7840–5. [PubMed: 9733820]
45. Pinter A, Honnen WJ, He Y, Gorny MK, Zolla-Pazner S, Kayman SC. The V1/V2 domain of gp120 is a global regulator of the sensitivity of primary human immunodeficiency virus type 1 isolates to neutralization by antibodies commonly induced upon infection. Journal of virology. 2004 May; 78(10):5205–15. [PubMed: 15113902]
46. Johnson WE, Sanford H, Schwall L, Burton DR, Parren PW, Robinson JE, et al. Assorted mutations in the envelope gene of simian immunodeficiency virus lead to loss of neutralization resistance against antibodies representing a broad spectrum of specificities. Journal of virology. 2003 Sep; 77(18):9993–10003. [PubMed: 12941910]
47. Cao J, Sullivan N, Desjardin E, Parolin C, Robinson J, Wyatt R, et al. Replication and neutralization of human immunodeficiency virus type 1 lacking the V1 and V2 variable loops of the gp120 envelope glycoprotein. Journal of virology. 1997 Dec; 71(12):9808–12. [PubMed: 9371651]
48. Lu S, Wyatt R, Richmond JF, Mustafa F, Wang S, Weng J, et al. Immunogenicity of DNA vaccines expressing human immunodeficiency virus type 1 envelope glycoprotein with and

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

without deletions in the V1/2 and V3 regions. AIDS research and human retroviruses. 1998; 14(2): 151–5. [PubMed: 9462925]

49. Barnett SW, Lu S, Srivastava I, Cherpelis S, Gettie A, Blanchard J, et al. The ability of an oligomeric human immunodeficiency virus type 1 (HIV-1) envelope antigen to elicit neutralizing antibodies against primary HIV-1 isolates is improved following partial deletion of the second hypervariable region. Journal of virology. 2001 Jun; 75(12):5526–40. [PubMed: 11356960]
50. Srivastava IK, VanDorsten K, Vojtech L, Barnett SW, Stamatatos L. Changes in the Immunogenic Properties of Soluble gp140 Human Immunodeficiency Virus Envelope Constructs upon Partial Deletion of the Second Hypervariable Region. Journal of virology. 2003 Feb; 77(4):2310–20. [PubMed: 12551968]
51. Lian Y, Srivastava I, Gomez-Roman VR, zur Megede JZ, Sun Y, Kan E, et al. Evaluation of Envelope Vaccines Derived from the South African SubtypeC Human Immunodeficiency Virus Type 1 TV1 Strain. Journal of virology. 2005 Nov; 79(21):13338–49. 2005. [PubMed: 16227256]
52. Derby NR, Kraft Z, Kan E, Crooks ET, Barnett SW, Srivastava IK, et al. Antibody responses elicited in macaques immunized with human immunodeficiency virus type 1 (HIV-1) SF162-derived gp140 envelope immunogens: comparison with those elicited during homologous simian/human immunodeficiency virus SHIVSF162P4 and heterologous HIV-1 infection. Journal of virology. 2006 Sep; 80(17):8745–62. [PubMed: 16912322]
53. Kim YB, Han DP, Cao C, Cho MW. Immunogenicity and ability of variable loop-deleted human immunodeficiency virus type 1 envelope glycoproteins to elicit neutralizing antibodies. Virology. 2003 Jan 5; 305(1):124–37. [PubMed: 12504547]
54. Back NK, Smit L, De Jong JJ, Keulen W, Schutten M, Goudsmit J, et al. An N-glycan within the human immunodeficiency virus type 1 gp120 V3 loop affects virus neutralization. Virology. 1994 Mar; 199(2):431–8. [PubMed: 8122371]
55. Kang SM, Quan FS, Huang C, Guo L, Ye L, Yang C, et al. Modified HIV envelope proteins with enhanced binding to neutralizing monoclonal antibodies. Virology. 2005 Jan 5; 331(1):20–32. [PubMed: 15582650]
56. McCaffrey RA, Saunders C, Hensel M, Stamatatos L. N-linked glycosylation of the V3 loop and the immunologically silent face of gp120 protects human immunodeficiency virus type 1 SF162 from neutralization by anti-gp120 and anti-gp41 antibodies. Journal of virology. 2004 Apr; 78(7): 3279–95. [PubMed: 15016849]
57. Reynard F, Fatmi A, Verrier B, Bedin F. HIV-1 acute infection env glycomutants designed from 3D model: effects on processing, antigenicity, and neutralization sensitivity. Virology. 2004 Jun 20; 324(1):90–102. [PubMed: 15183057]
58. Koch M, Pancera M, Kwong PD, Kolchinsky P, Grundner C, Wang L, et al. Structure-based, targeted deglycosylation of HIV-1 gp120 and effects on neutralization sensitivity and antibody recognition. Virology. 2003 Sep 1; 313(2):387–400. [PubMed: 12954207]
59. Huang Z, Chou A, Tanguay J, Shen S, Mboudjeka I, Chou TH, et al. Levels of N-linked glycosylation on the V1 loop of HIV-1 Env proteins and their relationship to the antigenicity of Env from primary viral isolates. Current HIV research. 2008 Jun; 6(4):296–305. [PubMed: 18691028]
60. Pantophlet R, Wilson IA, Burton DR. Hyperglycosylated mutants of human immunodeficiency virus (HIV) type 1 monomeric gp120 as novel antigens for HIV vaccine design. Journal of virology. 2003 May; 77(10):5889–901. [PubMed: 12719582]
61. Selvarajah S, Puffer B, Pantophlet R, Law M, Doms RW, Burton DR. Comparing antigenicity and immunogenicity of engineered gp120. Journal of virology. 2005 Oct; 79(19):12148–63. [PubMed: 16160142]
62. Selvarajah S, Puffer BA, Lee FH, Zhu P, Li Y, Wyatt R, et al. Focused dampening of antibody response to the immunodominant variable loops by engineered soluble gp140. AIDS research and human retroviruses. 2008 Feb; 24(2):301–14. [PubMed: 18284327]
63. Reitter JN, Means RE, Desrosiers RC. A role for carbohydrates in immune evasion in AIDS. Nat Med. 1998 Jun; 4(6):679–84. [PubMed: 9623976]
64. Li Y, Cleveland B, Klots I, Travis B, Richardson BA, Anderson D, et al. Removal of a single N-linked glycan in human immunodeficiency virus type 1 gp120 results in an enhanced ability to

induce neutralizing antibody responses. Journal of virology. 2008 Jan; 82(2):638–51. [PubMed: 17959660]

65. Bolmstedt A, Sjolander S, Hansen JE, Akerblom L, Hemming A, Hu SL, et al. Influence of N-linked glycans in V4-V5 region of human immunodeficiency virus type 1 glycoprotein gp160 on induction of a virus-neutralizing humoral response. J Acquir Immune Defic Syndr Hum Retrovirol. 1996 Jul; 12(3):213–20. [PubMed: 8673525]
66. Quinones-Kochs MI, Buonocore L, Rose JK. Role of N-Linked Glycans in a Human Immunodeficiency Virus Envelope Glycoprotein: Effects on Protein Function and the Neutralizing Antibody Response. Journal of virology. 2002 May; 76(9):4199–211. [PubMed: 11932385]
67. Earl PL, Sugiura W, Montefiori DC, Broder CC, Lee SA, Wild C, et al. Immunogenicity and protective efficacy of oligomeric human immunodeficiency virus type 1 gp140. Journal of virology. 2001 Jan; 75(2):645–53. [PubMed: 11134278]
68. Zhang PF, Cham F, Dong M, Choudhary A, Bouma P, Zhang Z, et al. Extensively cross-reactive anti-HIV-1 neutralizing antibodies induced by gp140 immunization. Proceedings of the National Academy of Sciences of the United States of America. 2007 Jun 12; 104(24):10193–8. [PubMed: 17540729]
69. Yang X, Wyatt R, Sodroski J. Improved Elicitation of Neutralizing Antibodies against Primary Human Immunodeficiency Viruses by Soluble Stabilized Envelope Glycoprotein Trimers. Journal of virology. 2001 Feb; 75(3):1165–71. [PubMed: 11152489]
70. Li Y, Svehla K, Mathy NL, Voss G, Mascola JR, Wyatt R. Characterization of Antibody Responses Elicited by Human Immunodeficiency Virus Type 1 Primary Isolate Trimeric and Monomeric Envelope Glycoproteins in Selected Adjuvants. Journal of virology. 2006 Feb; 80(3): 1414–26. 2006. [PubMed: 16415019]
71. Pancera M, Lebowitz J, Schon A, Zhu P, Freire E, Kwong PD, et al. Soluble Mimetics of Human Immunodeficiency Virus Type 1 Viral Spikes Produced by Replacement of the Native Trimerization Domain with a Heterologous Trimerization Motif: Characterization and Ligand Binding Analysis. Journal of virology. 2005 Aug; 79(15):9954–69. 2005. [PubMed: 16014956]
72. Dey B, Pancera M, Svehla K, Shu Y, Xiang SH, Vainshtein J, et al. Characterization of human immunodeficiency virus type 1 monomeric and trimeric gp120 glycoproteins stabilized in the CD4-bound state: antigenicity, biophysics, and immunogenicity. Journal of virology. 2007 Jun; 81(11):5579–93. [PubMed: 17360741]
73. Binley JM, Sanders RW, Clas B, Schuelke N, Master A, Guo Y, et al. A Recombinant Human Immunodeficiency Virus Type 1 Envelope Glycoprotein Complex Stabilized by an Intermolecular Disulfide Bond between the gp120 and gp41 Subunits Is an Antigenic Mimic of the Trimeric Virion-Associated Structure. Journal of virology. 2000; 74(2):627–43. [PubMed: 10623724]
74. Sanders RW, Vesanen M, Schuelke N, Master A, Schiffner L, Kalyanaraman R, et al. Stabilization of the soluble, cleaved, trimeric form of the envelope glycoprotein complex of human immunodeficiency virus type 1. Journal of virology. 2002 Sep; 76(17):8875–89. [PubMed: 12163607]
75. Beddows S, Schulke N, Kirschner M, Barnes K, Franti M, Michael E, et al. Evaluating the immunogenicity of a disulfide-stabilized, cleaved, trimeric form of the envelope glycoprotein complex of human immunodeficiency virus type 1. Journal of virology. 2005 Jul; 79(14):8812–27. [PubMed: 15994775]
76. Liang X, Munshi S, Shendure J, Mark IIIG, Davies ME, Freed DC, et al. Epitope insertion into variable loops of HIV-1 gp120 as a potential means to improve immunogenicity of viral envelope protein. Vaccine. 1999; 17:2862–72. [PubMed: 10438057]
77. Law M, Cardoso RM, Wilson IA, Burton DR. Antigenic and Immunogenic Study of Membrane-Proximal External Region-Grafted gp120 Antigens by a DNA Prime-Protein Boost Immunization Strategy. Journal of virology. 2007 Apr; 81(8):4272–85. [PubMed: 17267498]
78. Burioni R, Mancini N, De Marco D, Clementi N, Perotti M, Nitti G, et al. Anti-HIV-1 response elicited in rabbits by anti-idiotype monoclonal antibodies mimicking the CD4-binding site. PLoS ONE. 2008; 3(10):e3423. [PubMed: 18923648]
79. Catanzaro AT, Koup RA, Roederer M, Bailer RT, Enama ME, Moodie Z, et al. Phase 1 safety and immunogenicity evaluation of a multiclade HIV-1 candidate vaccine delivered by a replication-

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NIH-PA Author Manuscript

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defective recombinant adenovirus vector. The Journal of infectious diseases. 2006 Dec 15; 194(12):1638–49. [PubMed: 17109335]

80. Russell ND, Graham BS, Keefer MC, McElrath MJ, Self SG, Weinhold KJ, et al. Phase 2 study of an HIV-1 canarypox vaccine (vCP1452) alone and in combination with rgp120: negative results fail to trigger a phase 3 correlates trial. Journal of acquired immune deficiency syndromes (1999). 2007 Feb 1; 44(2):203–12. [PubMed: 17106277]
81. Gupta K, Hudgens M, Corey L, McElrath MJ, Weinhold K, Montefiori DC, et al. Safety and immunogenicity of a high-titered canarypox vaccine in combination with rgp120 in a diverse population of HIV-1-uninfected adults: AIDS Vaccine Evaluation Group Protocol 022A. Journal of acquired immune deficiency syndromes (1999). 2002 Mar 1; 29(3):254–61. [PubMed: 11873074]
82. Thongcharoen P, Suriyanon V, Paris RM, Khamboonruang C, de Souza MS, Ratto-Kim S, et al. A phase 1/2 comparative vaccine trial of the safety and immunogenicity of a CRF01_AE (subtype E) candidate vaccine: ALVAC-HIV (vCP1521) prime with oligomeric gp160 (92TH023/LAI-DID) or bivalent gp120 (CM235/SF2) boost. Journal of acquired immune deficiency syndromes (1999). 2007 Sep 1; 46(1):48–55. [PubMed: 17909315]
83. Evans TG, Keefer MC, Weinhold KJ, Wolff M, Montefiori D, Gorse GJ, et al. A canarypox vaccine expressing multiple human immunodeficiency virus type 1 genes given alone or with rgp120 elicits broad and durable CD8+ cytotoxic T lymphocyte responses in seronegative volunteers. The Journal of infectious diseases. 1999 Aug; 180(2):290–8. [PubMed: 10395842]
84. Belshe RB, Stevens C, Gorse GJ, Buchbinder S, Weinhold K, Sheppard H, et al. Safety and immunogenicity of a canarypox-vectored human immunodeficiency virus Type 1 vaccine with or without gp120: a phase 2 study in higher- and lower-risk volunteers. The Journal of infectious diseases. 2001 May 1; 183(9):1343–52. [PubMed: 11294665]
85. Catanzaro AT, Roederer M, Koup RA, Bailer RT, Enama ME, Nason MC, et al. Phase I clinical evaluation of a six-plasmid multiclade HIV-1 DNA candidate vaccine. Vaccine. 2007 May 16; 25(20):4085–92. [PubMed: 17391815]
86. Graham BS, Koup RA, Roederer M, Bailer RT, Enama ME, Moodie Z, et al. Phase 1 safety and immunogenicity evaluation of a multiclade HIV-1 DNA candidate vaccine. The Journal of infectious diseases. 2006 Dec 15; 194(12):1650–60. [PubMed: 17109336]
87. Mulligan MJ, Russell ND, Celum C, Kahn J, Noonan E, Montefiori DC, et al. Excellent safety and tolerability of the human immunodeficiency virus type 1 pGA2/JS2 plasmid DNA priming vector vaccine in HIV type 1 uninfected adults. AIDS research and human retroviruses. 2006 Jul; 22(7): 678–83. [PubMed: 16831092]
88. Chakrabarti BK, Kong WP, Wu BY, Yang ZY, Friborg J, Ling X, et al. Modifications of the Human Immunodeficiency Virus Envelope Glycoprotein Enhance Immunogenicity for Genetic Immunization. Journal of virology. 2002 Jun; 76(11):5357–68. [PubMed: 11991964]
89. Seaman MS, Xu L, Beaudry K, Martin KL, Beddall MH, Miura A, et al. Multiclade Human Immunodeficiency Virus Type 1 Envleope Immunogens Elicit Broad Cellular and Humoral Immunity in Rhesus Monkeys. Journal of virology. 2005 Mar; 79(5):2956–63. 2005. [PubMed: 15709015]
90. Mascola JR, Sambor A, Beaudry K, Santra S, Welcher B, Louder MK, et al. Neutralizing antibodies elicited by immunization of monkeys with DNA plasmids and recombinant adenoviral vectors expressing human immunodeficiency virus type 1 proteins. Journal of virology. 2005 Jan; 79(2):771–9. [PubMed: 15613305]
91. Wang B, Ugen KE, Srikantan V, Agadjanyan MG, Dang K, Refaeli Y, et al. Gene inoculation generates immune responses against human immunodeficiency virus type 1. Proceedings of the National Academy of Sciences of the United States of America. 1993 May 1; 90(9):4156–60. [PubMed: 8483929]
92. Lu S, Santoro JC, Fuller DH, Haynes JR, Robinson HL. Use of DNAs expressing HIV-1 Env and noninfectious HIV-1 particles to raise antibody responses in mice. Virology. 1995; 209(1):147–54. [PubMed: 7747464]
93. Boyer JD, Wang B, Ugen KE, Agadjanyan M, Javadian A, Frost P, et al. In vivo protective anti-HIV immune responses in non-human primates through DNA immunization. Journal of medical primatology. 1996 Jun; 25(3):242–50. [PubMed: 8892046]

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94. Roy MJ, Wu MS, Barr LJ, Fuller JT, Tussey LG, Speller S, et al. Induction of antigen-specific CD8+ T cells, T helper cells, and protective levels of antibody in humans by particle-mediated administration of a hepatitis B virus DNA vaccine. Vaccine. 2000 Nov 22; 19(7–8):764–78. [PubMed: 11115698]
95. Wang R, Doolan DL, Le TP, Hedstrom RC, Coonan KM, Charoenvit Y, et al. Induction of antigen-specific cytotoxic T lymphocytes in humans by a malaria DNA vaccine. Science (New York, NY). 1998; 282(5388):476–80.
96. Calarota S, Bratt G, Nordlund S, Hinkula J, Leandersson AC, Sandstrom E, et al. Cellular cytotoxic response induced by DNA vaccination in HIV-1-infected patients. Lancet. 1998 May 2; 351(9112):1320–5. [PubMed: 9643795]
97. MacGregor RR, Boyer JD, Ugen KE, Lacy KE, Gluckman SJ, Bagarazzi ML, et al. First human trial of a DNA-based vaccine for treatment of human immunodeficiency virus type 1 infection: safety and host response. The Journal of infectious diseases. 1998 Jul; 178(1):92–100. [PubMed: 9652427]
98. Ugen KE, Nyland SB, Boyer JD, Vidal C, Lera L, Rasheid S, et al. DNA vaccination with HIV-1 expressing constructs elicits immune responses in humans. Vaccine. 1998; 16(19):1818–21. [PubMed: 9795386]
99. Richmond JF, Mustafa F, Lu S, Santoro JC, Weng J, O'Connell M, et al. Screening of HIV-1 Env glycoproteins for the ability to raise neutralizing antibody using DNA immunization and recombinant vaccinia virus boosting. Virology. 1997 Apr 14; 230(2):265–74. [PubMed: 9143282]
100. Mustafa F, Richmond JF, Fernandez-Larsson R, Lu S, Fredriksson R, Fenyo EM, et al. HIV-1 Env glycoproteins from two series of primary isolates: replication phenotype and immunogenicity. Virology. 1997 Mar 3; 229(1):269–78. [PubMed: 9123870]
101. Wang S, Chou TH, Sakhatskyy PV, Huang S, Lawrence JM, Cao H, et al. Identification of two neutralizing regions on the severe acute respiratory syndrome coronavirus spike glycoprotein produced from the mammalian expression system. Journal of virology. 2005 Feb; 79(3):1906–10. [PubMed: 15650214]
102. Robinson HL, Montefiori DC, Johnson RP, Manson KH, Kalish ML, Lifson JD, et al. Neutralizing antibody-independent containment of immunodeficiency virus challenges by DNA priming and recombinant pox virus booster immunizations. Nature medicine. 1999; 5(5):526–34.
103. Heller LC, Ugen K, Heller R. Electroporation for targeted gene transfer. Expert Opin Drug Deliv. 2005 Mar; 2(2):255–68. [PubMed: 16296752]
104. Wang S, Zhang C, Zhang L, Li J, Huang Z, Lu S. The relative immunogenicity of DNA vaccines delivered by the intramuscular needle injection, electroporation and gene gun methods. Vaccine. 2008 Apr 16; 26(17):2100–10. [PubMed: 18378365]
105. Aguiar JC, Hedstrom RC, Rogers WO, Charoenvit Y, Sacci JB Jr, Lanar DE, et al. Enhancement of the immune response in rabbits to a malaria DNA vaccine by immunization with a needle-free jet device. Vaccine. 2001 Oct 12; 20(1–2):275–80. [PubMed: 11567774]
106. Haensler J, Verdelet C, Sanchez V, Girerd-Chambaz Y, Bonnin A, Trannoy E, et al. Intradermal DNA immunization by using jet-injectors in mice and monkeys. Vaccine. 1999 Feb 26; 17(7–8): 628–38. [PubMed: 10067667]
107. Trimble C, Lin CT, Hung CF, Pai S, Juang J, He L, et al. Comparison of the CD8+ T cell responses and antitumor effects generated by DNA vaccine administered through gene gun, biojector, and syringe. Vaccine. 2003 Sep 8; 21(25–26):4036–42. [PubMed: 12922140]
108. Prausnitz MR. Microneedles for transdermal drug delivery. Advanced drug delivery reviews. 2004 Mar 27; 56(5):581–7. [PubMed: 15019747]
109. Deml L, Bojak A, Steck S, Graf M, Wild J, Schirmbeck R, et al. Multiple effects of codon usage optimization on expression and immunogenicity of DNA candidate vaccines encoding the human immunodeficiency virus type 1 Gag protein. Journal of virology. 2001 Nov; 75(22):10991–1001. [PubMed: 11602739]
110. Wang S, Farfan-Arribas DJ, Shen S, Chou TH, Hirsch A, He F, et al. Relative contributions of codon usage, promoter efficiency and leader sequence to the antigen expression and immunogenicity of HIV-1 Env DNA vaccine. Vaccine. 2006 May 22; 24(21):4531–40. [PubMed: 16140431]

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111. zur Megede JZ, Chen MC, Doe B, Schaffer M, Greer CE, Selby M, et al. Increased Expression and Immunogenicity of Sequence-Modified Human Immunodeficiency Virus Type 1 *gag* Gene. Journal of virology. 2000; 74(6):2628–35. [PubMed: 10684277]
112. Lu S. Combination DNA plus protein HIV vaccines. Springer Semin Immunopathol. 2006 Nov; 28(3):255–65. [PubMed: 17021720]
113. Richmond JF, Lu S, Santoro JC, Weng J, Hu SL, Montefiori DC, et al. Studies of the neutralizing activity and avidity of anti-human immunodeficiency virus type 1 Env antibody elicited by DNA priming and protein boosting. Journal of virology. 1998; 72(11):9092–100. [PubMed: 9765454]
114. Barnett SW, Rajasekar S, Legg H, Doe B, Fuller DH, Haynes JR, et al. Vaccination with HIV-1 gp120 DNA induces immune responses that are boosted by a recombinant gp120 protein subunit. Vaccine. 1997 Jun; 15(8):869–73. [PubMed: 9234536]
115. Letvin NL, Montefiori DC, Yasutomi Y, Perry HC, Davies ME, Lekutis C, et al. Potent, protective anti-HIV immune responses generated by bimodal HIV envelope DNA plus protein vaccination. Proceedings of the National Academy of Sciences of the United States of America. 1997 Aug 19; 94(17):9378–83. [PubMed: 9256490]
116. Wang S, Arthos J, Lawrence JM, Van Ryk D, Mboudjeka I, Shen S, et al. Enhanced immunogenicity of gp120 protein when combined with recombinant DNA priming to generate antibodies that neutralize the JR-FL primary isolate of human immunodeficiency virus type 1. Journal of virology. 2005 Jun; 79(12):7933–7. [PubMed: 15919951]
117. Wang S, Pal R, Mascola JR, Chou TH, Mboudjeka I, Shen S, et al. Polyvalent HIV-1 Env vaccine formulations delivered by the DNA priming plus protein boosting approach are effective in generating neutralizing antibodies against primary human immunodeficiency virus type 1 isolates from subtypes A, B, C, D and E. Virology. 2006 Jun 20; 350(1):34–47. [PubMed: 16616287]
118. Pal R, Wang S, Kalyanaraman VS, Nair BC, Whitney S, Keen T, et al. Immunization of rhesus macaques with a polyvalent DNA prime/protein boost human immunodeficiency virus type 1 vaccine elicits protective antibody response against simian human immunodeficiency virus of R5 phenotype. Virology. 2006 May 10; 348(2):341–53. [PubMed: 16460776]
119. Rasmussen RA, Hofmann-Lehman R, Montefiori DC, Li PL, Liska V, Vlasak J, et al. DNA prime/protein boost vaccine strategy in neonatal macaques against simian human immunodeficiency virus. Journal of medical primatology. 2002 Feb; 31(1):40–60. [PubMed: 12076047]
120. Wang S, Kennedy J, West K, Montefiori DC, Bansal A, Goepfert P, et al. Balanced cellular and antibody responses induced by the polyvalent DNA prime-protein boost HIV-1 vaccine formulation DP6–001 in healthy human volunteers. Vaccine. 2008; 26:1098–110. [PubMed: 18243434]
121. Kennedy JS, Co M, Green S, Longtine K, Longtine J, O'Neill MA, et al. The safety and tolerability of an HIV-1 DNA prime-protein boost vaccine (DP6–001) in healthy adult volunteers. Vaccine. 2008 Aug 18; 26(35):4420–4. [PubMed: 18588934]
122. Vaine, M.; Wang, S.; Crooks, ET.; Jiang, P.; Montefiori, DC.; Binley, J., et al. Improved induction of antibodies against key neutralizing epitopes by HIV-1 gp120 DNA prime-protein boost vaccination compared to gp120 protein only vaccination. 2008. Submitted
123. Li Y, Migueles SA, Welcher B, Svehla K, Phogat A, Louder MK, et al. Broad HIV-1 neutralization mediated by CD4-binding site antibodies. Nat Med. 2007 Sep; 13(9):1032–4. [PubMed: 17721546]
124. Srivastava IK, Stamatatos L, Kan E, Vajdy M, Lian Y, Hilt S, et al. Purification, characterization, and immunogenicity of a soluble trimeric envelope protein containing a partial deletion of the V2 loop derived from SF162, an R5-tropic human immunodeficiency virus type 1 isolate. Journal of virology. 2003 Oct; 77(20):11244–59. [PubMed: 14512572]

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Table 1

Env immunogen designs and their effect on neutralizing antibody responses

Immunogen Design	Concept	Immunogens Tested	Result	References
Centralized Sequences	To reduce the genetic distance between immunogens and primary isolates to elicit a more cross reactive response	Group M Consensus	Increased Breadth of NAb Response	[37, 38]
		Subtype B Consensus	Increased NAb Potency Over Wild type Sequences	[41]
		Subtype C Consensus	No Appreciable Increase Nab Response	[39]
		Ancestral B	No Appreciable Increase Nab Response	[40]
		Ancestral C	No Appreciable Increase Nab Response	[39]
Variable Loop Deletions	To make functionally important domains more accessible	V1/V2, V3, & V4 Deletions	No Appreciable Increase Nab Response	[48, 52, 53]
		V2 Deletions	Increased potency and small increases in breadth of NAb Response	[49, 51, 52, 124]
Glycosylation Mutants	To shield irrelevant domains or expose important domains	Hyperglycosylation	Elimination of unwanted Ab specificities No improvement on overall NAb	[61, 62]
		Targeted deletion	Increased breadth and potency of NAb response is some isolates, no effect in others	[63–66]
Envelope Trimers	To better mimic the natural state of functional trimer	Eliminate Env cleavage site	Study and Isolate Dependent	[67, 68]
		Stabilized intermolecular interactions	Inconsistent increases in potency of NAb titers to homologous isolates	
		Env trimerized with heterologous motifs	Increased potency of NAb Response	[69, 70, 72]
Epitope Grafting	To make a neutralizable epitope more immunogenic when presented in a different context	MPER Grafts	No increases in NAbs with specificity for graft	[76, 77]

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Table 2

Vaccination strategies and resulting NAb response in clinical trials.

Vaccination Approach	Rationale	Result	Reference
Subunit Protein	Subunit based immunizations have been successfully used to raise antibody responses to a number of pathogens	Narrow NAb responses with no protective efficacy	[34, 35]
Viral Vectors	Raise a balanced T and B cell response	Narrow, low titer NAb responses elicited, when observed at all	[79, 80]
DNA Vaccines	Raise a balanced T and B cell response	No NAb responses elicited	[85–87]
Viral Vector + Protein	Viruses alone elicited only low quality antibody responses that may be boosted with a recombinant protein	Increase in NAb titers with administration of protein boost	[80]
DNA + Viral Vectors	Adenoviruses may be capable of boosting low titer immune responses resulting from DNA priming	Increased potency but still limited breadth of NAb	[90]
DNA + Protein	DNA immunizations can successfully prime a humoral response which can be augmented by boosting with a subunit protein	Low titer, but broad NAb responses against a wide range of primary isolates	[120]