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Polyvalent AIDS Vaccines

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Abstract

A major hurdle in the development of a global HIV-1 vaccine is viral diversity. For close to three decades, HIV vaccine development has focused on either the induction of T cell immune responses or antibody responses, and only rarely on both components. After the failure of the STEP trial, the scientific community concluded that a T cell-based vaccine would likely not be protective if the T cell immune responses were elicited against only a few dominant epitopes. Similarly, for vaccines focusing on antibody responses, one of the main criticisms after VaxGen's failed Phase III trials was on the limited antigen breadth included in the two formulations used. The successes of polyvalent vaccine approaches against other antigenically variable pathogens encourage implementation of the same approach for the design of HIV-1 vaccines. A review of the existing HIV-1 vaccination approaches based on the polyvalent principle is included here to provide a historical perspective for the current effort of developing a polyvalent HIV-1 vaccine. Results summarized in this review provide a clear indication that the polyvalent approach is a viable one for the future development of an effective HIV vaccine.

Keywords

HIV-1; vaccine; subtypes; neutralizing antibodies; cytotoxic T lymphocytes; polyvalent vaccine

INTRODUCTION

According to the 2008 Report on the Global AIDS Epidemic by the Joint United Nations Programme on HIV/AIDS (UNAIDS), 33–36 million people throughout the world were living with the human immunodeficiency virus type 1 (HIV-1) in 2007. Among many technical and political obstacles associated with the development of an effective vaccine against HIV-1, viral diversity may be considered the most challenging. HIV-1 is comprised of three distinct groups, designated as major (M), new (N), and outlier (O). In 2009, a new strain closely related to gorilla simian immunodeficiency virus (SIVgor) was discovered in a Cameroonian woman and has been designated HIV-1 Group P [1]. The predominant group, M, consists of 11 clades (i.e., subtypes A through K) and is responsible for the majority of all HIV infections throughout the world (approximately 99% of all HIV-1 infections) although some subtypes predominate in certain regions (for review, see [2]). A strain of simian immunodeficiency virus (SIVcpz) isolated from a subpopulation of chimpanzees (*Pan troglodytes troglodytes*) is closely related to all three groups of HIV-1 (M, N, and O) [3, 4] and, therefore, is believed to be the natural reservoir for human infection [5]. In addition to well defined subtypes, there are also circulating recombinant forms (CRFs), which are

hybrid HIV-1 viruses that contain genetic material from different clades, further increasing the diversity and complexity of HIV-1 infection. The development of a vaccine to encompass such viral diversity is a major hurdle in AIDS vaccine research.

For close to three decades, HIV vaccine development has focused on either the induction of T cell immune responses [6, 7] or antibody responses [8–10], and only rarely on both components [11]. After the failure of the STEP trial, the scientific community concluded that a T cell-based vaccine would likely not be protective if the T cell immune responses were elicited against only a few dominant epitopes [12–15]. Similarly, for vaccines focusing on antibody responses, one of the main criticisms after VaxGen's failed Phase III trials was on the limited antigen breadth included in the two formulations used (either two subtype B envelope (Env) antigens for the US-based trial, or one subtype B and one subtype E Env antigen for the Thailand-based trial).

One main strategy to overcome the high viral diversity is to develop an HIV antigen that can elicit broadly protective antibodies. The leading hypothesis in more than two decades suggests that an Env antigen with a trimer structure is the ideal antigen conformation that can mimic Env found on the surface of HIV-1 viral particles [16–19]. The early studies of several neutralizing monoclonal antibodies (mAbs) (b12, 2G12, 2F5 and 4E10) from HIV-1 infected patients [20–25] and the more recent discovery of additional mAbs with even broader neutralizing activities (PG9/PG16 and VRC1) [26–28] have indicated that there may be some common Env structures across very different HIV-1 isolates to render the viruses susceptible to neutralization, and thus, making it feasible to develop one HIV vaccine effective against many diverse strains.

However, such “antigenicity” analysis has not been translated into the design of optimal Env antigens for “immunogenicity” studies. Despite the success of neutralizing mAbs, there has been no real progress in inducing broadly neutralizing antibody responses through active immunization in animal or human hosts with structurally “optimized” immunogens. Surprisingly, in the recently reported RV144 clinical trial conducted in Thailand, which employed a prime immunization with recombinant canarypox vector vaccine vCP1521 (HIV-1 antigens expressed by ALVAC vector) plus booster injections of a bivalent recombinant gp120 subunit vaccine AIDSVAX B/E, a 31% decrease in HIV-1 infection was observed among those that received the vaccine compared to placebo [29]. Because this protection was identified in the early phase of HIV-1 infection, it is suspected that antibody responses may have played an important role [30, 31], which would challenge the prevailing concept that a gp120 monomer is not capable of eliciting protective antibody responses [32, 33]. At the same time, additional data from several groups have independently demonstrated that gp120 antigens, if delivered in a heterologous prime-boost format, may be able to elicit neutralizing antibodies against primary HIV-1 isolates that are not sensitive to neutralization [34–36]. Furthermore, the breadth of antibody responses can be significantly improved if multiple gp120 antigens are delivered in a “polyvalent formulation” [37]. Therefore, polyvalent Env vaccines may offer an alternative, and potentially complementary, approach to the long but unsuccessful effort of identifying an optimal Env antigen for the development of an effective AIDS vaccine to cover the broad diversity of HIV-1 isolates. The current review will highlight the key data in this area and outline the opportunities and challenges associated with the development of polyvalent HIV vaccines.

SUCCESS OF POLYVALENT VACCINES AGAINST OTHER PATHOGENS

The development and use of polyvalent vaccine strategies have been observed throughout the history of modern vaccinology. In the early 1900s, development of a polio vaccine was initially unsuccessful. It was later shown that the etiological agent of poliomyelitis could be

any one of three enteroviruses [38]. Two effective vaccine formulations were subsequently developed. The inactivated vaccine, formulated by mixing the three viral strains, was developed by Jonas Salk in 1952. Another was developed by Albert Sabin in 1957 and provided the advantages of a live, polyvalent attenuated oral vaccine [39] to cover major enteroviruses that are responsible for the outbreak of polio.

Polyvalent vaccines have also been successful against other viruses with more frequent mutations, such as the influenza virus. There are three types of influenza virus - Types A, B, and C. Influenza virus Types A and B have been responsible for epidemic human diseases whereas Type A viruses have been responsible for several past human pandemics. Vaccines against influenza virus incorporate new viral strains in each year's formulation based on the general design of trivalent vaccines: two Type A viruses (from H1N1 and H3N2), and a Type B virus. This polyvalent design can be used for both inactivated and live-attenuated forms of influenza vaccines. While this vaccination strategy has proven effective against seasonal influenza, it is still possible to further improve the coverage by adding another influenza vaccine component to cover new emerging viral strains. For example, with more infections with Type B viruses identified in recent years, there has been discussion on whether it is necessary to include a second Type B vaccine as the fourth component of expanded polyvalent influenza vaccines. Similarly, in the case of flu pandemics with avian or swine source viruses, a supplemental influenza vaccine can be developed and used in large scale in addition to routine seasonal influenza vaccines, such as during the 2009 outbreak of a new H1N1 swine origin virus [40, 41]. This supplement component proves the utility and flexibility of the polyvalent influenza vaccine strategy.

Polyvalent vaccines against bacterial infections, such as pneumococcus, have undergone various formulation changes throughout history including a 23-valent formulation as a vaccine with the highest number of antigens included in one formulation. The components of this 23-valent vaccine formulation were chosen to represent the majority of the individual serotypes that cause invasive pneumococcal infections in both developed and developing countries [42, 43] and a single inoculation results in IgM and IgG antibodies within a short period of time. However, since the 23-valent vaccine formulation is T cell-independent, it is less immunogenic in infants and in immunocompromised individuals and additional efforts have been made to convert the polysaccharide antigens to T cell-dependent antigens, leading to the development of a 7-valent conjugate pneumococcal vaccine licensed for clinical use to infants and young children since 2000 [44]. An improved 13-valent version has since been developed to provide coverage for children two months to five years against six additional strains of pneumococcal bacteria [45].

More recently, a quadravalent vaccine against human papillomavirus (HPV) has been developed against HPV types 6, 11, 16, and 18 [46–48]. The development of this vaccine has demonstrated two very important principles in vaccination technology. The first being that this 4-valent vaccine was able to broaden the scope of immune responses against four circulating strains of HPV and the second being that it afforded protection against two diseases - cervical cancer and genital warts, a new expansion on the concept of polyvalent vaccines.

The development of polyvalent vaccines against these very complicated viral and bacterial diseases provides a valuable lesson to researchers working in the field of HIV-1 vaccine development.

ARGUMENT FOR THE USE OF THE POLYVALENT STRATEGY FOR HIV-1 VACCINE DEVELOPMENT

A major challenge in developing an HIV vaccine is to identify immunogens and delivery methods that elicit balanced antibody and cell-mediated immunity against highly variant HIV primary isolates given that high titer neutralizing antibodies and broad cellular immunity are inversely correlated with HIV-1 disease progression in humans [49, 50]. The diversity of HIV-1 subtypes and the high frequency of viral mutations has almost eliminated the possibility of developing a vaccine based on a single natural HIV-1 Env antigen [51]. Significant effort has been made in various attempts to identify a unique Env structure that would induce broadly neutralizing antibodies against a wide array of HIV-1 isolates, such as the principal neutralizing domain (PND) based on the V3 loop [52], antigen sequence or structures of broadly neutralizing monoclonal antibodies [23, 24, 53–57], induction of fusion intermediates [58], and consensus *env* sequences [59, 60]. However, these attempts have not been successful because these approaches were not effective in eliciting broadly reactive neutralizing antibodies despite such ideas being very attractive in theory.

Furthermore, the development of polyvalent vaccines are more critical given the various mechanisms used by HIV to evade the host immune system, such as cytotoxic effects toward virus-specific T helper cells, escape from neutralizing antibodies and cytotoxic T lymphocytes, epitope masking through shielding by variable loops and glycosylation, downregulation of Major Histocompatibility Complex (MHC), and low-grade chronic infection through latently-infected cells [61]. An effective vaccine against HIV will likely need to circumvent these escape mechanisms [61]. The multifaceted nature of polyvalent vaccines (incorporating both T and B cell antigens) may be one solution to control HIV infection.

Facing the failure of eliciting broad antibody responses, researchers have tried to use different viral vectors to develop T cell immune responses with the hope that this strategy may overcome the diversity of viruses [62–74]. This strategy has been tested against various HIV-1 antigens (i.e., Gag, Pol, Tat, or Vpu) [73, 75–78] as a means to expand the coverage of HIV-1-directed vaccines. Unfortunately, most T cell alone vaccines could not completely protect non-human primates against simian human immunodeficiency viruses (SHIV) or simian immunodeficiency (SIV) challenges when sterilizing immunity is taken as the primary endpoint; however, many were able to produce a significant reduction in viremia and did provide protection from disease progression [65, 66, 71, 76, 77]. Gag-based T cell vaccines were able to achieve protection in non-human primates but one leading clinical trial of this strategy, the STEP trial, failed to elicit protection in humans, presumably due to limited numbers of CD8+ T cell epitopes elicited in individual volunteers by this vaccine [6, 7]. HIV-1 diversity continues to pose a significant threat to the development of an efficacious AIDS vaccine.

The idea of developing a polyvalent vaccine against HIV-1 has not been popular. Many HIV vaccine researchers will automatically rule out this strategy based on the concern that HIV-1, being a retrovirus, will continuously produce mutated viruses within infected individuals and in an endemic region, therefore, it will be difficult to protect against such rapid and broad viral sequence changes. While it is true that viral gene sequence complexity for HIV-1 over the past two decades is greater than that observed for other viruses, such as influenza, over a longer time frame, several studies have suggested the promising potential of a polyvalent approach for HIV vaccine development: 1) a combination of neutralizing monoclonal antibodies derived from several different sources has been shown to provide broad neutralizing activities *in vitro* and *in vivo* against heterologous isolates (for review, see [79]); 2) given that HIV protein structures are constrained by function, there exists some

restriction in structural diversity [80–82]; 3) although superinfection does occur for HIV-1, it is rare and patients that have been infected with HIV are often less susceptible to another HIV-1 infection [83]. Similarly macaques that have been infected with SIV or SHIV are protected from challenges with heterologous viruses [84, 85].

In fact, strategies of envelope selection for polyvalent Env vaccines have been directly or indirectly explored in the previous studies including the use of representatives from multiple clades, natural escape mutant viruses, and antigenically distinct envelope proteins as defined by antibody-antigen interaction and antibody-virus neutralization studies [86–89].

Initial research in primates demonstrated that although live-attenuated SIV vaccines could offer protection against subsequent infection in nonhuman primates [90–92], protection was observed only during challenge with the homologous virus and not when a heterologous challenge virus was used. Cho *et al.* subsequently showed that a recombinant vaccinia prime and subunit protein boost approach using a cocktail of HIV-1 Env glycoproteins was capable of eliciting neutralizing antibody responses against three or more viral strains of HIV [93]. However, the breadth of neutralization was still limited to the strains that were included in the vaccine formulation and vaccination did not afford protection from a heterologous SHIV challenge. A different type of polyvalent vaccine based on macaque simian immunodeficiency virus (SIVmac) regulatory genes, *rev*, *tat*, and *nef*, was demonstrated to be immunogenic in SIVmac-251-infected macaques as evidenced by an expansion of virus-specific CD8⁺ T cell virus responses but no protection was studied in this report [94]. Given the setback of the STEP trial, polyvalent vaccines with a focus on eliciting broad protective antibody responses are clearly indicated.

STUDIES WITH POLYVALENT ENV VACCINE FORMULATIONS

The discovery of the DNA-based vaccination approach has greatly expanded the technical flexibility of testing polyvalent HIV vaccines employing multiple HIV-1 Env antigens with sequence diversity. Candidate polyvalent Env formulations can be tested directly as DNA vaccines with *env* gene inserts to avoid the more complicated process of producing recombinant Env proteins. Several Env-expressing DNA vaccines can be mixed and delivered in one formulation without concern of any potential interactions among different recombinant Env protein antigens. Furthermore, a protein boost component can be added to further enhance the immunogenicity of polyvalent Env-expressing DNA vaccines.

DNA-based polyvalent vaccines composed of Env either from clades A, B, and C [95, 96] combined with homologous protein boost [97] or from clades A, B, C, D, and E combined with an homologous gp120 protein boost [37] have been tested for their ability to induce broad neutralizing antibody responses in guinea pigs, rabbits, and rhesus macaques with considerable success compared to immunization with a monovalent vaccine. More specifically, in this later study, it was shown that rabbit sera immunized with the DNA prime plus protein boosting approach, but not DNA vaccine alone or Env protein alone, were capable of neutralizing seven out of 10 viruses in a single round infection neutralization assay against a panel of 10 primary HIV-1 isolates of subtypes A, B, C, and E, and 12 out of 14 viruses in a PhenoSense assay against a panel of 12 pseudoviruses expressing primary HIV-1 Env antigens from subtypes A, B, C, D, and E. More importantly, sera immunized with the polyvalent Env antigens were able to neutralize a significantly higher percentage of viruses than the sera immunized with the monovalent antigens [37].

Additional studies have shown that immunization with a DNA vaccine encoding *env* genes from multiple clades of HIV-1 and a *gag* gene from a single clade of HIV-1, in combination with a gp120 protein boost homologous to the DNA vaccine, elicited neutralizing antibodies against homologous [98, 99] and, to a lesser extent, heterologous HIV-1 isolates, including

Env from clade A, which was not present in the vaccine formulation [100]. Furthermore, both Env- and Gag-specific cell-mediated immune responses were also observed and immunization with this vaccine platform was protective against a rectal challenge with a SHIV isolate encoding HIV-1_{Ba-L} *env* gene. Sterilizing immunity was achieved in four out of six immunized monkeys while all seven animals in the control group which did not receive any vaccines had high levels of viral infection after SHIV challenge. Further studies showed that this vaccine formulation induced Env-specific cellular immune responses and humoral immune responses in rhesus macaques and that in both macaques and mice, these effects were further augmented with administration of a protein boost [101]. Further dissection of cell-mediated responses showed that both CD8⁺ CTL and CD4⁺ T-helper cells were important contributors of the immune response.

Based on the results of the aforementioned preclinical trials, which employed a DNA vaccine encoding *env* genes from multiple clades of HIV-1 and a *gag* gene from a single clade of HIV-1 in combination with a gp120 protein boost homologous to the DNA vaccine, a Phase I clinical trial was carried out to determine the immunogenicity and safety of this multi-gene, polyvalent formulation in humans [102, 103]. Results from this clinical study demonstrated the immunogenicity of this vaccination platform as evidenced by robust cross-subtype HIV-1-specific T cell responses and high titer serum antibody responses that recognized a wide range of primary HIV-1 Env antigens and neutralized pseudotyped viruses that expressed the randomly selected primary Env antigens from HIV-1 subtypes A to E [11]. Approximately 60% of the volunteers in this study achieved such neutralizing activities against 50%–100% viruses in this 14-pseudotyped virus panel, while another 30% volunteers had neutralizing antibodies against 25–50% pseudotyped viruses. This finding with human immune sera is highly significant because it argues that at least a good percentage of primary HIV-1 isolates can be neutralized with by a five-valent Env vaccine formulation.

Recently, a more detailed analysis was conducted in rabbits to compare the relative immunogenicity between a monovalent DNA prime + monovalent protein boost and a monovalent DNA prime + polyvalent protein boost [104]. It was demonstrated that the use of a polyvalent protein boost was more effective than a monovalent protein boost in eliciting broader neutralizing activities against a panel of Tier 2 primary Env-based pseudotyped viruses. A similar finding was observed with V3 scaffold immunogens as the boost following DNA prime immunizations [105]. The V3 epitope is a well-known target on the HIV-1 Env antigen for the induction of neutralizing antibody (NAb) responses [89, 106]. V3-scaffold chimeric proteins were developed to fuse a V3 peptide with an unrelated protein to focus the body's antibody response to the V3 epitope. This fusion protein was effective in boosting HIV-1-specific neutralizing activities in rabbits primed with a gp120 DNA vaccine [107]. Compared to a single V3-scaffold fusion protein, a mixture of bi-valent V3-scaffold proteins, as a boost formulation following a monovalent gp120 DNA prime, provided better breadth and/or potency against V3 chimeric pseudotyped viruses, primary isolates from clades A, A/G, and B, and Tier 1 and Tier 2 viruses from clades B and C [105].

The use of the polyvalent concept purely for T cell immune responses has been rare but in one study, HIV-specific induction of cellular immunity has also been observed following immunization with polyvalent clade A, B, and C Env-based DNA vaccines; a response that was not observed following immunization with the monovalent vaccine formulation [95, 97]. A new way of producing polyvalent T cell-based vaccines is the design of mosaic vaccines: antigens comprised of sets of artificial proteins assembled from fragments of natural sequences *via* a computational optimization method [108]. This approach was subsequently shown effective in expanded coverage against known T cell epitopes, when compared with the use of natural HIV antigens in non-human primate models [109, 110]. It

is yet to be seen whether this approach is also immunogenic in humans and, ultimately, whether improved breadth of CD8+ T cell epitopes can lead to protection against HIV-1 infection in efficacy studies.

FUTURE DEVELOPMENT OF POLYVALENT AIDS VACCINES

While studies on the use of polyvalent formulations for AIDS vaccine development are still very limited, data collected from the above studies provide an important indication that there may be some unrecognized merit in the polyvalent approach. A lack of animal models to directly test protection efficacy against HIV-1 infection makes proving the utility of polyvalent AIDS vaccines difficult and studies may have to rely on biomarkers to measure its relative effectiveness, until a more advanced clinical efficacy trial can be conducted. Therefore, additional basic studies are needed to ask the following questions:

First, can primary Env antigens be grouped based on their neutralizing sensitivities or specificities to neutralizing antibodies/sera. Binley *et al.* showed that primary HIV-1 can be divided into five major groups based on their susceptibility to various neutralizing antibodies [111]. One question that is prompted from this study relates to the structural basis for such grouping and gaining insight into this aspect may further guide the development of representative Env antigens targeting each of these groups.

Second, with the discovery of new neutralizing mAbs targeting different epitopes [26–28], it will be very important for future studies to determine if mixing two or more of such mAb (i.e. mAbs that target different neutralizing epitopes) can achieve any synergistic effect against HIV-1, as measured by the breadth or potency of neutralizing antibody responses.

Third, the issue of how to group available primary Env antigens (from their genetic sequences) based on the information learned from the above two questions arises.

While such grouping may not be obvious based on the traditional primary sequences of proteins, hopefully, with more information from the above studies, algorithms can be developed to identify the subtle structure common ground for such groups.

Finally, more polyvalent Env small animal studies should be conducted to dissect any potential differences in antibody responses elicited by different individual Env antigens. Although it is possible to start this screening process in a random fashion, the results from the suggested neutralizing grouping studies and the more rational selection of Env with unique sequence of structural signatures may be used as studies advance. At the end, we can learn if one polyvalent formulation is more effective than the other polyvalent formulation when they are measured against different populations of HIV-1 isolates in the world.

T cell-based polyvalent vaccines are just being explored and it is a powerful approach because it is supported by sophisticated bioinformatics analysis. It will certainly provide an important supplemental approach to the traditional antibody-based polyvalent vaccination approach.

While the above studies can be done in small animals or in selected non-human primates to prove the general principles of polyvalent formulations for AIDS vaccine development, it is equally important to combine human immunogenicity studies to test any important information learned from animal studies. Such human studies do not need to be designed to test the efficacy of such formulations. Instead, analysis on the biological activities of human sera and lymphocytes either between volunteers receiving a monovalent and a polyvalent Env formulation, or between those receiving different polyvalent Env formulations, will greatly expand our understanding on the potential of such approaches.

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ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
CRFs	Circulating recombinant forms
Env	Envelope
HIV-1	Human immunodeficiency virus-1
HPV	Human papillomavirus
mAbs	Monoclonal antibodies
Nab	Neutralizing antibody
PND	Principal neutralizing domain
SHIV	Simian human immunodeficiency virus
SIV	Simian immunodeficiency virus
SIVgor	Gorilla simian immunodeficiency virus
SIVmac	Macaque simian immunodeficiency virus

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