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# Recent Advances on the Use of Structural Biology for the Design of Novel Envelope Immunogens of HIV-1

Shi-Hua Xiang
*University of Nebraska-Lincoln*, sxiang2@unl.edu

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# Recent Advances on the Use of Structural Biology for the Design of Novel Envelope Immunogens of HIV-1

**Shi-hua Xiang**[*,1,2]

[1]Nebraska Center for Virology, University of Nebraska-Lincoln, Lincoln, NE 68583, USA

[2]School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln, Lincoln, NE 68583, USA

## Abstract

Many efforts have been made in the worldwide quest for a prophylactic HIV vaccine to end the AIDS pandemic, but none has yet succeeded. The lessons learned have repeatedly informed us that the traditional or conventional approaches directly using the pathogens or subunits will not be sufficient for an effective HIV/AIDS vaccine. Recent advances in structure-based technology have shown some promise in the quest for a better immunogen in HIV vaccine development. According to the basic binding structural relationship of an antigen and an antibody, structure-based antigen design could bring some hope for the development of an effective vaccine against HIV.

### Keywords

HIV-1; Immunogen design; Vaccine development; Structure-based design; Envelope trimer; Antibody epitope; Germline B-cells

## Introduction

Human immunodeficiency virus type 1 (HIV-1) was identified as the causative agent of human acquired immunodeficiency syndrome (AIDS) about thirty years ago [1, 2], but unfortunately we still do not have an effective vaccine to stop this epidemic. Although highly active antiretroviral therapy (HAART) is effective in treating patients by controlling their viral load, it cannot completely eliminate the virus from the body. This is because the virus can reside latently in the resting memory $CD4^+$ T cells, where the latent virus is not sensitive to the drugs [3-8]. When the patients stop HAART, the virus generally rebounds to a high level within a few days. Once an individual is infected by HIV-1, it is almost



[*]Address correspondence to this author at the Nebraska Center for Virology, School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln, Morrison Center 143, 4240 Fair Street, Lincoln, NE 68583-0900, USA; Tel: (402) 472-4520; Fax: (402) 473-3323; sxiang2@unl.edu.

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invariably lifelong. Thus, preventing new infection is critical for ending the HIV/AIDS epidemic.

According to UNAIDS, there are about 34 million people who are currently living with HIV-1 and about 2.6 million people are newly infected each year [9]. Therefore, a vaccine is urgently required to curb the worldwide HIV/AIDS pandemic. Many efforts have been made in searching for an effective vaccine against HIV-1 infection in the past 30 years, but thus far, these efforts have not been successful. Several review articles have been published recently summarizing the basic research and clinical trials which have been conducted [10-13]. Notably, many approaches previously used in successful vaccine development for other pathogens have been applied to HIV/AIDS, but none has been successful [10]. These have included the use of killed or attenuated virus particles (14), HIV-1 subunits of envelope glycoproteins gp160 or gp120 [15-17] and envelope peptides [18-22]. So far the only HIV vaccine trial that has shown some efficacy is the Thai RV144 trial using the subunit gp120 recombinant proteins [23]. However, RV144 only showed 31% protection, which will not be effective enough for actual use as a vaccine, but was merely statistically significant when compared with the control group [23]. Nevertheless, RV144 is considered to be the most successful HIV-1 vaccine trial so far [23]. Therefore, traditional or conventional vaccine approaches by directly using the pathogens (killed or attenuated) or the subunits may not be suitable as an HIV/AIDS vaccine. The fact that broad neutralizing antibodies (bNAbs) have been found in only 10-25% HIV-1-infected people from recent large scale screening [24-28] underscores the difficulty of obtaining antibody-based vaccine-mediated protection using conventional approaches.

## Why do the Traditional or Conventional Approaches not Work for Developing an HIV/AIDS Vaccine?

During the past three decades, extensive studies on HIV-1 structure, pathogenicity, and evolution have concluded that the virus has evolved into an immunorecessive form, or so-called “decoy” form, to evade the human immune system surveillance [29-31]. This decoy form of the virus basically has two characteristics: one is that the virus cannot induce a robust response when presented to the human immune system; another is that the viral conserved immunogenic epitopes are not exposed. Since the neutralizing epitopes are hidden or not fully exposed on the surface of the virus, only a minimal level of neutralizing antibodies with no breadth of activity was induced in spite of having a strong antibody response to the HIV-1 envelope glycoproteins in HIV/AIDS patients. This could be one of the reasons why traditional or conventional vaccine immunogens directly using the viruses or their subunits have been ineffective.

HIV-1 is an enveloped retrovirus. The envelope spikes on the viral membrane surface consist of the glycoproteins gp120 and gp41, which are the most prominent viral proteins that directly interact with the human immune system. Studies have shown that Env spikes are sparsely scattered on the viral surface [32-34]. HIV-1 requires two receptors for its entry, i.e. the primary receptor CD4 and a co-receptor CCR5 or CXCR4. The binding of gp120 to the primary receptor CD4 induces a conformational change in gp120 to expose the binding sites in gp120 for coreceptor binding [29, 35, 36]. Receptor binding is a sequential process

and the coreceptor binding epitope is not exposed before CD4 binding. Thermodynamic analysis showed that the entropy and enthalpy changes of gp120 upon the binding of CD4 are remarkably large [37]. It was suggested that the gp120 molecule undergoes a major conformational change upon CD4 binding. This large conformational change of gp120 may cause some antibodies to lose their neutralizing activities or reduce their neutralizing capacities, by decreasing their binding affinity or preventing them from binding HIV during the viral entry process.

Extensive studies on the envelope spikes have been carried out to analyze their composition, structure, and immunogenicity. Recent structural studies on the trimeric envelope spikes have opened new avenues for Env trimer-based vaccine design. It has become much clearer that the major loops (V1, V2 and V3) of gp120 are involved in trimer stabilization (Fig. 1) [34, 38, 39]. For a long time, it was thought that the gp120 core structure would form a trimeric body from which the loops might extend. This assumption led to a number of studies in which the loops were eliminated in order to stabilize the structure of the monomeric gp120 or the Env trimer for vaccine design. In fact, the major variable loops (V1, V2, and V3) are packed into the central apex of the trimeric spike body, so changes in the loops can adjust the trimer stability to adapt to the host immune systems. The adjustment could occur through substituting amino acids in the loop region and also through changing the loop length [40-43]. It is apparent that this trimeric packing model also has great advantages for viral evolution and generation of viral diversity. The evolution and adaptation of this virus must have occurred during the passaging of simian immunodeficiency viruses (SIV) to chimpanzees (SIVcpz) and then finally to humans (HIV) [44-50]. It is likely that the virus has gone through an adjustment process to allow it to evade the human immune system.

Another characteristic of the HIV-1 immune evasion is the glycosylation of the envelope glycoprotein gp120. The gp120 is heavily glycosylated with about 25-30 glycan molecules, which are mostly of high-mannose forms. Thus, most of the envelope surface is covered by glycans that form a protective shield. This is a major barrier for HIV-1 vaccine development, because the heavy glycan shielding can protect the virus from neutralizing antibodies. Recently, however, some antibodies such as PGT128 were found to be able to penetrate the glycan shield [51-55].

Because traditional or conventional methods used the whole or part of the pathogen as immunogens for vaccination, they cannot overcome the various decoy forms of HIV-1. In order to solve these problems, other approaches must be sought to overcome immune evasion by the viruses. One such approach is the use of modern structural biology to design novel vaccines against HIV-1 infection. The structure-based immunogen design is an innovative and revolutionary method that could help with HIV/AIDS vaccine development.

## Structure-Based Immunogen Design for Developing an HIV/AIDS Vaccine

Structural modeling and simulation methods have been broadly used for protein design to study the structure and function of proteins. These could be used to structurally modify and design the immunogens to increase their immunogenicity and to induce specific neutralizing

antibodies. The structure-based design approaches may 1) expose the conserved sites, 2) stabilize a conformation of the immunogen, 3) mimic an antibody epitope. The structure-based design of a vaccine could be described as one form of reverse vaccinology [56, 57]. This approach does not involve the use of killed or attenuated viral particles or the subunits for the vaccine, but uses the structural information of the pathogens for the development of an effective vaccine. Structure-based design of HIV-1 vaccines can be classified into four major categories and they are described below, and also summarized in Fig. (2).

## Envelope Trimer Structure-Based Immunogen Design

It has been known for a long time that trimer-based immunogens generally produce better neutralizing antibodies than subunit-based immunogens [58]. However, it is much more challenging to generate trimer-based immunogens. The envelope trimer consists of the surface envelope glycoprotein gp120 and the transmembrane glycoprotein gp41, but there are no inter-molecule covalent bonds between gp120 and gp41, therefore, it is more difficult to generate a soluble Env trimer for vaccine design. Several methods have been tested for generating Env trimers, such as using a GCN4 trimeric motif [59-61], a T4 bacteriophage fibritin trimer motif (62), using the disulfide bonds to build the soluble cleaved gp140 trimers (63-65), or the V2-loop deleted modification gp140 trimers [66, 67]. The soluble gp140s were able to induce stronger immune responses in animal models than gp120 monomers, and the immune sera were confirmed to have higher neutralizing activities [64, 68-72] although this is not thought to be adequate for vaccine-induced HIV-1 protection.

With the advent of recent structural findings on the trimer architecture of the HIV-1 envelope trimeric spikes using the cryo-electron microscopy (cryo-EM) approaches [32, 38, 73-75], there is now more detailed information on how to stabilize an env-trimer for a vaccine immunogen. With the envelope trimer structure at 11Å resolution based on the cryo-EM, the locations of most domains and the loops can be distinguished (Fig. 1) [38], and a recently published report at 6Å resolution allows most of the secondary structure elements of the HIV-1 Env trimer to be located [34]. This has now provided more information on the structural basis in the stabilization of the molecule, such as the trimer association domain (TAD), which consists of the V1V2 loops and a central mini-barrel, making direct trimeric contacts from the three subunits of gp120. The TAD presumably includes the twin-cysteine motif of the V2-loop of simian immunodeficiency virus, which may form the disulfide bonds in the subunit (intra-) or between the subunits (inter-)(76). Stabilizing the trimer structure appears to be a good strategy for developing a trimer-based vaccine. There are two main methods for the design of a trimer-based vaccine. One is to stabilize the native trimer conformation without other modifications [62, 64, 68, 77, 78]. The rationale of this design is based on the findings that most of the trimer spikes are nonfunctional and unstable [38, 79]. Because the native trimer structure has an open and flexible architecture (Fig. 1), the gp120 subunits can easily fall apart from the gp41 subunits. It is estimated that there are very few particles with stable or functional envelope trimeric spikes [32, 33, 79]. Therefore, generating more stable trimers should result in better immunogens for HIV-1 vaccines. Another strategy for trimer-based immunogen design is to expose the hidden conserved epitope sites [29-31] and this may include removing some glycans to increase the exposure of the conserved epitopes for antibody targeting and to increase its antigenicity [80-83].

The virus-like particle (VLP) design is mainly for stabilizing the envelope trimer structures on a native particle resembling a virion [84, 85]. VLPs are generated essentially by mimicking the native viral particle structure. VLPs are able to be self-assembled, but are non-replicating and non-pathogenic due to the absence of viral genetic elements. VLP-based vaccines are much safer to use than attenuated HIV-1 forms, and can also be engineered easily by the structure-based approaches. Recently, HIV-1 VLPs have been successfully produced from Drosophila cells [86], and even in plants [87]. The VLP-based immunogen design offers a new approach for developing a safe and effective HIV-1 vaccine [85, 88, 89].

## Antibody Epitope Structure-Based Immunogen Design

The epitope structure-based design utilizes the antibody epitope for immunogen design. It is based on the notion that the epitope-based immunogen should elicit antibodies that can bind the same epitope on the viral spike. Recent advances in identifying neutralizing antibodies have provided renewed hope to develop an effective vaccine against HIV/AIDS. High through-put technology for large scale screening of neutralizing antibodies, in combination with the B-cell evolutionary genomics, have led to the isolation of a number of broad neutralizing antibodies (bNAbs) [25], such as VRC01-03 (90), PG04 (91), HJ16 (92), PG9 and PG16 (26), CH01-04 (93), and PGT121-137 [94]. For some of these bNAbs, the structures have also been solved with the binding epitopes of the envelope glycoproteins of HIV-1. The structural information from the co-crystalized structures of the binding complex of antibodies and antigens are especially valuable for epitope structure-based design [11, 95, 96].

The membrane-proximal external region (MPER) of gp41 is a well-known vulnerable region as target of neutralizing antibodies [97], such as 2F5 [98], 4E10 [99], 10E8 [100] and Z13e1 [99]. The MPER neutralizing antibodies 2F5 and 4E10 appear to be autoreactive [101, 102], but it is not clear whether the autoreactivity or polyreactivity is detrimental for vaccine purposes. It is interesting that another MPER monoclonal antibody 10E8 was not found to bind phospholipids and is not autoreactive [100]. Because the MPER-binding antibodies have broad neutralizing capacity, and their binding epitopes are linear, they represent a very attractive solution for structure-based immunogen design. Since the structures of some of the MPER antibodies and their epitopes have been solved [103-105], structure-based design for better immunogens can now be easily conducted. One of the first studies on epitope design was to constrain and stabilize the 2F5 epitope-based fragment as an immunogen [103, 106]. More recently, the protein scaffold grafting approach has been introduced to this design, and the 2F5 linear epitope was grafted onto a protein scaffold [107, 108]. This engineered 2F5 epitopescaffold was used for immunization and elicited antibodies that could recognize the 2F5 epitope and had significantly higher binding affinity. Unfortunately, the antibodies did not result in neutralization of the virus [95, 107, 109]. Other similar MPER neutralizing antibodies 4E10 [110] and Z13e1 [111], were also tested for epitope-based grafting design; the outcomes were similar as 2F5, and the antibodies generated did not possess any strong neutralizing activity. One explanation perhaps is that these antibodies bind to their epitopes in a different conformation from 2F5 and resulted in less potent neutralization activities.

Another target for epitope structure-based vaccine design is the primary receptor CD4-binding site (CD4-bs). A number of bNAbs target this site, such as the well-known monoclonal antibody b12 [112], and the recently discovered monoclonal antibodies VRC01-03 [90], PG04 [91] and HJ16 [92]. From the actual structural binding information of the CD4-bs antibody b12 [112] and VRC01 [113], it was found that their epitopes are mostly located on the outer domain of gp120. However the CD4-binding site is not a linear epitope, and it is discontinuous- or conformation-dependent. Therefore, the CD4-bs antibodies generated are also against discontinuous epitopes which have presented a challenge for structure-based immunogen design. The protein grafting approach was also tested with the CD4-bs antibody b12 epitopes. The designed b12 discontinuous epitope-scaffold from the backbone grafting was able to bind b12 antibody with high affinity. It was also shown that the designed epitope scaffold is structurally similar to the b12 discontinuous epitope [114]. The structure-based design using germ-line genes to target the CD4-binding site has also been tested, and such an approach is described in the following section on B cell-based immunogen design.

## Glycan Structure-Based Immunogen Design

The glycans on the surface of HIV-1 virions play an important role in shielding and protecting the virus from the immune response because they function as a steric barrier for the binding of neutralizing antibodies. However, the glycans of HIV-1 have been demonstrated to be immunogenic and immunogenicity [40, 118], and can also be a target for vaccine design. For example, it has been shown that bNAb 2G12 [115] can directly bind to the glycans on the outer domain of gp120. Furthermore, a number of other glycan-associated bNAbs have recently been identified, such as PGT121-137 [94]. These antibodies not only directly interact with HIV-1 glycans on the gp120, but can also penetrate the glycan shield to reach the short beta-strand segment of the gp120 V3-loop [51]. The antibodies of PGT141-145 [116] and PG9 [116] and PG16 (26) have also been found to share a specificity for an N-linked glycan at residue 160 in V1V2 of gp120 [116]. The glycans consist of mostly N-linked oligo-mannoses, and the Manalphal →2Man motif is the primary neutralization determinant (epitope) for antibody 2G12 [117]. The PG9 and PG16 antibodies have an unusually wide and long CDR H3 loop, and can potentially bind to both the glycan and protein backbone of the virus to mediate neutralization. Similarly, the epitopes of PGT121-137 consist of both the glycan and protein backbone. Thus, it is well established that the glycans can also be targeted by neutralizing antibodies. The loss of glycans in HIV-1 gp120 vaccine candidates may result in a loss of their antigenicity [119-125].

The glycan structure and its distribution pattern on the virion are critical for glycan structure-based immunogen design. Therefore, in order to target HIV-1 glycans by vaccine-induced antibodies, one must first determine the types of glycans which are specific on the virion surface and whether they are distinguishable from other glycans present on other proteins. A glyco-conjugate antigen based on the antibody 2G12 recognition motif was used to immunize rabbits, but the elicited antibodies failed to bind the glycans of gp120 [126]. However, using polysaccharide mimicry of the epitope of 2G12 from yeast, it was shown that they were able to elicit 2G12-like antibodies [117]. In addition, high-mannose oligosaccharides from yeast have also been used for immunization; they elicited antibodies

that could recognize the glycan structures. These antibodies generated unfortunately did not have neutralizing activities [127, 128]. An engineered triple mutant yeast strain elicited antibodies could bind to gp120 glycans but were not able to bind the cell surface-expressed trimeric envelope [129]. However, when the envelope was expressed in the presence of mannosidase inhibitor, the elicited antibodies gained the ability to bind the trimeric Env and neutralize the HIV-1 viruses. This suggests that the high-mannose glycans may be required for eliciting neutralizing antibodies against HIV-1 [129].

### Germline B-Cell Receptor-Based Immunogen Design

Structure-based vaccine design can be also applied to the germline B-cell receptor-based immunogen design. This is based on the experience gained from *in vitro* B-cell maturation studies to identify bNAbs. The basis of this approach is to stimulate or activate the germline B-cells using specific immunogens to induce them to enter a specific maturation pathway in order for them to produce more specific antibodies against HIV-1. Germline B-cell receptor-based immunogen design appears to be a more specific approach for inducing or educating the B-cells to make specific antibodies. Recent reports on germline B-cell based immunogen design have focused on modifying the gp120 immunogen for eliciting VRC01-like [130-133] and 2F5-like bNAbs [109, 134]. In addition, the complex-type N-glycan binding antibody PGT121 has also been investigated by inducing differentiation of the germline B-precursor cells [135].

## Challenges of Structure-Based Vaccine Design

The use of structural biology has shed some light on making better immunogens for HIV-1 vaccine development (13, 96, 136). The structure-based approach is mainly dependent on the binding structural relationship between an antigen and an antibody. However, one must be aware that it is the natural immune response that is important in generating antibodies specific against incoming antigens or pathogens, and the process of generating an immune response is complex and may not be simply mimicked by biophysical structural relationships [137]. The same antigen or even the same small epitope can induce many different types of antibodies. As mentioned above, with the 2F5 epitope design, there are some 2F5-like antibodies elicited that cannot neutralize HIV-1. This suggests that antibody synthesis or the generation of broadly neutralizing antibodies is a complicated *in vivo* process that may not be replicated readily by a simple biochemical synthesis process *in vitro*. That is why one has to carefully evaluate and validate any immunogen based-designed HIV vaccine in the context of an immune system within a host [138].

There are a number of challenges to designing HIV-1 immunogens, and they can be summarized as follows. First, it is important to note that the bNAbs are rare in HIV-1 infected patients, and they often have unusual features. For example, these features include a long CDR H3 loop (b12) or a long and wide CDR H3 loop (PG9, PG16); a sulfated tyrosine on the CDR H3 loop (412d); swapped heavy chains (2G12); or auto-reactive and poly-reactive activities (2F5, 4E10). Most of these structurally uncommon antibodies may not be easily elicited by a normal vaccine or *via* the normal immune response pathway [139]. Second, for the trimer structure-based design, there is still a need to obtain a higher-resolution structure at an atomic level of a native Env trimer. In addition, how can we

stabilize the native or mutant trimers? Since the interaction between gp120 and gp41 is non-covalent, it will always be a challenge in generating stable and cleaved soluble trimers. Third, for the epitope-structure-based design, stabilizing the epitope structure alone or presenting it on a carrier scaffold may be the key to success. However, it will be a challenge to induce the immune system to recognize primarily the neutralizing epitopes and in parallel reduce the induction of other antibodies that are usually non-neutralizing. Fourth, in glycan structure-based design, the binding characteristics of glycan-associated bNAbs are specific for a conformation on the HIV-1 virion. It has been suggested that glycan-targeting antibodies may still need to interact with viral protein epitopes. Some of these antibodies can penetrate the glycan layer and reach the viral protein backbone epitopes. Fifth, in the germline B-cell targeting approach, the engineered antigens should induce or activate B-cells to mature in order to make specific bNAbs against HIV-1. Using such an approach *in vitro* may not reflect the process *in vivo*, since B cell maturation in the context of the entire human immune system will be significantly more complex.

In conclusion, HIV-1 has evolved into an immune decoy form which includes glycan shielding, recessing of conserved epitopes and trimer flexibility. These unique Env trimer properties have rendered these proteins poorly immunogenic. This is one of the reasons why natural HIV-1 infection cannot induce a robust immune response by the host to control viral infection. It can also explain why conventional vaccines using the pathogen or subunits as immunogens have not shown any success thus far. The structure-based immunogen design has achieved some successes in eliciting structurally similar antibodies that are able to bind the epitopes, but the elicited antibodies still do not have strong neutralizing activities. The problem we are now facing is how one can use a highly modified antigen to induce broadly neutralizing antibodies against the native and unmodified incoming viral targets. This is currently one of the most challenging questions in the field of structure-based HIV-1 vaccine design.

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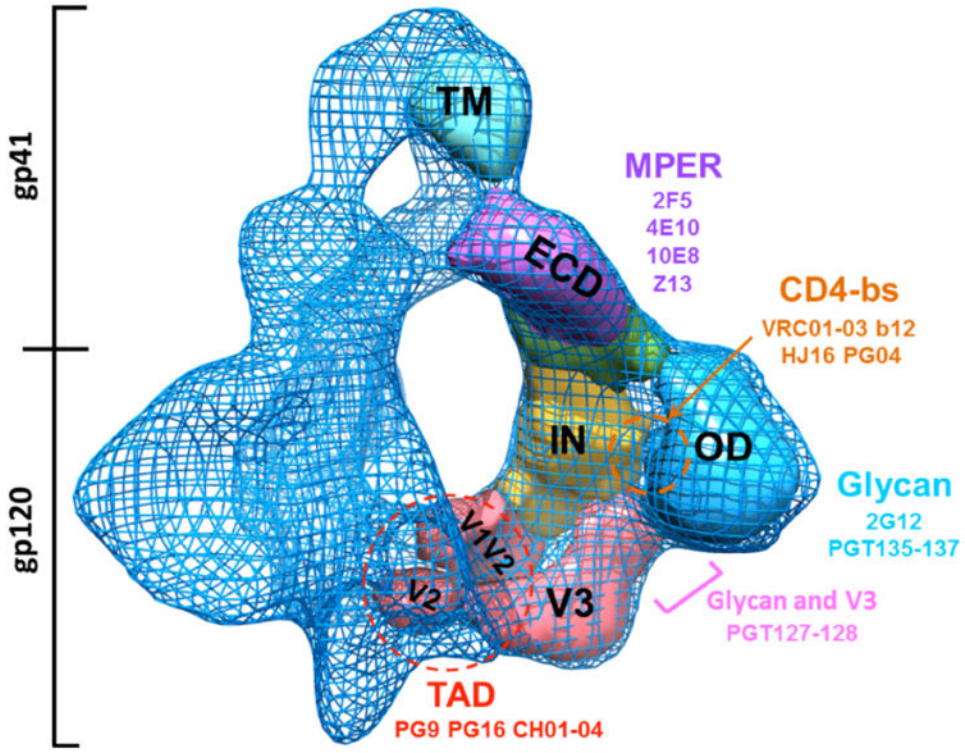


**Fig. (1).**

The HIV-1 Env trimer structure and the binding sites of broadly neutralizing antibodies (bNAbs) [13, 34, 140]. The picture is adapted from EMD-5418 at a resolution about 11Å in the Electron Microscopy Data Bank. The domain structures are shown in different colors. TM, transmembrane domain; ECD, Ectodomain of gp41; IN, inner domain of gp120; OD, outer domain of gp120, V1, V1 loop and etc.; TAD, trimer association domain. MPER, membrane-proximal external region; CD4-bs, CD4 binding sites; Glycan, glycosylation sites.

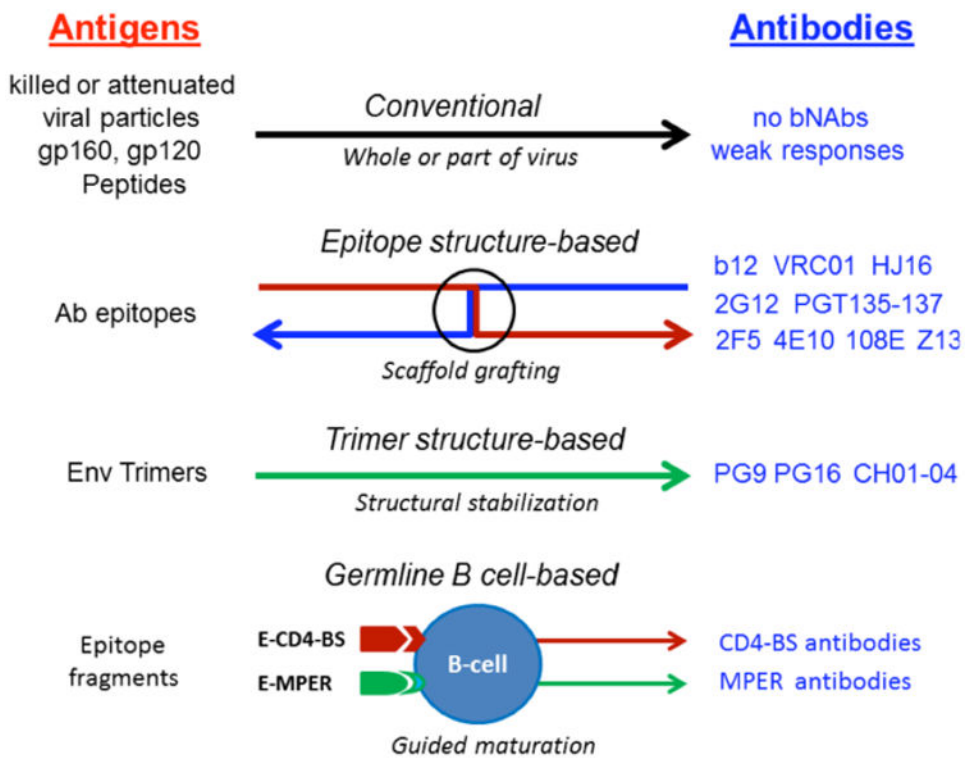


**Fig. (2).**

Current vaccine approaches for making preventive vaccines against HIV-1/AIDS [11, 13, 136]. The conventional vaccines directly use the whole pathogens or part of the pathogens as antigens for immunization, such as killed or attenuated viral particles, subunits (gp160 and gp120) of viral surface proteins, or peptides of the surface proteins. The epitope structure-based approach is based on reverse vaccinology [57] for developing vaccines, and it must utilize the known neutralizing epitopes for conducting antigen design. The trimer structure-based approach uses the envelope trimer structure for antigen design [58]. Some trimer-specific antibodies such as PG9 and PG16 could also provide useful information for designing the trimer-based antigens [26, 141]. The germline B cell-based approach is more relevant to the native immune response in inducing specific neutralizing antibodies against HIV-1 infection [132]. bNAbs, broadly neutralizing antibodies; Ab antibody, Env, envelope, E-CD4-bs, epitope of CD4-binding site; E-MPER, epitope of membrane proximal external region.