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Back to the future: covalent epitope-based HIV vaccine development

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Abstract

Traditional HIV vaccine approaches have proved ineffective because the immunodominant viral epitopes are mutable and the conserved epitopes necessary for infection are not sufficiently immunogenic. The CD4 binding site expressed by the HIV envelope protein of glycoprotein 120 is essential for viral entry into host cells. In this article, we review the B-cell superantigenic character of the CD4 binding site as the cause of its poor immunogenicity. We summarize evidence supporting development of covalent immunization as the first vaccine strategy with the potential to induce an antibody response to a conserved HIV epitope that neutralizes genetically divergent HIV strains.

Keywords

B-cell superantigen; catalytic antibodies; CD4 binding site; covalent antibodies; covalent immunization; electrophilic immunogen; gp120; nucleophilic antibodies; preventive HIV vaccine; therapeutic HIV vaccine

Is effective HIV vaccination feasible?

There is agreement that eradicating HIV will require development of an effective vaccine. In 2008, there were 2.7 million new cases of infection and 2 million people died of AIDS [1]. HIV mutates rapidly and thousands of HIV-1 strains have emerged [2]. Subtype C strains account for the majority of infections. The primary mode of HIV transmission worldwide is

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heterosexual vaginal intercourse. Most infections are initiated by strains that utilize chemokine coreceptor CCR5 for entry into host cells [3]. Coreceptor CXCR4-dependent strains emerge with time. Approximately 50% of patients develop CXCR4-dependent or dual tropic strains in approximately 10 years [4]. Both types of strain use CD4 as their primary host receptor.

The problem

Numerous HIV vaccine trials have attempted to induce neutralizing antibodies (Abs), cytotoxic T cells or both effector immunity arms [5]. Candidate vaccines tested in humans have been composed of the envelope proteins alone or combined with other HIV proteins. Multi-epitope synthetic peptides and polypeptides expressed by noninfectious vectors have been tested. Despite induction of robust immune responses, the VaxGen recombinant glycoprotein (gp)120 trial [6] and the Merck adenoviral *gag/pol/nef* (STEP) trial [7] did not reduce the risk of infection. The RV144 vaccine, composed of the full-length gp120 protein and a canary pox vector expressing the *gp120/gag/protease* genes, reduced the risk of infection marginally (by 31%) [8]. Risk reduction at this level is insufficient to stop the HIV pandemic. Furthermore, the risk reduction was insignificant for study subjects who completed the full course of immunizations. Similar to the ineffectual response induced by candidate vaccines, the natural immune response occurring after HIV infection generally does not control the spread of the virus.

The underlying problem is that HIV mutates rapidly, resulting in structural inconstancy of the viral envelope proteins [2]. The constituents of candidate vaccines are usually drawn from a unique HIV strain or at most a few strains, whereas genetically divergent virus strains cause the infection in different individuals and different parts of the world. The mutable regions of the HIV coat are also its immunodominant epitopes [9,10]. Consequently, previously tested candidate vaccines induced Ab and T-cell responses mostly directed to the variable coat protein regions, and vaccine efficacy against infecting strains expressing structurally divergent coat proteins was poor. Furthermore, the viral coat structure changes as the infection progresses. Viral escape mutants emerge and any immune protection conferred by the candidate vaccine is likely to be transient.

Potential solutions

Induction of neutralizing Abs has been the cornerstone of effective vaccination against microbes. The HIV surface is sparsely studded with noncovalently associated gp120–gp41 complexes [11]. These proteins are frequent targets of candidate vaccines. The failure of the whole virus and gp120/gp41 protein immunogens to induce Abs that neutralize genetically divergent HIV strains prompted a regrettable shift away from approaches designed to induce humoral immunity since the 1990s. Induction of cytotoxic T cells became the favored path to HIV vaccination. Cytotoxic T cells with the correct specificity can lyse infected cells and hold the potential of containing HIV after the infection has already occurred. However, T cells cannot prevent infection, as they do not inactivate the free virus. Moreover, cytotoxic T-cell responses suffer from the same problem as the humoral immune response – escape mutants resistant to candidate vaccine-induced immunity develop frequently [12]. With mounting pressure due to failure of the candidate vaccines, public health agencies have taken the position that combining the induction of humoral and cell-mediated immune responses will be needed for effective vaccination. As the two individual effector arms of the immune system were ineffective separately, it is hard to conceive that combining the traditional vaccine formulations will be useful beyond yielding an incrementally improved vaccine efficacy.

Although the virus mutates rapidly, it must keep certain surface protein epitopes as mostly constant structures to maintain its infectious capability. These regions are essential for virus–host recognition steps and virus propagation. Inducing a robust immune response to structurally conserved epitopes that are important in the viral life cycle is the logical route to a vaccine that is effective worldwide and minimizes the prospect of viral escape mutants. To prevent infection, the targeted region must be expressed on the surface of free virions in a form that is sterically accessible to Abs. The central problem is that the vulnerable HIV epitopes are poorly immunogenic, either because of physical occlusion or active immune suppression mechanisms. Similar to the full-length gp120 protein, peptide immunogens corresponding to its variable regions induce mostly strain-specific immune responses. Conformational mimicry of the discontinuous epitopes drawn from the conserved gp120 regions has been difficult because of limitations in contemporary physicochemical methods for accurately assessing protein structure and dynamics in the course of binding to Abs. Peptides with sequences identical to linear conserved epitopes of gp120 can be synthesized readily, but the conformation of such peptides can also diverge from the native epitope conformation. Non-native peptide conformational states will induce Abs with useless, non-neutralizing specificity. Consequently, no promising epitope-based vaccine candidate has emerged until recently. Here, we review the potential of developing a vaccine candidate that steers the humoral immune response to a vulnerable HIV epitope located in the host CD4 binding site (CD4BS) of the virus.

Neutralizing gp120 epitopes & their immunogenicity

Epitopes susceptible to neutralizing Abs (neutralizing epitopes) have been mapped using Abs produced after HIV infection or by experimental immunizations. Very few conserved neutralizing epitopes that are suitable for vaccine targeting have been identified (Table 1). Rare monoclonal Abs (mAbs) to gp120 and gp41 display comparatively broad neutralizing activity, for example: mAbs to a conformational gp120 V2–V3–C4 domain epitope [13], mAb b12 to a conformational epitope overlapping the CD4BS [14], mAb 2G12 to a carbohydrate epitope [15] and mAb 2F5 to the gp41 membrane-proximal external region [16]. Abs similar to these mAbs are not found at appreciable levels in the polyclonal Ab mixture present in the blood of infected humans or animals immunized with experimental immunogens. This indicates the poor immunogenicity of the neutralizing epitopes, consistent with heroic efforts required to identify the neutralizing mAbs. While locating a neutralizing epitope for vaccine targeting is a significant first step, effective vaccination will require development of an immunogen that induces a robust polyclonal Ab response with the ability to neutralize genetically diverse HIV strains.

Vaccine development strategies based on Abs to the gp120 variable (V) domains have been largely abandoned, as such Abs only express strain-specific neutralizing activity. Partial broadening of Ab specificity to encompass neutralization of additional strains may occur upon immunization with full-length gp120 DNA followed by V3-fusion proteins by a mechanism that remains unclear [17]. The small G-P-G-X tetrapeptide located at the tip of the gp120 V3 loop has been suggested as a vaccine target [18,19]. However, flanking residues on both sides of the tetra-peptide display high-level sequence variability, and it is uncertain whether the three comparatively conserved residues are sufficient to form a well-defined Ab-recognizable epitope.

Elements of the V3 loop along with peptide regions distant in the linear gp120 sequence are components of the conformational determinant that binds HIV coreceptors on host cells, CCR5 and CXCR4 (the coreceptor binding site). gp120 is a conformationally flexible protein. Certain coreceptor binding site epitopes that are sterically inaccessible to Abs become exposed after gp120 binds CD4, the primary HIV receptor. Vaccine targeting of

such coreceptor binding site epitopes presents the pitfall of insufficient sequence conservation, as important regions of the coreceptor binding site are located in the gp120 V domains. In addition, the transient presentation of the coreceptor binding site epitopes on the gp120 protein surface limits their exposure to Abs, as virus neutralization is dependent on successful engagement of the coreceptor binding site by Abs in the microscopic time interval between formation of the binary HIV–CD4 complex and its progress to the HIV–CD4–coreceptor ternary complex. The envelope proteins from a unique CD4-independent HIV strain have been tested as immunogens (subtype B strain R2). Immunization with the strain R2 gp120 protein was only partially effective in inducing Abs capable of neutralizing genetically divergent strains [20], but the gp120–gp41 fusion protein trimers from this strain induced Abs with improved breadth of neutralization [21]. The encouraging implication is that an exposed, conserved epitope in the coreceptor binding site of the CD4-independent form of gp120 is sufficiently immunogenic to induce broadly neutralizing Abs. The Abs were tested using pseudovirions and a genetically modified host cell line that reports HIV entry. As the CD4-dependent form of gp120 did not induce such neutralizing Abs, it is not self-evident that native CD4-dependent HIV strains express the epitope in a sufficiently exposed form. Confirmation of the neutralizing activity using clinical (native) HIV strains and unmodified host T cells and macrophages, the native HIV hosts, is awaited.

Initial HIV binding to host cell CD4 receptors is an obligatory step in the infection, except for the CD4-independent coreceptor CCR5-utilizing strain R2 and a few such coreceptor CXCR4-utilizing strains. This provides a selective pressure for structural conservation of the gp120 determinant responsible for binding CD4 in HIV strains that are otherwise highly divergent in structure (CD4BS). The CD4BS is a conformational determinant composed of residues in the 421–433 gp120 region along with residues 256, 257, 368–370 and 457 [22,23]. The CD4BS is a leading vaccine target, but certain challenges must be met. Several anti-CD4BS Abs neutralize HIV poorly [24,25]. Therefore, individual epitopes within the CD4BS are not equally suited for vaccine targeting. Furthermore, the CD4BS can undergo conformational transitions. For example, converting gp120 from an oligomeric to a monomeric state influences CD4BS recognition by Abs [26]. CD4 binding may itself induce changes in CD4BS structure [23,27]. Mutations remote from the CD4BS also influence the CD4BS conformation [28,29]. Immunization with full-length gp120 induces very weak anti-CD4BS Abs [30,31], consistent with the poor immunogenicity of the CD4BS described in the next section. Neutralizing anti-CD4 Abs have been identified in some subjects after prolonged HIV infection, providing hope that the human immune system is not completely powerless with respect to synthesizing Abs that might control HIV infection. Engineering of immunogens that induce high-level production of specific Abs to the CD4BS is a vigorous area of research. gp120–gp41 fusion proteins that tend to form non-covalent oligomers more readily than gp120 have been cloned based on the logic that the CD4BS of the oligomers may mimic the native CD4BS conformation [32]. Peptide epitopes have been designed to mimic the conformational epitope within the CD4BS recognized by mAb b12 [33]. However, accurate conformational mimicry of native proteins is a daunting task, and these approaches have not led to an immunogen that induces neutralizing anti-CD4BS Abs.

CD4BS 421–433 epitope properties

The linear 421–433 peptide region of gp120 is essential for maintenance of CD4BS integrity (Table 2). This region contains six amino acid residues that make direct stabilizing contacts with CD4 as determined by crystallography, and two additional residues in this region influence the conformation of the CD4BS as suggested by site-directed mutagenesis. Indirect arguments from the crystal studies have prompted the suggestion that weak initial contacts with CD4 occurring at CD4BS elements outside the 421–433 region might induce movements of the gp120 backbone, facilitating essential CD4 contacts with the 421–433

region required for high-affinity binding. Crystallography also indicates that most of the 421–433 region is expressed on the solvent-accessible gp120 surface [23,27,34,35]. The exposed area of this region is 971–1023 Å², exceeding the area needed for high affinity Ab binding (Figure 1) [36]. Similarly, the region of Simian immunodeficiency virus (SIV)gp120 corresponding to the HIV 421–433 epitope is located on the protein surface and has an accessible area of 951 Å² (PDB structure 2BF1) [27]. The evident physical exposure of the 421–433 region is consistent with the requirement for CD4 binding as the initiating step for HIV infection.

Table 3 summarizes information pertinent to Ab recognition of the 421–433 epitope. The epitope is not recognized by the anti-CD4BS mAb b12. The well-known mAbs 17b and 48d that recognize epitopes close to the CD4BS also do not bind the 421–433 region [37]. Several reports from independent groups have indicated that gp120 expresses a B-cell superantigen (SAg) determinant [38–42], a property expressed by a small group of microbial proteins. gp120 is recognized with modest strength by a noncovalent binding site located primarily in the framework regions (FRs) of preimmune Abs produced with no prior exposure to HIV, a defining feature of B-cell SAgS [38–42]. Synthetic peptide studies have indicated that the 421–433 region overlaps the gp120 SAg determinant recognized by preimmune Abs. A subset of the preimmune Abs that recognize the 421–433 region noncovalently proceeded to catalyze the hydrolysis of gp120 by a serine protease mechanism [43,44]. Both activities important in the catalytic reaction, noncovalent 421–433 region recognition and the subsequent nucleophilic attack on gp120 carbonyl groups resulting in peptide bond cleavage, are innate Ab properties expressed with no requirement for adaptive sequence diversification of the Ab V domains. The catalytic reaction occurred at a distinct Ab subsite that is spatially proximate to the noncovalent Ab binding site. Secretory IgA found in mucosal fluids of noninfected humans expressed catalytic activity superior to IgA/IgG class Abs present in the blood of the same subjects. HIV neutralization by these Ab preparations was proportional to their catalytic activity.

The reversible binding and catalytic properties of preimmune Abs may provide some level of innate protection against transmission of HIV infection [44,45]. However, such a protective role comes at a heavy cost. Traditional antigen binding at the Ab complementarity determining regions (CDRs) is a stimulatory signal for B-lymphocyte differentiation, driving clonal selection of cells that produce affinity-matured Abs specific for the antigen. By contrast, SAg binding at the FRs is thought to downregulate B-cell differentiation [46]. An impaired adaptive B-cell response to the 421–433 epitope is evident from the rare production of Abs that bind peptides spanning this region in HIV-infected patients and mice immunized with purified gp120 [30,31].

The CD4BS 421–433 region is the proverbial Achilles heel of the virus. Production of Abs to this region by traditional B-cell differentiation pathways is proscribed, but when sufficiently specific anti-421–433 Abs appear, they neutralize genetically diverse virus strains with exceptional potency [47,48]. Patients with the autoimmune disease systemic lupus erythematosus can mount Ab responses that are normally disfavored in healthy humans. HIV infection occurs rarely in lupus patients. Increased binding of the 421–433 peptide region by Abs from lupus patients without HIV infection and a mouse model of lupus was reported [49]. A recombinant Ab fragment specific for the 421–433 epitope cloned from the immune repertoire of lupus patients neutralized clinical viral isolates belonging to multiple group M HIV subtypes [47,50]. More recently, it was realized that production of neutralizing Abs to the 421–433 region is not limited to the humans with autoimmune dysfunction. Abs from patients who survived subtype B HIV infection for 19–21 years neutralized genetically heterologous clinical HIV isolates from other viral subtypes found worldwide (Figure 2) [48]. Immunochemical and mutational analysis indicated that

the neutralizing activity was due to specific recognition of the 421–433 epitope, and that Ab specificity derives from recognition of several individual amino acids important for CD4 binding [22,23,51]. The 421–433 epitope is largely but not fully conserved among group M HIV strain sequences available in the databanks (Table 2). Decreased Ab neutralization potency correlating with certain epitope sequence divergences was noted. However, the Abs neutralized all strains tested, and it is reasonable to believe that escape mutants will emerge under the selective pressure imposed by the Abs only if the viral infection can become a CD4-independent process. Taken together, these studies indicate that HIV is highly vulnerable to neutralization by specific Abs to the 421–433 CD4BS region, but the adaptive immune response to the region is insufficient to control infection under normal circumstances.

Covalent binary vaccination

This immunization strategy derives from novel concepts holding the potential of inducing protective Abs beyond the scope of the physiological immune response. Evidence for its potential utility in improving the Ab response to HIV antigens has been reported [37]. The central points in this strategy are:

- The highly energetic covalent immunogen-BCR reaction is hypothesized to induce favorable B-cell differentiation instead of B-cell downregulation occurring upon noncovalent recognition of the CD4BS 421–433 epitope by the Ab FRs;
- Simultaneous stimulatory binding of a second immunogen epitope at the Ab CDRs compensates for B-cell downregulation due to downregulatory CD4BS binding at the FRs.

In addition to induction of reversibly binding Abs, covalent immunization can stimulate adaptive improvement of the nucleophilic function of Abs, thereby strengthening their ability to inactivate HIV by covalent and catalytic effector mechanisms (Figure 3).

Electrophilic gp120 immunogen

The strategy entails B-cell stimulation by covalent binding of immunogens containing strongly electrophilic phosphonate groups to the naturally occurring nucleophilic sites of Abs. Such sites were originally identified in enzymes of the serine protease family as triads of Ser(Thr)–His–Asp(Glu) residues [52]. The serine/threonine side chain oxygen acquires enhanced nucleophilic reactivity due to intramolecular hydrogen bonding, becoming capable of forming a covalent intermediate with the weakly electrophilic carbonyl groups of polypeptide substrates. The nucleophilic sites are necessary but not sufficient for serine protease catalysis, as the participation of additional structural elements supporting water attack on the covalent intermediate is needed to complete the catalytic cycle. Thus, proteins expressing nucleophilic sites but no appreciable enzymatic activity have been identified [53]. Nucleophilic sites are ubiquitous in Abs, including the first IgM-class Abs expressed on the B-cell surface complexed to signal-transducing proteins (BCR) [54,55]. From the split combining site model [44,56–58], it appears that distinct subsites located in the Ab variable domains are responsible for initial noncovalent antigen binding and the ensuing nucleophilic attack on antigen electrophiles (Figure 4A). Based on this model, covalently reactive immunogens have been prepared by incorporating electrophilic phosphonate groups at the amino acid side chains of polypeptides. The electrophiles in such immunogens display covalent binding to nucleophilic BCRs in coordination with specific noncovalent binding of the peptide epitope [54,55].

We described a full-length electrophilic gp120 analog containing phosphonates at lysine side chains that oligomerizes by a self-assembly process (E-gp120) [56]. Alterations in the

topographic presentation of various epitopes on the E-gp120 surface were evident compared with chemically unmodified monomeric gp120. E-gp120 displayed enhanced binding of a single-chain Fv fragment specific for the CD4BS 421–433 epitope and reduced binding of mAbs to the gp120 V3 domain [37]. As noted previously, HIV infection or immunization with gp120 devoid of electrophilic groups fail to induce a rapid anti-CD4BS Ab response. Analysis of serum Abs suggested that E-gp120 immunization accelerates the rate-limiting step in the anti-CD4BS adaptive immune response, that is, deficient IgM→IgG/IgA class switching. Seven out of 17 monoclonal IgGs raised by immunization with E-gp120 displayed neutralizing activity attributable to 421–433 epitope recognition [37]. The mAbs neutralized genetically divergent clinical HIV isolates, including all ‘difficult-to-neutralize’ strains tested [37].

Insight into mechanisms underlying the immunogenicity of E-gp120 was obtained by additional epitope mapping, mutagenesis and crystallography (Figure 4B) [37]. The neutralizing mAbs are unique by virtue of their binary-epitope reactivity. The same mAb displayed binding to the CD4BS 421–433 epitope and a second spatially distant epitope composed of the mostly conserved residues 301–311. The latter epitope is evidently recognized by the traditional antigen-binding cavity formed by the CDRs, whereas the CD4BS is recognized by a neighboring cavity formed mainly by the V_H domain FR residues, which includes a putative nucleophilic site. Both binding sites displayed replacement/silent mutation ratios exceeding the values of a random mutational process, a classical sign of adaptive maturation of Ab binding sites. These analyses indicate the feasibility of directing the innate CD4BS recognition capability of B cells towards a favorable maturational pathway that eventually results in production of neutralizing anti-HIV Abs.

Focusing the Ab response at the CD4BS

The surface of gp120 expresses a plethora of linear and conformational epitopes [59]. As noted previously, Abs to most gp120 epitopes fail to neutralize genetically divergent HIV strains. Indeed, some Abs even enhance infection in tissue culture assay models by the complement-dependent and Fc receptor-dependent mechanisms [60,61]. CD4BS targeting alone may be conceived as a sufficient basis for effective vaccination if the anti-CD4BS Abs can neutralize the extant HIV strains and are not permissive for emergence of escape viral variants. To induce a focused anti-CD4BS-neutralizing Ab response, a CD4BS-based immunogen that mimics the native CD4BS conformation on the viral surface is needed. The CD4BS of purified monomeric gp120 mimics the conformation of native CD4BS imperfectly at best. Previous studies on Abs raised to peptide immunogens containing part or all of the 421–433 region indicated varying neutralization of laboratory- adapted HIV strains [31,62–64]. Clinical CCR5-dependent isolates were not tested (they were not widely available at the time). The pitfall of small-peptide immunogens is their ability to assume alternate conformations in varying microenvironments. This is exemplified by the finding of differing binding specificity of Abs raised by immunization with the 421–436 peptide conjugated to different polypeptides [65]. A flexible immunogen can acquire a shape complementary to the structure of the pre-existing BCR-combining site by the induced-fit mechanism, thereby losing its CD4BS-mimicking conformation (Figure 5). Such a non-native immunogen will not induce neutralizing Ab production. To stimulate the production of neutralizing Abs, a rigid immunogen with the correct conformation is necessary to recruit and expand the minority of preimmune B cells specific for the native CD4BS.

Firm pronouncements concerning the 421–433 region conformation supporting high-affinity CD4 binding have been difficult. Secondary structure predictions by the Chou–Fasman algorithm have suggested that synthetic gp120 peptides containing residues of the 418–430 region can assume alternate structures with pronounced α -helix or β -sheet content [66]. The

crystal structure of gp120 suggests that the 425–430 region, which contains critical CD4 binding residues, exists mostly as a β -sheet. By contrast, soluble CD4 binding by synthetic peptides corresponding to the 421–433 region is enhanced by organic compounds that stabilize the helical peptide conformation [67]. The conformational considerations are germane both for identifying the neutralizing Abs and inducing a focused anti-CD4BS Ab response. We employed synthetic peptide probes containing electrophilic phosphonate groups and residues 421–433 (E-421–433) or 416–433 (E-416–433) to identify neutralizing Abs from lupus patients, long-term survivors of HIV infection and mice immunized with E-gp120 described in the preceding sections [37,48]. E-416–433 contains the N-terminal pentapeptide Leu–Pro–Ser–Arg–Ile, which promotes folding of the 421–433 region into a helical conformation [66,67]. The pentapeptide corresponds to gp120 residues 416–420 with Cys418 replaced by Ser418 to preclude disulfide bond formation. In addition, placement of the phosphonates at the Lys side chains may impart rigidity to the peptide mimetics. E-421–433 and E-416–433 bind the HIV-neutralizing Abs specifically. E-416–433 displays CD4 binding activity approximately 100-fold superior to previously tested 421–433 region synthetic peptides [48]. Consequently, it is suitable for further immunogenicity studies designed to induce neutralizing Abs to the CD4BS.

Covalent & catalytic Abs

Reversible CD4BS binding by Abs alone is sufficient to neutralize HIV. Immunization with electrophilic immunogens offers the bonus of strengthened Ab nucleophilic reactivity by virtue of adaptive B-cell selection driven by covalent electrophile binding [56,68,69]. Enhancements of the nucleophilic reactivity may improve HIV inactivation as follows (Figure 3D). First, specific pairing of the Ab nucleophile with the weakly electrophilic carbonyls of gp120 forms stable immune complexes with covalent character. Covalently binding Abs were induced by immunization with the electrophilic analogs of full-length gp120 and a gp120 V3 peptide [68,69]. Unlike reversible immune complexes, the covalent complexes did not dissociate readily, increasing the HIV-neutralization potency [69]. Second, if the Ab-combining site supports water attack on the covalent acyl–Ab complex, catalytic gp120 cleavage occurs. Certain monoclonal IgG class Abs raised by immunization with E-gp120 displayed low-level ability to hydrolyze gp120 [56]. A more robust improvement of the catalytic function was observed by immunization with E-416–433. A single catalytic Ab molecule is reused to cleave thousands of gp120 molecules over its biological half-life in blood (1–3 weeks), permitting potent virus inactivation [44,70].

Autoimmunity/safety

As an HIV vaccine is needed urgently, approaches with potential side effects remain under consideration. For example, there is interest in vaccination against the gp41 membrane proximal external region despite the cross-reaction of Abs to this region with lipidic autoantigens, such as cardiolipin [71]. The CD4BS 421–433 region expresses no sequence similarity with human proteins listed in GenBank. Thus, the likelihood of inducing autoimmune damage is remote. The connection between the CD4BS and lupus, an autoimmune disease that is rarely coexistent with HIV infection [72], deserves mention. Lupus is associated with amplified Abs to the 421–433 epitope [49] and increased expression of human endogenous retroviral sequences (HERVs) [73]. We have noted the partial sequence homology of the 421–433 region with certain HERVs as a possible explanation for amplified production of the Abs [74]. However, as there is no evidence for a physiologically important HERV expressing structural similarity to the CD4BS, the possibility of autoimmune side effects induced by a CD4BS-based vaccine is small. Electrophiles can react with serine protease enzymes. However, the reaction of E-polypeptides with Abs is specific and rapid compared with enzymes because of the accelerant effect of noncovalent epitope recognition [75]. As only small amounts of

immunogen are required to stimulate a neutralizing Ab response, the likelihood of nonspecific enzyme inhibition by E-polypeptides is minimal. As to the catalytic function of Abs produced by covalent immunization, the Abs are specific for the target antigen and no promiscuous cleavage of irrelevant proteins was observed [43,44].

Preclinical vaccine testing

Despite success in preclinical tests, previous vaccine candidates were ineffective in human trials. This has raised concern about the predictive power of available tissue culture and animal models of HIV infection. The tissue culture infection assays are the only available means to determine whether a candidate vaccine induces Abs capable of neutralizing a sufficiently broad range of HIV strains. Panels of virus strains with varying genetic divergence and resistance to commonly studied Abs to gp120/gp41 have been assembled to assess neutralization breadth and potency [76–79]. CCR5-dependent primary strains are substantially more resistant to anti-HIV Abs compared with laboratory-adapted strains [80]. Abs to the 421–433 epitope neutralize highly divergent HIV strains drawn from different group M subtypes, including difficult-to-neutralize CCR5-dependent strains using the classical peripheral blood mononuclear cell (PBMC) assay (or clinical isolate infection assay) [47,48]. This assay utilizes the closest available tissue culture model to the natural infection process, for example, phytohemagglutinin-activated PBMCs pooled from humans without HIV infection and HIV isolates grown from the clinical specimens (usually serum) from infected patients that have not been subjected to tissue culture passage in cell lines. Minimizing tissue culture manipulations is advisable in view of changes in viral coat structure upon growth in different types of host cells [81,82]. Host cell effects on viral growth rates and susceptibility to neutralization by Abs have also been described [81,83,84]. The PBMCs are composed primarily of lymphocytes with variable levels of monocyte contamination. The natural host cells for HIV-1 are T lymphocytes and cells of the monocytic lineage.

To assess the reliability of the PBMC/clinical isolate assay, we conducted a retrospective analysis of neutralization data gathered using the PBMC/clinical isolate assay and two well-defined Abs to the 421–433 epitope, mIgG clone YZ23 isolated by immunization with E-gp120 and single chain Fv (scFv) clone JL427 isolated from a lupus Ab phage library. The extent of variability was judged from the Ab concentrations needed to reach 50% virus neutralization (IC_{50} values). The coreceptor CCR5-dependent subtype C strain 97ZA009 was neutralized by IgG YZ23 in 26 out of 26 assays (IC_{50} range: 0.5–59 μ g/ml) and by scFv JL427 in 34 out of 35 assays (IC_{50} range: 0.003–9 μ g/ml) conducted using various host PBMC batches, various virus batches and various purified Ab batches. The variability was reduced using the same infecting virus inoculum obtained from a single large-scale tissue culture passage in PBMCs (Figure 6A). The IC_{50} values for Ab clones YZ23 and JL427 were spread over a 13-fold and 150-fold concentration, respectively, range using different batches of pooled PBMCs as hosts. The IC_{50} spread for assays conducted using the same pooled PBMC host cells was smaller. Neutralization assays conducted in parallel using PBMCs isolated from four individual human donors indicated a small IC_{50} spread for clone YZ23 and a larger IC_{50} spread for clone scFv JL427 (Figures 6B & 6C). In future studies, it is important to determine whether activation of the cells by the mitogen (phytohaemagglutinin) or allogenic PBMCs is a factor governing the potency of Ab neutralization. Provided that the variability is taken into account by including a sufficiently large range of test Ab concentrations, the assay is a reproducible guide to the neutralizing activity of the Abs.

Endotoxin (lipopolysaccharide) can induce chemokine release from monocytes that may bind chemokine coreceptors and inhibit HIV infection [85,86]. Preparations of Abs to the

421–433 epitope-containing endotoxin at concentrations lower than required for HIV neutralization by the chemokine-release mechanism displayed readily detected virus neutralization [37,48]. Removal of trace endotoxin amounts in the Ab preparations by ion-exchange methods did not diminish the neutralizing activity [37,48]. Moreover, a wealth of immunochemical data and control studies are inconsistent with endotoxin contamination and other trivial causes of HIV neutralization [37,44,47,48], for example, removal of the neutralizing activity upon immunoadsorption with immobilized E-416–433 but not an irrelevant immunoadsorbent and induction of the neutralizing Abs by immunization with E-gp120.

Reporter cell lines and HIV pseudovirions have been developed for convenient analysis of large numbers of Ab samples, for example, the TZM-bl cell line/pseudovirion assay [87]. TZM-bl is a genetically engineered HeLa cell line that expresses the HIV receptors and contains the Tat-inducible luciferase gene. The pseudovirions are replication-incompetent particles expressing the HIV envelope proteins. This assay is unsuitable to detect Abs to the CD4BS 421–433 epitope. Homogeneous Abs to this epitope prepared in the authors' laboratory [37,47,48] did not impede infection of TZM-bl cells by several pseudovirus strains but neutralized the corresponding native HIV strains expressing gp120 with the same sequence (clinical virus isolates) in the PBMC assay in David Montefiori's laboratory (Figure 6D). The reference anti-CD4BS mAb b12, also displayed discrepant neutralization of one strain (undetectable neutralization of strain 98Du123 in the PBMC/clinical isolate assay and robust neutralization in the TZM-bl assay). Discrepant neutralization by other anti-HIV mAbs in these assays has been noted previously [24,88–92]. For example, the pseudovirion reporter assay does not detect the neutralizing activity of mAb 2G12 validated by *in vivo* passive transfer studies [91]. The level of discrepancy varies depending on the epitope recognized by the Abs. It is possible that excessive expression of the HIV coreceptor CCR5 on TZM-bl cells compared with PBMCs [91,93], and nonphysiological pseudovirion interaction with host membrane proteins/lipids permit infection with reduced dependency on the CD4BS 421–433 epitope. The conformational flexibility of gp120 in differing membrane microenvironments is another variable [26,35,94]. Epitope-specific variations in the conformations of gp120 expressed by native HIV versus pseudovirions are conceivable.

Animal model testing is desirable to predict the success of candidate human vaccines. HIV infects chimpanzees transiently. The infection does not progress to AIDS. Immunization of chimpanzees with recombinant gp120 suppressed HIV viremia, but human trials of the gp120 immunogen did not reduce HIV infection risk [6,95,96]. As the HIV and SIV envelope proteins are structurally divergent, direct testing of candidate HIV vaccines in the SIV-infection model is difficult. Hybrid simian–human virus strains (SHIV) containing the HIV envelope proteins grafted into SIV produce viremia in rhesus monkeys. Candidate vaccines that induced cytotoxic T cells protected monkeys from SHIV infection but did not protect humans from HIV infection [7]. The SHIV/rhesus monkey model was recently suggested to be a useful 'gatekeeper' to identify candidate vaccines that induce 'better immunity' compared with the failed immunogens [97]. However, as the precise laboratory tests constituting 'better immunity' have remained undefined, it is not possible to predict vaccine success in humans from this animal model.

Expert commentary

HIV is one of several modern microbes that have proved intractable to traditional vaccine approaches. The first step in developing effective vaccines to these microbes is to understand the evolutionary strategies permitting infection despite robust humoral and cell-mediated immune responses to the mutable microbial antigens. One such strategy is the ability of HIV to silence the adaptive immune response to vulnerable envelope epitopes,

which must be maintained in a mostly conserved form because they are essential to maintain virus infectivity. HIV has evolved a binding site for its primary host receptor, the CD4BS, that expresses B-cell SAg character. Empirical evidence indicating that the CD4BS 421–433 epitope meets the defining criteria of a SAg epitope has been documented by several groups, including recognition of this epitope by the FRs of reversibly binding and catalytic preimmune Abs [38,41,43,44]. Despite its physical exposure, the CD4BS does not provoke robust adaptive Ab responses. The CD4BS may induce a state of specific immune ‘tolerance’ due to its downregulatory contacts with the BCR, which drive B cells into a nonproductive differentiation pathway. Such an epitope-specific downregulatory effect diminishes the prospect of an anti-CD4BS-neutralizing Ab response by traditional vaccine approaches. Importantly, the hypothesis of an epitope-specific deficiency in the adaptive Ab response does not imply that the CD4BS-contacting B cells are deleted from the immune repertoire. Indeed, the immune system mounts robust adaptive Ab responses to other HIV epitopes and other infectious microbes until serious impairment of helper T-cell function develops at advanced stages of HIV infection. This suggests that there is no rapid, overall downregulation of B-cell adaptive immunity due to the SAg character of gp120 and its CD4BS.

There are no established means to render a microbial SAg site immunogenic in humans. If such means can be developed, neutralizing Abs to the CD4BS could be generated by amplifying the innate B-cell subset that recognizes the CD4BS. The innate CD4BS recognition site is located primarily in the FRs of Abs, particularly the V_H domain FRs. The somatic hypermutation process underlying adaptive affinity maturation of Abs occurs randomly over the entire length of their V domains. Replacement mutations that improve the binding affinity for conventional antigens tend to be concentrated in the CDRs, because the combining site for such antigens is formed mostly by the CDRs, and there is no selective pressure for survival of FR-replacement mutations. SAgS bind at the FRs, but the downregulatory signal transduction associated with SAg-FR precludes improvement of the innate SAg-recognition capability. Studies in the authors’ laboratories have suggested two mechanisms that can bypass the downregulatory signaling and induce neutralizing anti-CD4BS Ab production in experimental animals [37]: first, the highly energetic covalent stimulation of B cells with an electrophilic immunogen; and second, binary stimulation of the cells with an immunogen that simultaneously engages the CDRs and FRs. The anti-CD4BS Abs induced by E-gp120 displayed a mutational pattern supporting amplification and improvement of the innate CD4BS recognition capability of Abs. Consequently, the covalent immunization strategy holds promise in developing an effective HIV vaccine that may induce Abs capable of neutralizing diverse group M HIV strains responsible for the pandemic.

The well-known conformational flexibility of the CD4BS and small peptide mimetics is a major hurdle in developing an effective anti-CD4BS vaccine. This difficulty has been addressed at least in part by the identification of the E-416–433 mimetic capable of binding neutralizing anti-CD4BS Abs and CD4 at levels substantially superior to previously studied peptides [37,48]. E-416–433 offers the opportunity to induce an Ab response focused at the CD4BS, free of irrelevant Abs. Inclusion of a second peptide module in the immunogen, which engages the CDRs simultaneously with 421–433 engagements of the FRs, may help improve the Ab response.

Inducing an immune response in animals as a model for human HIV vaccination is meaningful only if it is monitored using assays that predict protection against infection in humans. Covalent immunization to the CD4BS 421–433 epitope has been validated by the PBMC/clinical isolate neutralization assay, the ‘gold standard’ for measuring HIV infection in tissue culture. Whether the covalent immunization approach can induce Abs with

sufficient strength and specificity to protect against infection *in vivo* should be tested further in nonhuman primates and humans.

Five-year view

The concept of covalent vaccination is based on novel scientific principles holding the potential for vaccination against HIV and other intractable microbes expressing virulence factors with B-cell SAg character. According to Thomas Kuhn in the '*Structure of Scientific Revolutions*', for a new paradigm candidate to be accepted by a scientific community, "First, the new candidate must seem to resolve some outstanding and generally recognized problem that can be met in no other way. Second, the new paradigm must promise to preserve a relatively large part of the concrete problem solving activity that has accrued to science through its predecessors."

The emergence of covalent vaccination as a medical paradigm will depend on reaching these objectives:

- Demonstration of protection against infection by passively transferred Abs to the CD4BS 421–433 epitope in a nonhuman primate model;
- Identification of adjuvants and covalent immunogens that can induce sufficiently strong mucosal and systemic neutralizing anti-CD4BS Ab responses, preferably in multiple animal models;
- Additional demonstration of the ability of anti-CD4BS Abs to neutralize diverse HIV-1 strains and the inability of HIV to develop escape mutants under the selective pressure imposed by the Abs;
- Human trials of covalent vaccination for therapy and prevention of HIV infection;
- Sufficient initial confidence in this approach in the scientific community and funding agencies to enable further development.

Five years is sufficient to meet the foregoing preclinical objectives, initiate human trials and know the outcome of therapeutic vaccination in infected humans. As preventive vaccination trials require large study cohorts and an extended observation duration, these will likely require a longer time span.

Key issues

- The mutability of the immunodominant envelope epitopes and poor immunogenicity of the conserved epitopes necessary for the viral propagation have thwarted effective HIV vaccination.
- The CD4 binding site (CD4BS) is a suitable target for HIV vaccination but its superantigenic character does not permit an effective adaptive antibody (Ab) response that neutralizes HIV.
- Electrophilic immunogens break the immune 'tolerance' state and induce anti-CD4BS Abs that neutralize genetically diverse HIV strains.
- Putative mechanisms for successful immunization are recruitment and improvement of the innate capacity of Abs to recognize the CD4BS due to the highly energetic covalent stimulation of B cells and simultaneous, binary immunogen binding to the complementarity-determining regions and framework regions.

- Focusing the Ab response at the CD4BS is desirable. An electrophilic peptide mimicking the native CD4BS conformation is available as a candidate vaccine.
- Imminent future studies will consist of candidate vaccine validation in a nonhuman primate model followed by human trials.

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Figure 1. Exposure of the 421–433 epitope

Surface representation of glycoprotein 120 crystal structure showing the 421–433 epitope in green (PDB 2B4C; after stripping away bound soluble CD4 and Fab X5). Of the total epitope surface area, approximately 85% is fully accessible. The neighboring $\beta 15/\beta 2$ strands are shown in purple.

Figure 2. Neutralization of genetically diverse primary HIV strains and SHIVSF162P3 by IgA purified from three long-term survivors of HIV infection (19–21 years, LTS patients 2857, 2866 and 2886)

(A) Neutralization potency scatter plots (IC_{50}) of the 3 IgA preparations and the reference anti-CD4 binding site IgG b12. Each point represents an individual virus strain. (B) Phylogenetic relationship of the strains neutralized by LTS IgA preparations and the autologous viruses from IgA donors (designated strains 2857.P1, 2866.P4 and 2886.P1). The bootstrap values of each node represent the percentage of 1,000 bootstrap replicates that support the branching order. Only bootstrap values at 80% or higher are shown. Horizontal branch lengths are drawn to scale. Bar indicates 0.02 nucleotide substitution per site. Percent infections caused by strains drawn from individual HIV subtypes is indicated on the right. Data for (A) from [48]. Data for (B) from [2].

IC_{50} : Half maximal inhibitory concentration; LTS: Long-term survivor; SHIV: Simian human immunodeficiency virus.

Figure 3. Covalent vaccine principle

(A) Traditional nonelectrophilic immunogens induce a transient Ab response to the 421–433 CD4 binding site epitope limited mostly to the IgM compartment because of deficient class-switching. **(B)** The electrophilic phosphonate of the prototype E-vaccine binds BCR nucleophiles (*Nu*) *via* the highly energetic covalent reaction, bypassing constraints on B-cell differentiation. This generates memory B cells and plasma cells producing neutralizing Abs. **(C)** An optional epitope 2 that generates a positive signal by binding the CDRs can be incorporated in the E-vaccine to counteract negative B-cell signaling due to 416–433 epitope binding at the FRs. **(D)** Electrophile-driven clonal selection of the B cells results in adaptive strengthening of Ab nucleophilic reactivity, improving the innate catalytic activity of Abs. Specificity is derived from noncovalent epitope–paratope binding. Covalent immune complex 1 is a resonant stable complex prior to expulsion of C-terminal antigen fragment. Covalent immune complex 2 is an acyl-Ab complex. Ag' and Ag'' are components of the epitope recognized by the Ab. Ag' Lys-OH is the N-terminal antigen fragment and NH₂-Ag'' is the C-terminal antigen fragment.

Ab: Antibody; BCR: B-cell receptor; CDR: Complementarity determining region; FR: Framework region; gp120: Glycoprotein 120.

Figure 4. Structural aspects of CD4 binding site 421–433 epitope recognition by antibodies
(A) Split-site model explaining proteolytic Ab epitope specificity. Two different Ab subsites are responsible for the initial noncovalent antigen binding and the subsequent peptide bond hydrolysis process. In the initial immune complex (left), the antigen region not involved in noncovalent Ab binding enjoys conformational flexibility. Consequently, peptide bonds remote from the noncovalent binding site that are in register with the Ab nucleophilic subsite can be hydrolyzed (right). If the antigen contains an electrophilic phosphonate group, it can form a covalent bond with the nucleophile (not shown). The triangle represents a nucleophile, the circle represents a neighboring general base that activates the nucleophile.
(B) Surface model of anti-E-glycoprotein 120 Fab YZ23 crystal solved at 2.5 Å resolution (PDB 3CLE). The V_L domain is shown in pink, the V_H domain in cyan. Complementarity-determining region (CDR)L1 and L3 are shown in yellow and orange, respectively, and CDRH1, H2 and H3 are shown in blue, light green and dark green, respectively. For clarity, the CDR cavity (CDR-cavity) and V_H framework region-dominated cavity (framework region-cavity) are fitted, respectively, with the gray-meshed object and white-meshed object. The inter-cavity centroid-to-centroid distance and cavity surface areas are indicated. For **(A)**, see [54,57] for further details; figure taken from [57]. For **(B)** data are from [69]. Ab: Antibody.

Figure 5. Importance of conformational rigidity in CD4BS mimicry

(A) An immunogen expressing a flexible CD4BS can adopt a conformation complementary to the BCR combining site by the induced-fit mechanism, thereby losing its CD4BS-mimicking conformation and resulting in induction of non-neutralizing Abs.

(B) A rigid CD4BS mimetic will induce the correct BCR conformation, which can be affinity-matured further by immunogen-driven selection, resulting in synthesis of neutralizing antibodies.

Ab: Antibody; BCR: B-cell receptor; CD4BS: CD4 binding site.

Figure 6. Reliability of antibody neutralization in tissue culture

(A) Variability of scFv JL427 and IgG YZ23 neutralization potency (IC_{50}) in PBMC/native HIV assay (clinical strain subtype C 97ZA009) using the same virus preparation batch and same Ab preparation batches. PBMC host cells were either from the same batch of frozen cells pooled from eight donors or different batches of cells pooled from different sets of eight donors each. Each symbol shows the IC_{50} extracted from the Ab dose-response curve of individual assays. The dashed line shows the arithmetic mean and the black line shows the geometric mean. (B) scFv JL427 dose-response using PBMCs isolated from four individual donors without pooling of the cells or the PBMC pool from the same four donors. HIV strain: subtype C 97ZA009. (C) IgG YZ23 dose-response using PBMCs isolated from four individual donors without pooling of the cells or the PBMC pool from the same four donors. HIV strain: subtype C 97ZA009. (D) Antibody-neutralizing potency observed in the PBMC/native HIV (clinical virus strains) and T2M-bl/pseudovirion assays. IC_{50} values extracted from dose-response curves are shown for one subtype B strain and one subtype C strain each for Abs to the 421–433 epitope (scFv JL427, IgG YZ23 and LTS IgA 2857) and the reference IgG b12. The native clinical HIV strains and pseudovirus strains express gp120 with identical sequence.

Ab: Antibody; IC_{50} : Half maximal inhibitory concentration; PBMC: Peripheral blood mononuclear cell; scFv: Single-chain variable fragment.

Table 1

Major glycoprotein 120 neutralizing epitopes.

Abs used to identify epitope	Epitope location	Function	Vaccine relevance		Ref.
			Epitope accessibility on HIV	Ab-neutralizing properties	
IgG b12	Conformational CD4BS epitope in exposed gp120 outer domain; consists of residues in β 15– α 3 CD4 binding loop (364–374) and outer domain exit loop between β 24 and α 5 (470–474)	May be initial CD4 contact site	No evidence for limiting epitope exposure. Dimeric IgA b12 form neutralizes comparably to monomer IgA and IgG forms	Neutralizes most subtype B strains; strains from other subtypes often neutralized poorly	[14,24,99]
IgG YZ23 scFv JL413 scFv JL427 IgA LTS	Linear CD4BS epitope consisting of residues 421–433; found in exposed β 20/ β 21 half of bridging sheet. Also exposed on SIV gp120	Binds CD4. Peptide mimetics replicate CD4 binding function	No evidence for limiting epitope exposure. Small scFv and full-length Abs neutralize HIV	Exceptionally potent neutralization of diverse native HIV strains from all subtypes tested. Abs do not neutralize pseudovirions in reporter assay	[37,47,48]
IgG 2G12	Carbohydrate; glycan substituents mainly at residues 332 and 392; some dependence on glycans at residues 295, 339 and 386	Not known	2G12 IgM neutralizes more potently than its IgG form	Neutralizes many subtype B strains; strains from other subtypes often neutralized poorly	[15,24,100]
IgG X5 IgG 17b IgG 48d	CD4-induced epitope composed of residues in β 2, β 3 and β 20– β 21 regions	Coreceptor binding	Exposure requires prior CD4 binding. Full-length Abs neutralize less potently than Ab fragments, suggesting limiting epitope exposure	Limited breadth of neutralization. Neutralizes a subset of subtype B strains	[24,101,102]
IgG 447–52D	V3 domain apex, residues GPXR	Coreceptor binding	Full exposure of coreceptor binding site exposure requires CD4	Limited breadth of neutralization	[24,103,104]
IgG PG9 IgG PG16	Conformational epitope composed of residues from V2, V3 and C4 domains	Not known	Potent neutralization suggests epitope exposure. Abs bind infected cells	Neutralizes broadly across HIV pseudovirion subtypes. Neutralization of native HIV strains not reported	[13]
Polyclonal Abs to CD4-independent gp140	Epitope not identified	Not known	Immunogenicity data imply recognition of exposed epitope expressed by CD4-independent HIV but not CD4-dependent HIV	Neutralizes broadly across HIV pseudovirion subtypes. Neutralization of native HIV strains not reported	[20,21]

Ab: Antibody; CD4BS: CD4 binding site; gp120: Glycoprotein 120; LTS: Long-term survivor; scFv: Single-chain variable region; SIV: Simian immunodeficiency virus.

Table 2

Conservation and exposure of glycoprotein 120, 421–433 region.

Amino acid	Conservation (%)	Exposure, HIV gp120 (%)	Exposure, SIV gp120 (%)	Binding, sCD4	Binding, LTS IgA
K421	98.8	24–38	32	OO	OO
Q422	99.2	24–26	28		
I423	94.3	59–68	54		OO
I424	99.4	6	17		OO
N425	90.1	20–22	31	XX	OO
M426	90.8	11–13	14	XX	
W427	99.2	19–21	36	XX	OO
Q428	99.1	20–21	40	XX	
E429	48.0	46–51	37	XX	
V430	91.8	79–91	94	XX	OO
G431	98.8	19–26	40		
K432	99.2	45–46	65	OO	OO
A433	99.1	3–4	24		

Source exposure information is from human gp120 complexed to sCD4 and Fab 412d or to sCD4 and Fab X5 (Protein Data Bank [PBD] 2QAD and 2B4C, respectively) or unliganded simian gp120 (PDB 2BF1).

gp120: Glycoprotein 120; LTS: Long-term survivor; OO: Amino acids determined by site-directed mutagenesis to reduce gp120 binding by CD4 by at least twofold [22,51] or the binding of electrophilic E-416–433 by long-term survivor IgA directed to the 421–433 epitope (LTS IgA) by at least 2.5-fold [48]; sCD4: Soluble CD4; SIV: Simian immunodeficiency virus; XX: gp120 residues that make direct contact with CD4 in the crystal structure (within 3.5Å from the CD4 surface).

Table 3

History of the 421–433 epitope.

Property	Observation	Conclusion	Ref.
Mutability	Highly conserved in group M strains	Excellent vaccine target	
Participation in CD4 binding	Overlaps CD4BS; residues 421, 425–430 and 432 essential for CD4 binding	Escape mutations unlikely without losing viral infectivity	[22,23]
Innate Ig recognition due to B-cell SAg character	Overlaps discontinuous gp120 SAg site	Weak/modest binding not requiring adaptive B-cell differentiation	[39,40]
	Binds preimmune Abs		[38–40]
	Binds FR-dominated preimmune Ab binding site		[41,42]
	Preimmune IgG binding to gp120 predicts resistance to HIV infection	Weak, innate systemic protection	[45]
	Preimmune Abs hydrolyze gp120 with potency mucosal IgA > serum IgM > IgA >> IgG; catalysis is rapid, binding is poor	Weak, innate mucosal protection	[43,44]
	Mucosal IgA neutralizes HIV		[44]
	Lupus and HIV rarely coexist	Suggests adaptive epitope- specific response in lupus – may protect against HIV	[72]
	421–433 binding Igs increased in lupus patients		[49]
Adaptive response to infection/ gp120 immunization	Recombinant Ab fragments recognize gp120 noncovalently, some hydrolyze gp120, some neutralize R5 strains with unprecedented potency/breadth		[47,50,105]
	Anti-421–433 Abs formed rarely. Impaired Ig class switching and very slow appearance of epitope-specific neutralizing IgAs after prolonged infection	Poor response due to SAg character. If produced, Abs neutralize diverse HIV strains	[48]
Vaccinogenicity of previously tested 421–433 peptides	Early synthetic peptide immunizations indicated conflicting ability to neutralize X4 strains. Varying fine specificity of Abs when conjugated to different carriers	Ab response directed to non-native peptide conformation	[65]
Vaccinogenicity of E-gp120/ potential of focused anti-CD4BS response	E-gp120 induces binary epitope-reactive neutralizing mAbs that bind HIV covalently and slowly catalyze monomer gp120 hydrolysis	Covalent immunization induces covalent/catalytic immunity	[37,56,68]
	E-416–433 mimetic recognized by CD4 and reference neutralizing Abs have been identified	Prototype vaccine identified	[37,48]

Ab: Antibody; CD4BS: CD4 binding site; E: Epitope; FR: Framework region; gp120: Glycoprotein 120; Ig: Immunoglobulin; mAb: Monoclonal antibody; SAg: Superantigen.