Published in final edited form as:

Future Virol. 2009 ; 4(1): 7–10. doi:10.2217/17460794.4.1.7.

A covalent HIV vaccine: is there hope for the future?

Sudhir Paul[†],

Chemical Immunology Research Center, Department of Pathology, University of Texas-Houston Medical School, 6431 Fannin, Houston, TX 77030, USA, Tel.: +1 713 500 5347; Fax: +1 713 500 0574; sudhir.paul@uth.tmc.edu

Stephanie A Planque,

Chemical Immunology Research Center, Department of Pathology, University of Texas-Houston Medical School, 6431 Fannin, Houston, TX 77030, USA, Tel.: +1 713 500 5329; Fax: +1 713 500 0574; stephanie.a.planque@uth.tmc.edu

Yasuhiro Nishiyama, and

Chemical Immunology Research Center, Department of Pathology, University of Texas-Houston Medical School, 6431 Fannin, Houston, TX 77030, USA, Tel: +1 713 500 5329; Fax: +1 713 500 0574; yasuhiko.nishiyama@uth.tmc.edu

Carl V Hanson

Center for Infectious Diseases, California Department of Public Health, 850 Marina Bay Parkway, Richmond, CA 94804, USA, Tel: +1 510 307 8540; Fax: +1 510 307 8955; carl.hanson@cdph.ca.gov

A vaccine is necessary to arrest the HIV pandemic. Inducing the synthesis of neutralizing antibodies by B lymphocytes is the cornerstone for successful vaccination against other viruses. The envelope protein gp120, a central player in the lifecycle of HIV, is a major target for vaccine development. However, the infection was not prevented in clinical trials of a gp120 candidate vaccine that induced robust anti-gp120 antibodies. Candidate vaccines that induce cytotoxic T-cell responses to infected cells were also ineffective. HIV-1 diversifies the structure of its envelope rapidly, thereby eluding host immunity. Like the natural immune responses occurring after HIV infection, the antibodies induced by various experimental vaccines were directed mostly at mutable envelope epitopes. gp120 contains structurally conserved neutralizing epitopes that are essential for maintenance of viral infectivity, but they do not stimulate the production of sufficiently neutralizing antibodies. Evidently, the virus hides its vulnerable components from the immune system. Yet, these are the components that must be targeted to successfully treat and prevent infections caused by diverse HIV-1 strains across the world. Hope for developing an effective HIV vaccination can be found if these milestones are met: a structurally constant gp120 epitope recognized by antibodies is identified and an immunogen that selectively induces synthesis of neutralizing antibodies to the epitope is developed.

To maintain its infectivity, HIV must keep the gp120 regions that bind host cells as comparatively constant structures, such as the binding site for CD4 receptors (CD4bs) and, to

© 2009 Future Medicine

[†]Author for correspondence: Chemical Immunology Research Center, Department of Pathology, University of Texas-Houston Medical School, 6431 Fannin, Houston, TX 77030, USA, Tel.: +1 713 500 5347, Fax: +1 713 500 0574, sudhir.paul@uth.tmc.edu.

Financial & competing interests disclosure

The authors' research was funded by NIH grants R21 AI071951, R01 AI058865, R01AI067020 and UL1 RR024148 (CTSA). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

a lesser extent, the binding site for chemokine coreceptors. Useful neutralizing epitopes are located by measuring the potency and breadth with which specific antibodies inhibit infection of diverse HIV strains. By this token, neutralizing epitopes within the CD4bs hold promise. The monoclonal antibody IgG b12 binds an epitope overlapping the CD4bs and neutralizes a broader array of HIV-1 strains than antibodies to hypermutable gp120 epitopes [1]. HIV disease progression is slow since the median duration to AIDS is approximately 10 years. Sera from some infected patients contain anti-CD4bs antibodies with epitope specificity different from IgG b12 [2]. The sera and monoclonal IgG b12 do not neutralize many HIV-1 strains, suggesting that the virus can change the target epitope structures sufficiently to escape antibody neutralization while retaining CD4-binding activity necessary for infection. Alternatively, the epitope may be occluded to variable extents in different virus strains. This is feasible because of the remarkable conformational flexibility of gp120 and its variable glycosylation status. Differing CD4bs conformational states in trimeric gp120 expressed on the viral surface and the shed monomer have been acknowledged. CD4 binding induces further gp120 conformational transitions, exemplified by changes in its recognition by antibodies to the chemokine receptor binding sites. However, even a partially effective vaccine might slow the pandemic significantly, prompting efforts to develop immunogens that mimic the b12 epitope. The CD4bs and IgG b12 epitopes are conformational determinants formed by spatially distant amino acids. No CD4bs mimetic that induces broadly neutralizing antibodies has been found.

The linear epitope formed by residues 421–433 is a shared component of the CD4bs and the B-cell superantigen (B-SAg) site of gp120. The immune system produces antibodies to B-SAg with no prior antigenic exposure, suggesting development of recognition specificity for ancestral B-SAg homologs over the course of antibody germline gene evolution. The extent of gp120 B-SAg site binding by serum IgG from noninfected individuals predicts weak but significant resistance to contracting HIV infection [3]. Purified mucosal and serum antibodies from noninfected humans recognize the 421–433 residues weakly by traditional noncovalent means, proceed to catalyze the hydrolysis of a peptide bond within the B-SAg site and neutralize clinical HIV-1 isolates with modest potency [4,5]. The catalytic function is interesting since it imparts improved neutralizing potency to antibodies by virtue of repeated use of a single catalyst molecule to hydrolyze multiple gp120 molecules. CD4bs recognition by reversibly binding and catalytic antibodies, therefore, appears to be an innate HIV resistance mechanism. Importantly, the B-SAg character of the CD4bs may confer a decisive advantage to HIV in eluding adaptive immune defenses. B-SAg binding to antibodies found within B-cell receptors (BCRs) induces B-cell death, aborting cellular differentiation necessary for adaptive improvement of the HIV-neutralizing antibodies. This precludes efficient protection against the virus. Initial indications that the desired adaptive immune response to the 421–433 epitope is not absolutely prohibited came from findings of increased 421–433 binding antibodies in the autoimmune disease lupus [6]. Recombinant antibody fragments from lupus patients neutralize diverse chemokine receptor R5-dependent HIV strains [7,8]. Lupus and HIV infection coexist rarely. Breakage of immune tolerance to self antigens in lupus is conceptually analogous to acquisition of cellular responsiveness to B-SAg. The genesis of neutralizing antibody synthesis in lupus is nebulous, but increased immune stimulation by polypeptides with structural similarity to the 421–433 epitope may be a factor; for example, epitopes corresponding to expressed endogenous retroviral sequences or bacterial products [9].

Unambiguous evidence for adaptive improvement of the neutralizing activity has emerged from hemophilic patients who survived infection for 19–21 years without developing AIDS [10]. Purified polyclonal IgAs from these patients neutralized diverse HIV-1 strains found across the world with exceptional potencies. The neutralizing activity was lost by immuno-adsorption with a synthetic 421–433 analog, and the IgAs recognized the epitope analogs in immunochemical assays. Prolonged HIV infection, therefore, stimulates B-cell adaptive

differentiation culminating in production of broadly neutralizing antibodies. An effective vaccine must induce an adaptive humoral response more rapidly, usually over a time span of weeks to months, entailing an initial primary immunization followed by one or more booster immunizations that stimulate memory B cells. Experimental covalent immunization procedures offer hope for such a vaccine [11–13]. The synthetic immunogens contain strong electrophiles that bind covalently to enzyme-like nucleophiles expressed by BCRs. The covalent immunization procedure drives adaptive improvement of the nucleophilic reactivity, resulting in synthesis of antibodies that recognize gp120 with covalent character and catalyze hydrolysis of the protein. Immunization with a synthetic electrophilic peptide corresponding to the gp120 V3 loop generated a polyclonal antibody response with improved strain-specific neutralizing activity, validating covalent immunization as a future vaccine strategy. An electrophilic full-length gp120 analog induced broadly neutralizing monoclonal antibodies in mice directed, in part, to the 421–433 epitope, but irrelevant epitope specificities dominated the overall antibody response. The use of electrophilic 421–433 peptide immunogens focused the neutralizing antibody response at the desired neutralizing epitope. The experimental vaccines, therefore, are capable of bypassing the B-SAg character of the CD4bs and inducing adaptive improvement of the neutralizing activity.

Traditional antigens drive B-cell differentiation by engaging immunoglobulin complementarity determining regions in the BCRs. B-SAGs establish contacts mainly at immunoglobulin framework regions, which could underlie the poor immunogenicity of the CD4bs. Noncovalent BCR stimulation and covalent BCR binding are exothermic reactions. The covalent reaction, however, releases approximately tenfold greater free energy that is available for BCR-driven cellular stimulation. The highly energetic signal generated by covalent immunogen–BCR engagement may explain the ability of electrophilic immunogens to direct B cells into the desired differentiation pathway and overcome the problem of CD4bs immunogenicity. Adaptive acquisition of covalent HIV binding and specific catalytic activities are important advantages of the strategy. These mechanisms can confer neutralization potencies upon antibodies outside the range of traditional reversibly-binding reagents. A concern is the induction of antibodies that do not recognize the native 421–433 epitope on the viral surface since small peptide immunogens are flexible and can adopt alternate conformations. The electrophilic peptide analogs, however, bind efficiently to antibodies that neutralize HIV via recognition of the 421–433 epitope, indicating their ability to mimic the native epitope conformation.

Previous failures of candidate HIV vaccines highlight the importance of defining preclinical tests to predict the efficacy of covalent vaccination. The anti-421–433 antibodies meet the important criterion of broad neutralization of diverse clinical HIV isolates using cultured human peripheral blood mononuclear cell (PBMC) hosts, an assay system that simulates infection *in vivo*. Pseudovirions expressing HIV envelope proteins and recombinant host cells expressing HIV receptors are useful to assess reduced viral entry, but discordant antibody neutralization activities compared with the PBMC clinical isolate system [14] might indicate a divergence from the native virus infection mechanism.

Animal HIV models are not fully predictive. Certain monkeys are susceptible to infection with engineered versions of the HIV ancestor, simian immunodeficiency virus expressing the HIV envelope (SHIV). Human HIV-1 infection is a lingering process, facilitating emergence of antibody-resistant mutants. However, SHIV is usually cleared too rapidly to assess vaccine failure resulting from viral escape mutations. The anti-HIV efficacy of passively administered antibodies can be measured using immune-incompetent mice populated with human lymphocytes and challenged with HIV. Transgenic mice producing HIV constitutively have been developed, but their predictive value for vaccine efficacy remains to be assessed.

Urgent problems require prompt and innovative solutions. The problem of HIV has been with us for over 25 years. Resistance to paradigm-changing concepts is justifiable as such concepts often turn out to be incorrect. While certain details must still be gathered, empirical evidence securing the scientific foundation for the covalent vaccination strategy is in place. It is time for further careful evaluation of the strategy in treating and preventing HIV infection.

Biographies

Acknowledgments

The authors wish to thank D Montefiore for discussions and neutralization assays done in his laboratory.

Future Virol. Author manuscript; available in PMC 2010 March 22.

Bibliography

1. Binley JM, Wrin T, Korber B, et al. Comprehensive cross-clade neutralization analysis of a panel of antihuman immunodeficiency virus type 1 monoclonal antibodies. *J. Virol* 2004;78:13232–13252. [PubMed: 15542675]
2. Li Y, Migueles SA, Welcher B, et al. Broad HIV-1 neutralization mediated by CD4-binding site antibodies. *Nat. Med* 2007;13:1032–1034. [PubMed: 17721546]
3. Townsley-Fuchs J, Kam L, Fairhurst R, et al. Human immunodeficiency virus-1 (HIV-1) gp120 superantigen-binding serum antibodies. A host factor in homosexual HIV-1 transmission. *J. Clin Invest* 1996;98:1794–1801. [PubMed: 8878430]
4. Paul S, Karle S, Planque S, et al. Naturally occurring proteolytic antibodies: selective immunoglobulin M-catalyzed hydrolysis of HIVgp120. *J. Biol. Chem* 2004;279:39611–39619. [PubMed: 15269209]
5. Planque S, Mitsuda Y, Taguchi H, et al. Characterization of gp120 hydrolysis by IgA antibodies from humans without HIV infection. *AIDS Res. Hum. Retroviruses* 2007;23:1541–1554. [PubMed: 18160012]
6. Bermas BL, Petri M, Berzofsky JA, et al. Binding of glycoprotein 120 and peptides from the HIV-1 envelope by autoantibodies in mice with experimentally induced systemic lupus erythematosus and in patients with the disease. *AIDS Res. Hum. Retroviruses* 1994;10:1071–1077. [PubMed: 7826694]
7. Karle S, Planque S, Nishiyama Y, et al. Cross-clade HIV-1 neutralization by an antibody fragment from a lupus phage display library. *AIDS* 2004;18:329–331. [PubMed: 15075554]
8. Planque S, Siencyk M, Nishiyama Y, et al. Prospective clinical utility and evolutionary implication of broadly neutralizing antibody fragments to HIV gp120 superantigenic epitope. Presented at: AIDS 2008 – XVII International AIDS Conference; 3–8 August 2008; México City, México.
9. Planque S, Nishiyama Y, Taguchi H, et al. Catalytic antibodies to HIV: physiological role and potential clinical utility. *Autoimmun. Rev* 2008;7:473–479. [PubMed: 18558365]
10. Planque S, Salas M, Escobar M, et al. Survivors of HIV infection produce potent, broadly neutralizing IgAs directed to the superantigenic region of the gp120 CD4 binding site. Presented at: AIDS 2008 – XVII International AIDS Conference; 3–8 August 2008; México City, México.
11. Paul S, Planque S, Zhou YX, et al. Specific HIV gp120-cleaving antibodies induced by covalently reactive analog of gp120. *J. Biol. Chem* 2003;278:20429–20435. [PubMed: 12665517]
12. Nishiyama Y, Karle S, Mitsuda Y, et al. Towards irreversible HIV inactivation: stable gp120 binding by nucleophilic antibodies. *J. Mol. Recognit* 2006;19:423–431. [PubMed: 16838382]
13. Nishiyama Y, Mitsuda Y, Taguchi H, et al. Towards covalent vaccination: improved polyclonal HIV neutralizing antibody response induced by an electrophilic gp120 V3 peptide analog. *J. Biol. Chem* 2007;282:31250–31256. [PubMed: 17728243]
14. Brown BK, Wieczorek L, Sanders-Buell E, et al. Cross-clade neutralization patterns among HIV-1 strains from the six major clades of the pandemic evaluated and compared in two different models. *Virology* 2008;375:529–538. [PubMed: 18433824]