Published in final edited form as:

Vaccine. 2008 June 13; 26(25): 3138–3141.

Retracing Our STEP towards a Successful CTL-Based HIV-1 Vaccine

Otto O. Yang

Division of Infectious Diseases, Department of Medicine, and Department of Microbiology, Immunology, and Molecular Genetics and UCLA AIDS Institute, David Geffen School of Medicine at UCLA, Los Angeles, California, 90095, USA

Failure in the STEP study: Vaccine failure versus disproof-of-concept?

The September 21st announcement that the STEP study (of the recombinant Adenovirus serotype 5, rAd5 HIV-1 vaccine) was being halted for futility [1] was a serious setback in the quest for a vaccine to prevent or attenuate HIV-1 infection. The rAd5 vaccine (with or without DNA vaccine priming) had been the *only* strategy demonstrating apparently consistent immunogenicity for HIV-1-specific CD8⁺ T lymphocyte (CTL) responses in humans. This vaccine approach was held as the major hope for a strategy that would show some efficacy in human trials, to serve as a proof-of-concept working prototype vaccine to be refined and improved. This hope was dashed when interim analysis revealed 21/762 versus 24/741 infections in the placebo and vaccine groups respectively, with set-point viremia levels also being similar (37,000 versus 40,000 genomes/ml plasma respectively).

Does this result indicate that a CTL-based vaccine is not the right approach? To date, the CTL response has been the only arm of adaptive immunity that has been clearly linked to control (albeit partial) of HIV-1 replication *in vivo*, as demonstrated by CTL being the major selective force for viral sequence evolution [2–4], and suggested by an inverse correlation of bulk CTL levels with viremia during CD8 depletion in the SIV-macaque model [5–7]. Such observations have been the basis for pursuing the CTL-based approach. The STEP study was pursued based on the apparent ability of the rAd5 vaccine to elicit HIV-1-specific CTL responses. Does the negative finding in this study indicate that a CTL-based approach is not a viable solution for a vaccine? Alternatively, is this a failure of the current vaccine to elicit functional CTL?

Requirements for CTL antiviral function

Unfortunately, a clear “correlate of immunity” based on any existing CTL measurements is lacking. To date, the interferon- γ ELISpot is the only validated assay for HIV-1 vaccine trials [8,9], by nature of its ease, reproducibility, and quantitative precision in mapping the targeting and magnitude of CTL responses. This assay provided the data that served as the basis for advancement of rAd5 into phase II human trials. However, ELISpot does not provide measurements that clearly correlate to immune control within infected persons, likely because it does not reflect antiviral function [10,11]. Measurements of CTL “polyfunctionality” via

Otto O. Yang, Division of Infectious Diseases, 37-121 CHS, UCLA Medical Center, 10833 LeConte Ave, Los Angeles, CA, 90095, USA, E-mail: oyang@mednet.ucla.edu, Phone: 310-794-9491, Fax: 310-825-3632.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

intracellular cytokine staining for multiple cytokines are under development for vaccine trial evaluation because polyfunctionality correlates to good immune control in HIV-1-infected persons [12], but it remains to be determined whether this is cause or effect, and whether polyfunctionality of CTL would predict protection by a vaccine [13].

Despite this muddy understanding of CTL and measurement for vaccine studies, some important points are apparent, offering potential alternative explanations for failure in the STEP trial other than futility of the overall approach of eliciting CTL:

1. CTL elicited by the vaccine must recognize HIV-1-infected cells to exert antiviral activity.
2. To attenuate disease, CTL elicited by the vaccine must avoid mechanisms of CTL failure in natural infection.

Recognition of excess peptide-loaded target cells versus HIV-1-infected target cells by CTL: a blind spot in vaccine testing

Evidence suggests the importance of direct cytolytic activity of CTL against HIV-1-infected cells for their antiviral effect. *In vitro* studies have demonstrated that while CTL release viral-inhibitory cytokines, killing activity is required for potent HIV-1 suppression [14,15], and the ability of CTL to suppress HIV-1 replication is directly correlated to efficiency of infected cell killing [16]. Assuming that HIV-1 suppression is the goal of vaccine-induced CTL *in vivo*, did the rAd5 vaccine tested in STEP induce CTL responses with this activity?

There are no data on the interaction of vaccine-elicited CTL with HIV-1-infected cells, but is quite possible that the vaccine did not achieve this goal. All data on CTL were derived from assays utilizing exogenously peptide-loaded target cells to detect CTL, via cytokine release (ELISpot, intracellular cytokine flow cytometry) or direct binding (MHC-peptide tetramer flow cytometry). These assays therefore detected HIV-1 *peptide*-specific CTL, under conditions of excess added peptide. However, to date there has been no evaluation of HIV-1-infected *cell*-recognizing CTL responses in rAd5 vaccinees. This is more than an academic distinction; it has been demonstrated that CTL can recognize exogenously-loaded HIV-1 peptides despite being unable to recognize HIV-1-infected cells with the same epitope sequences [11,16,17]. The mechanism relates to the difference between excess peptides used for detection assays versus lower levels of endogenously-produced epitopes on HIV-1-infected cells, with insufficient CTL avidity to recognize physiologic levels of epitopes [16,17].

The design of the rAd5 vaccines from Merck (and the similar vaccine from the NIH Vaccine Research Center) could actually favor CTL that trigger responses by assays such as ELISpot, yet which lack sufficient avidity to recognize HIV-1-infected cells. Emphasis was placed on driving high expression of HIV-1 epitopes by maximizing protein expression with the constitutive CMV immediate-early promoter and “codon-optimizing” insert genes [18]. Supra-physiologic levels of cell surface epitopes compared to natural HIV-1 infection could therefore have selected for CTL without regard for avidity, allowing preferential expansion of CTL with relatively low avidity [19–21] that was insufficient to recognize HIV-1-infected cells.

Trafficking of CTL where they are needed

Of necessity, most studies of HIV-1-specific CTL responses have examined peripheral blood. It is clear that viral replication and antiviral immunity are predominately tissue-based. Furthermore, early infection, when the most severe loss of CD4⁺ T helper cells occurs and the course of disease is set, is marked by vigorous viral replication in the gut compartment regardless of route of infection [22–24]. It stands to reason that a preventive or disease attenuating vaccine should elicit CTL responses that access this compartment. It has been

observed that HIV-1-specific CTL are found among gut lymphocytes in chronically-infected persons [25,26], but whether rAd5 vaccination elicits CTL in the gut remains unknown. Data comparing gut shedding of poliovirus in persons who had previously received inactivated versus live-attenuated poliovirus vaccine suggested that mucosally administered replicating vaccines may yield better mucosal immunity [27,28]. Whether a peripherally administered non-replicating vaccine such as rAd5 yields gut immunity remains to be determined, but developing delivery strategies to promote such responses may be important.

The need to improve on nature

Historically, successful vaccines have been derived by empiric attempts to mimic successful natural responses to infections. HIV-1 poses a greater challenge, however, in that natural responses typically fail to clear or contain infection. Although it seems clear that CTL provide a modest degree of protection from disease during natural infection, thus far the mechanisms limiting CTL efficacy and allowing long term failure of the CTL response remain poorly understood. Two processes that almost certainly contribute to CTL failure are the loss of CD4⁺ T helper cells [29,30], and the high replication and mutation rates of HIV-1 [31,32]. Vaccination in the absence of viral replication may assist in preventing the former, giving the CTL response a head start compared to HIV-1 infection.

However, the variability of HIV-1 sequence is a more formidable problem that may be overwhelming. The great mutation rate of the virus poses at least two major impediments to vaccine-elicited CTL. First, there may be significant mismatching of the epitope sequences in the vaccine compared to the challenge virus. Recent data suggest that the cross-reactivity of CTL for epitope variants (such as between strains from different clades) has been vastly overestimated by exogenous peptide-based assays [16,17]. While the use of clade B consensus sequences in rAd5 vaccines minimizes the average genetic distance of the vaccine from any given infecting clade B strain of HIV-1 (compared to using any other fixed sequence), it is likely that substantial intra-clade genetic variability of circulating strains is not adequately addressed by this approach. Second, even if CTL responses can recognize infected cells, HIV-1 has the capability to develop escape mutations to evade CTL [32]. Even single epitope point mutations can allow complete escape [33], which is daunting in the context of replication and mutation rates that predict the random generation of every possible single or double point mutant *in vivo* daily [31,34].

The solution to the problem of HIV-1 sequence variability must lie in the choice of sequences for vaccine inclusion. Current vaccine approaches follow a whole protein-based approach for including viral sequences; the rAd5 vaccines administered in STEP included HIV-1 Gag, Pol, and Nef [18]. However, expressing whole HIV-1 proteins is likely to mimic natural patterns of CTL targeting, with recognition of more variable epitopes in acute infection [35,36] which allows widespread escape that is frequently noted during early infection [37–42]. The antigenic units of CTL are linear epitopes; maintaining entire reading frames for proteins in the vaccine is unnecessary. For a CTL-based vaccine, it may be beneficial to include stretches of sequences with desired properties and exclude those with undesired properties (e.g. including highly conserved sequences, and excluding variable sequences, to minimize the risk of epitope escape mutation), without the constraint of considering proteins as all-or-nothing units. The debate thus far has been regarding which proteins to include, but perhaps the focus should be on which stretches of sequences to include, because the antigenic unit of CTL is the epitope, and not the whole protein.

Taking the next STEPs?

The failure of the STEP study to demonstrate vaccine benefit is a disappointment in the quest for a CTL-based HIV-1 vaccine, but instructs us about the importance of considering cellular

immunopathogenesis concepts in vaccine design. Steps required for a successful vaccine include generating CTL that can recognize and kill HIV-1-infected cells, ensuring that these CTL traffic appropriately to important sites of viral replication, and finding methods to overcome the massive genetic inter-host diversity and intra-host diversification of HIV-1 sequences. In looking where to step next, it will be crucial to assess these pre-requisites for a successful vaccine. At this point, it is simply not possible to know why the rAd5 approach failed, but there are many potential shortcomings of the vaccine that must be considered before a CTL-based approach is discounted. It would be premature to consider the STEP result as a disproof-of-concept.

References

1. Vaccination and Enrollment Are Discontinued in Phase II Trials of Merck's Investigational HIV Vaccine Candidate. In: Press release from Merck and the HIV Vaccine Trials Network. Whitehouse Station NJ and Seattle WA, 2007.
2. Moore CB, John M, James IR, Christiansen FT, Witt CS, Mallal SA. Evidence of HIV-1 adaptation to HLA-restricted immune responses at a population level. *Science* 2002;296(5572):1439–43. [PubMed: 12029127]
3. Allen TM, Altfeld M, Geer SC, Kalife ET, Moore C, O'Sullivan KM, et al. Selective escape from CD8 + T-cell responses represents a major driving force of human immunodeficiency virus type 1 (HIV-1) sequence diversity and reveals constraints on HIV-1 evolution. *J Virol* 2005;79(21):13239–49. [PubMed: 16227247]
4. Liu Y, McNevin J, Cao J, Zhao H, Genowati I, Wong K, et al. Selection on the human immunodeficiency virus type 1 proteome following primary infection. *J Virol* 2006;80(19):9519–29. [PubMed: 16973556]
5. Matano T, Shibata R, Siemon C, Connors M, Lane HC, Martin MA. Administration of an anti-CD8 monoclonal antibody interferes with the clearance of chimeric simian/human immunodeficiency virus during primary infections of rhesus macaques. *J Virol* 1998;72(1):164–9. [PubMed: 9420212]
6. Jin X, Bauer DE, Tuttleton SE, Lewin S, Gettie A, Blanchard J, et al. Dramatic rise in plasma viremia after CD8(+) T cell depletion in simian immunodeficiency virus-infected macaques. *J Exp Med* 1999;189(6):991–8. [PubMed: 10075982]
7. Schmitz JE, Kuroda MJ, Santra S, Sasseville VG, Simon MA, Lifton MA, et al. Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. *Science* 1999;283(5403):857–60. [PubMed: 9933172]
8. Dubey S, Clair J, Fu TM, Guan L, Long R, Mogg R, et al. Detection of HIV vaccine-induced cell-mediated immunity in HIV-seronegative clinical trial participants using an optimized and validated enzyme-linked immunospot assay. *J Acquir Immune Defic Syndr* 2007;45(1):20–7. [PubMed: 17310936]
9. Fu TM, Dubey SA, Mehrotra DV, Freed DC, Trigona WL, Adams-Muhler L, et al. Evaluation of cellular immune responses in subjects chronically infected with HIV type 1. *AIDS Res Hum Retroviruses* 2007;23(1):67–76. [PubMed: 17263635]
10. Yang OO. Will we be able to 'spot' an effective HIV-1 vaccine? *Trends Immunol* 2003;24(2):67–72. [PubMed: 12547502]
11. Valentine LE, Piaskowski SM, Rakasz EG, Henry NL, Wilson NA, Watkins DI. Recognition of escape variants in ELISPOT does not always predict CD8+ T-cell recognition of simian immunodeficiency virus-infected cells expressing the same variant sequences. *J Virol* 2008;82(1):575–81. [PubMed: 17959674]
12. Pantaleo G, Koup RA. Correlates of immune protection in HIV-1 infection: what we know, what we don't know, what we should know. *Nat Med* 2004;10(8):806–10. [PubMed: 15286782]
13. Betts MR, Exley B, Price DA, Bansal A, Camacho ZT, Teaberry V, et al. Characterization of functional and phenotypic changes in anti-Gag vaccine-induced T cell responses and their role in protection after HIV-1 infection. *Proc Natl Acad Sci U S A* 2005;102(12):4512–7. [PubMed: 15753288]

14. Buseyne F, Fevrier M, Garcia S, Gougeon ML, Riviere Y. Dual function of a human immunodeficiency virus (HIV)-specific cytotoxic T-lymphocyte clone: inhibition of HIV replication by noncytolytic mechanisms and lysis of HIV-infected CD4+ cells. *Virology* 1996;225(1):248–53. [PubMed: 8918556]
15. Yang OO, Kalams SA, Trocha A, Cao H, Luster A, Johnson RP, et al. Suppression of human immunodeficiency virus type 1 replication by CD8+ cells: evidence for HLA class I-restricted triggering of cytolytic and noncytolytic mechanisms. *J Virol* 1997;71(4):3120–8. [PubMed: 9060675]
16. Bennett MS, Ng HL, Dagarag M, Ali A, Yang OO. Epitope-dependent avidity thresholds for cytotoxic T-lymphocyte clearance of virus-infected cells. *J Virol* 2007;81(10):4973–80. [PubMed: 17329324]
17. Bennett MS, Ng H, Ali A, Yang OO. Cross-Clade Detection of HIV-1-Specific CTL Does Not Reflect Cross-Clade Antiviral Activity. *J Infect Dis.* in press
18. Shiver JW, Emini EA. Recent advances in the development of HIV-1 vaccines using replication-incompetent adenovirus vectors. *Annu Rev Med* 2004;55:355–72. [PubMed: 14746526]
19. Kim M, Moon HB, Kim K, Lee KY. Antigen dose governs the shaping of CTL repertoires in vitro and in vivo. *Int Immunol* 2006;18(3):435–44. [PubMed: 16431877]
20. Alexander-Miller MA, Leggatt GR, Berzofsky JA. Selective expansion of high- or low-avidity cytotoxic T lymphocytes and efficacy for adoptive immunotherapy. *Proc Natl Acad Sci U S A* 1996;93(9):4102–7. [PubMed: 8633023]
21. Byers DE, Lindahl KF. Peptide affinity and concentration affect the sensitivity of M3-restricted CTLs induced in vitro. *J Immunol* 1999;163(6):3022–8. [PubMed: 10477565]
22. Brenchley JM, Schacker TW, Ruff LE, Price DA, Taylor JH, Beilman GJ, et al. CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. *J Exp Med* 2004;200(6):749–59. [PubMed: 15365096]
23. Veazey RS, DeMaria M, Chalifoux LV, Shvetz DE, Pauley DR, Knight HL, et al. Gastrointestinal tract as a major site of CD4+ T cell depletion and viral replication in SIV infection. *Science* 1998;280(5362):427–31. [PubMed: 9545219]
24. Mehandru S, Poles MA, Tenner-Racz K, Horowitz A, Hurley A, Hogan C, et al. Primary HIV-1 infection is associated with preferential depletion of CD4+ T lymphocytes from effector sites in the gastrointestinal tract. *J Exp Med* 2004;200(6):761–70. [PubMed: 15365095]
25. Ibarrondo FJ, Anton PA, Fuerst M, Ng HL, Wong JT, Matud J, et al. Parallel human immunodeficiency virus type 1-specific CD8+ T-lymphocyte responses in blood and mucosa during chronic infection. *J Virol* 2005;79(7):4289–97. [PubMed: 15767429]
26. Critchfield JW, Lemongello D, Walker DH, Garcia JC, Asmuth DM, Pollard RB, et al. Multifunctional human immunodeficiency virus (HIV) gag-specific CD8+ T-cell responses in rectal mucosa and peripheral blood mononuclear cells during chronic HIV type 1 infection. *J Virol* 2007;81(11):5460–71. [PubMed: 17344302]
27. Ghendon Y, Robertson SE. Interrupting the transmission of wild polioviruses with vaccines: immunological considerations. *Bull World Health Organ* 1994;72(6):973–83. [PubMed: 7867144]
28. Onorato IM, Modlin JF, McBean AM, Thoms ML, Losonsky GA, Bernier RH. Mucosal immunity induced by enhance-potency inactivated and oral polio vaccines. *J Infect Dis* 1991;163(1):1–6. [PubMed: 1845806]
29. Rosenberg ES, Billingsley JM, Caliendo AM, Boswell SL, Sax PE, Kalams SA, et al. Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. *Science* 1997;278(5342):1447–50. [PubMed: 9367954]
30. Kalams SA, Buchbinder SP, Rosenberg ES, Billingsley JM, Colbert DS, Jones NG, et al. Association between virus-specific cytotoxic T-lymphocyte and helper responses in human immunodeficiency virus type 1 infection. *J Virol* 1999;73(8):6715–20. [PubMed: 10400769]
31. McMichael AJ, Phillips RE. Escape of human immunodeficiency virus from immune control. *Annu Rev Immunol* 1997;15:271–96. [PubMed: 9143689]
32. Yang OO. CTL ontogeny and viral escape: implications for HIV-1 vaccine design. *Trends Immunol* 2004;25(3):138–42. [PubMed: 15036041]

33. Yang OO, Sarkis PT, Ali A, Harlow JD, Brander C, Kalams SA, et al. Determinants of HIV-1 mutational escape from cytotoxic T lymphocytes. *J Exp Med* 2003;197(10):1365–75. [PubMed: 12743169]
34. Perelson AS, Essunger P, Ho DD. Dynamics of HIV-1 and CD4+ lymphocytes in vivo. *Aids* 1997;11 (Suppl A):S17–24. [PubMed: 9451962]
35. Brander C, Riviere Y. Early and late cytotoxic T lymphocyte responses in HIV infection. *Aids* 2002;16 (Suppl 4):S97–103. [PubMed: 12699006]
36. Goulder PJ, Altfield MA, Rosenberg ES, Nguyen T, Tang Y, Eldridge RL, et al. Substantial differences in specificity of HIV-specific cytotoxic T cells in acute and chronic HIV infection. *Journal of Experimental Medicine* 2001;193(2):181–94. [PubMed: 11148222]
37. Cao J, McNeven J, Malhotra U, McElrath MJ. Evolution of CD8+ T cell immunity and viral escape following acute HIV-1 infection. *J Immunol* 2003;171(7):3837–46. [PubMed: 14500685]
38. Borrow P, Lewicki H, Wei X, Horwitz MS, Pfeffer N, Meyers H, et al. Antiviral pressure exerted by HIV-1-specific cytotoxic T lymphocytes (CTLs) during primary infection demonstrated by rapid selection of CTL escape virus. *Nat Med* 1997;3(2):205–11. [PubMed: 9018240]
39. Oxenius A, Price DA, Trkola A, Edwards C, Gostick E, Zhang HT, et al. Loss of viral control in early HIV-1 infection is temporally associated with sequential escape from CD8+ T cell responses and decrease in HIV-1-specific CD4+ and CD8+ T cell frequencies. *J Infect Dis* 2004;190(4):713–21. [PubMed: 15272399]
40. Price DA, Goulder PJ, Klennerman P, Sewell AK, Easterbrook PJ, Troop M, et al. Positive selection of HIV-1 cytotoxic T lymphocyte escape variants during primary infection. *Proc Natl Acad Sci U S A* 1997;94(5):1890–5. [PubMed: 9050875]
41. Jones NA, Wei X, Flower DR, Wong M, Michor F, Saag MS, et al. Determinants of human immunodeficiency virus type 1 escape from the primary CD8+ cytotoxic T lymphocyte response. *J Exp Med* 2004;200(10):1243–56. [PubMed: 15545352]
42. Karlsson AC, Iversen AK, Chapman JM, de Oliveira T, Spotts G, McMichael AJ, et al. Sequential broadening of CTL responses in early HIV-1 infection is associated with viral escape. *PLoS ONE* 2007;2:e225. [PubMed: 17311088]