The failed HIV Merck vaccine study: a step back or a launching point for future vaccine development?

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The world of human immunodeficiency virus (HIV) vaccines has suffered a baffling setback. The first trial of a vaccine designed to elicit strong cellular immunity has shown no protection against infection. More alarmingly, the vaccine appeared to increase the rate of HIV infection in individuals with prior immunity against the adenovirus vector used in the vaccine. A new study in this issue suggests that a different vaccine approach—using a DNA prime/poxvirus boost strategy—induces polyfunctional immune responses to an HIV immunogen. The disappointing results of the recent vaccine trial suggest that a more thorough assessment of vaccine–induced immune responses is urgently needed, and that more emphasis should be placed on primate models before efficacy trials are undertaken.

The pathway to an HIV vaccine has never been considered easy or straightforward (1, 2). The challenges involved in developing a successful vaccine have accumulated from the time of the first clinical trials of the Microgenesis vaccine to the highly publicized Vaxgen trial (1, 3, 4). These HIV envelope–based vaccines were aimed at inducing neutralizing antibody responses, as several groups had shown that passive transfer of large amounts of neutralizing antibodies could protect primates against infection. Unfortunately, these first trials failed in large part because inducing neutralizing antibodies is a daunting task at which more than one group has failed (5). Other reasons for these failures include the genetic variability of the viral envelope proteins, which allows the virus to escape neutralizing antibodies, and the difficulty in identifying immunogens and immunization platforms that consistently induce antibodies that can neutralize several HIV clades (6).

A shift in focus
In light of the difficulties in eliciting neutralizing antibodies, the field has switched its focus away from vaccines that induce sterilizing immunity (still the ultimate goal) toward those that control viral load after infection and thus reduce secondary transmission (7–11). This shift was prompted by data showing that T cell–mediated immunity was critical for resistance to immunodeficiency viruses. For example, depletion of $CD8^+$ cells in simian immunodeficiency virus (SIV)-infected macaques led to a resurgence of viral load (12), and in HIV-infected individuals known as "elite controllers," the control of viral load was associated with potent and broad cellular immune responses (13, 14).

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These findings have helped move the field toward the development of vaccines designed to elicit strong cellular immunity (15).

One approach to generating robust T cell responses is to express HIV antigens in replication–defective recombinant adenoviral vaccines (16). These vectors elicit protection in some primate models of HIV infection and induce detectable $CD8^+$ T cell responses in humans, suggesting that this approach could help limit viral load and disease progression (16–19). But this approach is not without its problems. There have been several concerns about the use of recombinant adenovirus serotype 5 (rAD5) as a vaccine vector, including preexisting vector-specific immunity and uncertainty about whether these vectors can induce long-lasting, broad, and protective immune responses (20–22). The scientific community has recently learned that the STEP HIV vaccine trial, which used an rAd5-based vaccine developed by Merck, failed to protect Ad5-seronegative individuals against infection and may even have enhanced infection in vaccinees with prior immunity to adenoviruses (23–26). In this issue, Harari et al. (p. 63; reference 27) report on an alternative heterologous prime-boost strategy in which DNA priming improved the immunogenicity of a recombinant viral vector, in this case the vaccinia virus NYVAC (27). In this Commentary, I will discuss the STEP trial and the new report from Harari et al. from the perspective of someone engaged in research on human T cell function and the immune monitoring of vaccination protocols.

Reflections on the STEP trial
The STEP trial involved the immunization of almost 3,000 healthy uninfected volunteers with three rAD5 vectors, each expressing an HIV gene: Ad5–gag, Ad5–pol, and Ad5–Nef (http://www.hvtn.org/media/pr/step111307.html). This proof-of-concept trial was intended to test the capacity of the vaccine to reduce infection and to reduce the viral load (or "set point") in vaccinated individuals who nevertheless became infected. Each individual in the trial received three injections of the three

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rAD5 vectors, with the last two injections spaced 6 months apart. The same vaccine was being administered in South Africa to 3,000 individuals in the Phambilli trial when the initial results of the STEP trial were made public.

The path to the development of the STEP trial included 12 Phase I trials with more than 1,300 volunteers, which

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showed that the vaccine was safe and immunogenic as measured by a standardized interferon (IFN)-γ enzyme-linked immunospot (ELISPOT) assay. Experiments in primates also showed some protection. Immunization with the defective rAD5 vector followed by challenge with a hybrid SHIV (SIV with an HIV envelope) lead to a 1–3 log decrease in viral load (16). However, these experiments were performed on only a limited number of macaques. And a more stringent challenge with the SIV mac239 strain resulted in a more modest decrease of viral load (~1 log, leaving an average of 10^5 copies of RNA/ml plasma in vaccinated animals) (17, 28). In addition, this immunization strategy was effective only in monkeys carrying a specific human histocompatibility leukocyte antigen (HLA) allele known to present a dominant epitope from the gag protein. Thus, the Merck vaccine had limited efficacy in a stringent primate model.

The foremost issue facing any rAD5-based vaccine is the high prevalence of adenovirus-specific antibodies as a result of prior exposure to the virus, particularly in sub-Saharan Africa. Adenovirus vectors, and many other viral vectors currently used in HIV vaccines, will induce a rapid memory immune response against the vector. The resulting elimination of the vector was anticipated to be an impediment to the development of a T cell response against the inserted antigen. What was completely unexpected, however, was the possibility that previous adenovirus infection might enhance

susceptibility to HIV infection in vaccinated subjects. Although the statistical analysis has not been completed, the initial results show that vaccinated subjects who had high titers of antibodies against adenovirus tended to have a higher incidence of HIV infection than those without anti-adenovirus antibodies. One possible explanation for this outcome is that the presence of both antibody and virus could lead to the activation of T cells, thus providing an environment that favors HIV replication.

These results raise several critical questions. Was the failure of this Ad5-based trial attributable to something specific about the Merck rAd5 vector, or would all rAd vectors face similar problems? Of note, the Merck rAD5 vector is missing only one adenovirus protein (E1) and thus expresses more viral genes than other adenovirus vectors. The vector used by National Institutes of Health's Vaccine Research Center, for example, lacks both the E1 and E3 protein. Minimizing the number of viral genes in a vaccine vector helps strip the virus of its natural ability to evade the immune system. The STEP trial also included three shots of the vaccine, and it is unclear whether the increased rate of infection would have occurred if only a single shot of rAd5-based vaccine had been used. The fundamental question, however, is whether the problem was with the specific vector or immunization regimen used in the STEP trial, or whether there is a more general danger of using viral vectors to immunize against HIV. In light of the immunomodulatory properties of viruses and attenuated viral vectors, we should systematically investigate vector-specific immunity and, more importantly, its impact on qualitative and quantitative parameters of the HIV-specific immune response. One cannot exclude the possibility that the rAd5 vector and the immune response to the vector (whether innate or adaptive) prevented the full development of an HIV-specific immune response.

Immune responses to vaccines: quality versus quantity
The Merck candidate vaccine showed good HIV-specific immunogenicity in

Phase I and II studies (see http://www.hvtn.org/science/1107.html for the recently released STEP trial results) as measured mostly by a single parameter: the IFN-γ ELISPOT assay. The rAd vaccine also induced long-lasting, multifunctional responses as monitored by polychromatic flow cytometry (http://www.hvtn.org/fgm/1107slides/McElrath.pdf). Indeed, after homologous prime-boost immunization with a replication-defective adenovirus-based vaccine, a majority of responders had HIV-specific CD8+ T lymphocytes that were capable of producing CD107, macrophage inflammatory protein 1β, IFN-γ, and TNF, and antigen-specific CD4+ T cells that were able to produce IFN-γ, interleukin (IL)-2, and TNF (Casimiro, D., personal communication). The CD8 T cell responses to HIV antigens, however, were not particularly broad. A median of three peptide pools, each consisting of overlapping 9–amino acid peptides spanning a 16–amino acid region of gag, nef, or pol, was recognized by vaccinated subjects (Casimiro, D., personal communication). Thus, by all accounts, the STEP

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vaccine was safe, immunogenic (by the aforementioned criteria), and induced some level of protection in primate studies. Although some may have predicted disappointing results based on the relatively weak protection in macaques and the lack of a broad T cell response, no one could have predicted the correlation between preexisting vector-specific immunity and an increase in susceptibility to infection—a result that lead to the immediate halting of both the STEP and Phambilli trials.

The new study by Harari et al. clearly shows that the DNA/NYVAC prime-boost regimen induces HIV-specific CD4+ and CD8+ T cell responses (27). In this regimen, DNA priming was essential for the induction of a strong response to the recombinant virus. The T

cell responses were also polyfunctional; nearly 50% of the HIV-specific CD4$^+$ and CD8$^+$ T cells induced in vaccinated subjects produced more than three cytokines. It is also interesting to note that this regimen induced strong T cell proliferation and, more importantly, robust production of the T cell growth factor IL-2 by HIV-specific CD4$^+$ and CD8$^+$ cells (29). These responses were also persistent, as some T cell responses were still detectable after 96 weeks (Pantaleo, G., personal communication). A big effort is now being made to standardize immune-monitoring assays in humans and in preclinical models, but it is currently difficult to compare the relative immunogenicity of two vectors outside of a single trial where the two vaccines are compared head-to-head using a common, standardized set of assays to monitor the resulting immune response. Alternatively, immune responses from both trials should be tested in a single assay platform.

One notable difference between the vaccines used in the STEP trial and by Harari et al. was the detection in the latter study of higher frequencies of CD4$^+$ and CD8$^+$ T cells producing a high level of IL-2 or both IL-2 and IFN-γ. These dual-producing cells have previously been associated with better control of viral load after vaccination and in natural history studies (30). Polyfunctional T cell responses have also been associated with protection against *Leishmania major* infection (31) and are a feature of immune responses in HIV-infected elite controllers (32). Moreover, while rAd5-based vaccines elicit strong CD8 responses to gag, the DNA/NYVAC leads to the development of strong env-specific responses, whereas gag-specific responses are poor. Of note, the rAd5 vaccine tested in the STEP trial did not include env constructs.

The DNA/poxvirus immunization strategy may thus provide a promising alternative to adenoviral vector–based approaches. This strategy is also advantageous in that most individuals born after 1974 have not been immunized against smallpox and hence will have little preexisting immunity to these viruses—an important consideration given

the results of the STEP trial. In addition, the DNA-priming step appears to enhance the immune response against the poxvirus, as it also does with other vectors, including adenoviruses (33–36). Hence, the DNA prime NYVAC–boost strategy not only might bypass the requirement for multiple booster injections with viral vector, but it might also favorably modulate the immune response against the immunogen. Finally, the immunogenicty data obtained with the DNA/poxvirus strategy show that this vector combination can induce potent, multifunctional immune responses, including a large fraction of IL-2–producing cells, which are often endowed with superior memory functions.

In response to the STEP failure, however, it is important to continue to research the criteria that best predict protective T cell immunity against pathogens. It must now be determined whether the potential improvements afforded by DNA prime virus–boost regimens warrant large-scale clinical studies to evaluate protective immunity. More importantly, we should reflect on whether the assays we are currently using to guide vaccine development should be expanded to include additional surrogate markers of efficacy, or whether they need only provide a simple indicator of immunogenicity. The field should quickly develop assays that will help in predicting the development of protective immunity in response to vaccines.

Polyfunctionality and its scope

Polyfunctional T helper (Th)1 cells that make higher levels of cytokines on an individual cell basis (e.g., as assessed by mean fluorescence intensity for IFN-γ staining) have been associated with protection in vaccine trials against other microbes as well as in HIV-infected elite controllers (31, 32). Both adenovirus-based vaccines and the DNA/NYVAC vaccine trigger T cells that produce at least three different cytokines in response to the HIV immunogen. The DNA/NYVAC vaccine elicited a greater frequency of CD8 cells and Th1 cells secreting IL-2, TNF, and IFN-γ, whereas the rAD-based vaccines typically elicited little IL-2 and mostly IFN-γ and

TNF. Hence, at first glance it appears that pox and rAD5 vectors elicit overlapping but qualitatively different Th1 responses. Current immune-monitoring strategies are focused on measuring effector T cell responses. They do not, however, measure memory and its renewal or persistence, despite the fact that absolute numbers of memory T cells and, in particular, central memory T cells have been associated with protection in primate models (9). Nor do the current strategies assess other immune parameters, such as Th2 cytokines and innate immune responses. In light of the STEP trial outcome, one has to keep an open mind on the kind of polyfunctionality that will provide protection. The term polyfunctionality might also imply more than just the induction of CD8$^+$ and CD4$^+$ cells that produce multiple cytokines; it could also reflect an integrated immune response that includes

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different types of T cells (Th1 and Th2), B cells, and other innate immune cells, including dendritic cells and natural killer (NK) cells. Our current assays also do not allow us to evaluate the homing of T cells to mucosal sites, the primary site of HIV infection.

How should we measure the success of a vaccine? Successful, well-tested vaccines such as the smallpox, hepatitis A, and yellow fever vaccines all elicit broad integrated responses that encompass all effector arms of immune response, including innate (Toll-like receptor–mediated) responses, NK cell responses, and Th1/Th2 responses (37–40). With the knowledge that the innate immune response influences both qualitative and quantitative features of adaptive immunity, one should make every attempt to identify innate responses that correlate with protection (38). Information is currently lacking on the innate responses elicited by the different vectors, including

adenovirus and poxviruses early after immunization and in ex vivo systems. The adaptation of system biology approaches, including genomics, proteomics, and bioinformatic tools, to understand vaccine-induced innate and adaptive immune responses should become a priority for the field (41, 42).

Measuring vector–specific responses

As it is likely that viral vectors will continue to be used, at least in the near future, it will be critical to understand whether immunological memory to infectious viruses is different than memory to vaccines. This is likely because immune responses to infections are influenced by the high viral loads characteristic of acute infections, whereas viral vectors are usually attenuated viruses (like the rAd5 vector) and thus do not

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lead to high viremia. This is likely to influence the immune response, as high antigen load can result in the deletion of high affinity T cells and clonal exhaustion via, for example, the activation of the negative PD-1 pathway (43–45). One must keep in mind that there is little evidence that adenoviral infection in humans induces broadly cross-reactive protection against adenoviral infection itself (19). Thus, a better understanding of the differences between infection- and vaccine-mediated immunity, especially in the context of viruses used to generate vaccine vectors, is warranted. Several novel viral vectors will soon be tested in humans (http://www.iavi.org/), including other forms of adenoviruses (PAVE) and poxviruses, as well as flaviviruses, alphaviruses, measles virus, and replication-competent viruses (46). In light of the STEP results, we need to consider the fact that the vector-specific immune response and the immuno-modulatory function of vector-encoded proteins could compete or bias HIV-specific responses (47).

Animal models and minimal requirements

Can we learn more from animal models, since many investigators were concerned that the STEP trial was based on a vaccine that showed minimal protection against SIV infection in macaques? Animal studies offer many distinct advantages (48–50). They can be optimized in terms of the viral strain used, as well as the route and dose of challenge. Animals can also be challenged with heterologous viral isolates, thereby mimicking the situation in humans where it is very unlikely that one will be naturally exposed to the same virus strain used in a vaccine. Hence, proof that viral infection is attenuated in a heterologous infection model should be a minimal requirement for a vaccine to be tested for efficacy in humans. Preclinical trials of vaccines should also include macaques that express a spectrum of HLA haplotypes because HLA can clearly influence the quality of the T cell response that develops against the vaccine and against the challenge virus. Lastly, the field should come up with a consensus on a strategy for SIV challenge. Currently, different groups are challenging with different viruses making it problematic to compare the relative efficacy of the vectors and immunization strategies. Animal models should also be used to search for immunological readouts that can predict protection, using various multiparametric approaches. It might also be useful to contemplate the development of new animal models. A large effort has been put forward to develop humanized mice, but these are still not ready to be used to test immune responses to vaccines (51, 52).

What next?

For some, the Merck vaccine begs the question of whether there is still a strong rationale for the development of vaccines that trigger only Th1-type immunity. Lessons should be learned from this trial, and a vigorous effort is underway to gather the information required to understand the biological reasons behind the surprising results of the STEP trial. It has become evident that vaccines that induce neutralizing antibody

responses are still far from the clinic. Hence, pursuing the T cell approach is a viable alternative, provided we can optimize the vaccines and develop a coordinated strategy to evaluate immune responses in greater depth. The tools to do this are emerging through initiatives like the Global Enterprise for an HIV vaccine. As we are still far from understanding the ingredients required for effective vaccines, attempts to better understand the components of the immune response to licensed, efficacious vaccines should be a research priority. This research will help define the elusive correlates of T cell immunity to vaccines in humans.

REFERENCES

1. Letvin, N.L. 2006. Progress and obstacles in the development of an AIDS vaccine. *Nat. Rev. Immunol.* 6:930–939.
2. Berkley, S.F., and W.C. Koff. 2007. Scientific and policy challenges to development of an AIDS vaccine. *Lancet.* 370:94–101.
3. Robinson, H.L. 2007. HIV/AIDS vaccines: 2007. *Clin. Pharmacol. Ther.* 82:686–693.
4. Billich, A. 2004. AIDSVAX VaxGen. *Curr. Opin. Investig. Drugs.* 5:214–221.
5. Burton, D.R., R.C. Desrosiers, R.W. Doms, W.C. Koff, P.D. Kwong, J.P. Moore, G.J. Nabel, J. Sodroski, I.A. Wilson, and R.T. Wyatt. 2004. HIV vaccine design and the neutralizing antibody problem. *Nat. Immunol.* 5:233–236.
6. Phogat, S., R.T. Wyatt, and G.B. Karlsson Hedestam. 2007. Inhibition of HIV-1 entry by antibodies: potential viral and cellular targets. *J. Intern. Med.* 262:26–43.
7. McMichael, A.J. 2006. HIV vaccines. *Annu. Rev. Immunol.* 24:227–255.
8. Emini, E.A., and W.C. Koff. 2004. AIDS/HIV. Developing an AIDS vaccine: need, uncertainty, hope. *Science.* 304:1913–1914.
9. Letvin, N.L., J.R. Mascola, Y. Sun, D.A. Gorgone, A.P. Buzby, L. Xu, Z.Y. Yang, B. Chakrabarti, S.S. Rao, J.E. Schmitz, et al. 2006. Preserved CD4+ central memory T cells and survival in vaccinated SIV-challenged monkeys. *Science.* 312:1530–1533.
10. Thorner, A.R., and D.H. Barouch. 2007. HIV-1 vaccine development: progress and prospects. *Curr. Infect. Dis. Rep.* 9:71–75.
11. Robinson, H.L., and R.R. Amara. 2005. T cell vaccines for microbial infections. *Nat. Med.* 11:S25–S32.

12. Schmitz, J.E., M.J. Kuroda, S. Santra, V.G. Sasseville, M.A. Simon, M.A. Lifton, P. Racz, K. Tenner-Racz, M. Dalesandro, B.J. Scallon, et al. 1999. Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. *Science*. 283:857–860.

13. Bernard, N.F., K. Pederson, F. Chung, L. Ouellet, M.A. Wainberg, and C.M. Tsoukas. 1998. HIV-specific cytotoxic T-lymphocyte activity in immunologically normal HIV-infected persons. *AIDS*. 12:2125–2139.

14. Pontesilli, O., M.R. Klein, S.R. Kerkhof-Garde, N.G. Pakker, F. de Wolf, H. Schuitemaker, and F. Miedema. 1998. Longitudinal analysis of human immunodeficiency virus type 1-specific cytotoxic T lymphocyte responses: a predominant gag-specific response is associated with nonprogressive infection. *J. Infect. Dis*. 178:1008–1018.

15. Pantaleo, G., and R.A. Koup. 2004. Correlates of immune protection in HIV-1 infection: what we know, what we don't know, what we should know. *Nat. Med*. 10:806–810.

16. Shiver, J.W., T.M. Fu, L. Chen, D.R. Casimiro, M.E. Davies, R.K. Evans, Z.Q. Zhang, A.J. Simon, W.L. Trigona, S.A. Dubey, et al. 2002. Replication-incompetent adenoviral vaccine vector elicits effective anti-immunodeficiency-virus immunity. *Nature*. 415:331–335.

17. Casimiro, D.R., F. Wang, W.A. Schleif, X. Liang, Z.Q. Zhang, T.W. Tobery, M.E. Davies, A.B. McDermott, D.H. O'Connor, A. Fridman, et al. 2005. Attenuation of simian immunodeficiency virus SIVmac239 infection by prophylactic immunization with dna and recombinant adenoviral vaccine vectors expressing Gag. *J. Virol*. 79: 15547–15555.

18. Gomez-Roman, V.R., R.H. Florese, B. Peng, D.C. Montefiori, V.S. Kalyanaraman, D. Venzon, I. Srivastava, S.W. Barnett, and M. Robert-Guroff. 2006. An adenovirus-based HIV subtype B prime/boost vaccine regimen elicits antibodies mediating broad antibody-dependent cellular cytotoxicity against non-subtype B HIV strains. *J. Acquir. Immune Defic. Syndr*. 43:270–277.

19. Barouch, D.H., and G.J. Nabel. 2005. Adenovirus vector-based vaccines for human immunodeficiency virus type 1. *Hum. Gene Ther*. 16:149–156.

20. Kostense, S., W. Koudstaal, M. Sprangers, G.J. Weverling, G. Penders, N. Helmus, R. Vogels, M. Bakker, B. Berkhout, M. Havenga, and J. Goudsmit. 2004. Adenovirus types 5 and 35 seroprevalence in AIDS risk groups supports type 35 as a vaccine vector. *AIDS*. 18:1213–1216.

21. Sumida, S.M., D.M. Truitt, A.A. Lemckert, R. Vogels, J.H. Custers, M.M. Addo, S. Lockman, T. Peter, F.W. Peyerl, M.G. Kishko, et al. 2005. Neutralizing antibodies to adenovirus serotype 5 vaccine vectors are directed primarily against the adenovirus hexon protein. *J. Immunol*. 174:7179–7185.

22. Roberts, D.M., A. Nanda, M.J. Havenga, P. Abbink, D.M. Lynch, B.A. Ewald, J. Liu, A.R. Thorner, P.E. Swanson, D.A. Gorgone, et al. 2006. Hexon-chimaeric adenovirus serotype 5 vectors circumvent pre-existing anti-vector immunity. *Nature*. 441:239–243.

23. Pantaleo, G. 2007. HIV-1 T-cell vaccines: evaluating the next step. *Lancet Infect. Dis*. In press.

24. Cohen, J. 2007. AIDS research. Did Merck's failed HIV vaccine cause harm? *Science*. 318:1048–1049.

25. Ledford, H. 2007. HIV vaccine may raise risk. *Nature*. 450:325.

26. Cohen, J. 2007. AIDS research. Promising AIDS vaccine's failure leaves field reeling. *Science*. 318:28–29.

27. Harari A., M.P. Bart, W. Stohr, G. Tapia, M. Garcia, E. Medjitna-Rais, S. Burnet, C. Cellerai, O. Erlwein, T. Barber, et al. An HIV-1 clade C DNA prime, NYVAC boost vaccine regimen induces reliable, polyfunctional, and long-lasting T cell responses. *J. Exp. Med*. 205:63–77.

28. Liang, X., D.R. Casimiro, W.A. Schleif, F. Wang, M.E. Davies, Z.Q. Zhang, T.M. Fu, A.C. Finnefrock, L. Handt, M.P. Citron, et al. 2005. Vectored Gag and Env but not Tat show efficacy against simian-human immunodeficiency virus 89.6P challenge in Mamu-A*01-negative rhesus monkeys. *J. Virol*. 79: 12321–12331.

29. Migueles, S.A., A.C. Laborico, W.L. Shupert, M.S. Sabbaghian, R. Rabin, C.W. Hallahan, D. Van Baarle, S. Kostense, F. Miedema, M. McLaughlin, et al. 2002. HIV-specific CD8+ T cell proliferation is coupled to perforin expression and is maintained in nonprogressors. *Nat. Immunol*. 3:1061–1068.

30. Harari, A., F. Vallelian, P.R. Meylan, and G. Pantaleo. 2005. Functional heterogeneity of memory CD4 T cell responses in different conditions of antigen exposure and persistence. *J. Immunol*. 174:1037–1045.

31. Darrah, P.A., D.T. Patel, P.M. De Luca, R.W. Lindsay, D.F. Davey, B.J. Flynn, S.T. Hoff, P. Andersen, S.G. Reed, S.L. Morris, et al. 2007. Multifunctional TH1 cells define a correlate of vaccine-mediated protection against Leishmania major. *Nat. Med*. 13: 843–850.

32. Betts, M.R., M.C. Nason, S.M. West, S.C. De Rosa, S.A. Migueles, J. Abraham, M.M. Lederman, J.M. Benito, P.A. Goepfert, M. Connors, et al. 2006. HIV nonprogressors preferentially maintain highly functional HIV-specific CD8+ T cells. *Blood*. 107: 4781–4789.

33. Yang, Z.Y., L.S. Wyatt, W.P. Kong, Z. Moodie, B. Moss, and G.J. Nabel. 2003. Overcoming immunity to a viral vaccine by DNA priming before vector boosting. *J. Virol*. 77:799–803.

34. Santra, S., D.H. Barouch, B. Korioth-Schmitz, C.I. Lord, G.R. Krivulka, F. Yu, M.H. Beddall, D.A. Gorgone, M.A. Lifton, A. Miura, et al. 2004. Recombinant poxvirus boosting of DNA-primed rhesus monkeys augments peak but not memory T lymphocyte responses. *Proc. Natl. Acad. Sci. USA*. 101:11088–11093.

35. Hel, Z., W.P. Tsai, A. Thornton, J. Nacsa, L. Giuliani, E. Tryniszewska, M. Poudyal, D. Venzon, X. Wang, J. Altman, et al. 2001. Potentiation of simian immunodeficiency virus (SIV)-specific CD4(+) and CD8(+) T cell responses by a DNA-SIV and NYVAC-SIV prime/boost regimen. *J. Immunol*. 167: 7180–7191.

36. Weiss, W.R., A. Kumar, G. Jiang, J. Williams, A. Bostick, S. Conteh, D. Fryauff, J. Aguiar, M. Singh, D.T. O'Hagan, et al. 2007. Protection of rhesus monkeys by a DNA prime/poxvirus boost malaria vaccine depends on optimal DNA priming and inclusion of blood stage antigens. *PLoS ONE*. 2:e1063.

37. Amanna, I.J., M.K. Slifka, and S. Crotty. 2006. Immunity and immunological memory following smallpox vaccination. *Immunol. Rev*. 211:320–337.

38. Pulendran, B., and R. Ahmed. 2006. Translating innate immunity into immunological memory: implications for vaccine development. *Cell*. 124:849–863.

39. Querec, T., S. Bennouna, S. Alkan, Y. Laouar, K. Gorden, R. Flavell, S. Akira, R. Ahmed, and B. Pulendran. 2006. Yellow fever vaccine YF-17D activates multiple dendritic cell subsets via TLR2, 7, 8, and 9 to stimulate polyvalent immunity. *J. Exp. Med*. 203:413–424.

40. Lambert, P.H., M. Liu, and C.A. Siegrist. 2005. Can successful vaccines teach us how to induce efficient protective immune responses? *Nat. Med*. 11:S54–S62.

41. Gilchrist, M., V. Thorsson, B. Li, A.G. Rust, M. Korb, K. Kennedy, T. Hai, H. Bolouri, and A. Aderem. 2006. Systems biology approaches identify ATF3 as a negative regulator of Toll-like receptor 4. *Nature*. 441:173–178.

42. Aderem, A. 2005. Systems biology: its practice and challenges. *Cell*. 121:511–513.

43. Barber, D.L., E.J. Wherry, D. Masopust, B. Zhu, J.P. Allison, A.H. Sharpe, G.J. Freeman, and R. Ahmed. 2006. Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature*. 439:682–687.

44. Trautmann, L., L. Janbazian, N. Chomont, E.A. Said, S. Gimmig, B. Bessette, M.R. Boulassel, E. Delwart, H. Sepulveda, R.S. Balderas, et al. 2006. Upregulation of PD-1 expression on HIV-specific CD8+ T cells leads to reversible immune dysfunction. *Nat. Med*. 12:1198–1202.

45. Day, C.L., D.E. Kaufmann, P. Kiepiela, J.A. Brown, E.S. Moodley, S. Reddy, E.W. Mackey, J.D. Miller, A.J. Leslie, C. DePierres, et al. 2006. PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. *Nature*. 443: 350–354.

46. Robert-Guroff, M. 2007. Replicating and non-replicating viral vectors for vaccine development. *Curr. Opin. Biotechnol*. In press.

47. Welsh, R.M., and R.S. Fujinami. 2007. Pathogenic epitopes, heterologous immunity

and vaccine design. *Nat. Rev. Microbiol*. 5: 555–563.

48. Ling, B., R.S. Veazey, A. Luckay, C. Penedo, K. Xu, J.D. Lifson, and P.A. Marx. 2002. SIV(mac) pathogenesis in rhesus macaques of Chinese and Indian origin compared with primary HIV infections in humans. *AIDS*. 16:1489–1496.

49. Borkow, G. 2005. Mouse models for HIV-1 infection. *IUBMB Life*. 57:819–823.

50. Hu, S.L. 2005. Non-human primate models for AIDS vaccine research. *Curr. Drug Targets Infect. Disord*. 5:193–201.

51. Zhang, L., G.I. Kovalev, and L. Su. 2007. HIV-1 infection and pathogenesis in a novel humanized mouse model. *Blood*. 109:2978–2981.

52. Sun, Z., P.W. Denton, J.D. Estes, F.A. Othieno, B.L. Wei, A.K. Wege, M.W. Melkus, A. Padgett-Thomas, M. Zupancic, A.T. Haase, and J.V. Garcia. 2007. Intra-rectal transmission, systemic infection, and CD4^{+} T cell depletion in humanized mice infected with HIV-1. *J. Exp. Med*. 204: 705–714.