REPORT

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Immunogens and Antigen Processing: Report from a Global HIV Vaccine Enterprise Working Group

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The Global HIV Vaccine Enterprise (the Enterprise) convened a meeting of a Working Group in July 2009 to discuss recent progress in rational design of the components of an HIV vaccine, such as inserts, vectors and adjuvants, and in understanding antigen processing and presentation to T and B cells. This Report summarizes the key points of that discussion, and subsequent discussions with the Chairs of the other Enterprise Working Groups, the Enterprise Science Committee, the Enterprise Council and the broader scientific community during open sessions at scientific conferences.

A. INTRODUCTION

An effective HIV vaccine will need to induce durable immune responses that prevent the acquisition of infection and/or reduce viral replication to levels necessary to minimize HIV disease and viral transmission[1–4]. Designing an immunogen capable of eliciting and maintaining such immune responses remains a major obstacle to the development of an HIV vaccine[2,5–7]. Experiments in non-human primate (NHP) models have demonstrated that neutralizing antibodies are able to prevent infection[8–11] and that cellular immune responses can control viral replication[2,12–14]. However, the immune correlates of protection against HIV in humans are not known[4,15]. The findings of the RV144 vaccine trial suggest that vaccine-elicited immune responses can decrease the acquisition rate of HIV[16]. Further analysis of this trial is currently underway and it is not yet clear if immune correlates of protection will emerge[17]. A better understanding of protective immune responses, coupled with an improved ability to induce robust anti-HIV responses by immunization, are key to future success in the design and testing of HIV vaccines. We need to improve our mechanistic understanding of the intertwined functions of T-cells, B-cells, dendritic cells and other innate immune elements in controlling adaptive immunity[18–20]. In parallel, we need to learn how different vaccine platforms can induce protective responses and engender long-term effector populations by the different arms of the immune system. Studies of vaccine immunogenicity and efficacy in humans are essential to understand immune responses and to establish immune correlates of vaccine-induced protection. We propose that the Enterprise vigorously support a new era of HIV vaccine research and development in which discovery science is the engine driving our efforts to identify protective mechanisms and create new vaccine immunogens and immunization strategies.

This new era of vaccine science will require a programmatic mix that recognizes the complementary and distinct roles of individual scientists and multidisciplinary groups of researchers focusing on problems that require collaborative efforts. Teams are especially important in clinical trials, where a wide ranging set of skills is required. Knowledge gained from carefully conducted immunogenicity and protection studies in non-human primates is also essential to provide fundamental scientific insights and influence the design of human clinical trials.

B. SCIENTIFIC PRIORITIES

The Working Group identified key scientific gaps in immunology, virology and vaccine design. Addressing these gaps presents an opportunity to significantly advance our understanding of vaccine science and accelerate the path to a safe and effective HIV vaccine. These scientific gaps were encompassed into three main priorities.

Priority 1: Study immune mechanisms of protection against HIV infection and disease progression

Factors contributing to the development of neutralizing antibodies. Recent studies have shown that 10–25% of HIV- infected individuals generate broadly cross-reactive neutralizing antibodies against HIV (bNAbs)[21,22]. While such NAbs may appear too late during infection to play a protective role, data from NHP studies[8,23] show that bNAbs present before virus exposure protect against infection, suggesting that bNAbs might also prevent HIV infection in humans if present at the right time. Current vaccine immunogens generate low levels of NAbs that are reactive with a narrow subset of HIV strains. It is thus imperative to: (i) understand the evolution of the bNAb response during HIV infection; (ii) identify viral epitopes targeted by these

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antibodies; and (iii) learn how to elicit bNABs more efficiently (discussed in Priority 2). The first question is best studied in longitudinal HIV seroconversion cohorts, where time of HIV infection can be established and evolution of the antibody response can be tracked, including the roles of antibody affinity maturation, T-cell help, and genetics of virus and the host.

Need for robust animal models to study the in vivo role of HIV specific antibodies. NHP models can provide key information about the role of antibodies in protection against HIV. Monkey challenges with chimeric simian-human immunodeficiency viruses (SHIVs) allow the evaluation of HIV-specific antibody responses. However, the SHIV model is currently limited by the small number of biologically relevant viral strains that use the CCR5 co-receptor. Several of the currently used SHIVs do not cause persistent infection and induction of AIDS-like disease[8–11]. In addition, the SHIV model doesn't address the challenge of viral diversity in humans since only a narrow panel of strains is available. Thus, replication-competent SHIVs from several major genetic subtypes should be constructed in order to study the role of antibodies against diverse viral strains. The generation of polyclonal SHIV stocks consisting of "swarms" of virus would also be valuable to better mimic human-to-human transmission. One strategy to improve the NHP model would be to generate altered forms of HIV resistant to host restriction factors present in monkeys cells, and, therefore, capable of infection and replication in NHPs. In addition to NHP studies, there was considerable discussion about proof of concept studies in humans to demonstrate the role of antibodies in protection against HIV-1 infection. Such passive antibody studies could provide key information about the character and level of protective antibodies *in vivo*.

CD8+ and CD4+ protective T-cell responses. There is accumulating evidence in both humans and NHPs of a protective role for CD8+ T cells in the control of HIV/SIV infection[13,24–28]. It is critical to define a metric to identify optimal types of CD8+ T cell responses required for protection. At the same time, a possible role for CD4+ T cells has been relatively neglected in the search for protective mechanisms and their precise roles in orchestrating the immune response against HIV has not been elucidated. CD4+ T cells have many potential roles in protection, from providing help to B and CD8+ T cells to directly killing MHC II+ HIV-infected T cells.

Studies of NHP challenge models and human elite controllers are essential to understand the role of antigen-specific T-cells in conferring protection. By using systems biology approaches it is now feasible to study antigen-specific T cells elicited by different vaccines using small volumes of blood. Reliable *ex vivo* assays of T-cell-mediated protection, in which T cells recognize naturally processed SIV and HIV antigens in infected CD4+ T cells, are needed. New technologies, for example, single cell assays, to detect the heterogeneity of T cell responses, are under development. Data on the full array of functional, antigen-specific CD4+ T cell populations [Treg, Th1, Th2, Th17] during vaccination and infection are needed and will greatly benefit from development of MHC II tetramers. All of these approaches will depend on strategies for the deposition, access and analysis of large amounts of data.

Another major question is whether the breadth of T cell response, i.e. the number of recognized HIV peptides, will be critical for vaccine efficacy. In addition, we need to investigate the feasibility of directing immune responses to specific T-cell epitopes, which cannot easily undergo mutation due to significant fitness cost to the virus. The role of select MHC alleles that mediate long-term control of viral load and disease progression in NHPs and humans also needs to be explored.

Priority 2: Explore pathways to eliciting protective responses through vaccination

Structure-based design to improve B-cell immunogens. Well defined clinical cohorts provide the opportunity to derive new bNAbs against HIV. Examples of such antibodies include those known to neutralize via binding to gp41 (2F5, 4E10), to the CD4 binding site of gp120 (b12, VRC01) and those that bind to quaternary epitopes present on mature viral Env (PG9 and PG16)[3,5,7,29,30]. These antibodies define conserved viral epitopes that could serve as the basis for improved immunogen design. A systematic effort using complementary methodologies to clone numerous NAbs from B-cells of humans infected with HIV, including non-clade B strains, would provide a better understanding of the nature of the bNAb response. Isolation of Nabs should be coupled with systematic efforts to define the atomic level structure of targeted viral epitopes and the integration of structural data with computational modeling to develop strategies for translating neutralization epitope data into vaccine immunogen design. The overall effort should be linked with efforts related to iterative immunogenicity testing of novel immunogens and with development of improved SHIV models.

Understand and modulate the B-cell response to overcome current limitations of vaccine immunogens and vaccination strategies, and better elicit protective antibodies. During HIV infection, there is a dominant type-specific NAb response to the autologous virus during the first months to years of infection; broadly reactive NAbs generally do not arise until 1-3 years of ongoing infection[3]. Similarly, vaccination with most Env immunogens results in dominant responses to some Env regions, such as the variable loops, that are poorly neutralizing and minimal responses to potential neutralization regions such as the CD4bs or the membrane-proximal external region. The mechanistic explanation for the poor immune responses to key regions of Env during natural infection and after primary immunization should be a major focus of study.

While it is appropriate to focus on the design of improved vaccine immunogens, it is likely that the method of antigen delivery and associated innate pathways engaged by immunization will impact the type, potency or breadth of NAb response elicited by a vaccine. For example, it may be possible to elicit NAbs to specific epitopes by modulating the method of antigen delivery or by stimulation of specific innate pathways. Studies in this area seek to understand: 1) why antigens reactive with bNAb don't generate bNAbs when used as immunogens; 2) the impact of multimeric immunogens on the B-cell response; 3) the mechanism of immunodominance in the evolution of the natural NAb response and how it impacts the vaccine-elicited NAb responses. These studies are high-risk, and, thus, require teams of investigators with necessary expertise and funding schemes that support the scientific considerations.

Define pathways for vaccines to induce protective T-cells. There has been insufficient attention to the basic immunobiology of vaccination, i.e. how best to initiate and direct appropriate responses for the control of HIV. The data on mechanisms and correlates of protection will inform vaccine design. In addition, we need to understand how to elicit and direct protective adaptive immunity. Therefore, at the same time that information on immune correlates is being generated, a central focus should be the innate immune system, especially cells that capture and present vaccine antigens and dictate the quality and memory of the immune response. Briefly, immunization requires antigen/vaccine capture and presentation, especially by dendritic cells and follicular dendritic cells, to T and B cells, followed by differentiation of the latter into protective cells. Dendritic cell science has been underutilized in vaccine design, but

dendritic cell function early on during vaccination serves to initiate and "imprint" T and B cell immunity (including in mucosa), controlling its quality and memory.

Understanding early events in immunization elicited by a variety of vectors, formulations and adjuvants will help uncover strategies to achieve protection. We need to learn what organs and cell types are accessed by vaccines and where immunogens are retained and presented. For example, information on the longevity of intact vaccine antigens on follicular dendritic cells and processed, presented antigens [peptide/MHC] on dendritic cells and their subsets, will be invaluable for vaccine design. An emphasis on early events also needs to include analyses of how adjuvants enhance immunity and control its quality. Advances in the science of pattern recognition receptors have provided major opportunities for rational adjuvant design, but the field needs to move beyond *in vitro* approaches in order to understand the roles of these receptors as potential adjuvants to dendritic cells and other cells *in vivo*. Finally, NK cells also are potentially important modulators of dendritic cell activation.

Our ability to elicit protective immune responses depends on detailed understanding of the role of the mucosal immune system in protection against HIV. To induce protective T cells at defined mucosal sites, we should determine how different routes of mucosal delivery influence T cell generation. Following mucosal routes of immunization, a thorough assessment of the innate response should be performed at mucosal surfaces, including epithelial cells, NK cells, phagocytes and dendritic cells. Regardless of route, a fundamental parameter of the response is likely to be the ability of vaccines to improve effector T and B cell development in, or traffic to, mucosal sites. This will require data on the level of vaccine antigen and location that leads to persistent T cell expansion without exhaustive signals. We need to learn how different prime-boost strategies and the timing of vaccine administration affect the quality of T cells. These areas of research should expand beyond model antigens in mice and focus on HIV/SIV antigens in humans/NHPs[31].

This research requires better reagents (for example, monoclonal antibodies) and methods (for example, high resolution imaging techniques to visualize vaccines and cells *in vivo*) to identify and isolate distinct types of immune cells in NHPs and humans. More effort is needed to learn how vaccines and adjuvants could be better formulated and delivered. A mechanism for centralized manufacturing and distribution of key vaccine components (adjuvants, gene inserts, proteins) would accelerate the research and allow standardization and comparability of results.

In summary, we have made considerable progress in advancing our understanding of the immune response. We now need to build on this understanding and learn to direct, augment and speed up the response to relevant epitopes of the virus.

Priority 3: Explore a diversity of vaccination strategies taking into account the need for comparability of data

Develop protein vaccines for priming and/or repeated boosting. Protein vaccines have been relatively neglected, with the exception of boosting with viral envelopes to elicit antibodies. This neglect logically stems from the insufficient immunogenicity of proteins observed previosuly[3]. On the other hand, proteins offer advantages in terms of lower manufacturing costs, ease of distribution, and smaller number of complications that arise through the use of vectors (for example, prior immunity). Therefore, we should assess viral vector prime - protein boost vaccination strategies in parallel with protein-only approaches to reduce the effect of vector competition and preexisting immunity.

Recent advances have elucidated a number of principles that make it feasible to reconsider the potential value of protein vaccines. To develop proteins for immune priming and/or repeated boosting, protein capture by dendritic cells must be optimized, together with the use of adjuvants appropriate to stimulate dendritic and other cells that will elicit protective T and B cell immunity.

Improve immune responses for vector-based vaccines. The use of vectors to deliver antigen-encoding genes is a leading strategy for the generation of an effective HIV vaccine[32–34]. The advantages of this approach include: exposure of the immune system to the antigen in a way that partially mimics a viral infection, flexibility of vector systems allowing control of their biological activity, track record of safety and immunogenicity of many vectors, proven methods for manufacture of many vectors for clinical trial, and ease of manipulation of inserts in the vector to improve the immune response. Unfortunately, we have only limited understanding of the mechanisms by which particular vectors/insert technologies induce an immunological response and the nature of the required stimulus for protection against HIV infection.

It is clear that alterations in vector change the responses to a given insert. Furthermore, the design of inserts for a specific vector can clearly direct the immune response to distinct T-cell and B-cell epitopes[35,36]. Consequently, there should be a close interplay between our understanding of the immunological mechanisms of protection as outlined in Priorities 1 and 2 and the development of both vectors and inserts. Important parameters to be investigated include the study of vector tropism and mode of delivery, the impact of vector replication and persistence, and the importance of anti-vector immune responses. New designs of insert genes should be explored, especially those that allow for increased breadth of T-cell responses, the control of epitope-specific responses and the elicitation of bNAbs. The path forward will require detailed testing of new and existing vector/insert combinations, identification of improved methods for evaluating vector performance, and judicious use of NHP models and clinical trials to both advance existing candidates and inform design of succeeding generations of vaccines.

Compare performance properties of vector-based vaccines. A key challenge to the field is the large array of vector/insert combinations. Without an understanding of the underlying biological mechanisms that determine the immunological behavior of different vector/insert combinations, and in the absence of broadly agreed upon methods of assessment, the field is at great risk of spending considerable scarce resources on the generation of data which is not comparable and therefore does not allow for the advancement of the most promising candidates.

A harmonized and standardized set of assays is needed to allow comparison of vaccine performance in both NHPs and humans. An array of shared validated reagents, assays and methods is necessary to ensure that experiments conducted in different laboratories can be compared. Developers of vector/insert combinations and candidate vaccines should then be strongly encouraged to use these resources and publish data using these systems.

Where possible, clinical trials should be conducted that allow direct comparison of data obtained with different vector/insert technologies. The field has already implemented systems for standardization of immunological measurements obtained in human clinical testing allowing comparison across trials, and this should be continued and expanded. As data emerges on possible correlates of protection, the relevant assays should be validated and made widely available to facilitate comparisons.

C. STRATEGIC RECOMMENDATIONS

In addition to the scientific priorities identified above, the Working Group put forward three strategic Recommendations to facilitate HIV vaccine research and development.

Focus NHP studies on understanding the immunological principles that underlie the induction of robust T- and B-cell responses to vaccine immunogens, on investigating immune mechanisms associated with protection from infection, and on early events responsible for control of viral replication

Studies of vaccine immunogens, antigen processing and resulting immune responses can be performed in NHPs without the expense and time delay associated with production of clinical-grade vaccine materials. In addition, the NHP model allows the intensive study of local, systemic and mucosal immune responses, which are difficult to study in humans. Moreover, viral challenge studies can be performed in NHPs to elucidate mechanisms of immune protection, especially those that contain the nidus of incoming virus immediately after infection[14,37] and lead to the genetic bottleneck of the virus that has been documented in both humans and NHPs[38,39]. These studies can provide fundamental knowledge that is critical to drive the design of novel vaccine concepts and inform the more efficient design of human clinical trials (**Table 1**).

Table 1. Examples of areas in which NHP studies have the best opportunity to contribute to HIV vaccine research and development.

- Identifying sites where vaccine antigens are retained and presented
- Development of improved markers for immune cells in lymphoid and mucosal tissues
- Use of *in vivo* imaging to study the induction of immune responses to vaccine antigens
- Studying mechanisms of adjuvant effect on T and B cell immunity
- Studying mechanisms to sustain effector cells at mucosal surfaces
- Discovering properties of T-cells *ex vivo* that are associated with control of viral replication *in vivo*
- Comparing ability of different vectors to present antigen and induce immunity
- Studying the influence of antigen structure on neutralizing antibody responses
- Describing evolution of B-cell responses, including affinity maturation
- Studying effects of passive transfer of neutralizing or non-neutralizing antibodies on protection
- Studying the relative contribution of neutralizing, ADCC and other Fc-mediated effector Ab functions in mediating protection
- Determining the level of Env-specific antibody required to impact acquisition
- Studying whether polyclonal antibodies with low or moderate neutralizing activity can mediate protection
- Studying whether there are antibodies that mediate antibody-dependent cytotoxicity without cell-free virus neutralization, and what effect they have on acquisition and disease progression
- Measuring the relative contribution of IgG vs IgA antibodies at the mucosal surface
- Exploring whether antibodies work to block initial infection of mucosal target cells or impact later events of viral spread

Explore in phase I human trials and longitudinal cohorts of HIV-1 infection, mechanisms of adaptive immune responses and their links with innate immunity

Clinical trials present unique opportunities to gather critical information about human immune responses to vaccines (**Table 2**). It is imperative that these opportunities are fully explored in each trial. In addition, there is much to be learned about the role of adaptive immune responses in controlling viral replication from studies of HIV infection. Effective T-cell responses and neutralizing antibodies develop in some HIV infected subjects, and if such responses could be induced by a vaccine before viral exposure, HIV infection might be prevented or fully controlled.

Table 2. Examples of areas of focus for research in humans.

- Exploring innate responses to adjuvants and vectors and their relationship with adaptive immunity
- Studying mechanisms of immune control of viral replication in long-term non-progressors
- Studying the role of T cell help in eliciting improved B and CD8+ T-cell immunity
- Studying strategies for mucosal immunization
- Exploring approaches to increase breadth of T-cell responses or to direct them to specific epitopes
- Establishing seroconversion cohorts to study evolution of antibody responses
- Isolating novel neutralizing and binding mAbs from HIV infected subjects
- Comparing diverse vectors and prime-boost approaches
- Studying basic B-cell biology: evolution of B-cell response to HIV and the role of affinity maturation
- Developing *ex vivo* assays to mimic human immune responses in artificial lymph nodes

Encourage investigators from within and outside the field of HIV vaccines to form teams targeting fundamental immunological questions and exploring novel approaches to vaccine discovery

HIV vaccine research and development would benefit from a far greater interaction between those studying HIV vaccines and scientists from outside the field of HIV research (**Table 3**). New funding mechanisms should be created to facilitate the entry and continued involvement of new investigators, and groups of investigators, in the field of HIV immunology and vaccine development.

Table 3. Examples of areas of HIV vaccine research in which expertise from the fundamental human immunology might accelerate progress.

- Understanding evolution of B-cell response to HIV and the role of affinity maturation
- Isolating novel anti-envelope Abs using new technologies
- Applying structure-based immunogen design to elicit neutralizing and protective antibodies
- Studying the role of mobilization of Fc receptors in protection
- Exploring the role of select MHC alleles in NHP and humans in resisting SIV/HIV
- Studying the role of CD4+ helper T cells in resistance to SIV/HIV
- Investigating mechanisms to sustain effector cells at mucosal surfaces
- Developing strategies to improve dendritic cell capture of vaccines
- Overcoming tolerogenic dendritic cell pathways, especially at mucosal surfaces
- Developing *ex vivo* assays for human immune responses (for example, artificial lymph nodes)
- Learning to assess the immune system and adaptive response of newborns

Table 4. Summary of priorities and recommendations

Priority 1. Study immune mechanisms of protection against HIV infection and disease progression

- Establish multidisciplinary teams to study the evolution of B-cell responses in longitudinal cohorts
- Develop NHP models to study the *in vivo* immune responses to vaccination and infection
- Explore the role of breadth of responses in mediating protection against HIV

Priority 2. Explore pathways to eliciting protective responses through vaccination

- Use structure-based design to improve B-cell immunogens
- Study the differences in immune responses to vaccines associated with different methods of antigen delivery
- Focus on the innate immune system, especially on cells that capture and present vaccine antigens dictating the quality of the immune response
- Focus on delivering vaccines to mucosal surfaces and measuring mucosal immune responses
- Encourage standardization by developing and making available vaccine components (proteins, inserts, adjuvants, etc.)

Priority 3. Explore a diversity of vaccination strategies taking into account the need for comparability of data

- Develop protein vaccines for priming and/or repeated boosting
- Improve the immune responses for vector-based vaccines
- Compare performance properties of vector-based vaccines

Strategic Recommendations

- Focus NHP studies on understanding the immunological principles that underlie the induction of robust T- and B-cell responses to vaccine immunogens, on investigating immune mechanisms associated with protection from infection, and on early events responsible for control of viral replication.
- Explore in phase I human trials and longitudinal cohorts, mechanisms of adaptive immune responses and their links with innate immunity.
- Encourage investigators from within and outside the field of HIV vaccines to form teams targeting fundamental immunological questions and exploring novel approaches to vaccine discovery.

1. McMichael, A.J. HIV vaccines. *Annu. Rev. Immunol.* **24**, 227–255 (2006).
2. Letvin, N.L. Progress and obstacles in the development of an AIDS vaccine. *Nat. Rev. Immunol.* **6**, 930–939 (2006).
3. Mascola, J.R. & Montefiori, D.C. The role of antibodies in HIV vaccines. *Annu. Rev. Immunol.* **28**, 413–444 (2010).
4. Johnston, M.I. & Fauci, A.S. An HIV vaccine–evolving concepts. *N. Engl. J. Med.* **356**, 2073–2081 (2007).
5. Burton, D.R. *et al.* HIV vaccine design and the neutralizing antibody problem. *Nat. Immunol.* **5**, 233–236 (2004).
6. Douek, D.C., Kwong, P.D. & Nabel, G.J. The rational design of an AIDS vaccine. *Cell* **124**, 677–681 (2006).
7. Phogat, S. & Wyatt, R. Rational modifications of HIV-1 envelope glycoproteins for immunogen design. *Curr. Pharm. Des.* **13**, 213–227 (2007).
8. Hessell, A.J. *et al.* Effective, low-titer antibody protection against low-dose repeated mucosal SHIV challenge in macaques. *Nat. Med.* **15**, 951–954 (2009).
9. Baba, T.W. *et al.* Human neutralizing monoclonal antibodies of the IgG1 subtype protect against mucosal simian-human immunodeficiency virus infection. *Nat. Med.* **6**, 200–206 (2000).
10. Shibata, R. *et al.* Neutralizing antibody directed against the HIV-1 envelope glycoprotein can completely block HIV-1/SIV chimeric virus infections of macaque monkeys. *Nat. Med.* **5**, 204–210 (1999).
11. Mascola, J.R. *et al.* Protection of macaques against vaginal transmission of a pathogenic HIV-1/SIV chimeric virus by passive infusion of neutralizing antibodies. *Nat. Med.* **6**, 207–210 (2000).
12. Schmitz, J.E. *et al.* Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. *Science* **283**, 857–860 (1999).
13. Wilson, N.A. *et al.* Vaccine-induced cellular immune responses reduce plasma viral concentrations after repeated low-dose challenge with pathogenic simian immunodeficiency virus SIVmac239. *J. Virol.* **80**, 5875–5885 (2006).
14. Hansen, S.G. *et al.* Effector memory T cell responses are associated with protection of rhesus monkeys from mucosal simian immunodeficiency virus challenge. *Nat. Med.* **15**, 293–299 (2009).
15. Pantaleo, G. & Koup, R.A. 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 (2004).
16. Rerks-Ngarm, S. *et al.* Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in thailand. *N. Engl. J. Med.* **361**, 2209–2220 (2009).
17. Dolin, R. HIV vaccine trial results–an opening for further research. *N. Engl. J. Med.* **361**, 2279–2280 (2009).
18. Steinman, R.M. & Banchereau, J. Taking dendritic cells into medicine. *Nature* **449**, 419–426 (2007).
19. Ueno, H. *et al.* Harnessing human dendritic cell subsets for medicine. *Immunol. Rev.* **234**, 199–212 (2010).
20. Lanzavecchia, A. & Sallusto, F. Toll-like receptors and innate immunity in B-cell activation and antibody responses. *Curr. Opin. Immunol.* **19**, 268–274 (2007).
21. Doria-Rose, N.A. *et al.* Breadth of human immunodeficiency virus-specific neutralizing activity in sera: clustering analysis and association with clinical variables. *J. Virol.* **84**, 1631–1636 (2010).
22. Simek, M.D. *et al.* Human immunodeficiency virus type 1 elite neutralizers: individuals with broad and potent neutralizing activity identified by using a high-throughput neutralization assay together with an analytical selection algorithm. *J. Virol.* **83**, 7337–7348 (2009).
23. Mascola, J.R. Passive transfer studies to elucidate the role of antibody-mediated protection against HIV-1. *Vaccine* **20**, 1922–1925 (2002).
24. Betts, M.R. & Harari, A. Phenotype and function of protective T cell immune responses in HIV. *Curr. Opin. HIV AIDS* **3**, 349–355 (2008).
25. Blankson, J.N. Control of HIV-1 replication in elite suppressors. *Discov. Med.* **9**, 261–266 (2010).
26. Friedrich, T.C. & Watkins, D.I. Wanted: correlates of vaccine-induced protection against simian immunodeficiency virus. *Curr. Opin. HIV AIDS* **3**, 393–398 (2008).
27. Liu, J. *et al.* Immune control of an SIV challenge by a T-cell-based vaccine in rhesus monkeys. *Nature* **457**, 87–91 (2009).
28. Korber, B.T., Letvin, N.L. & Haynes, B.F. T-cell vaccine strategies for human immunodeficiency virus, the virus with a thousand faces. *J. Virol.* **83**, 8300–8314 (2009).
29. Walker, L.M. *et al.* Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target. *Science* **326**, 285–289 (2009).
30. Wu, X. *et al.* Rational design of envelope identifies broadly neutralizing human monoclonal antibodies to HIV-1. *Science* doi:10.1126/science.1187659 (8 July 2010).
31. Davis, M.M. A prescription for human immunology. *Immunity* **29**, 835–838 (2008).
32. Plotkin, S.A. Vaccines: the fourth century. *Clin. Vaccine Immunol.* **16**, 1709–1719 (2009).
33. Barouch, D.H. & Nabel, G.J. Adenovirus vector-based vaccines for human immunodeficiency virus type 1. *Hum. Gene Ther.* **16**, 149–156 (2005).
34. Excler, J.L. et al. Replicating viral vectors as HIV vaccines: Summary report from the IAVI-sponsored satellite symposium at the AIDS vaccine 2009 conference. *Biologicals* 10.1016/j.biologicals.2010.03.005 (2010).
35. Honda, M. et al. Different vaccine vectors delivering the same antigen elicit CD8+ T cell responses with distinct clonotype and epitope specificity. *J. Immunol.* **183**, 2425–2434 (2009).
36. Koup, R.A. et al. Priming immunization with DNA augments immunogenicity of recombinant adenoviral vectors for both HIV-1 specific antibody and T-cell responses. *PLoS ONE* **5**, e9015 (2010).
37. Deeks, S.G. & Walker, B.D. Human immunodeficiency virus controllers: mechanisms of durable virus control in the absence of antiretroviral therapy. *Immunity* **27**, 406–416 (2007).
38. Stone, M. et al. A limited number of simian immunodeficiency virus (SIV) env variants are transmitted to rhesus macaques vaginally inoculated with SIVmac251. *J. Virol.* **84**, 7083–7095 (2010).
39. Keele, B.F. et al. Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection. *Proc. Natl. Acad. Sci. USA* **105**, 7552–7557 (2008).