OPINION

The quest for a T cell-based immune correlate of protection against HIV: a story of trials and errors

Richard A. Koup, Barney S. Graham and Daniel C. Douek

Abstract | Even before the partial success of a preventive HIV vaccine in a recent Phase III clinical trial, there had been an active research effort to determine one or more immune correlates of protection for HIV infection. This effort has been hampered by the lack of natural protective immunity against HIV. As a result, most of the studies have focused on long-term non-progressive infection or other clinical situations, none of which fully recapitulates protective immunity against HIV. Although this effort has been successful in defining characteristics of T cells in acute and non-progressive HIV infection, and has therefore greatly expanded our knowledge of the immunopathogenesis of AIDS, its success in defining immune correlates of protection is less clear. In this Opinion article we offer a perspective on how successful this effort has been in defining immune correlates of protection that have been, or will be, of use in the development of an HIV vaccine. Our view is that investing in an iterative approach to human vaccine efficacy trials of sufficient size and sampling frequency will improve the likelihood that an immune correlate of vaccine protection will be defined.

Only a few potential HIV vaccines have undergone testing for efficacy in humans[1–3], and only the one tested in the recent RV144 vaccine trial has shown partial protection against infection[4] (TABLE 1). Despite the absence of a vaccine that can clearly protect against HIV, we and numerous others have spent the past several years trying to define correlates of protection against HIV. The obvious hope was that by knowing what correlated with protection, we could predict which vaccines would and would not work in efficacy trials, even before the trials were performed. Although trying to define such a correlate of protection has been a worthwhile endeavour, achieving evidence of efficacy in the RV144 vaccine trial changes the outlook of the field and compels an appraisal of the rationale for this effort and an assessment of whether the findings proved useful in the quest for an HIV vaccine.

Why do we need an immune correlate?

Most vaccines for viral infections have been developed empirically by testing the efficacy of inactivated or attenuated virus preparations in protecting against infection or disease in the absence of a known mechanism of immune protection (although this is almost always an antibody response)[5,6]. Once protective efficacy of the vaccine is shown, an immune correlate can be established statistically and can then be used as a surrogate for efficacy as vaccine production methods and processes are updated, thereby avoiding the need to re-test efficacy with every change or improvement in a vaccine (BOX 1). This approach is supported by the fact that for most viral illnesses there are individuals who have survived natural infection, are protected against reinfection and in whom the ongoing reliance on a given immune correlate can be validated. Unfortunately, such individuals do not exist in the case of HIV infection, rendering unrealistic the prospect of validating an immune correlate based on protection afforded by clearance of natural infection. Therefore, the precedent for defining an immune correlate (especially a T cell-based correlate) before proving vaccine efficacy is a weak one at best, and certainly does not exist for an infection in which there are no individuals who have cleared infection and are subsequently immune to reinfection. Nevertheless, this problem did not deter researchers from searching for an immune correlate of protection against HIV as no reasonable alternative strategy was readily apparent.

Alternative definitions of protection

In the absence of natural protection against infection, many investigators sought other clinical situations in which to evaluate protection against HIV. In human populations, these included studies of highly exposed but uninfected individuals[7], long-term non-progressors or elite controllers of HIV-1 infection[8], subjects with HIV-2 infection who do not progress to AIDS[9], and acute HIV-1 seroconverters (that is, those who convert from an HIV-specific antibody negative state to an HIV-specific antibody positive state)[10,11]. Unfortunately, none of these clinical situations accurately models pre-existing immune protection against HIV. Specifically, despite years of study, the mechanisms underlying lack of infection in the majority of exposed but uninfected individuals are unknown. The data are equally consistent with an innate or genetic predisposition to non-infection[12] and with protection through the generation of a systemic or local immune response[13], and the data do not predict the effector functions mediated by pre-existing adaptive immunity.

In an effort to understand how a vaccine-induced T cell response could control viral replication in the absence of sterilizing immunity, the greatest emphasis has been on the study of long-term non-progressors, elite controllers and HIV-2-infected non-progressors. It is important to remember that the T cells that are evaluated in these studies are derived from chronically infected individuals who have some degree of ongoing viral replication and continued mutation of the virus, allowing escape from the T cell response. The scope of this effort has been too great to be fully covered in this Opinion article, and so we provide a selective list of optimal characteristics for a T cell vaccine based on these efforts (BOX 2). A protective vaccine should stimulate $CD4^+$ T cells[14] and/or $CD8^+$ T cells[15,16] that: have a central[17] or effector[18,19] memory phenotype; can proliferate[20]; kill HIV-infected targets[21–23] or at least inhibit HIV replication *in vitro*[24–26]; can secrete several cytokines[27]; express a diverse T cell receptor repertoire[28,29]; do not express markers of exhaustion[30–32]; and target several epitopes in HIV group-specific antigen (Gag) but do not target the envelope protein (Env)[33–35]. The Gag epitopes that should be targeted are those that are restricted by certain HLA alleles[36–39], are conserved across strains[40,41] and in which mutation is detrimental to viral fitness[42,43]. Although such investigations have been an incredibly rich and valuable source of information for the study of HIV disease

Table 1 | **Past and current efficacy trials for HIV vaccines***

Vaccine trial	Schedule (months)					Population	Immunity	Rate of infection		Efficacy
	0	1	2	3	6			Vaccine	Placebo	
Vax003	Env protein	Env protein			Env protein	MSM and IVDU	Antibody and $CD4^+$ T cells	191/3,330 (5.7%)	98/1,679 (5.8%)	0%
STEP	Adenoviral vector	Adenoviral vector			Adenoviral vector	MSM	$CD8^+$ and $CD4^+$ T cells	65/893 (7.3%)	45/894 (5.0%)	–31.5% (NS)
RV144	ALVAC vector	ALVAC vector		ALVAC vector and Env protein	ALVAC vector and Env protein	General population	Antibody, $CD8^+$ and $CD4^+$ T cells	51/8,197 (0.6%)	74/8,198 (0.9%)	+31.2% (p = 0.4)
HVTN 505	Plasmid DNA	Plasmid DNA	Plasmid DNA		Adenoviral vector	MSM	Antibody, $CD8^+$ and $CD4^+$ T cells	Ongoing	Ongoing	Unknown

IVDU, intravenous drug user; MSM, men who have sex with men; NS, not significant. *Not included in the table are the Vax004 vaccine trial, which was similar to the Vax003 vaccine trial and failed to show efficacy, and the Phambili trial, which was similar to the STEP trial but was terminated before full enrolment after the STEP trial was halted.

pathogenesis, the fact that these data were drawn from the study of chronically infected individuals means it is difficult to know which attributes would be crucial for an HIV-specific T cell that was present in the individual before infection with the virus. In fact, studies have shown that many of these characteristics of vaccine-induced T cell responses rapidly convert to those seen during chronic progressive HIV infection once the subject becomes infected[44], and they have not prevented secondary infection or the generation of viral variants through recombination. In the absence of a T cell vaccine with proven efficacy against HIV, these characteristics need to be considered solely as correlates of lower viral loads in chronic HIV infection. How any of these characteristics could or should be effectively applied to the development of a vaccine against HIV is unclear.

Another clinical situation that is being applied to the search for a T cell correlate is the study of acute HIV infection[11,45]. Based on elegant studies of the viruses and immune responses that are generated during acute HIV infection, we now know that: the viral epitopes targeted by the early T cell response often differ from those that are targeted later in infection[46]; the early response helps to control virus replication[47], but the virus rapidly escapes from this control[48]; and reversion of mutated 'escaped' epitopes to their original sequence may or may not occur when viruses are transmitted to individuals whose T cell response does not target the escaped epitopes[49–51]. However, it remains unclear whether a vaccine should stimulate responses against epitopes that are targeted early in the infection or those that are targeted late. The answer to that question has to await the testing of an efficacious T cell vaccine, in which targeting of early or late epitopes can be evaluated as a potential correlate.

Nonhuman primate studies

An obvious setting to evaluate immune correlates is in animal models of human infections in which vaccines can be shown to be protective and immune analyses can be performed. Unfortunately, HIV does not adequately infect and cause disease in any readily available small animal model, so researchers have had to resort to the use of a similar virus (simian immunodeficiency virus (SIV) or simian–HIV (SHIV)) in nonhuman primates. Although disease pathogenesis in SIV-infected nonhuman primates is remarkably similar to HIV disease in humans, and there are only subtle differences in the specific immune responses generated in both infections, the main source of difficulty in using this model to evaluate immune correlates is in defining how well the model recapitulates human sexual transmission of HIV. We know that mucosal transmission of HIV in humans is inefficient[52,53] and usually leads to the establishment of a single clone of replicating virus from the numerous viruses present in the inoculum[54]. Recent studies have shown that models involving the use of repeated low-dose mucosal virus challenge show transmission of single SIV variants across a mucosal surface[55]. However, appropriate application of this model has greatly increased the number (and cost) of nonhuman primates needed in each study to show protection by a given vaccine[56] and still does not equate to the human setting in which dozens of encounters with virus are typically required for mucosal transmission to occur even during the time of peak viraemia in the infected partner[52,53]. In addition, there are limited numbers of SIV isolates that can be used to test protection across genetically diverse virus strains, a major obstacle for any field test of an HIV vaccine. Finally, because antibodies may be an important component of the protective response, and SIV Env differs substantially from HIV Env, SHIV recombinant viruses, which have HIV Env proteins and an SIV backbone have been derived; however, SHIVs are an imperfect solution. Differences in how they are transmitted, which $CD4^+$ T cells they infect and how they induce disease in nonhuman primates make many investigators wary of relying on them as a predictor of vaccine efficacy in humans[57–59]. Because of such limitations, some have argued that showing protection in nonhuman primates should not be a gatekeeper for advancement of a particular vaccine product or approach into human efficacy trials.

Box 1 | **Definition and use of immune correlates in vaccine development**

An immune correlate of protection is an immune response that is statistically correlated with protection against infection or disease. Ideally, an immune correlate of protection can be quantified and associated with a threshold value that can be measured in the blood or other easily accessible samples. An immune correlate is strongest when it is also an immunological determinant of protection, which means that the assay measures a biological function that is necessary and sufficient for achieving protection against the pathogen. If a determinant or correlate of protection can be defined, that measurement can potentially become a surrogate endpoint for future clinical efficacy trials. In other words, defining immune responses associated with protection from infection or clinical disease allows subsequent studies to substitute the immune response measurement for the clinical endpoint, which would substantially reduce the study sizes, cost and time that are required for completing vaccine development.

Another concern is that most T cell vaccines that are tested in nonhuman primate models have shown effects not on viral transmission but on viral load, but they did not predict the lack of impact on viral load that was seen in the STEP vaccine trial (Merck & Co.)[1,60,61] (TABLE 1). The type of unique protection against acquisition of infection afforded by vaccines using a rhesus macaque cytomegalovirus vector has yet to be evaluated in human clinical trials, and is probably years from such efficacy testing[62]. However, the successful efficacy testing of a vaccine in humans may help to direct future studies in nonhuman primates. Specifically, vaccines based on approaches that have been shown to protect humans from HIV can now be used to evaluate, improve, select and validate existing nonhuman primate models so that they more accurately reflect the protective outcomes observed in humans. At that point, they may become useful as gatekeepers of future vaccine concepts.

Determining immune correlates

From failed trials? Among the handful of HIV vaccines that have been tested for efficacy, one was based on a hypothesis that T cells provide protection. The vaccine tested by Merck & Co. in the STEP and Phambili trials contained genes encoding HIV Gag, Pol and Nef, but not Env, and therefore stimulated neither virion-binding antibodies nor neutralizing antibodies[1,60]. Protection was intended to be produced by stimulation of $CD8^+$ T cell responses that would control viral replication following infection, and preclinical testing of the vaccine in nonhuman primates supported this assertion[61]. Although the vaccine was shown to stimulate T cells with several of the characteristics associated with long-term non-progression (each of which was touted as a mechanism of immune protection), the vaccine failed to protect volunteers from acquisition of infection and to reduce viral loads after infection[1,60]. The main measure of T cell immunogenicity that led to the clinical testing of this vaccine was the enumeration of interferon-γ (IFNγ)-producing T cells using enzyme-linked immunosorbent spot (ELISPOT) assays. The lack of vaccine efficacy in the trial called into question the use of this assay as a correlate of protection, and indicated that newer and better measures of T cell function were needed[63,64]. Although such questioning of the importance of IFNγ as an antiviral effector is justifiable, its measurement nevertheless reveals the ability of T cells to recognize and respond to HIV-derived peptides[65]. More importantly, this raises the question of whether it is valid to reject a specific T cell function as an immune correlate based on a vaccine trial that did not show protection. Indeed, a correlate is a statistical term, and a potential correlate can only be eliminated from consideration when it is shown to be unable to discriminate protected from unprotected recipients of a partially protective vaccine (BOX 1). Thus, the vaccine that was tested in the STEP and Phambili trials is an example of a failed vaccine, and although these studies suggest that something quantitatively or qualitatively different is needed, they do not provide the basis for defining or eliminating a particular immune measurement as a potential correlate of protection. Rather than advocating for more T cell-based vaccines or the use of ELISPOT assays as a sole measurement of vaccine immunogenicity, this specific example illustrates the general principle that the analyses of correlates should be evaluated in the context of an at least partially effective vaccine. In the absence of protection, any measure of an immune response will fail to be a correlate, irrespective of its potential role in protection.

From current and future trials? For pathogens such as HIV, which are likely to require an iterative approach to vaccine design with many generations of product modifications and improvements, focusing on the identification of immune correlates of protection deserves substantial investment. Current HIV vaccine efficacy trials should be designed to identify a correlate of protection and should not focus on direct and immediate development of a product. Performing a test-of-concept efficacy trial to define a correlate of protection requires three major factors. The first is the clinical trial infrastructure and use of well-characterized risk cohorts with a high enough incidence of HIV infection to make accumulation of >100 infection endpoints feasible. Second, the candidate vaccine needs to induce the immune response of interest at a sufficient frequency to support a correlates analysis (30–70%). Third, there has to be clinical efficacy. Identifying and maintaining high-risk cohorts and the clinical infrastructure to conduct HIV vaccine efficacy trials is expensive and dynamic. Many factors other than the vaccine intervention tend to reduce HIV incidence in relevant study populations[66–68]. Education, counselling, self-esteem and access to medical care for trial participants all contribute to reduce incidence of infection, and over time new options for prevention, such as circumcision, pre-exposure prophylaxis or microbicides, will change the constituency of study populations that are appropriate for HIV vaccine clinical trials. This will add to the expense and complexity of performing the trials that are needed to establish a correlate of protection.

There are several candidate vaccine regimens in development, but few immunological hypotheses for how vaccine-induced immunity might protect. Candidate vaccine regimens with immunological endpoints that have distinct specificity or functional properties should be considered for analysis in test-of-concept efficacy trials to define a correlate of protection. Currently, it is typical in Phase I and Phase II clinical trials of candidate HIV vaccines to exclude individuals who are at high risk of HIV infection,

Box 2 | T cell immune correlates

Several characteristics of T cells have been investigated as potential correlates of immune protection in HIV.

T cell phenotype
- $CD4^+$ or $CD8^+$ T cells
- Central memory, effector memory or effector T cells
- Expression of homing markers
- Expression of exhaustion markers

T cell function
- Expression of individual cytokines and chemokines
- Production of single versus several cytokines and chemokines
- Killing capacity or perforin expression
- Inhibition of virus *in vitro*
- Proliferation

T cell antigen specificity
- Responses to Gag, Env, Pol, Nef and accessory proteins
- Number of epitopes targeted
- Sequence conservation of epitopes
- Ease of sequence escape within epitopes
- Effect of sequence escape on viral fitness

HLA restriction
- Frequency in human population
- Association with lack of disease progression

T cell receptor
- Diverse or restricted
- Ability to cover several clades
- Ability to cover potential escape variants
- Public T cell receptors (dominant in multiple individuals) or private T cell receptors (rarely present in multiple individuals)

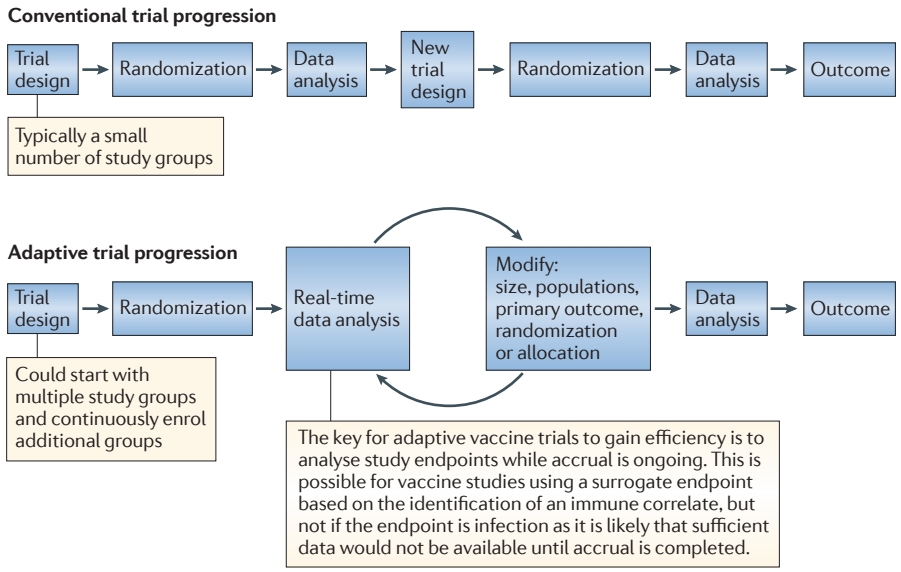

Figure 1 | **Conventional and adaptive trial design.** Conventional clinical trials explicitly follow a protocol that is designed to provide an unbiased answer to a discrete question stated as the primary objective. Achieving the predetermined number of study endpoints should then provide a statistically robust conclusion. Adaptive trial designs may be useful when there are multiple objectives or multiple treatment arms to test, or both. Adaptive designs provide an unbiased approach to shift from one study objective to another or shift the emphasis from many treatment groups to a few. For example, if a vaccine study using a 1/1 allocation of vaccine to placebo was discovered to have efficacy during a proscribed interim analysis, the allocation could be changed to a 2/1 ratio to shift the primary focus towards defining an immune correlate. Alternatively, if an immune correlate was known, it could be used as a surrogate endpoint in a study that tests multiple vaccine concepts. When a particular vaccine achieved the predetermined immunological endpoint, it would trigger an altered randomization scheme to assure more subjects would be enrolled in study arms that achieve the immunogenicity objective. This would allow the emphasis to shift from immunogenicity to efficacy evaluation.

so there are relatively few exposures and breakthrough infection cases to evaluate. The inclusion of subjects who are at risk of HIV infection in early Phase clinical trials would be one way of improving our knowledge of vaccine-induced immunity and would provide another parameter of safety to assess before commencing larger test-of-concept efficacy trials. Importantly, robust and frequent sample collection is crucial. Extensive sample collection may not be necessary for a candidate vaccine being developed for licensure, but is essential for defining a correlate of protection. Frequent sampling allows the assessment of immunity closer to the time of infection and helps to define the timing of infection more precisely. The measurement of peak immunogenicity time points is useful for product validation but is much less likely to provide a correlate with efficacy than estimates of immunity near the time of HIV exposure that may be distant from the time of immunization. The use of similar collection time points and uniform assays across a series of clinical efficacy trials will improve the likelihood of identifying a correlate of protection and would ultimately help to generalize findings by providing data for subsequent meta-analysis[69].

From trials that show partial protection?

It is crucial for funding bodies and decision-makers to appreciate that an immune correlate of protection can be defined from trials of vaccines that show partial efficacy. If properly designed with enough clinical endpoints and sufficient sample collection, efficacy as low as 10–15% may allow identification of an immune correlate of protection. This would then allow the subsequent evaluation of several vaccine delivery platforms and antigen concepts, and product development can proceed in a more logical and systematic way.

The concept of using 'adaptive' trial designs for HIV vaccine evaluation has recently been a topic of considerable discussion. Adaptive trial design implies that flexibility to accommodate changes in study design in response to accumulating data is incorporated into the protocol (FIG. 1). Adaptive trial designs have gained some momentum in treatment-intervention studies in which a clinical endpoint can be detected soon after treatment initiation. The theoretical advantage of an adaptive approach is that it provides a mechanism to evaluate many approaches in a shorter amount of time. However, the practical advantage is lost if events that trigger adaptation are infrequent and distant from the time of subject randomization. There may be ways in which adaptive trial designs facilitate the identification of correlates of vaccine-induced immunity. Although vaccine efficacy is determined by comparing the frequency of study endpoints in recipients of the vaccine and the placebo, to detect immune correlates a case-controlled comparison is performed between vaccine recipients who become infected and those who do not. Therefore, enrolling additional vaccine recipients in trials that show early evidence of efficacy would improve the chances of defining immune correlates. However, this would not provide a notable time advantage. To attain the time efficiency promised by adaptive trial designs for defining efficacy, adjustments to group allocation would need to be done in real-time based on an immunological 'surrogate' endpoint (BOX 1). For example, if an antibody response with a particular specificity and function or a T cell response with certain phenotypic characteristics was found to be a correlate of protection, then parallel trials with several product concepts could be initiated, and every time an immunized subject achieved the 'surrogate' endpoint or the immune correlate of protection, that group would gain an advantage in future subject allocations. In that way, enrolment could proceed for many concepts, but only the ones achieving the correlate at a high frequency would accrue enough subjects to determine clinical efficacy.

Defining a correlate of protection to use as a surrogate endpoint is the crucial step that would allow the best use of adaptive trial designs and improve the likelihood of eventually achieving a significant level of clinical efficacy. Adaptive vaccine clinical trials that use a clinical endpoint of infection or disease progression require too much time and clinical trial capacity to remain relevant. Performing efficacy trials that are designed to detect high levels of clinical efficacy (>50%) but are of insufficient size or intensity to define a correlate of protection may result in a fortuitous breakthrough that could support the further development of a selected product. However, they are much less likely to support incremental scientific advances that would lead to a highly effective HIV vaccine. As we have witnessed in the two

Box 3 | **Antibody immune correlates**

Characteristics of antibodies that will be investigated as potential correlates of immune protection in the RV144 vaccine trial.

Antibody titre
- Binding by enzyme-linked immunosorbent assay
- Duration

Antibody function
- Neutralization (compared with several panels of isolates)
- Antibody-dependent cell-mediated cytotoxicity
- Antibody-dependent cell-mediated virus inhibition
- Fc binding
- Effect on viral mobility in mucous
- Affinity and avidity

Antibody specificity
- Clade specificity of antibody functions
- Cross-competition with known neutralizing antibodies
- Linear epitope mapping

Antibody phenotype
- Immunoglobulin class and subclass
- Fc modifications (sialylation and glycosylation)

Antibody location
- Serum
- Mucosal samples

most recent efficacy trials, it is hard to guess based on established scientific paradigms what the outcome of a vaccine trial will be. The STEP trial focused on inducing Gag-specific $CD8^+$ T cell responses and was considered promising by the scientific community but resulted in vaccine-enhanced infection rates, whereas the vaccine tested in the RV144 trial, which induced relatively weak $CD8^+$ T cell responses and non-neutralizing antibodies and was highly criticized by the scientific community, showed partial efficacy. Unfortunately, neither trial was specifically designed to define an immune correlate of protection, and the retrospective analysis of available samples is not likely to reach a definitive answer. The current HVTN 505 efficacy trial has relatively extensive sample collection, but is not large enough to accumulate a sufficient number of subjects in the vaccine group to be sure of defining a correlate of protection if partial efficacy is achieved. It should also be noted that in the absence of a strong T cell response in the RV144 trial, an antibody correlate is most likely to derive from the correlates analysis. This does not, however, diminish the complexity of the investigation. Similarly to the T cell response, there are many aspects of the antibody response that can and will be evaluated in the search for an antibody correlate of protection in the RV144 vaccine trial (BOX 3). Until there is sufficient investment in the process to define a correlate of protection to allow the establishment of surrogate immunological endpoints for efficacy trials, development of a vaccine for HIV will remain a distant hope, a point made recently by the Global HIV Vaccine Enterprise[70].

Conclusion

The challenge presented in developing an HIV vaccine is both new and unique. Infected individuals do not clear the virus, are not immune to subsequent reinfection and do not typically survive in the absence of antiretroviral therapy. This separates the quest for an HIV vaccine from other vaccine efforts in which correlates of protection may arise from an empirical approach, instead of being prerequisites for the rational design of the vaccine. The search for correlates of protection in cohorts such as long-term non-progressors seemed to be a reasonable approach but may ultimately have led us astray. Although polyfunctional Gag-specific cytotoxic T cells were heralded as the goal to be achieved in a successful vaccine, in fact they may be only a correlate of lower viral load in chronically infected people. Observations in highly exposed uninfected people are equally consistent with innate rather than adaptive host immune factors as underlying mechanisms for protection, and it is unclear how these mechanisms may be relevant to a vaccine. Furthermore, although there has been much progress in the elicitation of Env-specific antibodies by vaccines, studies in infected individuals certainly did not reveal them as correlates of protection and, in fact, suggested that stimulating T cell responses to Env would be harmful. Thus the focus of our studies should shift to the establishment of correlates of protection in uninfected people in large vaccine trials, rather than protection from virus replication or disease progression in chronically infected people. When we embark on such trials we must bear in mind that should a trial fail, certain response thresholds may be found to be inadequate, but assays themselves cannot be formally negated. Only in a trial that has partial efficacy can a correlate be disregarded if it fails to distinguish protected from unprotected individuals. As transmission incidence is generally low, defining correlates is inherently difficult, and therefore we should be guided in this endeavour by the necessity for the recruitment of large numbers of volunteers who should be sampled frequently. The path to a successful vaccine for HIV is likely to be an iterative one, driven forward by a process of successive approximation or, as it is more colloquially termed, trial and error.

Richard A. Koup, Barney S. Graham and Daniel C. Douek are at the Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20892-3017, USA.
Correspondence to D.C.D.
e-mail: ddouek@mail.nih.gov

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Competing interests statement

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