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# HIV vaccines 1983–2003

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**Twenty years after the discovery of HIV, there is still no vaccine. This year, an envelope vaccine aimed at stimulating neutralizing antibodies was unable to protect against infection in phase 3 trials. But more than 20 HIV vaccines designed to stimulate T-cell responses are being developed. Will any of them work?**

Earlier this year, the results of the first phase 3 efficacy trial of a vaccine against AIDS were announced (see the VaxGen website; http://www.vaxgen.com). The gp120 vaccine, tested in 5,000 at-risk volunteers, showed no protective effect. This result, although not unexpected, cast some gloom in the vaccine development field and raised fundamental questions: is a vaccine against AIDS possible at all? Will it ever be able to cope with HIV variability? Will it offer sterilizing immunity or only partial protection? Are there alternative approaches to stimulating neutralizing antibodies? Although it is not unusual for the development of a vaccine to take 20 years, this goal still seems a long way off for HIV.

The most essential of these questions is whether a vaccine will be possible at all. Throughout the development of previous vaccines for other viruses, it was clear that people who recovered from acute viral infections were immune from a subsequent attack by the same virus. This is not so for HIV because no one is known to have recovered from, and completely cleared, acute infection. HIV causes a chronic infection with reservoirs of virus in T-cell, macrophage and monocyte compartments, where some of it is integrated as a silent provirus[1]. The virus diversifies during the infection, with repeated selection of mutants that escape both antibody and T-cell immune responses[2,3]. Control of the infection by T cells seems to determine the progression of the infection, and it is possible that some infected people can control infection indefinitely, especially if helped by antiretroviral drugs[4].

A very relevant issue is whether superinfection occurs in infected people who are exposed repeatedly to the virus. This can happen[5], but it is not known how often. If superinfection is rare, it means that the immune response to HIV, although unable to control established infection completely, may be able to increase the threshold for new infections. Support for this conclusion comes from macaques that are chronically infected with attenuated SIV but resist superinfection with more aggressive virus[6,7]. Similarly, macaques that are infected with SIV and then immediately treated briefly with antiretroviral drugs control their infection and resist superinfection[8]; this resistance is abrogated by the removal of $CD8^+$ T cells[9]. Thus, there is some evidence that the immune response can, under certain circumstances, prevent HIV or SIV infection.

At the time that HIV was identified, our understanding of the immune response was relatively poor. Cytotoxic T cells were not known to recognize viral peptides until 1986 (ref. 10), the T-cell receptor had not been discovered and the distinction between T-helper type 1 ($T_H1$) and $T_H2$ cells had not been made. Attempts to design an HIV vaccine during this period should therefore be viewed alongside these and other advances in basic immunology. **Table 1** shows the principal steps, not all of them forward, in HIV vaccine design in the past 20 years. These steps will be reviewed in greater detail below.

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## Antibody immunity

Studies of vaccines that protect macaques against SIV infection indicate that antibody-mediated protection is possible. It has been shown repeatedly that vaccines based on the viral envelope can protect nonhuman primates challenged with homologous virus[11–16]. But the numbers of animals used in such studies are small, and the studies may have limited relevance to humans[17]. It was disconcerting to find that unlike viruses adapted to laboratory culture, primary HIV isolates from infected patients were resistant to neutralization[18]. These isolates were later shown to use different coreceptors[19], although this fact alone does not account for the difficulty in neutralization. Two recent studies have shown that neutralizing antibodies directed at the envelope are made during HIV infection, but as they appear they immediately select for viral escape mutants, thereby becoming irrelevant[20,21].

Sera from individuals infected with HIV have been analyzed extensively for the presence of neutralizing antibodies. Five human monoclonal antibodies have been found that are capable of neutralizing a broad range of primary B-clade HIV isolates[22]. Two of these require CD4 to alter the conformation of gp120; the other three have been characterized in detail. The first antibody binds to the CD4 binding site on the gp120 domain (**Fig. 1**), but it needs an unusually long complementarity-determining region-3 loop to access the deeply recessed site. The second antibody recognizes a complex polymannose epitope, but it has extraordinary structural features rarely seen on other antibody molecules (D. Burton, personal communication). The third antibody binds to a site on gp41 on the native spike that remains hidden before CD4 binding. These antibodies can protect severe combined immunodeficient mice that have been reconstituted with human lymphoid cells against challenge with HIV[23] and can also protect monkeys against challenge with an SIV/HIV (SHIV) hybrid virus[24–26]. The titers required are high, however, and might be difficult to achieve by active immunization, even if it were possible to devise ways in which to persuade the immune system to generate antibodies of these specificities.

Most antibodies that neutralize can be easily evaded by mutation, and those that bind gp120 monomers seem to be irrelevant. The gp120 crystal structure indicates why neutralization is difficult[27]. The envelope is a trimer of gp120-gp41 heterodimers. The trimer is held together by interactions involving conserved gp120 surfaces that are not exposed on the virion surface but, on gp120 shedding, act as a decoy to stimulate largely irrelevant antibodies. Hypervariable loops mask the critical receptor-binding sites. The exposed surface is covered in asparagine-linked carbohydrates. The importance of these carbohydrates is clear from studies in infected individuals, which show that viral escape is facilitated by changes in glycosylation[20]. Similarly, in macaques, challenge with SIV that has been deliberately mutated to remove glycosylation around the V1 loop results in an effective antibody response that can control the virus, but only until mutations repair the glycosylation deficits[28]. Although not unreasonable at the time, the first vaccines to be tested in the 1980s were unfortunately based on monomeric gp120.

**Table 1 Principal steps towards an HIV vaccine since 1983**

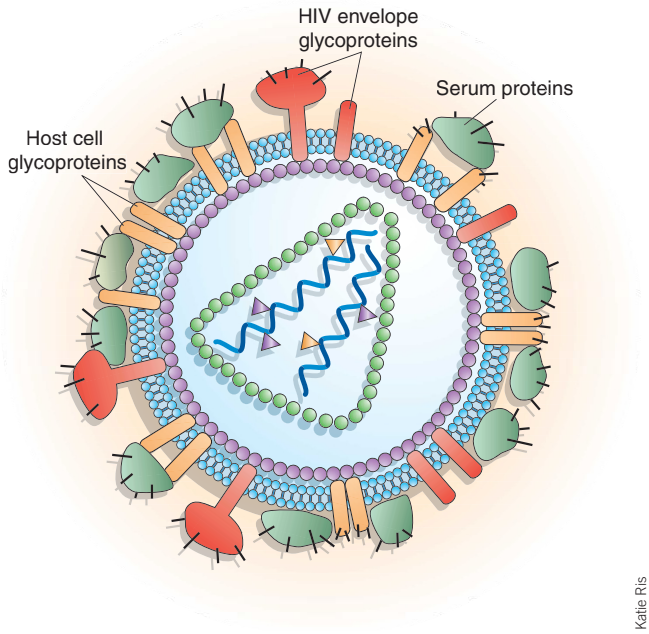
| Year | Event |
|---|---|
| 1983 | Recombinant vaccinia virus as a candidate vaccine[103,104] |
| 1984– 1996 | Identification of HIV cell receptors[105,106] |
| 1990 | Envelope-based vaccines protect chimpanzees against homologous HIV challenge[11] |
| 1991 | Protection of macaques by inactivated SIV is dependent on cells in which vaccine is grown[31] |
| 1991– 2002 | HIV/SIV can escape from $CD8^+$ T cells and vaccines[41–46,60] |
| 1992 | Attenuated SIV protects against challenge with wild-type SIV[6] |
| 1993 | CD4 binding alters conformation of gp120 (ref. 107) |
| 1993 | DNA vaccine can protect against viral infection[52] |
| 1995 | Resistance of primary HIV isolates to neutralization[18] |
| 1996– 2003 | Characterization of binding sites for neutralizing monoclonal antibodies[108–110] |
| 1998 | Structure of gp120 (ref. 26) |
| 1998 | Development of common immunogen prime-boost strategies[58,59,76,77,79] |
| 1999 | $CD8^+$ T cells central to controlling acute and chronic SIV infection[111,112] |
| 2000 | $CD8^+$ T-cell-inducing vaccines protect against SHIV89.6P challenge[55–59] |
| 2003 | Virus escapes antibody neutralization *in vivo*[20,21] |
| 2003 | First phase 3 vaccine trial completed but no protection observed[93] |

Ten years ago, it was claimed that formalin-fixed SIV could effectively protect against SIV challenge in macaques[29,30]. But Stott *et al.*[31] showed that this protection was not virus-specific; in their study, protection was seen only when vaccine virus (before inactivation) and challenge virus were grown in human T-cell lines. Macaques challenged with virus grown in macaque cells were unprotected[31]. SIV and HIV acquire large amounts of major histocompatibility complex (MHC) class I and II molecules, as well as other surface proteins, when they bud from the surface of T cells or macrophages, so the protective immune response might be directed against these acquired human proteins. The original result[31] was regarded as an artifact, although the observed protection was better than any seen in an experimental SIV or SHIV vaccine system. Some attempts have been made to show that similar protection might occur if the virus is grown in cells of a different MHC type, but the results have been inconclusive. The enigma remains and may deserve renewed attention.

Finding vaccines that stimulate antibodies capable of neutralizing primary HIV-1 isolates must still take the highest priority. The challenge is to find ways of inducing such antibodies reliably and in sufficiently high titers, but this may require advances in basic immunology. Structural information should help to find ways of stabilizing envelope proteins in vaccines in conformations that, for example, expose the binding site for the chemokine receptor[32]. Targeting gp41 also may be an option. T20, a peptide that interferes with the hairpin loop formation in gp41 that is necessary for membrane fusion and viral entry, was recently introduced to therapy[33], offering hope for approaches based on a better understanding of the structure-function relationships of viral molecules.

## T-cell approaches

Traditionally, the stimulation of a good neutralizing antibody is sufficient for a vaccine. But live attenuated vaccines, which stimulate strong $CD4^+$ and $CD8^+$ T-cell responses and neutralizing antibodies, are more efficient than are inactivated virus or purified protein subunit vaccines, which are poor at stimulating $CD8^+$ T cells. Because the stimulation of neutralizing antibodies is problematic in HIV infection, nearly all current vaccine approaches (**Table 2**) are aimed at stimulating T-cell




Katie Ris

**Figure 1** The HIV target for antibodies (not drawn to scale). The textbooks have imprinted into our minds a picture of a perfect HIV virion with a core wrapped in a membrane containing nicely shaped trimeric gp160 spikes on its surface. The reality of what the immune system actually faces is quite different. Thus, only 1 in about 1,000–10,000 HIV particles is not defective and can productively infect host cells, which may, but often does not, leave the relevant antigenic determinants intact. In addition to the HIV envelope spikes (red), HIV particles carry in their membranes numerous host cell–derived glycoproteins (orange) and an array of serum proteins nonspecifically attached to the virion surface (green). Many of the original (approximately) 72 functional spikes have shed their gp120 subunits and may display a conformationally irrelevant postfusion gp41 (red). The remaining intact spikes are highly glycosylated, flexible on the surface and may differ by up to 10% of amino acids between different HIV virions within an individual at a particular time point, thus interfering with the affinity maturation of antibodies.

responses. They are based on the assumption that the induction of a strong $CD8^+$ and $CD4^+$ T-cell response by vaccination will abort or control early HIV infection.

HIV stimulates a strong $CD8^+$ T-cell response during acute viremia and usually persists through the chronic phase of infection[4,34]. A $CD4^+$ T-cell response is also generated early on but is susceptible to damage by the virus, which preferentially infects HIV-specific $CD4^+$ T cells[35–37]. In mice, $CD4^+$ T-cell help is crucial for priming an effective memory $CD8^+$ T-cell response; in mice deficient in $CD4^+$ T cells, pathogen exposure generates normal numbers of memory $CD8^+$ T cells, but these have poor replicative capacity when re-exposed to the microbe[38,39]. In HIV infection, therefore, the initial $CD8^+$ T-cell response may be effective in inducing memory T cells capable of regeneration and a full range of functions, whereas T cells primed later in the infection may be defective even if they are detectable in some assays[40]. Because $CD8^+$ T cells select for HIV escape mutants[41–49], new immune responses are needed during the infection, but if $CD4^+$ T-cell function becomes impaired, these 'secondary' T cells may be less capable of controlling the virus.

Whereas neutralizing antibodies can prevent infection, $CD8^+$ T-cell responses cannot. These cytotoxic T lymphocytes (CTLs) react to other cells of the body that are infected by HIV and present peptide fragments of viral proteins bound to MHC class I proteins[10]. $CD8^+$ T cells kill the infected cells, thereby reducing the production of new HIV virions. They can also inhibit entry of HIV-1 by releasing the β-chemokines RANTES, MIP-1α and MIP-1β, which compete for the CCR5 receptor[50], and other cytokines with antiviral activity. A vaccine should stimulate high numbers of $CD8^+$ T memory cells, which rapidly release cytokines and chemokines on subsequent antigen contact and start killing target cells (**Fig. 2**). But these cells may need to be expanded to outnumber the virus-infected cells and distributed to several sites around the body. Thus, full antiviral activity may take days to develop and will only control, rather than prevent, viral infection.

Even though $CD8^+$ T cells cannot neutralize virus, there is ample experimental evidence that vaccination to stimulate these T cells can protect mice against high-dose challenges with several viruses[51–54]. Vaccinated mice become infected, but have lower titers of virus as compared with unvaccinated controls. The immune system of the mouse can then cope well with the low amounts of virus and no disease develops.

Recently, vaccines designed to stimulate $CD8^+$ and $CD4^+$ T-cell immunity have protected macaques from challenge with the aggressive strain SHIV89.6P[55–59], which causes a rapid decline in $CD4^+$ T-cell numbers and fatal immunodeficiency. The vaccinated macaques were infected but had a viral load that was 1,000 times less than that in unvaccinated controls. These studies provide the strongest experimental rationale for the current vaccine approach based on $CD8^+$ T cells.

But there are reasons to be cautious. Barouch *et al.*[60] have shown that this SHIV strain can escape vaccine-induced immune control by the mutation of a single amino acid—a process that is facilitated by the focus of the T-cell response on a dominant epitope. If this is not an

**Table 2 Prophylactic HIV vaccines in clinical trials**

| Vaccine | Immunogen | Clade | Sponsor | Country | Phase |
|---|---|---|---|---|---|
| AIDSVAX B/E | gp120 | B, E | VaxGen | Thailand | 3 |
| AIDSVAX B/B | gp120 | B | VaxGen | USA | 3 |
| ALVAC vCP1452 | Env-Gag-Pol-CTL | B | NIAID | USA | 2b |
| AIDSVAX B/B | gp120 | B | | | |
| ALVAC vCP1452 | Env-Gag-Pol-CTL | B | NIAID | Brazil, Haiti, Peru | 2b |
| AIDSVAX B/B | gp120 | B | | Trinidad & Tobago | |
| DNA.HIVA | Gag-CTL | A | IAVI/MRC | UK | 2a |
| MVA.HIVA | Gag-CTL | A | | Uganda | |
| ALVAC vCP205 or vCP1452 | Env-Gag-Pol-CTL | B | NIAID | USA | 2a |
| AIDSVAX B/B | gp120 | B | | | |
| ALVAC vCP205 | Env-Gag-Pol | B | WRAIR | USA | 1 |
| ALVAC vCP1452 | Env-Gag-Pol-CTL | B | NIAID | USA | 1 |
| DNA.HIVA | Gag-CTL | A | IAVI/MRC | Kenya | 1 |
| MVA.HIVA | Gag-CTL | A | | | |
| MRKAd5 | Gag | B | Merck | USA | 1 |
| Poly-env1 vaccinia | Env | A, B, C D, E | St Jude's | USA | 1 |
| VCR-HIVDNA009-99-VP | Env Gag-Pol-Nef | A, B, C B | NIAID/VRC | USA | 1 |
| GTU-Nef DNA | Nef | B | FIT Biotech | Finland | 1 |
| VCR4302 DNA | Gag-Pol | B | NIAID/VRC | USA | 1 |
| Gag DNA | Gag | B | Merck | USA | 1 |
| PGA2/JS2 DNA | Gag, RT, Env, Tat, Rev, Vpu | B | NIAID | USA | 1 |
| NefTat fusion/gp120 | Nef-Tat, gp120 | B | NIAID | USA | 1 |
| LIPO-4T lipopeptide | Gag-Pol-Nef-TT-CD4 | B | ANRS | France | 1 |
| ALVAC vCP1452 | Env-Gag-Pol-CTL | B | ANRS/Aventis | France | 1 |
| LIPO-5T or LIPO-6T lipopeptide | Gag-Pol-Nef-TT-CD4 | B | | | |

All trials are being conducted in HIV-negative volunteers at either low risk (phases 1 and 2a) or high risk (phases 2b or 3). CTL denotes CTL epitopes. CD4 denotes T-helper epitopes. ANRS, National Agency for AIDS Research; IAVI, International AIDS Vaccine Initiative; MRC, Medical Research Council of the United Kingdom; NIAID, National Institute for Allergy and Infectious Diseases; VRC, Vaccine Research Center; WRAIR, Walter Reed Army Institute of Research. Table adapted from the International AIDS Vaccine Initiative website (http://www.iavi.org).

isolated incident, such escape could be a real concern when immunity allows low levels of virus to persist. In addition, it might be, paradoxically, relatively easy to protect against SHIV89.6P despite its virulence: it has proved more difficult to protect with similar vaccinations against $SIV_{MAC239}$, which is possibly closer to HIV in pathogenicity[61]. Such differences need explanation.

The reservations can be tempered by the fact that the dose of HIV encountered in a single exposure during sexual contact in humans is about 100 times less than the dose of SIV typically used to challenge vaccinated macaques. The studies discussed above use high-dose challenges to guarantee that all control macaques are infected. By contrast, many sexual exposures to HIV may be necessary before humans become infected[62,63]; consequently, it may be easier to protect against infection with a vaccine that stimulates T-cell immunity. If humans are repeatedly exposed, however, they will eventually become infected. Vaccination might raise the threshold for infection, reducing the absolute risk of infection from a single exposure to HIV and delaying infection in those who are repeatedly exposed. Those who are infected may have lower amounts of virus, similar to the vaccinated macaques challenged with SHIV89.6P. Although not ideal, these features could offer the benefits of a reduction in primary viremia and viral set point, with slower progression to AIDS and reduced chances of transmitting the virus.

Support for the idea that vaccination might prevent or abort early infection in humans comes from studies of rare individuals who are highly exposed to HIV but remain uninfected for prolonged periods of time; such individuals account for about 5% of the exposed population. $CD8^+$ T-cell responses have been observed in highly exposed but uninfected sex workers and HIV-discordant couples[64,65]. These individuals can also have $CD4^+$ T-cell responses to HIV but no serum antibodies. Whether the T cells are protecting them is uncertain, but direct genetic causes have been so far excluded. In Nairobi, some sex workers became susceptible when they changed their lifestyle, which suggests that immune protection needs continuous antigen exposure[66].

### Priming of $CD8^+$ T-cell immune responses by vaccines

The stimulation of $CD4^+$ T-cell responses is relatively easy to achieve: any vaccine that stimulates antibodies will stimulate T-helper cells. As antibody-producing B cells exert their antiviral effect at long range, $CD4^+$ T-helper cells need to act only in lymphoid organs. By contrast, $CD8^+$ T cells are more fastidious. They require antigen-presenting dendritic cells for priming. Their effector function is exerted at short range through contact with infected cells that express peptides derived from viral proteins bound to human leukocyte antigen (HLA) class I molecules. They respond to all viral proteins, with a preference in HIV infection for Gag and Nef[34,67].

Priming of $CD8^+$ T cells is normally achieved by dendritic cells that either are infected or contain reprocessed viral antigen, and that enter lymph nodes to stimulate $CD8^+$ T cells directly. $CD8^+$ T-cell priming in natural viral infections is highly efficient. In acute infections of Epstein-Barr virus (EBV), 40% of blood $CD8^+$ T cells can become specific for a dominant epitope within weeks of first viral contact[68]. This represents nearly 20 cell divisions from the rare EBV-specific naive T cells. The strength of the acute $CD8^+$ T-cell response in HIV infection is smaller, comprising 1–10% of peripheral blood $CD8^+$ T cells, but still represents about 15 divisions from the naive T cells[69–72]. Ideally, experimental vaccines should achieve a similar priming of $CD8^+$ T cells.

In macaques, immunization with plasmid DNA encoding SIV Gag, followed by a boost with recombinant modified vaccinia virus Ankara (MVA; a replication-defective vaccinia virus) expressing SIV Gag, stimulated strong $CD8^+$ T-cell responses comprising up to 20% of T cells to a dominant epitope[59,73,74]. Similar immunity was achieved by a DNA prime and recombinant adenoviral boost[58], or by priming with a combination of DNA and interleukin-2 (ref. 75). Thus, vaccination can achieve early $CD8^+$ T-cell responses that are comparable to natural infection. The problem is that these immunogens do not persist and the T-cell response falls away rapidly as the T cells mature to memory T cells[73].

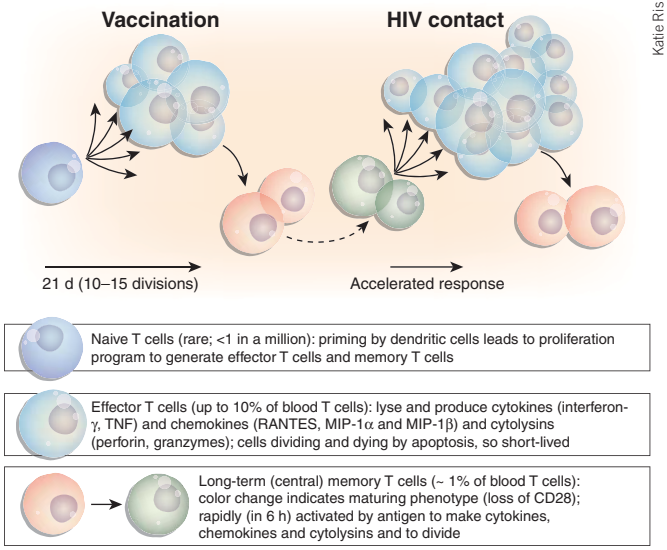



**Figure 2** Expansion of $CD8^+$ T cells by vaccination and subsequent response to HIV contact. Shown is the initial expansion of naive cells into effector and memory T cells. Because the vaccine does not persist, the primary immune response is short lived and decays rapidly, leaving memory T cells that further mature over several months. When the memory T cells are exposed to HIV-infected cells, a rapid secondary response ensues.

DNA alone stimulates weak acute $CD8^+$ T-cell responses in macaques, but primes for subsequent responses to a recombinant viral vaccine that are better than the responses to each vaccine alone[58,59,73,74]. The DNA may focus the T cells, ensuring that the same response is boosted after a subsequent immunization with the recombinant virus[76,77]. The virus may have around 200 antigenic proteins; without the priming step, it may not provoke a response to the inserted protein because of immunodominance. Priming and boosting with two vaccines that share a key passenger immunogen is clearly better than using either component alone in mice and macaques, but it has not been confirmed whether this procedure has an advantage over simple priming with recombinant virus in humans (see accompanying review in this issue[78]). DNA may provoke stronger T-cell responses if cytokines such as interleukin-2 are added, either as plasmid DNA or protein[55]. This approach has greatly enhanced $CD8^+$ T-cell responses against SIV Gag in macaques. The use of either adjuvants or other types of immunogens, such as recombinant virus-like particles, to improve responses to DNA also might be useful.

Many viral vectors are being developed as recombinant HIV vaccines, including fowlpox[79], canarypox[80,81], replication-deficient adenovirus-5 (ref. 58), Semliki Forest virus[82] and Venezuelan equine encephalitis virus[83]. Although each may stimulate similar immune responses, they provide opportunities for prime-boost strategies. In some cases, however, pre-existing immunity to the viral vector may limit its usefulness. In addition to the vectors mentioned above, vectors that can persist in the host, such as adeno-associated virus[84], are under consideration. Although the resulting prolonged T-cell responses would be desirable, the consequences of chronic or repeated exposure to foreign antigens on

the immune responses will need to be evaluated carefully. Recombinant bacterial vectors are also being developed as HIV vaccines, including bacillus Calmette-Guérin (BCG)[85] and salmonella[86,87]. BCG has the advantages that it is already known to stimulate T-cell immunity and can be given to newborn babies.

### Design of HIV-derived immunogens

There are many possible designs for an HIV-derived immunogen. Gag is usually included because it seems to be most immunogenic in HIV-infected individuals and contains important helper epitopes[34,67]. Similarly, Nef is often part of the construct, although its gene is quite variable and needs to be inactivated for safety reasons when inserted into cells *in vivo*. Reverse transcriptase is conserved but should also be inactivated, perhaps by 'scrambling', because there are conserved epitopes across the active site that should be preserved. Env is also often a component of the formulation, even though it is the most variable protein. In the absence of an efficient strategy for inducing neutralizing antibodies, Env is often included to generate T-cell responses, but there is an argument that it should be left out to leave a 'gap' for the later addition of a neutralizing antibody–inducing immunogen, when one becomes available. Decisions have to be made about what sequence to use; synthetic genes offer the possibility of optimizing amino acid codons to enhance expression in human cells and of using consensus or ancestral sequences[88].

The size of the construct may also be important. The trend is towards making polyprotein vaccines. The T-cell immune response to a fixed-sequence immunogen tends, however, to focus on small numbers of immunodominant epitopes. Thus, a polyprotein vaccine may not necessarily stimulate a broader T-cell response compared with a single protein vaccine. It may be better to break the vaccine up into smaller separate components, thereby forcing the immune response to treat each as a separate invading antigen against which to react.

An important issue is how closely to match the virus and the vaccine[89,90]. This argument generally revolves around clades—for example, is a clade C vaccine needed for South Africa? Intuitively, it seems preferable to match the virus and vaccine as closely as possible, even if the gains are small. The clades differ by 10% to more than 25%, depending on the viral protein. $CD8^+$ T cells respond to peptide fragments of 8–11 amino acids bound to HLA class I proteins, so that on average there is more than one amino acid change per epitope among clades. In an epitope, two-thirds of the amino acid side chains are involved in specific interactions, either with the presenting HLA molecule or with the T-cell receptor (reviewed in ref. 89). As these interactions are very sensitive to change[91], in theory there is only a one in three chance that the T-cell response stimulated by a vaccine of one clade will recognize the epitope from another clade. This problem can be offset if there is a multiepitope response; for example, a five-epitope response to the 'wrong' clade should have an 85% chance or greater of cross-reacting with another clade, but a third of those responses would be to only one epitope.

The danger of too narrow a response is that escape mutants could be easily selected for by the vaccine, particularly if protection is incomplete. This has been observed in macaques vaccinated with DNA for SIV Gag and then challenged with SHIV89.6P (ref. 60). This problem could be a formidable obstacle for T-cell-inducing vaccines: even if the clades of the vaccine and circulating virus are matched, the variability of sequence within a clade (4–10%) may produce similar problems unless the tendency of the T-cell response to focus on immunodominant epitopes is overcome.

### Early trials of T-cell vaccines in humans

Several HIV vaccines have entered phase 1 and 2 clinical trials in uninfected volunteers in the United States, Europe, Uganda and Kenya (**Table 2**). The vaccines include HIV-derived immunogens as adjuvant-associated peptides and proteins; as DNA in plasmid form[90,92]; and as inserts in recombinant canarypox[93], MVA (ref. 94 and M. Mwau *et al.*, unpublished data) and adenovirus (see E.A. Emini: http://63.126.3.84/2002/prelim.htm). So far the immune responses have been small as compared with responses in macaques to the same vaccines, possibly because the doses used are lower and the assays are different. But it is clear that these constructs are immunogenic and that improvement must be possible, for example, by increasing the dose and number of immunizations or by testing different routes of immunization. Combinations of these vaccines in prime-boost approaches may show additive effects. It is too early to say how broad and long-lasting the T-cell responses are in these early trials, but such data will undoubtedly be obtained over the next year.

The assays used to measure immune responses also need research. Currently, enzyme-linked immunospot assays—in which the T cells that make interferon-γ on peptide challenge are counted[95]—are the standard. Although the assay is robust and reliable, it may have limitations when used to measure relatively weak acute T-cell responses. Most of the validation for this assay has been done on well-established, large T-cell responses to EBV, cytomegalovirus or HIV in chronically infected people[96–98], whereas early vaccine-induced responses are likely to be weaker and more fragile. As results start coming in, it will be possible to validate the assays on vaccine-induced responses and improve them. Interferon-γ may not be the best cytokine to measure, given that it has little anti-HIV effect[99]. The use of flow cytometry to measure intracellular cytokine production in T cells stimulated with peptide or antigen *in vitro* might be a better option[100]. This is potentially more sensitive, and additional data on phenotypes of T cells can be gained.

Because most exposures to HIV in vaccine recipients will occur many months after vaccination, the most important measurement will be to quantify long-term memory T cells, especially their proliferative and functional potential[38,39]. The duration of such memory is important[101]. Experiments in mice indicate that memory can be maintained without further antigenic stimulation[102], and Amara *et al.*[59] have seen protection in their immunized macaques seven months after the last vaccination. Protection may be better, however, if the T cells are in a partially or wholly activated state[53]. The apparent necessity for continuous exposure to antigen to maintain protection for the sex workers discussed above suggests that this might be the case[66].

### Challenges ahead

There are three main challenges to developing an effective HIV vaccine. The first is to find a vaccine that can stimulate the equivalents of the five known monoclonal neutralizing antibodies in high titers in most or all individuals who are immunized. This may require conceptual breakthroughs in protein engineering and an understanding of how predetermined B cells can be preferentially stimulated and selected. Identifying more monoclonal antibodies that react with other broadly neutralizing epitopes on gp120 and gp41 would also be invaluable.

The second challenge is to find a way to optimize the T-cell-inducing vaccines so that some of them can be taken into phase 2 and phase 3 trials in high-risk volunteers. Studies of $CD8^+$ T-cell-inducing vaccines in animals[51–59] provide real hope that this approach can work, but the difficulties will be formidable. The current crop of vaccines need to be improved to generate bigger responses. Combinations in prime-boost regimes should increase the T-cell responses[59,76,77,79], as should the use of adjuvants and cytokines. Some viral or bacterial vectors may prove to be superior, although it is likely that the current replication-defective vectors will be roughly equivalent. The vaccine will have to stimulate a

long-term memory T-cell response that is broad enough to cope with variability within clades.

The third challenge is to increase the capacity to carry out phase 3 trials in developing countries. These trials will need to be designed so that viral infection, or seroconversion, is the primary end point and reduced viral load is the secondary end point, and an agreed measure of success will have to be decided beforehand. In addition, it must be recognized that finding a useful protective vaccine may take several phase 3 trials with gradually increasing efficacy, as opposed to being realized in a single trial. Those who are testing vaccines should be prepared to mix vaccines and, if necessary, to share intellectual property. A major step forward might be the combination of a T-cell vaccine and a good antibody-stimulating vaccine.

Finally, there are manufacturing issues. Ideally, vaccines should be tested in phase 3 trials only if it will be possible to manufacture them in quantities sufficient to immunize tens of millions of people. But it may be worth taking the first candidates through trials more rapidly to establish that a vaccine can indeed protect humans against HIV. All of this will require huge commitment and very large-scale international collaborations. There are signs that this will be possible.

ACKNOWLEDGMENTS

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