longer able to replicate in human cells, although they were infected[101,102]. It was given to more than 100,000 people as a smallpox vaccine, with no reported side effects[101,102]. These attenuated and avipox viruses, which are recombinant for HIV proteins, show promise in macaques[68,98,103,104]. Recombinant MVA has entered trials for both HIV and malaria. In the former, some strong (>500 ELISPOT spot-forming units per million PBMCs) responses were seen, but not in all vaccine recipients (M. Mwau *et al.*, unpublished observations).

An inconsistency in the use of recombinant poxviruses is that not all recipients in outbred species respond. Not all macaques make a CD8$^+$ T-cell response (for example, REF. 105) unless the response is deliberately focused on known immunodominant epitopes. This is typical of immunodominance, whereby the CTL response, which is determined by MHC type, focuses on few epitopes and might not respond to the inserted sequence despite a good response to the whole virus.

The relative inefficiency of single-vaccine modalities in humans and primates has led to methods of augmenting vaccine immunogenicity. Barouch *et al.*[85] reported good responses with 5 mg DNA plus IL-2–Fc fusion protein (IL-2 fused to the Fc fragment of immunoglobulin to greatly increase its half-life *in vivo*) or DNA that encodes IL-2–Fc. Immunization with DNA-coated microparticles can target the dermal–epidermal junction region of the skin, which is rich in Langerhans cells, and this has stimulated CTL responses at low DNA doses[106,107]. When mice were primed with plasmid DNA and then boosted with MVA recombinant for the same DNA sequence, a CTL response tenfold greater than for either vaccine alone was observed[80,98]. The DNA might prime a focused response that is then amplified by the virus boost. This approach works well in macaques[68,98,99,107] (TABLE 3) and is now in phase II trials in humans.

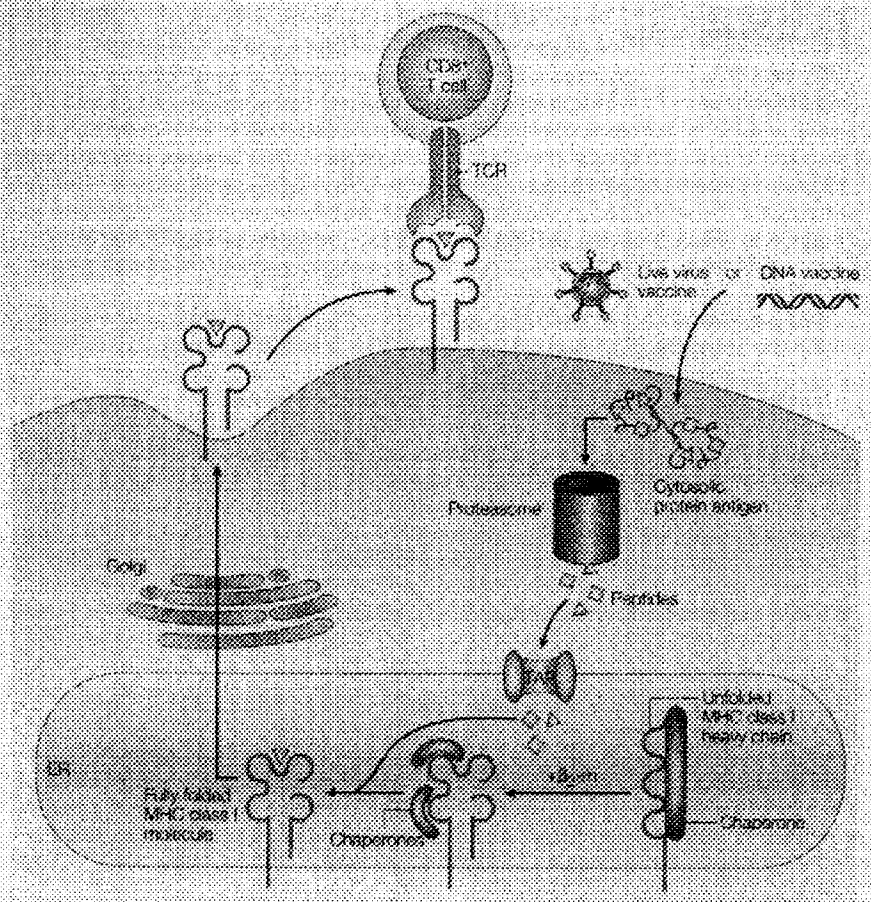


Figure 2 | **MHC class I processing and presentation of antigens.** CD8$^+$ T-cell vaccines enter the class I antigen processing pathway to generate HLA-class-I–peptide complexes on the surface of antigen-presenting cells. Cytosolic protein antigens are generated as a result of vaccination. These are degraded into peptides by the proteasome and transported into the ER. In the ER, peptides become associated with newly generated MHC class I molecules and are transported to the cell surface where they stimulate CD8$^+$ T cells. β2-m, β2-microglobulin; HLA, human leucocyte antigen; ER, endoplasmic reticulum; Tap, transporter for antigen processing; TCR, T-cell receptor.

What is a good CTL response?

Very little attention has been focused on what constitutes a good CTL response. There are few data on the level of CTL response that is needed to protect. Sex workers who are exposed but uninfected are protected from HIV infection and make CD8$^+$ T-cell ELISPOT responses of around 50–100 spot-forming units per 10^6 PBMCs — a level that should be easily achievable with a vaccine[57,108,109]. However, they need continuing exposure to virus to maintain their protection, so for a non-persisting vaccine, higher levels of CTLs are likely to be needed[109]. Also, it is not clear how broad the response in the sex workers is, and their total response might be two or three times higher than susceptible individuals; it is intriguing that they seem to respond to different epitopes than infected people, which indicates that not all epitopes are equal in this regard[57].

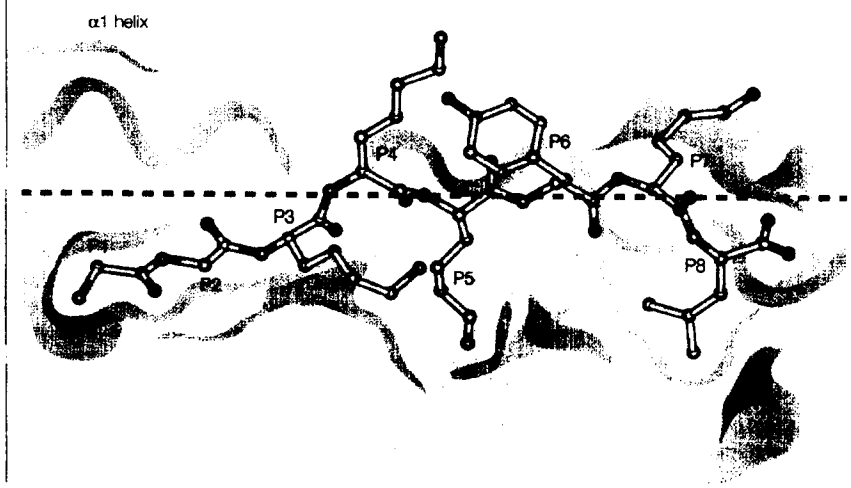
Macaques that were immunized using a prime-boost protocol with DNA and MVA Gag, which induced a very large CD8$^+$ T-cell response to a single epitope (up to 5% of all CD8$^+$ T cells specific for p11C, C-M presented by Mamu A*01) were not protected against a challenge with a high dose of moderately aggressive SIV[98] (D. Watkins also has similar data with the same epitope vaccine; personal communication). So, a response to more than one epitope might be needed and, again, choice of epitope could be crucial. The animals that were protected against SHIV-89.6P challenge generally had peak specific CD8$^+$ T-cell levels between 1% and 10% for known epitopes — by tetramer staining — and probably further responses to other epitopes[85,94,98], but these responses did not persist at this level before challenge.

The studies on virus challenge might show what level of response is needed for each macaque model, but these experiments do not mimic repeated low HIV dose mucosal exposure with a range of variant viruses. In the absence of relevant information, we can only make guesses on the basis of the above models and what we know about control of HIV infection in chronically infected humans. We propose that peak responses in excess of 300 IFN-γ ELISPOTS per million PBMCs to more than one epitope should be achievable and comparative to the macaque data. It is a concern that trials of vaccines that stimulate only a weak CD8$^+$ T-cell response might fail.

Box 1 | **Clades matter**

HIV clades differ by 7–15% in their amino-acid sequences and within the clades, there is significant variation[119]. The length of a cytotoxic T lymphocyte (CTL) epitope is eight to ten amino acids. So, on average, each epitope will differ by one amino acid between clades. Although there are claims for clustering of epitopes in conserved regions of HIV proteins, there has been a bias in ascertainment in identifying conserved epitopes, because the reagents used are based on consensus amino-acid sequences and might not match the infecting virus. Because HIV can escape from CTLs by mutation, and then be transmitted[120], epitopes could even cluster in the more variable parts of the virus. Here, it is reasonable to assume a roughly even distribution of variability in epitopes; that is, one amino acid per epitope.

The figure illustrates how a peptide binds to a human leukocyte antigen (HLA) class I molecule. In this typical example, HIV Gag p17 24–31 GGKKKYKL is situated in the groove of HLA-B8 (REF. 114). Three amino-acid side chains at P4, P6 and P7 point out towards the T-cell receptor (TCR) (above the dashed line), three — P3, P5 and P8 — bind into the groove and two — P1 and P2 — are neutral. Mutations of the TCR-interacting or the HLA-binding amino acids affect T-cell recognition[114]. Burrows *et al.*[112] thoroughly examined the effect of mutation for another peptide in Epstein–Barr virus EBNA3A-specific CTLs; he made all 171 single amino-acid changes in the nonamer peptide; those in the six HLA-binding and TCR-interaction positions severely damaged T-cell recognition by the T cells. This pattern is typical for HLA-B8, but the principles are the same for peptides that bind to other HLA class I molecules. Therefore, it should be expected that two thirds of epitope mutants affect T-cell recognition and that CD8+ T cells will cross react poorly across clades. Reproduced, with permission, from REF. 114 © (1996) The Rockefeller University Press.



Another unknown quantity is the T_H1 CD4+ T-cell response that will accompany a vaccine-induced CD8+ T-cell response. This is likely to be better than that induced by natural HIV infection, during which CD4+ T cells are preferentially infected by HIV and destroyed[36,110], and so might work in the vaccine's favour. In general, T_H1 responses to acute virus infection are present but smaller than the CD8+ T-cell responses[12]. A good vaccine would stimulate this type of response, which might be useful for the vaccine recipient who becomes infected with HIV. The rare non-progressors or very slow progressors after HIV infection have good T_H1 responses[110]. Therefore, any T_H1 response that is stimulated by the vaccine is likely to be beneficial. This could be offset by the particular susceptibility of these T cells to HIV infection, but this is generally discounted.

Clades

The design of an HIV vaccine is complicated by the virus variability in amino-acid sequence. Most studies in macaques have not addressed this issue, and have matched challenge virus to the vaccine. There is some confusion as to the importance of CLADE differences in the CTL response. The data that are available on cross-clade recognition by T cells are limited to a small number of T-cell clones and lines, which are specific for a tiny fraction (<1%) of the total repertoire of HIV epitopes that are seen by human CTLs. Therefore, reports of good cross-reaction or non-cross-reaction can not be representative. Until vaccine recipients can be thoroughly tested for cross-clade reactivity in their T-cell responses over a range of peptide concentrations, a more theoretical approach is appropriate. As argued in BOX 1, two thirds of point mutations in an epitope are likely to adversely affect T-cell recognition. If epitopes are more or less evenly distributed across virus proteins, each will have one or more changes when two clades are compared. There have been theoretical arguments for epitope clustering[111], but experimentally the question is still open. If most epitopes differ between clades, this would have serious implications for vaccine design.

As shown in BOX 1, mutations can interfere with peptide binding to the human leukocyte antigen (HLA) molecule. Such binding is very sensitive to change, and mutation removes the epitope. Changes that affect T-cell recognition are more subtle. Depending on the affinity of the interaction, there might be no effect (which is rare)[112], reduced affinity leading to T-cell antagonism and impaired responses[113,114], or no recognition. In the last case, we might expect a new response that can recognize the peptide to take over, but this often does not happen — a concept that is referred to as 'original antigenic sin'[115].

Therefore, a vaccine that stimulates a response to a single epitope would be very vulnerable to variation in the virus sequence. It would give only poor protection against a different clade of HIV — 33% of that seen with homologous virus exposure, and even virus with the same sequence as that recognized by the induced CTLs would be susceptible to escape by mutation. The latter would be a significant problem if the infection was not controlled before much virus replication occurred. If the vaccine could generate a response to several epitopes the problem would be lessened: a five-epitope response would have an 87% chance of cross-clade recognition of at least one epitope. However, in such a five-epitope response, a third of the responders would recognize only a single cross-reactive epitope and would therefore be very susceptible to virus escape. This is a real problem that has to be addressed in vaccine design.

Virus escape

Escape of SIV mutants after vaccination has already been reported[67,116]. In macaques that were protected against SHIV-89.6P infection by a vaccine, such escape was associated with rapid progression to AIDS[67]. In acute SIV infection, escape mutations at several epitopes are selected by the CTL response[52]. Escape from CTLs also occurs in acute HIV infection[51,117,118] and must be important in the failure of immune control.

Such escape could seriously undermine vaccine prophylaxis of HIV. The only defence is to induce responses to several epitopes at the same time. It is encouraging that