Design and tests of an HIV vaccine

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It is likely that a successful vaccine against HIV will need to stimulate the innate immune system, generate high levels of neutralising antibody, strong cellular immune responses, and mucosal immunity. Early efforts to develop HIV vaccines attempted to use the virus glycoprotein, gp120, to induce neutralising antibody, but did not take into account the trimeric structure of the native glycoprotein or the complex nature of the CD4 and chemokine receptor binding sites. Recently, attention has been focused on cellular immune responses, particularly T-cell cytotoxicity, based on evidence from the SIV model and from exposed and uninfected humans. Recent experiments in macaques and man suggest that a prime boost regimen using DNA and recombinant pox virus is highly effective at stimulating cellular immunity. However, in addition to the problems of generating neutralising antibodies and mucosal immunity, the difficulty of inducing broad cellular responses able to protect against all clades of HIV, remains an important issue.

HIV infection is out of control. Latest World Health Organization (WHO) figures (http://www.unaids.org/) show that there are more than 25 million people infected in sub-Saharan Africa alone and last year there were estimated to be more than 5 million new infections. For most of the world, treatment with anti-retroviral drugs, which effectively contain the infection in industrialised countries, are not available and are unlikely to become affordable. The consequences are drastic lowering of life expectancy and huge human, social and economic problems. A vaccine is much needed, therefore.

An ideal vaccine stimulates four components of the immune system (Fig. 1). It should: (i) elicit neutralising antibody at high titre; (ii) stimulate a cellular (T-cell) immune response, especially cytotoxic T-cells; (iii) stimulate mucosal immunity; and (iv) provoke the innate immune system. The live, attenuated virus vaccines that have been so successful in preventing many devastating diseases around the world probably stimulate all of these immune responses. Unfortunately, a live attenuated HIV is not an option because, though effective in the macaque – SIV system, the ‘vaccine’ causes persisting infection and some animals develop AIDS. Safer, killed-virus vaccines stimulate only neutralising
antibody and are effective in some cases, but it is generally agreed that live virus vaccines are more effective. Because the live virus option is barred for HIV, we are being forced to design a new type of vaccine that can safely stimulate protective immunity.

Early attempts to develop a vaccine focused on trying to stimulate neutralising antibody using preparations of the virus glycoprotein gp120 which is responsible for attaching the virus to CD4 and CCR4 or CXCR4, the virus receptors on cells. Although it is possible to stimulate neutralising antibody alone is enough to give good protection; in other cases, most or all responses are needed. A designed HIV vaccine should ideally stimulate all these types of immune response and it may be necessary to build several distinct vaccine components to achieve full protection.

Fig. 1 The immune response stimulated by an ideal vaccine. Live attenuated vaccines come closest to fulfilling these conditions, but are too risky to use in humans for HIV. With currently used live attenuated vaccines, such as MMR or polio, the relative proportions of each type of response varies with vaccine. In some cases, neutralising antibody alone is enough to give good protection; in other cases, most or all responses are needed. A designed HIV vaccine should ideally stimulate all these types of immune response and it may be necessary to build several distinct vaccine components to achieve full protection.
evade any antibody response. The chemokine receptor binding site is not normally exposed and only appears for fractions of a second after CD4 binding before locking on to CCR5 or CXCR4. Thus, the envelope of this virus has evolved in an extraordinary way to effectively avoid neutralisation by antibodies.

It is not surprising, therefore, that gp120 vaccine recipients who made antibody could still become infected with HIV. There are phase 3 trials of a gp120 vaccine in progress in the US and Thailand, but expectations for success are not high. This has stimulated an interest in vaccines that stimulate T-cell immune responses. At the same time, it is imperative that attempts to find a way of stimulating good neutralising antibodies continue.

**T-cell immune responses to HIV**

HIV infects cells that carry CD4 and either CCR5 or CXCR4 on their surface, T helper cells and macrophages. Dendritic cells are probably not infected, but can bind virus on their surface and infect T-cells that are in contact. Studies of the reservoirs of HIV infected cells show that most of the virus is rapidly replicating in activated CD4+ T-cells, with a half-life of productively virus-infected cells of about a day. There is a smaller longer-lived reservoir in tissue macrophages with a half-life of around 6 weeks. Then there is a small, but problematical, reservoir of memory T-cells that has a half-life of many months. Many of these cells carry an integrated virus sequence in their genome, but are silently infected. These cells are problematic because it has so far proved impossible to eliminate virus from this population by drugs or the immune response.

HIV infected CD4+ T-cells are damaged and dysfunctional. Because they are likely to come into contact with HIV infected or associated antigen presenting dendritic cells, the CD4+ T-cell response to HIV is weak and lost – many of these T-cells appear to be deleted early in the infection. When virus replication is active, there is often a non-specific activation of both CD4 and CD8 T-cells with loss of these cells by fas-induced apoptosis. In this environment, it was some surprise that the CD8+ T-cell (or cytotoxic T lymphocyte, CTL) response to HIV is vigorous and similar in quantity to those against other persisting viruses, such as Epstein-Barr virus (EBV) and cytomegalovirus (CMV).

There is good evidence that the CD8+ T-cell response to HIV plays an important role in controlling the infection. If these T-cells are removed by anti-CD8 antibody infusion, in macaques infected with SIV, the virus titre rises sharply, only to fall when the effect of the antibody in removing CD8+ T-cells wears off. If the antibody is given in the acute phase of infection, the virus is not controlled after the initial spike of viraemia. Further evidence from both humans and macaques, that the
virus escapes from CD8+ T-cell responses, implies that the T-cells exert selective pressure.\(^\text{14}\)

The CD8 T-cell response is thought to be largely responsible for the partial control of the virus during untreated chronic infection. However, the control ultimately fails possibly because of the almost total loss of any CD4+ T-cell help as these cells are decimated. The continuing escape of virus from CD8+ T-cell control is also thought to play a part, continually challenging the CD8+ T-cells to respond to new epitopes. Nevertheless, it is remarkable that infected persons can survive for several years, in some cases more than 20 years.

**Cytotoxic T lymphocyte vaccines**

Given that CTLs can control the on-going infection fairly well and are well known to control other persistent virus infections (e.g. EBV and CMV), attention has turned to vaccines which can stimulate CD8+ T-cell responses. The rationale is not quite as obvious as for neutralising antibody that can bind to virus envelope and prevent cell attachment or fusion. CTLs cannot prevent virus entering cells, except by secreting the cytokines that bind to CCR5, but this is thought not to be of great impact. CTLs can kill virus-infected cells before they start to replicate virus – it takes about 24 h from virus entry to the onset of production; in this time, virus proteins are made and once that occurs some will be degraded and peptide fragments routed to the endoplasmic reticulum and then into HLA class I molecules to be presented at the cell surface and stimulate CTLs. If cells are killed by CTLs before producing virus, the infection is controlled. Thus a vaccine-induced CTL population will probably allow cells to be infected and then kill them, possibly eliminating the infection.

The same principles arise for other viruses and there is good evidence in mice that vaccine-induced CTLs can control infections. In experiments with viruses such as LCMV\(^\text{17,18}\), RSV, and influenza\(^\text{19}\), it is clear that CTLs alone can protect mice so that the vaccinated animals survive whilst the controls die. The protected animals usually show evidence of infection with several logs reduction of virus titre and ultimate clearing of the virus. In effect, the vaccine reduces the challenge dose of the virus to one that the host can control by making an amplified immune response – note that the host will respond by making antibody responses as well as CTLs.

The proof of principle is confirmed for SIV in macaques. Several studies have shown partial protection against SIV challenge after immunisations with vaccines that stimulate CTLs, in some cases only CTLs\(^\text{20,21}\). Most impressive are four studies\(^\text{22-25}\) where the challenge was with the very aggressive SIV/HIV hybrid virus SHIV89.6P which destroys CD4 T-cells.
within weeks of infection and causes rapid death from AIDS. Animals immunised with vaccines that stimulate CTL responses to virus proteins, such as gag, were infected but had virus titres 1000-fold lower than the unvaccinated controls and survived while the controls died\(^\text{22–25}\). One vaccinated animal did succumb later when the CTL response was undermined by mutation in the epitope so that the dominant CTL response was no longer effective\(^\text{26}\). This is good evidence that the CD8\(^+\) T-cells were controlling the infection.

The above data are encouraging that a similar approach in humans might be effective. It is noteworthy that neither in the mice nor in the macaque studies were animals wholly protected against the virus tested. However, usually massive doses of virus were given, in order to ensure that all controls in small studies were fully infected. Humans are exposed to low doses of HIV through sexual contact and it may take many contacts to become infected even in high risk populations\(^\text{27}\). Thus the human ‘challenge’ dose of virus may be many fold lower than these challenges and the threshold for infection could be moved so that some may be completely protected.

**Exposed uninfected humans**

There are well-founded reports of humans who have remained uninfected despite definite and regular contact with HIV. The most striking are the sex workers in Kenya where around 5% remain uninfected despite very high levels of contact – in the whole sex worker population around 90% are infected. Rowland-Jones et al\(^\text{28}\) have shown that these women make CD8\(^+\) T-cell responses to HIV in their blood and genital mucosa\(^\text{29}\). They do not make neutralising IgG antibody responses\(^\text{30}\). They do not have the genetic polymorphisms in chemokine receptors that protect and their cells are fully infectible by HIV \textit{in vitro}\(^\text{28}\). This suggests that they are protected by the CD8\(^+\) T-cell response. Indeed they are not infected so this suggests that, under some circumstances, CD8\(^+\) T-cells can completely protect. Kaul et al\(^\text{31}\) have shown that some of the women who have changed their life-styles and ceased sex work have later become infected. This feels immunological, suggesting that they need regular contact with the virus to maintain their protection. But this might mean that it is necessary to maintain the CTLs in a state of activation to offer best protection, something that present vaccines might not be able to do.

**Generating CD8\(^+\) T-cell responses with a vaccine**

Live virus vaccines elicit immune responses that are similar to natural virus infections – both humoral, neutralising antibody, and CD4\(^+\) and
CD8+ T-cells. Killed virus vaccines and viral subunit protein vaccines are poor at stimulating CD8+ T-cells. Thus the early gp120 vaccines did not stimulate CTL responses.

For a vaccine to elicit a CTL response, the vaccine has to get into the class I antigen processing pathway that delivers peptides to newly folding class I HLA molecules in the endoplasmic reticulum (ER)\textsuperscript{32}. Essentially any cytoplasmic protein, especially if unstable, will be degraded by the ubiquitin–proteasome pathway, generating many peptide fragments. These are transported by the specialised transporter associated with antigen processing (TAP) into the ER where the peptides are delivered to chaperoned class I HLA molecules. Those that meet specific sequence criteria – different for different HLA types – bind in the peptide binding groove and exit to the cell surface. There those that are foreign stimulate CD8+ T-cells. For reasons that are not well understood, there is a hierarchy of immunodominance such that some peptides are more efficient in stimulating a CD8+ T-cell response than others.

Vaccines that deliver protein to the cytosol stimulate good CD8+ T-cell responses, particularly effective are transfected DNA and recombinant viruses\textsuperscript{21,23-25,33}. The former though good in mice is less efficient in larger animals and primates, but at least the vaccine can be wholly designed. The latter are very effective at stimulating CD8+ T-cell responses, but often to the virus rather than the insert – a reflection of immunodominance. Recently, it has become clear that combinations of DNA followed by a virus recombinant for the same DNA sequence is an effective way to stimulate good CD8+ T-cell responses in macaques and possibly humans\textsuperscript{21,23-25,33}. Another approach is to enhance the response to the DNA by putting it on adjuvant beads or adding cytokines\textsuperscript{34}.

**Phase 1 trials in humans**

Our approach has been to design a vaccine for East and Central Africa based on the A clade of HIV\textsuperscript{35}. This is the predominant virus strain in that part of Africa. It differs from the B strains of industrialised countries by 7–20\% in virus protein sequence. For reasons discussed below, we think it is important to match the clade of the virus to the circulating virus in the country. Because gag stimulates the strongest CTL responses in infected persons and is more conserved, we chose gag for the vaccine. We also added a string of 23 epitopes from other virus proteins presented by common HLA molecules\textsuperscript{35}. The DNA was entirely synthetic and was optimised to use the codons most often used in mammalian cells. The DNA was put into a plasmid pTHr which could be selected without the use of antibiotics. The same DNA was put into modified vaccinia virus Ankara (MVA), an attenuated form of vaccinia.
which was passaged extensively on chick embryo fibroblasts so that while it can infect human cells it does not replicate. MVA has an excellent safety profile.

Both constructs stimulate good CTL responses in mice and in macaques. In primates, better responses were seen when the animals were primed with DNA and then boosted with rMVA. After extensive safety, distribution, and persistence studies in mice, the vaccine was approved by the MCA for trials in humans after appropriate ethical and other permissions were obtained. The trials started in late 2000.

The DNA immunisations gave measurable CD8+ T-cell responses in most volunteers. Surprisingly, the responses got better with time and the strongest seen were 6 months and 1 year after the immunisations. The level of response, detectable by counting T-cells that produced IFN-γ after brief culture in vitro with antigenic peptides based on the vaccine sequence, was generally 100–1000 cells per million peripheral blood mononuclear cells. Some volunteers, however, did not respond. After MVA, the responses were similar though somewhat stronger. Studies of DNA plus MVA are in progress.

Recently Emini et al. have presented data on trials of a B clade gag vaccine, DNA and recombinant adenovirus expressing the same DNA. Their findings were similar. Quite good responses to DNA alone were seen with better late responses though they continued to immunise over several months. Responses to the adenovirus were stronger but not seen in all volunteers.

In both studies, the safety profiles looked good. These preliminary phase 1 trials are, therefore, encouraging. Given the animal data that DNA priming followed by recombinant virus boost works significantly better, it seems likely that this approach in humans could enhance both the level of response and the proportion of responders. If this is found to be correct, it would be appropriate to move these vaccines forward to phase 3 efficacy trials.

**Do clades matter?**

An issue that is becoming contentious is whether it is necessary to match the clade of the vaccine with that of the prevailing virus in populations to be vaccinated. If so, a B clade vaccine made for industrialised countries would not be suitable for non-industrialised countries where the vaccine is most needed. Commercial pressures favour production of B clade vaccines.

Emini et al. addressed this issue by looking at the cross reactivity of their vaccine-induced responses on peptides matched to the other major clades A and C. Some responses
were cross-reactive and some were not; a more detailed analysis is needed because the data presented were generated with peptide pools and so may have over-estimated the degree of cross reactivity. Also, the use of a relatively high peptide concentration in the Elispot assay used could mask important differences in recognition of variant epitopes.

The protein sequence differences between the clades mean that each epitope is likely to differ by 1–2 amino acids. An epitope is 8–10 amino acids long and, of those, 3 are normally involved in binding to the HLA molecule, 2 or 3 interact with the T-cell receptor, and the rest are relatively neutral. Changing amino acids involved in either HLA binding or T-cell receptor binding has adverse effects on the T-cell response. Thus a single amino acid change in an epitope could have a 66% chance of not being recognised. Experimental data where each position is changed to every possible alternative bear this out. Even conservative amino acid changes, which are more likely, have these strong effects.

This would be devastating if the vaccine-induced T-cell response is focused on one epitope, or if one dominated much more than the others. This is certainly seen in some natural virus infections and after some vaccinations in macaques. The problem could be solved by designing the vaccines to stimulate T-cell responses equally to several epitopes. If each epitope differs in one amino acid between clades, a 1-epitope response has a 66% chance of not working against a different clade, a 5-epitope response has only a 13% chance of not working. However, in a 5-epitope response, nearly a third would only respond to one epitope. This opens the possibility of virus escape – selection of new mutants of HIV that would no longer be controlled by the vaccine. This has been reported in two trials in macaques where the vaccine induced CD8+ T-cell response was evaded by exactly that kind of mutation.

**Broadening the T-cell response**

The virus sequence variability can, to some extent, be dealt with by broadening the CTL response induced by the vaccine. However, this is not so easy to achieve. It is encouraging that preliminary results in the phase 1 trials of DNA and recombinant viruses suggest that some immunised volunteers respond to more than one epitope. One possibility is to add more proteins to the construct – both Emini et al. and Mwau et al. (manuscript submitted for publication) used gag-based vaccines. However, this will not necessarily result in CTL responses to more epitopes. The CTL response tends to focus on a few epitopes, best exemplified by the acute CTL response to EBV in people who have HLA B8 – more that 40% of CD8+ T-cells are specific for a single epitope in one of the hundreds of virus proteins available. This suggests that simply adding more proteins to
a vaccine construct may not be the way to broaden the immune response. It might be better to immunise with several different constructs each expressing a different virus protein. That way the immune system might deal with each as if it was a separate invader.

**Will the vaccine give complete protection?**

Animal studies where mice were immunised to generate anti-virus CTL and then challenged with the virus (e.g. influenza, respiratory syncitial virus, lymphocytic choriomeningitis virus) showed that animals were protected and survived. However, they were usually infected but with a much lower virus level and were able to clear the virus. Similar results are reported for SIV in macaques where CTLs induced by vaccines do not prevent infection, but virus loads can be reduced by 1000-fold compared to controls and the animals survive with normal CD4 T-cell counts, while the controls die. In all of these studies, doses of challenge virus were very high. Given the apparent complete protection in the Nairobi sex workers, it is possible that CTLs induced by a vaccine could give complete protection by moving the threshold of the amount of virus that establishes infection (Fig. 2). It may be necessary, however, to maintain the memory T-cells in an active ‘effector’ state. This could require repeated immunisations or a persisting virus antigen. The latter seems the obvious way to go, but raises safety issues as there

![Graph showing the hypothetical probability of infection over a range of virus exposure doses.](image)

**Fig. 2** Vaccination could shift the threshold for infection with HIV. The figure shows the hypothetical probability of infection over a range of virus exposure doses. In macaque challenge experiments, the dose of SIV given is chosen to guarantee 100% infection so approximates to the right hand arrow. In human sexual exposure, the chances of a single contact resulting in infection are low, approximating to the left hand arrow. Vaccination could move the curve to the right and thus only give partial protection in macaques, manifest by low virus load after infection, but the same vaccination could completely protect against infection at the lower dose.
is a theoretical risk as long as the antigen persists – it will be very hard to prove safety in a realistic time frame.

If the vaccine does not protect completely, it will be hard to establish efficacy as this will only be possible by measuring virus levels after infection. Although a lot less than perfect, reduced virus levels could be worthwhile in prolonging survival and reducing transmission.

Conclusions

The design of an HIV vaccine is a formidable challenge. The main issues are the level of T-cell response obtained, safety, ensuring that the response is broad enough to cope with the virus variability, and keeping the T-cells as active as possible. If these are attended to, the vaccine has a good chance of offering some benefit. The vaccine will be short of the ideal vaccine, but it would be possible to add a neutralising antibody producing vaccine at some point to enhance the chances of both working. Induction of innate immune responses and mucosal immunity can also be added later. This requires a huge research effort, but the ultimate benefit would be beyond measurement.

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