Prospects for an AIDS vaccine

Andrew McMichael

Acquired immunodeficiency syndrome (AIDS) was first identified at the end of 1981.[1] In 1983, the causative virus, human immunodeficiency virus (HIV-1), was identified[2], almost certainly having entered humans from a reservoir in chimpanzees, possibly on several occasions.[3] Since 1981 the virus has spread to cause possibly the most serious epidemic infection in human history. The virus has diversified into seven major subtypes or clades, the splits probably occurring either early in the epidemic or before it transferred from chimpanzees to humans. Currently, more than 40 million people are infected with HIV, with five million new infections annually (http://www.unaids.org/hivaidsinfo/). Three-quarters of the infections occur in sub-Saharan Africa, but there is also an alarming spread in the Indian subcontinent and China. Drug treatments have made a major difference to life expectancy of infected persons in Western countries, but they are not widely available in Africa and unlikely ever to be accessible to those who most need them. The drugs do not cure the infection, while side effects that limit their use and drug-resistant viruses are gradually emerging.

Vaccines against human immunodeficiency virus

Glycoprotein vaccine

There is a desperate need for a vaccine, but progress so far has been slow. The earliest target was the virus envelope which plays a key role in virus attachment to and entry into target cells (Fig 1). Various forms of glycoprotein (gp)120 were made using recombinant DNA techniques, but it proved difficult to stimulate antibodies which neutralised primary virus isolates.[4] The reasons became clear when the structure of gp120 was elucidated.[5,6] Native gp120 is a trimer and heavily glycosylated, which effectively makes it poorly immunogenic. Highly variable loops on the exposed surface can easily mutate to evade antibody immune responses and guard the highly conserved sites that bind to the virus cell receptors, CD4 and CCR5 (or CXCR4). The CD4 binding site is deeply recessed and inaccessible to most antibodies, and the CCR5 binding site is only exposed once CD4 has bound gp120, probably only for milliseconds.

HIV has thus evolved so well to escape from neutralising antibodies that it may be impossible to make a vaccine that can effectively prevent infection by this virus. A phase III efficacy trial of a gp120 vaccine recently reported as offering no protection (www.vaxgen.com).

Cytotoxic T lymphocytes

In the pessimistic expectation that the gp120 vaccine would not offer significant protection against primary HIV infection, attention turned to other arms of the immune response, in particular vaccines that can stimulate CD8 T cells (cytotoxic T lymphocytes (CTL)). Several lines of evidence indicate that CTLs are important in controlling virus in HIV infected people:[7]

- the kinetics of the early CTL response peak as the early viraemia falls[8]
- the adverse effects of removing CD8 T cells in macaques in simian immunodeficiency virus (SIV) infection when virus control fails[9,10]
- the selection of escape mutants by CTL[11,12]
- given the key role of HLA types in selecting the epitopes seen by CTL, the clear protective (eg HLA B57 and HLA B27) or enhancing (eg HLA B35) effects of different HLA types on progression to AIDS.[13,14]

CTL work by killing virus infected cells before they have a chance to replicate new virus particles. This may be enhanced by release of various cytokines (eg interferon (IFN) γ, tumour necrosis factor a, macrophage inflammatory protein (MIP)-1α and MIP-1β.

Vaccines that stimulate CTL cannot protect against actual virus infection unless they kill incoming infected cells that match HLA type. Most likely, the infection occurs but the CTL can rapidly clear the infection before it takes off. There is good evidence in mice with other virus challenges that CTL induced by vaccines can offer substantial protection, though not sterilising immunity (reviewed in ref 15). Similarly, infection with aggressive SIV or SIV-HIV hybrid viruses could not be prevented in recent studies in macaques with CTL-inducing vaccines, but the infection was greatly attenuated – more than a 1,000-fold reduction of virus level and lack of pathogenic effects such as destruction of CD4 T cells .[16,19]

These studies challenge animals with virus doses far greater than those in human sexual exposure,

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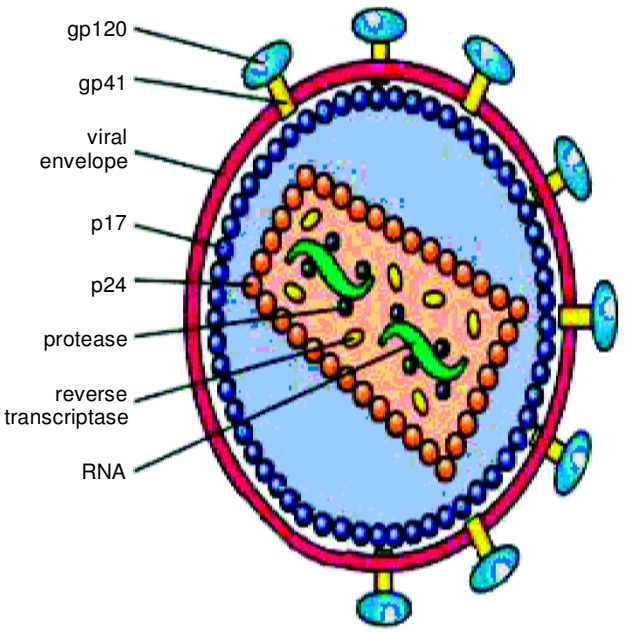

Fig 1. Human immunodeficiency virus showing the structural proteins of the virus. The gag proteins p17 and p24 form the virus matrix and capsid, respectively (gp = glycoprotein) (from www.avert.org).

which may be encouraging, and it is perhaps even possible that vaccinated humans could repel virus. Some support for this comes from studies in highly HIV-exposed sex workers, about 5% of whom appear to resist infection.[20] These women make HIV-specific CTL responses, which may be what protects them.[20]

New approaches

Induction of CTL by classical vaccines has not previously been studied in detail. Live attenuated virus vaccines can stimulate good CTL responses as well as neutralising antibodies, but inactivated and subunit vaccines cannot.[21] Inactivated HIV is not an option as a vaccine for safety reasons, so new approaches have had to be devised to focus on this type of immune response. The key is to enter the antigen processing pathway by which virus proteins are normally degraded to peptides, a few of which are selected to bind to the HLA class I proteins and so stimulate CTL. Plasmid DNA and recombinant viruses can stimulate CTL responses in mice, with particular combinations of prime and boost most effective.[22] One of these is to prime with plasmid DNA encoding the virus protein and then boost with an attenuated vaccinia virus, modified vaccinia virus Ankara (MVA), recombinant for the same segment of DNA (Fig 2).[23,24] This approach has also been shown to be particularly effective at stimulating CTL in macaques, and indeed can protect them from the lethal infection with SIV/HIV hybrid virus.[17]

We designed a vaccine based on the consensus amino acid sequence of A clade virus gag proteins p24 and p17.[25] The DNA was made synthetically using human preferred codons (which should enhance expression in transfected cells). DNA encoding 25 epitopes from other HIV proteins presented by common HLA types was also added. Finally, an epitope recognised by T cells in BALB/c mice and another presented by the common rhesus macaque major histocompatibility complex type Mamu A*01 were added, so that the vaccine could be tested in mice and rhesus monkeys as well as humans.[25] The same DNA sequence was inserted into the thymidine kinase gene of MVA. Both the DNA and MVA vaccines were made to good manufacturing practice quality and tested for toxicity, distribution and persistence after immunisation of mice. The vaccines were shown to be capable of stimulating CTL responses in mice and macaques.

Phase I trials Initial phase I trials (conducted under a Doctors and Dentists Exemption (DDX) from the Medicines Control Agency and with Oxford Ethical Committee approval) tested DNA alone, MVA alone and the DNA prime MVA boost combination in small numbers of HIV-negative, low-HIV risk volunteers. Both vaccines were generally well tolerated. One volunteer suffered a febrile illness with vomiting two days after her MVA immunisation. It is uncertain whether this was caused by the vaccine, and it was found in no other volunteer.

Nearly all volunteers made measurable CTL responses to HIV,

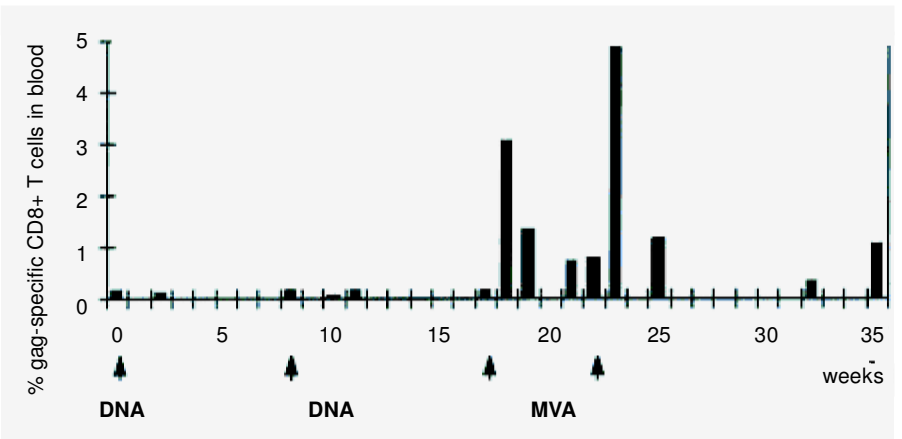

Fig 2. An example of priming with a plasmid DNA vaccine and boosting with a modified vaccinia virus Ankara (MVA) recombinant for the same segment of DNA. A rhesus macaque was immunised by intradermal injection at the times shown. The immune response was measured from peripheral blood mononuclear cells using a tetrameric construct of the simian immunodeficiency gag peptide bound to the HLA-like class I molecule Mamu A*01 to quantitate antigen-specific T cells by flow cytometry (from ref 24, with permission).

detected by the elispot technique which counts T cells in peripheral blood lymphocytes that make IFNγ during incubation for 24 hours, with pools of peptides representing the gag protein sequence in the vaccine. The MVA responses were stronger than the DNA responses and almost entirely mediated by CD8+ T cells.[26] In volunteers given MVA after DNA priming there were good responses, but in this case both CD8+ and CD4+ T cells responded. (These early preliminary results will be described in detail elsewhere.[26]) Similar trials have been conducted in Nairobi with generally comparable findings, and broadly similar results have been reported by Emini *et al*[27] in humans using DNA and recombinant adenovirus immunisation. A more extensive, double-blind, placebo-controlled trial involving 120 volunteers is currently in progress in Oxford and Imperial College at St Mary's, London.

It is encouraging that the vaccines appear to generate CTL immune responses in humans. There is optimism that it will be possible to improve on the level of response by manipulating vaccine dose and route of injection.

Issues in vaccine development

Given that approaches of this type will be capable of stimulating the desired type of immunity, various issues have to be addressed if the vaccine is to have a chance of protecting against HIV infection.

Virus variability

A major issue concerns virus variability. We have targeted HIV in East and Central Africa where the A clade is predominant. Others have suggested that clade matching may not matter[27] but we disagree. The clades differ by 10–25% in their protein amino acid sequences, so each epitope of nine (8–11) amino acids is highly likely to contain at least one amino acid difference.

This is borne out by inspection of the Los Alamos HIV epitope sequence database (http://hiv-web.lanl.gov/content/immunology/). As a general rule, about six of the nine amino acids in an epitope make close and specific contact through their side chains with either the presenting HLA molecule or the T cell receptor. These contacts are extremely sensitive to change, and many such mutations are either not presented by the HLA molecule or not recognised by T cells specific for the original sequence. Thus, approximately two-thirds of epitopes with a single amino acid change are no longer recognised. This has been confirmed experimentally[28] and makes a strong argument for matching a vaccine to the predominant virus clade.

The problem would be less critical if the vaccine could stimulate a response to several epitopes simultaneously, but this seems unpredictable at present. However, even a five-epitope response could leave a third of people immunised with a non-clade matched vaccine dependent on only one epitope. This, in turn, gives the virus an easy route to escape by mutating a single epitope – which has been seen in the macaque DNA vaccine SIV-HIV challenge model.[29]

Clade matching

Clade matching is important, but there is still considerable variability within a clade so there is a need to generate broad T cell responses to many epitopes. A serious problem is the phenomenon of immunodominance, where CTL tend to respond to few epitopes,[30] sometimes even only one, when many may be available. To overcome this difficulty it may be necessary to break up the vaccine into several separated parts which will then fool the immune system into responding to each of them.

T cell response duration

Another major issue is the duration of the T cell response and the state of T cell activation. A vaccine should simulate the T cell response in acute infection: that is, a massive expansion of reacting T cells, normally followed by a fall in T cell numbers and a change in the state of activation (memory T cells).[31] Some consider that for a CTL vaccine to work, T cells will have to be in a highly activated state.[32] In this condition, the T cells might be able to kill virus infected cells within hours of the initial infection. This could be difficult to achieve unless the vaccine antigen persists and gives a continuous stimulus to the T cells. It has led to suggestions of using persisting viruses (such as recombinant herpes viruses) as vectors for vaccine, but there are safety concerns.

Repeated boosting immunisations may be an option, but would not be practicable for intervals of less than 12 months. DNA persists at the site of injection and probably draining lymph glands (T Hanke *et al*; unpublished data) but may not be sufficiently immunogenic to maintain a strongly activated T cell population. It could be relevant that the highly exposed, but uninfected sex workers need to maintain their antigenic exposure to stay resistant to HIV infection.

Conclusion

There are good reasons to think that a vaccine that stimulates a strong CTL response could protect against HIV infection, possibly only partially but allowing better control of the virus and slower progression to AIDS. Another view is that it may be easier to protect against the low-dose virus in human sexual contacts.

Virus variability is likely severely to limit the effectiveness of a vaccine-induced T cell response. This can be partly controlled by matching vaccine to predominant virus strains but could still be problematic, especially if the immune response is too focused, allowing virus escape. Major efforts may be needed to ensure that vaccine-stimulated responses are broad – to several epitopes.

These objectives are achievable, and should be addressed before a vaccine is tested in phase III clinical trials. There is then a good chance that the vaccine would work.

References

1 Gottlieb MS, Schroff R, Schanker HM, Weisman JD *et al*. *Pneumocystis carinii* pneumonia and mucosal candidiasis in previously healthy

homosexual men: evidence of a new acquired cellular immunodeficiency. *N Engl J Med* 1981;**305**:1425–31.
2 Barre-Sinoussi F, Chermann JC, Rey F, Nugeyre MT *et al*. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* 1983;**220**:868–71.
3 Korber B, Gaschen B, Yusim K, Thakallapally R *et al*. Evolutionary and immunological implications of contemporary HIV-1 variation. Review. *Br Med Bull* 2001;**58**:19–42.
4 Connor RI, Korber BT, Graham BS, Hahn BH *et al*. Immunological and virological analyses of persons infected by human immunodeficiency virus type 1 while participating in trials of recombinant gp120 subunit vaccines. *J Virol* 1998;**72**:1552–76.
5 Wyatt R, Kwong PD, Desjardins E, Sweet RW *et al*. The antigenic structure of the HIV gp120 envelope glycoprotein. *Nature* 1998;**393**:705–11.
6 Kwong PD, Wyatt R, Robinson J, Sweet RW *et al*. Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. *Nature* 1998;**393**:648–59.
7 McMichael AJ, Rowland-Jones SL. Cellular immune responses to HIV. Review. *Nature* 2001;**410**:980–7.
8 Wilson JD, Ogg GS, Allen RL, Davis C *et al*. Direct visualization of HIV-1-specific cytotoxic T lymphocytes during primary infection. *Aids* 2000;**14**:225–33.
9 Schmitz JE, Kuroda MJ, Santra S, Sasseville VG *et al*. Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. *Science* 1999;**283**:857–60.
10 Jin X, Bauer DE, Tuttleton SE, Lewin S *et al*. Dramatic rise in plasma viremia after CD8(+) T cell depletion in simian immunodeficiency virus-infected macaques. *J Exp Med* 1999;**189**:991–8.
11 Phillips RE, Rowland-Jones S, Nixon DF, Gotch FM *et al*. Human immunodeficiency virus genetic variation that can escape cytotoxic T cell recognition. *Nature* 1991;**354**:453–9.
12 McMichael A. T cell responses and viral escape. Review. *Cell* 1998;**93**:673–6.
13 Carrington M, Nelson GW, Martin MP, Kissner T *et al*. HLA and HIV-1: heterozygote advantage and B*35-Cw*04 disadvantage. *Science* 1999;**283**:1748–52.
14 Gao X, Nelson GW, Karacki P, Martin MP *et al*. Effect of a single amino acid change in MHC class I molecules on the rate of progression to AIDS. *N Engl J Med* 2001;**344**:1668–75.
15 McMichael A, Hanke T. The quest for an AIDS vaccine: is the CD8+ T-cell approach feasible? Review. *Nat Rev Immunol* 2002;**2**:283–91.
16 Barouch DH, Santra S, Kuroda MJ, Schmitz JE *et al*. Reduction of simian-human immunodeficiency virus 89.6P viremia in rhesus monkeys by recombinant modified vaccinia virus Ankara vaccination. *J Virol* 2001;**75**:5151–8.
17 Amara RR, Villinger F, Altman JD, Lydy SL *et al*. Control of a mucosal challenge and prevention of AIDS by a multiprotein DNA/MVA vaccine. *Science* 2001;**292**:69–74.
18 Shiver JW, Fu TM, Chen L, Casimiro DR *et al*. Replication-incompetent adenoviral vaccine vector elicits effective anti-immunodeficiency-virus immunity. *Nature* 2002;**415**:331–5.
19 Rose NF, Marx PA, Luckay A, Nixon DF *et al*. An effective AIDS vaccine based on live attenuated vesicular stomatitis virus recombinants. *Cell* 2001;**106**:539–49.
20 Rowland-Jones SL, Dong T, Fowke KR, Kimani J *et al*. Cytotoxic T cell responses to multiple conserved HIV epitopes in HIV-resistant prostitutes in Nairobi. *J Clin Invest* 1998;**102**:1758–65.
21 Webster RG, Askonas BA. Cross-protection and cross-reactive cytotoxic T cells induced by influenza virus vaccines in mice. *Eur J Immunol* 1980;**10**:396–401.
22 Schneider J, Gilbert SC, Blanchard TJ, Hanke T *et al*. Enhanced immunogenicity for CD8+ T cell induction and complete protective efficacy of malaria DNA vaccination by boosting with modified vaccinia virus Ankara. *Nat Med* 1998;**4**:397–402.
23 Hanke T, Blanchard TJ, Schneider J, Hannan CM *et al*. Enhancement of MHC class I-restricted peptide-specific T cell induction by a DNA prime/MVA boost vaccination regime. *Vaccine* 1998;**16**:439–45.
24 Hanke T, Samuel RV, Blanchard TJ, Neumann VC *et al*. Effective induction of simian immunodeficiency virus-specific cytotoxic T lymphocytes in macaques by using a multiepitope gene and DNA prime-modified vaccinia virus Ankara boost vaccination regimen. *J Virol* 1999;**73**:7524–32.
25 Hanke T, McMichael AJ. Design and construction of an experimental HIV-1 vaccine for a year-2000 clinical trial in Kenya. *Nat Med* 2000;**6**:951–5.
26 Mwau *et al* (submitted).
27 Emini EA. http://www.retroconference.org/2002/about.htm
28 Burrows SR, Silins SL, Moss DJ, Khanna R *et al*. T cell receptor repertoire for a viral epitope in humans is diversified by tolerance to a background major histocompatibility complex antigen. *J Exp Med* 1995;**182**:1703–15.
29 Barouch DH, Kunstman J, Kuroda MJ, Schmitz JE *et al*. Eventual AIDS vaccine failure in a rhesus monkey by viral escape from cytotoxic T lymphocytes. *Nature* 2002;**415**:335–9.
30 Yewdell JW, Bennink JR. Immunodominance in major histocompatibility complex class I-restricted T lymphocyte responses. Review. *Annu Rev Immunol* 1999;**17**:51–88.
31 Appay V, Papagno L, Spina CA, Hansasuta P *et al*. Dynamics of T cell responses in HIV infection. *J Immunol* 2002;**168**:3660–6.
32 Zinkernagel RM. On immunological memory. Review. *Philos Trans R Soc Lond B Biol Sci* 2000;**355**:369–71.