Strategies for AIDS vaccines

E. J. Stott and G. C. Schild

National Institute for Biological Standards and Control Potters Bar, Hertfordshire EN6 3QG, UK

In the global AIDS epidemic, over half of all infections have occurred in people less than 25 years old resulting in profound social, economic and demographic consequences. Current estimates indicate that the present 15 million HIV infections will increase to over 30 million by the end of the millennium. For most countries a safe and effective vaccine offers the only hope of controlling the spread of this disease.

The development of an effective vaccine against HIV is beset with formidable obstacles. Despite these difficulties, substantial progress has been made towards developing effective strategies for vaccination. Human clinical trials and animal models for AIDS, particularly simian immunodeficiency virus (SIV) infection of macaques, have proved invaluable in this quest. Inactivated virus vaccines induced potent protection in this model, but subsequent studies revealed that protection was mediated by antibody to cellular proteins present in the vaccine preparations and on the surface of infecting virions. This surprising observation has provided an alternative and complementary approach to the development of vaccines against HIV in man which is still being pursued. Live attenuated vaccines were initially dismissed as far too hazardous. However, the concept has recently been reexamined in the light of powerful evidence that attenuated SIV induces potent protection against a wide variety of viruses administered by intravenous or mucosal routes and even against challenge with viable virus-infected spleen cells. Efforts are now underway to understand the mechanism of this protection and to attempt to reproduce it by less hazardous means. Considerable effort has been devoted to the development of subunit HIV vaccines, predominantly based on the envelope glycoproteins of the virus. Extensive clinical trials in human volunteers have established that these vaccines are safe and antigenic. However, the immune responses appear to be transient and the antibodies induced do not neutralize the primary isolates of HIV which are circulating in the population. There are now three possible approaches to an AIDS vaccine which are being actively pursued.

Introduction

Since the beginning of the AIDS epidemic approximately 15 million men, women and children have been infected with HIV worldwide (Mertens et al., 1994). Over half of all infections have occurred in people less than 25 years old. Although the ratio of infected men to women is currently 3 to 1, by the end of the decade the number of infections among women is expected to approach that among men. The rising infection rates in women are accompanied by a corresponding rise in the number of children with HIV
infection transmitted before, during or after birth, currently estimated at around 1 million cumulative infections. Vertical transmission of HIV from an infected mother to her child is around 30%. The remaining 70% of infants who are uninfected are potential future orphans. Because the virus effectively targets young adults, the epidemic is already having profound social, economic and demographic consequences, particularly in sub-Saharan Africa and South East Asia. The relentless spread of this global epidemic is unlikely to be halted by currently available control measures. The World Health Organisation estimate that between 30-40 million people will be infected by the end of the millennium (Mertens et al., 1994). For many countries, a safe and effective prophylactic vaccine is likely to be the only realistic approach to controlling this threat to public health.

Obstacles to an effective vaccine for HIV

Vaccines have effectively controlled many virus diseases. Smallpox has been eradicated from the globe, poliomyelitis has been eliminated from the Americas and the impact of measles has been reduced in many countries; all achieved by well planned immunization programmes. Effective vaccines are also available for influenza and hepatitis B virus. This history of successful vaccination led to early optimism that an effective vaccine against HIV would be developed quickly. However, as research progressed a series of formidable obstacles to successful vaccination began to emerge. First, HIV is a retrovirus, thus, during its growth cycle, proviral DNA is integrated in the host genome. In this form the virus is effectively protected from the immune response of the host and this feature of the virus suggests that effective vaccination must ideally prevent the initial virus-cell interaction following transmission. Few, if any, of the currently available successful viral vaccines against other infections achieve this level of protection. Secondly, HIV specifically targets and destroys T-helper lymphocytes, which form an essential component of the immune response. Thirdly, the virus is capable of extremely rapid antigenic variation which permits escape of the virus from immune responses. Fourthly, the majority of infections are acquired sexually via the genital or rectal mucosae, and infections by this route are generally considered difficult to prevent by vaccination. Finally, infection may be transmitted by virus-infected cells in which the proviral DNA is integrated and viral antigens are not expressed. Such a cell would not be recognised by immune responses to viral proteins and would therefore pass undetected. Few data are available to indicate how significant this mode of transmission is in the overall epidemiology of HIV-1. Nevertheless, it represents a potential route and one which some authorities believe cannot be blocked by vaccination (Sabin, 1992).

As this catalogue of difficulties began to emerge, the initial mood of optimism that a vaccine would rapidly be developed, was replaced by despair as some declared the task was hopeless. In reality, over the last 7 years steady progress had been made in evaluating a wide variety of vaccine strategies against immunodeficiency viruses. A large body of definitive information about protective antigens, immune responses and efficacy now exists as a result of studies in animal models and HIV-infected individuals and clinical trials in volunteers.
Potential uses of AIDS vaccines

The usual course of HIV infection involves an initial rapid replication of virus which appears at first to be controlled by the host's immune responses. This is followed by a prolonged latent period in which levels of virus in the blood decline and the infected individual remains healthy. Nevertheless, recent studies indicate that rapid replication of the virus and destruction of CD4 cells in the lymphoid tissue continue throughout this period (Ho et al., 1995; Wei et al., 1995). The latent period is followed by the reappearance of high levels of virus in the blood often accompanied by a decline in antibodies to the internal gag protein and in cytotoxic T-cell activity. At this stage the patient develops the classical signs of Acquired Immunodeficiency Disease Syndrome (AIDS). The prolonged latent period between initial infection and the development of AIDS has led to speculation that vaccines might be used after infection to prevent the development of disease. There is no precedent for the successful use of vaccines in this way, with the possible exception of rabies vaccine. Nevertheless, were this option to exist it would significantly extend the possible uses for AIDS vaccines.

The primary use of vaccines is in naive, uninfected individuals with the aim of preventing infection or disease. The prevention of an initial infection by vaccination is a formidable objective. Few, if any of the currently available successful viral vaccines achieve the level of protection. Usually there is a limited replication of the virus which is curtailed by the already primed immune response before the development of disease. However, if an HIV vaccine merely prevents disease without eliminating the HIV infection, or at least significantly reducing the virus load and, thus, preventing transmission, the effect of such a vaccine could potentially increase the number of healthy infected individuals with the consequent risk of accelerating the spread of the epidemic.

An alternative use of an HIV vaccine is in individuals already infected with HIV. Mothers infected with HIV might be vaccinated in order to prevent the transmission of virus to their offspring. Other HIV-infected individuals might be vaccinated with the objective of preventing progression to disease, or simply of reducing the virus load in order to prevent transmission, thus slowing the spread of the epidemic.

It is unlikely that a single vaccine would be appropriate for all these different uses described above. The safety requirements of a vaccine for use in naive, uninfected individuals are different from those for a vaccine to be used in infected persons. The immune responses required to prevent an initial infection may be quite different from those which are required to prevent the progression of infection to disease. This diversity of potential uses requires that vaccines are tested in a variety of contexts to evaluate their effectiveness against infection and disease.

Uses of animal models in AIDS vaccine development

The efficacy of vaccines can only be evaluated against an appropriate challenge. Experimental challenge of humans with a lethal pathogen such as HIV is unacceptable. Therefore, animal models are required in which the effectiveness of vaccine strategies can be evaluated. Unfortunately, apart from humans, only gibbon apes and chimpanzees are susceptible to infection by HIV-1. Both these species are rare and protected, and therefore only available in limited numbers. Since they can not be killed, HIV-infected chimpanzees must be kept in containment for the remainder of their
natural lives, which is a costly procedure. Furthermore, HIV replicates to relatively low levels in chimpanzees and does not cause disease in this species. These observations have led some to question whether this is an appropriate model for HIV infection in man. In any event, chimpanzees cannot be used to assess the ability of vaccines to prevent the development of disease.

An alternative animal model which has been widely used in AIDS vaccine development is the infection of old world monkeys by simian immunodeficiency viruses (SIV). Several strains of SIV have been identified which cause a clinical disease in macaques remarkably similar to AIDS in man. The SIVs, like HIVs, are lentiviruses and have the same morphology and morphogenesis. They bind to the equivalent CD4 receptor on T-helper cells and induce a similar destruction of the T-helper sub-population. Strains of SIV are closely related to HIV-2 sharing over 80% homology in the polymerase gene. They are less closely related to HIV-1 (60% homology in the polymerase gene) but have a similar, although not identical, genomic organisation (Kestler et al., 1988). These simian viruses are closer to HIV-1 than any other known vertebrate lentiviruses. An additional advantage of the simian model is that many of the human cytokines and cell surface makers are equally effective on simian cells.

The use of these animal models has provided important insights and some surprises which have been crucial in the development of AIDS vaccines.

Strategies for vaccination against AIDS

Initially, analogies were made between HIV and other enveloped viruses such as influenza, mumps and rubella. As a result, considerable efforts were directed to developing envelope glycoprotein as a vaccine designed to generate neutralizing antibodies. Recently, emphasis has shifted towards cytotoxic T-cell responses directed to internal structural or regulatory virus proteins. The progress which has been made towards AIDS vaccines will be reviewed under the types of vaccines which have been studied, namely inactivated virus, live attenuated virus and subunit proteins or peptides.

Data have accumulated on the safety and efficacy of each type of vaccine from experiments conducted in primate models and from human clinical trials.

Inactivated virus vaccines

The first evidence that vaccination against immunodeficiency viruses was feasible came from early experiments using simple inactivated virus vaccines which delayed or even prevented the onset of disease when vaccinated animals were subsequently challenged (Desrosiers et al., 1989; Sutjipto et al., 1990). These results were confirmed and extended by Murphey-Corb et al. (1989) who showed that most animals immunized with formalin-inactivated virus were protected against infection with SIV. Similar results were subsequently obtained by several laboratories using virus-infected cells (Stott et al., 1990) or partially purified virus, inactivated by aldehydes (Putkonen et al., 1991, 1992; Johnson et al., 1992a; Le Grand et al., 1992), β-propiolactone (Stott et al., 1990), detergent (Osterhaus et al., 1992) or psoralin and UV light (Carlson et al., 1990). Several different isolates of SIV or infectious molecular clones derived from them were used to prepare the vaccine and challenge viruses. A wide variety of adjuvants were also employed. On every occasion vaccinated macaques were protected against infection by intravenous challenge of between 10–50 MID50 (50% monkey infectious doses).
Infectious virus could not be recovered from the blood or tissues of the protected animals even when they were followed for prolonged periods of over 1 year. Even more impressive was the failure to detect proviral DNA in the lymphocytes of protected animals, indicating that there had been no integration of the challenge virus (Stott et al., 1990; Johnson et al., 1992a). It was thus clear that inactivated virus vaccines induced a powerful protective response in macaques.

The apparent success of inactivated virus vaccine in these studies was further exploited by the demonstration that parenteral immunization conferred complete protection against challenge via the intrarectal route, indicating that these vaccines also protected against challenge via the mucosae (Cranage et al., 1992b). Inactivated vaccines prepared from SIVmac or SIVsm were also shown to protect against certain heterologous viruses, using SIVmac, SIVsm and HIV-2 for the challenge (Gardner & Murphey-Corb, 1990; Cranage et al., 1992a; Johnson et al., 1992a; Stott et al., 1992). These isolates share approximately 80% nucleotide sequence homology in the envelope glycoproteins but are antigenically distinct when tested with a panel of monoclonal antibodies (Kent et al., 1992). The results of these experiments indicated that inactivated vaccines protected against heterologous strains of SIV but not against HIV-2.

The success of inactivated vaccines given prophylactically encouraged experiments in which the same vaccines were given to macaques already infected with SIV. However, there was no evidence that therapeutic vaccination altered the course of the virus infection, its clinical outcome or the time to death (Gardner et al., 1989; Stott et al., 1990; Murphey-Corb et al., personal communication).

The protection induced by vaccination with inactivated SIV in macaques led to considerable optimism that purified viral components could be identified which would reproduce the same protective immune responses. However, there were disconcerting features to the protection induced in macaques. Firstly, it was difficult to identify immune responses which correlated with the protection. Although neutralizing antibodies were detected in vaccinated animals concentrations were often low and did not correlate with protection (Murphey-Corb et al., 1989; Carlson et al., 1990; Stott et al., 1990, 1991). Similarly, the T-helper cell responses to virus-specific proteins were not significantly different between protected and unprotected animals (Stott et al., 1990; Mills et al., 1992). Furthermore, the protection induced by inactivated SIV in macaques was not reproduced in chimpanzees vaccinated with inactivated virus and challenged with HIV-1 (Warren & Doltshahi, 1993).

The role of host cell proteins

The explanation for these anomalies came with the observations that protection appeared to correlate with antibody to host cell components contaminating the vaccine and that a proportion of macaques could also be protected by immunization with uninfected human T-cells (Stott et al., 1991).

Further confirmation for the role of host cell antigens in the protection induced by inactivated vaccines was provided by the finding that when vaccinated animals were challenged with SIV grown in simian cells, there was no evidence of protection against infection (Cranage et al., 1992a; Johnson et al., 1992b; Le Grand et al., 1992; European Community Concerted Action, 1995). There are only two exceptions. First, a proportion of cynomolgous macaques, after prolonged immunization with inactivated
HIV-2 resisted challenge with HIV-2 grown in simian cells (Putkonen et al., 1991). Secondly, four of eight vaccinated animals were protected against challenge with SIV infected simian peripheral blood mononuclear cells (Osterhaus et al., 1992). Nevertheless, there is little evidence that inactivated SIV vaccines induce specific antiviral responses which can protect against cell-free virus grown in simian cells, and this has discouraged further development of inactivated HIV vaccines for man.

Over 200 macaques have been protected by inactivated SIV vaccines and attempts have been made to further exploit this potent protection by defining the cellular antigens which are responsible. Retrovirus particles incorporate MHC antigens into their surface as first described for murine oncornaviruses (Bubbers & Lilly, 1977; Azocar & Essex, 1979). The MHC Class II and HLA-DR has been found in both HIV and SIV particles (Gelderblom et al., 1987; Henderson et al., 1987; Hoxie et al., 1987). Furthermore, the heavy chain of HLA Class I and both alpha and beta chains of HLA Class II DR have been found in vaccine preparations (Cranage et al., 1993). Analysis of immunodeficiency viruses purified by high pressure liquid chromatography indicated that molecules of HLA-DR outnumber gp120 on the surface of the virion by a ratio of three to one (Arthur et al., 1992). Antibodies to human MHC Class I were found in the sera of protected macaques which had been vaccinated using inactivated virus, but there was no evidence of antibody to HLA-DR (Chan et al., 1992; Cranage et al., 1993). In order to define the roles of MHC Class I and II in protection, macaques have been vaccinated either with purified MHC molecules or with mouse L-cell lines expressing human MHC Class I or II. These experiments indicate that immunization with MHC Class I or II is capable of inducing protection against SIV grown in human cells (Arthur et al., 1995; Chan et al., 1995; Stott et al., 1994). All of this work has involved the xenogeneic immunization of macaques with human T-cell antigens. If this is to have relevance to HIV-1 vaccines in man, allogeneic immunization must also be effective. Macaques immunized with allogeneic simian peripheral blood mononuclear cells, and subsequently challenged with SIV grown in simian cells, were shown to be protected. Furthermore, this protection did not appear to be MHC type-specific (Stott et al., 1994). This result implies that allogeneic immunization of humans may prevent infection by HIV. There is no direct evidence to support this hypothesis, but the recent finding that multiparous women have a reduced risk of HIV infection is at least consistent with the idea that allogeneic antibodies are protective (Chao et al., 1994).

There are no human clinical trials of allogeneic immunization against AIDS, although this has been used as a treatment for infertility. Nevertheless, inactivated HIV-1 vaccines prepared in human T-cell lines are currently undergoing phase I and II clinical trials in HIV-infected individuals (Walker & Fast, 1994). The use of inactivated HIV inevitably carries some risk that viable virus may escape the inactivation process. To circumvent this problem, pseudovirions have been produced using vaccinia or baculovirus vectors, in mammalian or insect cells, to generate virus-like particles composed of HIV-1 envelope, gag and pol genes which contain multiple genetic deletions for increased safety. These products have also entered human clinical trials. Whatever the outcome of the current safety and immunogenicity trials, the implication of the available data from chimpanzee and macaque models is that inactivated viruses or pseudovirions are unlikely to be protective unless the particles carry allogeneic human antigens.
Live attenuated virus vaccines

The concept of using live attenuated HIV as a vaccine has been dismissed by most experts as being far too hazardous. However, the first evidence that an attenuated lentivirus might modify a subsequent infection was provided by Marthas et al. (1990). Macaques were infected by an infectious molecular clone (1A11) which did not induce clinical disease. They were subsequently challenged with a high dose of pathogenic SIVmac. Although all animals became infected, illness was delayed in the immunized monkeys. Similar observations were made in cynomolgus monkeys infected with an apathogenic strain of HIV-2. Five months after infection, the animals were challenged with SIVsm3. At the time of challenge with SIV, HIV-2 could no longer be recovered from the blood and no clinical signs of the disease were observed. Following SIV challenge, no disease developed in macaques previously infected with HIV-2. Furthermore, in lymph node biopsies there was abundant viral antigen in control monkeys, but none in the animals previously infected with HIV-2 (Putkonen et al., 1990). In these two examples, the attenuated virus did not prevent superinfection by virulent virus but appeared to delay or prevent subsequent disease. Interest was further stimulated by the finding that a molecular clone of SIV which contained a large 150 base pair frame-shift truncation in the nef gene continued to replicate in macaques, but with an attenuated phenotype (Kestler et al., 1991). Furthermore, when macaques persistently infected with this attenuated virus were subsequently challenged with high doses of virulent virus, they were completely protected against infection (Daniel et al., 1992). These observations have been independently confirmed and extended using a different infectious molecular clone with only a 12 base pair deletion in the nef gene (Rud et al., 1994). This virus has been shown to protect, not only against uncloned SIV, but also against chimeric SHIV virus (Li et al., 1992) in which the envelope protein of SIV has been replaced by that of HIV-1. This implies that antigenic relatedness of the envelope protein is not crucial in the protection mediated by the attenuated virus (Stott et al., 1994). Furthermore, the attenuated virus also protects against challenge with intact viable virus infected cells (Almond et al., 1995). Thus, attenuated SIV appears to induce protection which is far more potent than anything previously tested in the macaque model.

The mechanisms by which live attenuated SIV protect are not understood. Neutralizing antibodies are unlikely to be significant since the levels induced by attenuated viruses are far lower than those induced by recombinant proteins which failed to confer protection. Furthermore, the passive transfer of antibodies from macaques infected with SIVmac has consistently failed to protect recipients against challenge (Kent et al., 1994). High levels of cytotoxic T-cells, particularly against the nef protein, may be induced by attenuated SIV but this is not consistent (Gallimore, A., & Gotch, F., personal communication). Some form of viral interference phenomenon similar to that described for murine retroviruses (Mitchell & Risser, 1992) may also play a part in the protection. Defining the mechanism by which attenuated SIV protects macaques is essential if this potent immunity is to be reproduced by less hazardous means.

Attempts to reproduce these observations in chimpanzees using a clone of HIV-1 with deletions in the regulatory genes have so far given equivocal results (Desrosier, R., personal communication). There are substantial ethical obstacles against administering attenuated HIV to uninfected human volunteers without additional information on the
long term stability and safety of these materials. Currently the only population in which these viruses can reasonably be tested are HIV-infected individuals. In such cases it is at least theoretically possible that the attenuated virus could outgrow a previously established virulent virus (Bonhoeffer & Nowak, 1995). Unfortunately, currently available data from the SIV macaque model suggest that individuals already infected will be resistant to superinfection.

The major obstacle to the use of live attenuated HIV vaccines remains the concern over their safety. The vaccine virus could remain infectious within the recipient for their entire life span and could be integrated into the host chromosomal DNA. Under these circumstances, reversion to virulence and insertional mutagenesis altering the expression of normal genes or activating oncogenes are major concerns. Furthermore, the recent observation that a strain of SIV with multiple deletions which was attenuated in juvenile macaques, was nevertheless lethal when inoculated orally into neonatal animals, is a further cause for concern (Baba et al., 1995). Nevertheless, if the AIDS epidemic continues to spread unabated and alternative vaccine strategies fail to deliver significant protection, the use of live attenuated virus will have to be reconsidered and the safety issues addressed, in order to assess the risk-to-benefit ratio.

**Virus subunit vaccines**

Enormous effort and huge resources have been devoted to production and testing of virus subunit vaccines. The attractions of this approach are first, that all the latest powerful tools of biotechnology may be employed to produce a defined and purified product, and secondly, that the vaccine would be safe and free of the risk of infectious retrovirus associated with inactivated or attenuated virus vaccine. Unfortunately, the approach presupposes that the essential protective protein of the virus has been defined, but this is still far from the case with immunodeficiency viruses. By analogy with other enveloped viruses, most effort has been devoted to the envelope glycoproteins of HIV and SIV, and a wide variety of preparations including purified proteins, peptides or proteins expressed by replicating vectors have been used. Despite extensive studies in macaques, chimpanzees and man, consistent significant protection against infection or disease has rarely been demonstrated. Much less effort has been devoted to vaccines incorporating core proteins or regulatory proteins of the virus. Nevertheless, where these approaches have been used in the animal model, there has been no consistent protection against infection which approaches the potency of inactivated or live attenuated virus.

In the SIV macaque model, the strongest protection induced by envelope glycoprotein was against the SIVmne strain (Hu et al., 1992). Animals were first immunized with a recombinant vaccinia virus expressing gp160, and boosted 12 months later with gp160 produced from insect cells infected with a recombinant baculovirus. Vaccinated animals were protected against infection by a biological clone of SIVmne which was genetically closely related to the recombinant vaccines. Protection was less impressive when the animals were challenged with a genetically diverse pool of SIVmne. Attempts to protect macaques against SIVmac infection using recombinant proteins have proved uniformly unsuccessful. Macaques immunized with SIV p27 expressed on the surface of yeast Ty virus-like particles produced strong T-helper cell responses and high titres of antibody to the SIV protein, but they were not protected against challenge with SIVmac (Mills et al., 1991). Similarly, the generation of high levels of precursor
cytotoxic T-lymphocytes (CTL) against a specific epitope recognised by the MaMu A1 allele in rhesus macaques also failed to protect (Yasutomi et al., 1995). Animals immunized with gp160 and gag p55, produced, respectively, by vaccinia recombinant and baculovirus expression systems, developed strong T-helper responses and high titres of neutralizing and binding antibodies, but were not protected against infection with SIVmac (Mills et al., 1992, 1993). Recombinant gp130 produced in mammalian cells, gp140 produced in insect cells, or virus-like particles carrying gag and env proteins and generated by vaccinia viruses, have been used as immunogens with a variety of adjuvants. None of these preparations protected against challenge with SIV, despite the induction of significant cellular responses and high titres of neutralizing antibodies (Ohkawa et al., 1994; Silvera et al., 1994). Attempts to reproduce the success of Hu et al. (1992) by priming animals with live vaccinia virus expressing envelope proteins and boosting with purified recombinant envelope protein have also failed to induce protection against infection with SIVmac (Giavedoni et al., 1993; Stott et al., 1994). Even when this approach has been used and vaccine and challenge virus have been closely matched genetically using the infectious molecular clones SIVmac239, SIVmac BK28 and SIVmac J5, protection against infection has not been obtained (Ahmed et al., 1994; Israel et al., 1994; Stott et al., 1994). However, in the recent studies there was a significant suppression of virus load in the peripheral blood lymphocytes of vaccinated animals. This is likely to be a beneficial effect, but it has still not been shown that it significantly delays or prevents subsequent development of disease.

Vaccination of macaques with peptides analogous to four conserved domains in the envelope proteins of HIV-1 and SIV has given intriguing results. After challenge with SIVmne, virus was recovered from two of three vaccinated animals, but all remained healthy for 36 months during which time three controls had developed disease or died. Infection could not be transferred to naive recipients by inoculation of viable lymph node cells and peripheral blood lymphocytes from the three vaccinated animals, although proviral DNA was detectable in the lymph nodes (Shafferman et al., 1993). Furthermore, when serum was transferred from macaques vaccinated with peptide to naive recipients, three of six recipients were protected against challenge with SIVmne (Lewis et al., 1993).

Overall the immunization of macaques with vaccines based on the envelope proteins of SIV has rarely induced protection against infection. Whether the significant reductions in early virus load which have been seen in some vaccinated animals will translate into subsequent protection from disease and death remains to be established.

In contrast, vaccination of chimpanzees with recombinant proteins and peptides based on the envelope protein of HAV-1 IIIB strain has been more rewarding. In studies in the USA, two chimpanzees were protected against infection by immunization with gp120, but a preparation of gp160 was ineffective (Berman et al., 1990). In a more recent experiment, gp160 produced in mammalian cells protected one of two chimpanzees, whereas gp120 produced in insect cells failed to protect against infection, although there was evidence of a reduction in virus load (Bruck et al., 1994). More complex immunizations incorporating recombinant envelope proteins and peptides protected not only against cell-free virus challenge (Girard et al., 1991), but also against challenge with virus-infected peripheral blood mononuclear cells (Fultz et al., 1992). A proportion of chimpanzees have also been protected by immunization with gp160 (Mannhalter et al., 1991). In earlier experiments, vaccination with gp120 failed to protect against challenge (Arthur et al., 1989). Protection, therefore, has been demonstrated in a proportion of
chimpanzees, but it is by no means consistent. Although protection has been induced by the passive transfer of a monoclonal antibody to the V3 loop (Emini et al., 1992), and neutralizing antibodies have been implicated in the protection induced by envelope vaccines, it is still unclear whether neutralizing antibodies are primarily responsible for the protection induced by envelope vaccines in chimpanzees. Furthermore, all the chimpanzee challenges so far published have used the IIIB strain of virus which is significantly different, particularly in its neutralization properties, from primary isolates of HIV.

There are continuing uncertainties about the efficacy of vaccines based on envelope proteins and peptides, however, human clinical trials continue to concentrate on this approach. A wide variety of products have been used, based on LAI, MN or SF2 strains and produced in insect, simian or rodent cells. A variety of adjuvants, including alum, incomplete Freunds or muranyl tripeptide phosphatidylethanolamine have been used (Walker & Fast, 1994). The vaccines are safe and few significant adverse reactions have been observed. Most have induced varying levels of neutralizing antibodies, but these have been transient and ineffective against primary isolates of HIV. All vaccine candidates so far evaluated have induced proliferative responses, but cytotoxic T-cell activity has only been found in a proportion of volunteers after priming with recombinant pox virus expressing gp160. This activity has only been demonstrated after in vitro restimulation.

**Conclusions**

Much effort has been devoted to the pursuit of AIDS vaccines based on the envelope glycoproteins. Evidence from animal models in which such vaccines have been tested indicate that they are unlikely to be adequate to control the AIDS epidemic and there is a need to explore other options. Current evidence indicates that inactivated and live attenuated virus vaccines are likely to induce the most potent protection. However, the protection induced by the inactivated virus appears to be related to the cellular antigens on the surface of the virion and further research is required to elucidate the mechanism and define ways in which this phenomenon might be exploited in AIDS vaccine development. The major obstacle to the use of live attenuated virus remains concern about safety. Therefore, substantial effort should be invested in defining the mechanisms by which attenuated lentiviruses protect their hosts against subsequent superinfection, and to develop ways in which this potent protection can be induced by less hazardous means.

**References**


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