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AIDS vaccine: present problems and future perspectives

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Summary: Vaccination has proved to be an effective means for the prevention of infectious diseases. Advances in our understanding of the human immunodeficiency virus (HIV) and the immune system of the host may lay the foundation for the development of an AIDS vaccine. Current attempts to develop vaccines focus on the development of substances that will produce a different type of immune response from that which occurs naturally. Progress has been made in understanding the mechanisms by which the AIDS virus stimulates an neutralizing antibody response and triggers specific cytotoxic T lymphocytes in the host. The pressing need for a vaccine has prompted the testing of several candidate vaccines based on the simian immunodeficiency virus (closely related to HIV) in the macaque animal model for AIDS. The lessons learned from these trials will be valuable for developing future vaccines.

Key words: Acquired immunodeficiency syndrome – Human immunodeficiency virus – Simian immunodeficiency virus – Vaccine

Introduction

The acquired immunodeficiency syndrome (AIDS) is caused by an infectious microorganism, the human immunodeficiency virus type 1 (HIV-1). Infection by the virus initiates a slow progressive degenerative disease of the immune system and affects the central nervous system, with 7–10 years usually separating infection from the onset of serious disease. During the disease-free period, when the infection may remain unnoticed, the virus can be transmitted to others, predominantly during sexual intercourse. It has been estimated by the World Health Organization that by 1996 10–20 million people worldwide will be infected with HIV.

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Vaccination has proved to be the most effective means of preventing the spread of infectious diseases. Vaccines stimulate the immune system to recognize microorganisms by mimicking a prior infection. Once successfully stimulated by a vaccine, the immune system responds to natural infection with a strong and rapid secondary protective response. One major advantage of a vaccine is that it can be administered to the general population before risk of infection arises.

Vaccines have been developed to combat a number of viral diseases including smallpox, polio, measles, mumps, rubella, and hepatitis B. Efforts to develop an AIDS vaccine have been hindered by an unexpected number of difficulties and setbacks. Without doubt, limitation of the worldwide AIDS epidemic in the future will depend on the successful development of an efficacious, safe, and inexpensive AIDS vaccine.

Obstacles to AIDS vaccine development

HIV-1 is a member of the lentivirus subfamily of retroviruses and in humans it is able to infect different cells of the immune system, undergoing a long period of latency before clinical symptoms occur. The receptor for HIV is the CD4 molecule expressed predominantly on the surface of T-lymphocytes and monocytes/macrophages. Once infected, these cells provide a stable vehicle for the virus to be transported to different tissues and organs of the body, including the central nervous system. The progression to AIDS disease is accompanied by a profound depletion of CD4-positive T-cells and the suppression of various immune functions. Total recovery from natural HIV infection or from AIDS has never been observed, indicating that the natural immune response mechanism(s) in humans are not effective. In addition, stable integration of the HIV provirus into the host cell DNA following infection allows a period of viral latency to occur during which time the immune system, no matter how strong, cannot eliminate the virus-harboring cell.

The development of a suitable AIDS vaccine is hampered: (1) by our lack of understanding of the immune mechanisms (humoral vs cellular, or both) which are able to prevent establishment of infection; (2) by our ignorance of the epitopes of the virus which may stimulate both a systemic and a mucosal protective immune response; (3) by the absence of an inexpensive and practical animal model to study immune prophylaxis.

In this paper, we will review the current knowledge of the viral epitopes which may mediate protection and of the role that neutralizing antibodies and cytotoxic T-lymphocytes (CTLs) may play in protection. In addition, we will summarize the successful vaccination studies performed to date in the HIV/chimpanzee and the simian immunodeficiency virus (SIV)/macaque animal models.

Neutralizing antibodies

HIV is covered by an outer lipid membrane, acquired during budding from the host cell, in which the viral glycoproteins gp120 and gp41 are embedded. The main structure on the virus to which neutralizing antibodies are directed is a disulfide-bonded loop (the third *variable* region or "V3-loop") of the gp120 [13, 23]. The specific role of this epitope during infection remains unclear, but it is known that antibodies directed to this V3-loop neutralize infectivity and block syncytium formation in vitro. It is also clear that antibodies to the V3-loop do *not* interfere with the interaction of gp120 with CD4 molecules on the target cell. Interestingly, both mechanisms appear to work independently, as neutralization by antibodies can occur *after* HIV binds to the cell, indicating interference with a post-binding event which is necessary for infection to proceed.

Although antibodies develop (to variable titers) in HIV-infected individuals which are able to neutralize laboratory strains of HIV [21], these antibodies clearly do not prevent progression to disease. There is evidence, however, that neutralizing antibodies can protect against infection in vivo. Emini et al. [9] reported successful protection of chimpanzees against infection with HIV when the virus was incubated with neutralizing antibodies in vitro before inoculation of the mixture into the animal. In addition, passive transfer of a humanized neutralizing monoclonal antibody in large quantities to chimpanzees, either immediately before or immediately after injection of HIV, protected the chimps from infection. Also, cynomolgus macaques receiving, by passive transfer, whole sera from SIV-infected animals were protected against subsequent challenge with live SIV [19]. There is therefore mounting evidence that a sufficient titer of neutralizing antibodies may be protective, although other antibody-mediated immune mechanisms (e.g., antibody-dependent cellular cytotoxicity) could also be the responsible mechanism in the experiments described above.

As the name suggests, the V3 loop is not a *constant* element of HIV, showing extensive variation from one isolate to another, even within the same patient. Assuming that the immune response to the V3 loop is able to suppress or limit the spread of the virus, high variability in this region may represent the development of escape mutants able to avoid immune elimination. This hypervariability exacerbates the problems involved in the successful development of a general AIDS vaccine effective against all existing (and future) strains and variants of HIV. However, the tip of the loop is formed by a sequence of amino acids (GPGRAF) which is relatively well conserved amongst North American and European HIV isolates [13]. Furthermore, most HIV-infected individuals develop antibodies reactive with an epidemiologically dominant variant of the V3 loop (typified by the MN isolate) [8]. It is reasonable to suppose that a vaccine should include the most common variants of the loop.

In addition to mutations within the V3 loop itself, it is possible for changes outside the V3 loop to influence the binding of V3-specific antibodies (presumably by altering the conformation). It was reported, for example, that escape variants develop in HIV-infected chimpanzees which are no longer neutralized by antibodies specific for the "initial" V3 loop, although no changes had occurred in the V3 loop itself [15]. Further studies performed by Robert-Guroff et al. [22] and Reitz et al. [20] indicate that changes outside of the V3 loop affecting conformation of the gp120 may be as important as sequence variations in the V3 loop itself in influencing the effect of neutralizing antibodies.

Cytotoxic T-lymphocytes

CTLs represent one component of the anti-HIV defense mechanism which is directed against and can eliminate virus-infected cells. Experiments performed by Walker et al. [29] and others [27] show that CD8-positive CTLs can eliminate virus-infected cells and can limit the spread of virus in cultures of peripheral blood mononuclear cells derived from HIV-infected individuals. As for other virus systems, CTLs almost certainly play a very important role in limiting infection in vivo.

The early recognition (via viral epitopes expressed on the cell membrane in association with MHC class I molecules) and elimination by CTLs of infected cells may be instrumental in preventing the establishment of latency. For this reason, the stimulation of a strong CTL response is presently considered a prerequisite of a putative vaccine.

The expression of viral protein segments in conjunction with the MHC-I usually occurs when the cell is undergoing active viral replication. How then can a synthetic vaccine able to stimulate the CTL response be developed? First, it is necessary to precisely define the epitopes on the various HIV proteins which stimulate and serve as targets for CTLs [16, 26, 28]. The conserved and immunodominant CTL sites should be selected, as the development of HIV variants able to escape from CTL attack can occur [18]. Second, these epitopes must be presented to the immune system in such a way that the usual requirement for endogenous intracellular processing and reexpression is avoided or artificially initiated. To this end the use of modern adjuvant formulations (e.g., ISCOMS or lipopeptides) seems promising.

Animal models

HIV/chimpanzees

Chimpanzees (and gibbons) are the only known species other than man which can be infected with HIV, although these animals do not develop immunodeficiency. The number of chimpanzees available for vaccine research is very limited, and for ecological and economic reasons alternative animal models are essential. Initial vaccination trials in chimpanzees failed [2], but two recent reports demonstrate that immunization of chimpanzees with recombinant gp120 [3] or with a cocktail of different HIV immunogen preparations [10] can protect against a subsequent challenge with live HIV-1. These successes possibly reflect the purity and authenticity of the viral proteins used for immunization and the use of a low virus dose for challenge. The results are an encouraging sign that it is possible to protect against HIV infection. However, for testing large numbers of vaccine candidates only the SIV/macaque model is practical and chimpanzees will probably only be used for final testing of promising vaccines before trials in human volunteers are initiated.

SIV/macaques

Infection of macaques with pathogenic strains of SIV results, in a relatively short period of time (6 months to about 1 year), in a disease with symptoms similar to those observed in humans after infection with HIV. SIV_{mac} is genetically similar to HIV-2 and, as all SIV_{mac} isolates have been isolated in primate centers and not from wild-caught animals, it appears that rhesus macaques are not the natural host. This may explain why the animals succumb to disease for, in contrast, the SIV of African green monkeys (AGM) can be isolated from 40%–60% of wild AGMs [12] and, although related equidistantly to HIV-1 and HIV-2 [1], SIV_{agm} does not induce any symptoms of immunodeficiency [12]. This benign virus-host relationship probably reflects interaction and adaptation of SIV_{agm} to the host over millenia [17]. Regardless of the origin of SIV_{mac}, this animal model remains a valuable tool in AIDS vaccine research.

In 1989–1990, three laboratories reported the successful protection of macaques against homologous SIV infection using an inactivated whole virus vaccine [5, 7, 14]. These first successes were of great significance as they indicated that a protective anti-SIV immunity in the animals could be stimulated by a vaccine.

A number of the studies with the inactivated whole virus vaccine showed protection from infection [5, 7, 11, 14], whereas others "only" prevented or delayed disease development [25]. Normally it is enough for a vaccine to protect from disease development but for HIV, with its ability to integrate unseen in the host cells, a vaccine able to prevent primary infection, or at least integration, would be preferable.

Since HIV is normally transmitted during sexual intercourse over mucosal surfaces, a first-line immune protection at these sites will probably play a crucial role. In the SIV/macaque model protection against vaginal challenge has so far not been achieved by vaccination of animals with inactivated SIV, although protection against rectal challenge has [6]. Great efforts will be required to devise vaccine strategies able to stimulate a generally protective mucosal immunity.

It has recently been necessary to reevaluate the results of the whole-inactivated SIV_{mac} vaccine trials performed to date. Stott [24] reported in 1991 that protection against SIV_{mac} challenge could be achieved by immunizing monkeys with fixed, *uninfected* human cells. In addition they found, by analyzing all available sera from previous trials, that a strong correlation existed between the titer of anti-cellular antibodies on the day of challenge and the outcome of the trial (i.e., protection vs infection). In nearly all vaccine experiments of this type both the whole virus immunogen and the challenge virus were grown in human T-lymphocyte cell lines (see Fig. 1). It is known that retroviruses budding through the host cell membrane take with them cellular proteins, hence immunization with formaldehyde-fixed virus induces both antibodies specific for viral and for cellular proteins. Hence it is conceivable that anti-cell antibodies present in the sera of the experimental animals recognize human cell proteins on the challenge virus and thus prevent infection from occurring. Many groups are now performing vaccine studies using challenge virus grown in monkey peripheral blood mononuclear cells, as such a virus would have no human proteins in its membrane and would not be recognized by anti-human cell antibodies (Fig. 1).

Although the validity of the whole-inactivated virus and fixed cell immunogen experiments (which form the majority of successful "AIDS vaccine" experiments to date) is presently open to question, the fact that an SIV-specific immune response can protect against infection has been shown by the passive transfer experiments of Putkonen et al. [19] and by the recombinant SIV im-

Table 1. The simian immunodeficiency virus (SIV)/macaque animal model

Fact	Fancy	Future
Protection from infection (homologous, heterologous) by immunization with fixed SIV	Stimulation of an anti-SIV immunity	Role of anti-cellular antibodies: challenge with PBMC-grown virus or SIV-infected PBMCs
Protection from infection via i.v. challenge, but not via mucosal (i.e., vaginal) route	Broad anti-SIV immunity	Immunization and quantification of mucosal anti-SIV immunity
High neutralizing antibody titers can be stimulated by an inactivated SIV vaccine	Neutralizing antibodies protect against infection with free virus particles and cell-associated virus	Prove role of neutralizing antibodies and of cytotoxic T-lymphocytes in protection
Dead virus vaccines successfully protect	Long-lasting protection	Improvement of long-term immunity and alternative vaccine trials (live recombinant virus vaccines)

PBMC, Peripheal blood mononuclear cell

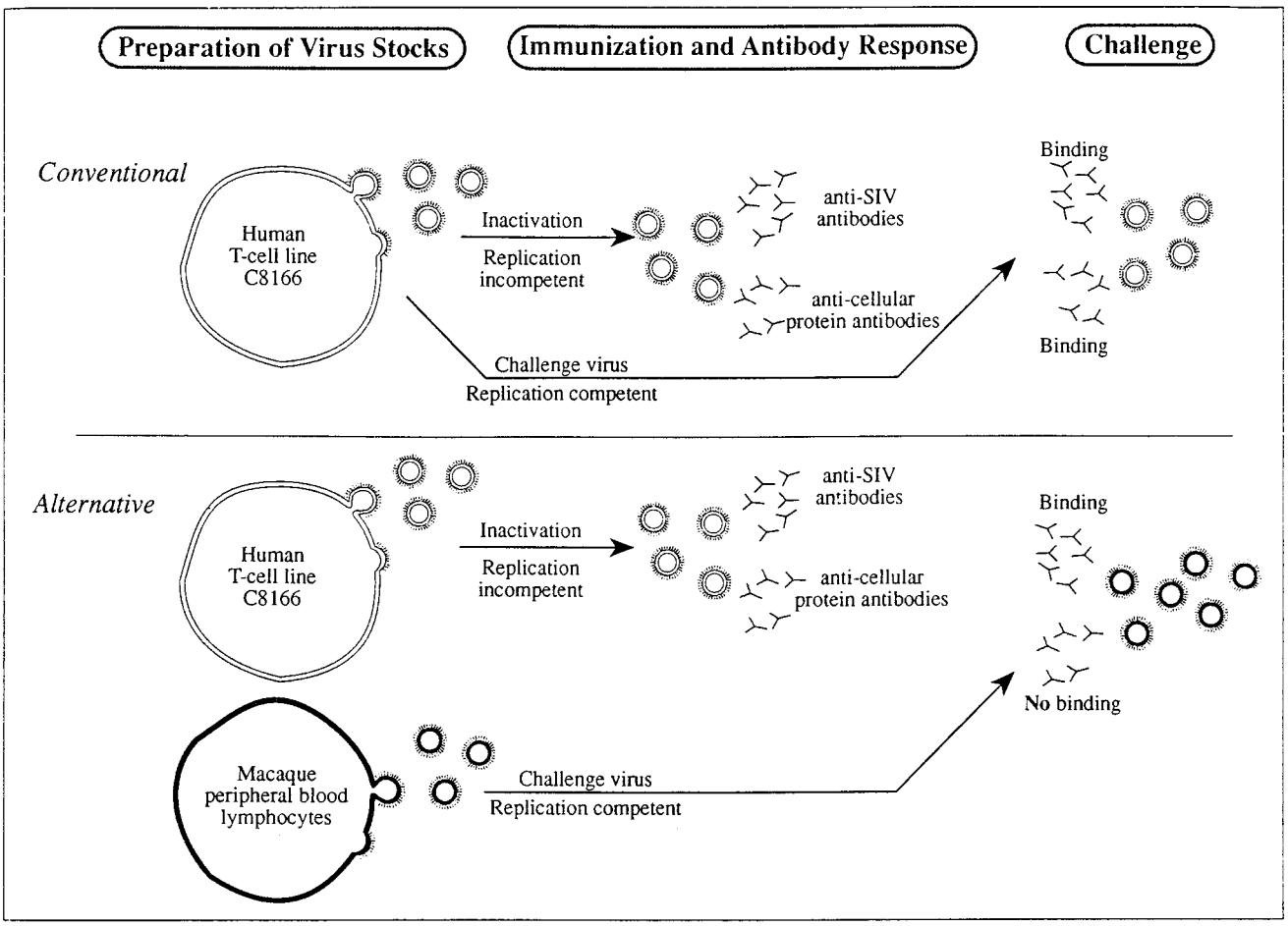

Fig. 1. Comparison of conventional and alternative strategies for propagation of virus for immunization and challenge. In conventional protocols virus stocks for immunization and for challenge are both grown in a human T-cell line (e.g., C8166). Immunization with inactivated whole simian immunodeficiency virus (*SIV*) vaccine induces anti-SIV antibodies *and* anti-cellular protein antibodies in the animal. The challenge is performed with replication-component SIV, and anti-SIV and anti-cellular antibodies will bind to the virus particle. An alternative strategy is to grow virus for immunization in a human T-cell line but to propagate the challenge virus in primary macaque mononuclear cells. After inoculation of replication-competent virus, antibodies directed to SIV proteins will bind whereas antibodies directed to human cellular proteins will not

munogen studies. In these experiments a role for anti-cell immunity can be excluded, although the nature of the protective anti-SIV response has yet to be determined.

Perspectives

The lack of an animal model for HIV-1 infection in monkeys means that vaccine or therapy studies will continue to be performed in monkeys using SIV strains or HIV-2. Although highly successful, it is clear that new vaccines besides the inactivated SIV have to be considered (see Table 1). The problem of the possible role for anti-cellular antibodies in protection against SIV infection in the vaccine model has to be solved. In addition, no real progress in the development of an AIDS vaccine will be possible until the duration of protection can be significantly extended. So far, virus challenges have usually been performed within a few weeks or months of the final immunization (when the immune response is at its peak) and, although infection was in these cases prevented, a longer interval before challenge usually resulted in vaccine failure. Such frequent immunization would not be practical for an AIDS vaccine and additional adjuvants or vaccination regimens have to be developed which induce a long-lasting immunity in the vaccinated host. In the near future, methods to simulate in the vaccine models the natural routes of infection, particularly transmucosal, have to be further developed. There is an obvious need to standardize protocols for mucosal challenge and for measurement of immune reactivity at mucosal sites.

The successful protection against SIV infection in monkeys using the simplest of vaccines, whole-inactivated virus, should not detract from efforts to develop alternative strategies of immunization and vaccination. Although successful, inactivated SIV vaccines usually give only short-term protection from infection. In addition, the risks of incomplete inactivation, and the problems potentially associated with an intact retroviral genome, make the general use of an inactivated HIV vaccine in humans unlikely. Live recombinant microorganisms (e.g., vaccinia-HIV constructs), recombinant

viral proteins, synthetic peptide immunogens, and attenuated strains of SIV or HIV unable to integrate in the host cell genome (e.g., through mutations in the *nef* [4], *vif*, or *int* genes) should be (and are being) evaluated experimentally.

The success of the inactivated SIV vaccine forms the cornerstone of AIDS vaccine development. Now the immunological parameters of protection and the proteins/epitopes responsible for protection must be identified and this knowledge applied to the design and production of an effective AIDS vaccine.

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