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HIV Vaccine Development in the Nonhuman Primate Model of AIDS

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Key Words

HIV · Vaccine · Macaque · Simian immunodeficiency virus

Abstract

Development of a prophylactic human immunodeficiency virus type 1 (HIV-1) vaccine is a leading priority in biomedical research. Much of this work has been done with the nonhuman primate model of AIDS. In a historical context, vaccine studies, which use this model, are summarized and discussed.

Nonhuman Primate Model

The most successful approach in controlling viral infections has been the preventative or prophylactic vaccine. Of the many viruses for which vaccines have been designed, some outstanding examples include the measles, polio and hepatitis B viruses [reviewed in [4]. Each of these vaccines stimulates long-lasting, protective immunity in the host, but each does so in very different ways. The measles vaccine is a live, attenuated version of the measles virus (rubeola). The Salk form of the poliovirus vaccine is inactivated (killed) wild-type virus. Finally, the hepatitis B vaccine consists of the hepatitis B surface protein. Despite the varied approaches, each of these vaccines confers dramatic protection against infection and disease by the wild-type pathogen.

For human immunodeficiency virus type 1 (HIV-1) infection, each approach described above has been or is being investigated. However, HIV-1 infection is much different from the viral infections mentioned above, in that there are no well-described examples of long-lasting immunity to natural infection. Prior to the development of vaccines for measles, polio, and hepatitis B, it was well known that survival of natural, acute infection with each of these viruses conferred durable, protective immunity to reinfection [4]. HIV-1 is not known to cause transient infection [39]. All human infections appear to be chronic and lifelong. Additionally, in chronic HIV-1 infection, the host appears to mount a strong, anti-HIV-1 immune response [32]. Yet, this immune response appears to be dysregulated and is unable to clear the virus or, in most cases, to significantly control virus replication. Consequently, HIV-1 vaccine researchers 'started from scratch', since no approach seemed more likely to succeed than another.

In this review, I describe HIV-1 prophylactic vaccine development in experiments, which use the nonhuman primate (NHP) model of acquired immunodeficiency syndrome (AIDS). As for a background, I will first describe the macaque/simian immunodeficiency virus (SIV) model. SIV was discovered coincidentally and shortly after HIV-1 was discovered [53, 59, 70]. In separate primate centers in the USA, macaques were dying of an AIDS-like illness. Researchers independently determined that strains of SIV, which are naturally found in sooty mangabeys *(Cercocebus atys)* from Africa, had spread to captive macaques, which are from Asia. These SIVs were found to be the causative agent of simian AIDS in the

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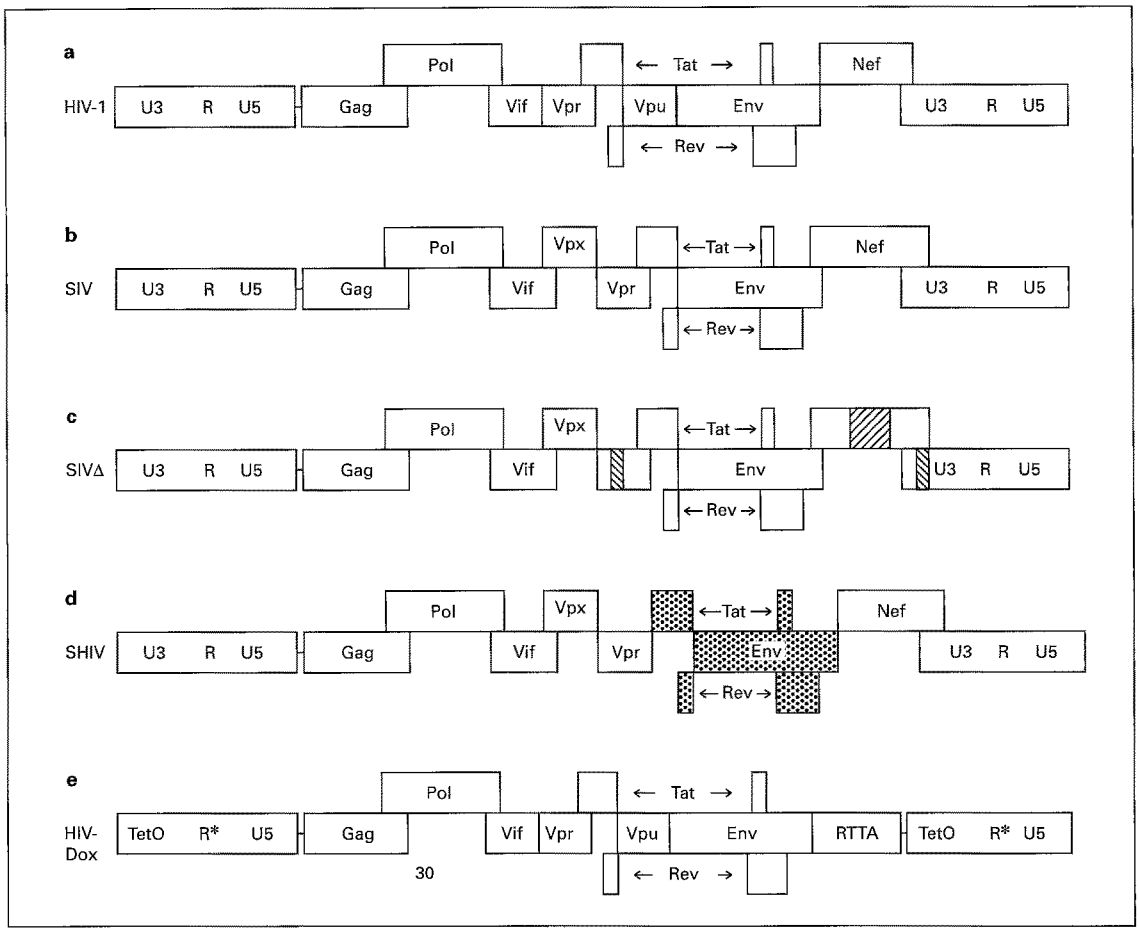

Fig. 1. Genome organization of viruses used in the NHP model of AIDS. **a** HIV-1. **b** SIV (representative of SIVmac, SIVsm and SIVmne strains). **c** SIVΔ with deletion in SIVΔnef (▨) and additional deletions (vpr and part of U3) in SIVΔ3 (▨). **d** SHIV with HIV-1 genes (tat, rev and env) shaded (▨); other genes and LTRs are from SIV. **e** HIV-Dox, which has mutations in U3 and R and the transactivator, RTTA, inserted in the nef reading frame.

macaques. As a result of the simultaneous codiscoveries, several different, but closely related strains (including SIVmac, SIVsm, and SIVmne) of SIV were developed for research purposes. Additionally, researchers found that these SIVs could cause simian AIDS in three species of macaques, rhesus macaques *(Macaca mulatta)*, cynomolgus macaques *(Macaca fascicularis)*, and pigtailed macaques *(Macaca nemestrina)*. These species differ substantially. Since each of these SIV strains produced similar disease in a given macaque species and no macaque/SIV model was clearly more relevant than another, researchers chose to study different SIV strains in different species of macaques. The resulting experiments, of course, often make direct comparison impossible.

Additionally, researchers, who use the NHP model of AIDS, have now begun using simian-human immunodeficiency virus (SHIV) as the challenge virus. SHIVs are manufactured lentiviruses, composed of part HIV-1 and part SIV (fig. 1). The SHIVs, which are used in vaccine studies, have gag, pol, vif, vpr and the LTR of SIV and tat, rev, and env of HIV-1 [63]. Pathogenic SHIVs (some strains are nonpathogenic) cause rapid immunodeficiency in macaques and a disease, which has less similarity to AIDS than do the diseases caused by the commonly used strains of SIV. Still, since the envelope protein is from HIV, many feel SHIV is a better challenge virus. Finally, macaque studies are expensive, and the supply of macaques is limited. Therefore, most macaque studies involve small numbers, usually well less than 20 total, of animals.

A few researchers study chimpanzees *(Pan troglodytes)*, since chimps are the only NHP, which can be infected with HIV-1. Most chimps do not develop disease following infection with HIV-1 and do not have significant viremia. Chimpanzee studies are, of course, limited by legal and ethical issues. The number of chimps used in a given study is typically four to five and makes statistical comparison impossible [60].

Inactivated Virus

Since inactivated virus had been used successfully as a vaccine for diseases such as polio, many researchers initially focused on this approach. Early efforts appeared to be quite effective. Using SIVmac in rhesus macaques, Desrosiers et al. [40] found that SIVmac inactivated with detergent conferred protection in 2 of 6 vaccinated animals, while all 4 control animals became infected. Similarly, formalin-fixed SIVdelta, a strain closely related to SIVsm, provided significant protection to a lower challenge dose of virus in rhesus macaques [85]. However, it soon became apparent that the protective immune response was directed against human antigens present in the vaccine preparation and in challenge virus [2, 55, 73, 81, 112]. Similar vaccines protected macaques against SIV propagated in human cells, but not SIV propagated in macaque cells. In a revealing experiment, macaques immunized with human HLA-DR (major histocompatibility complex class II) proteins were protected against SIV grown in human cells, but were readily infected by SIV grown in macaque cells [10]. The finding that the protective response induced by inactivated SIV vaccines was secondary to xenoimmunization greatly reduced the interest and pursuit for inactivated HIV-1 vaccines. Yet, the observations have led to speculation that alloimmunization may be used as a strategy for protecting against HIV-1 infection [75, 78, 103]. Concerns about inducing autoimmune processes have slowed research in this area.

Passive Immunization

While passive immunization is not practical as a long-term strategy for protection against HIV-1 infection, studies using this approach can at least confirm the feasibility of HIV-1 vaccines. In a limited study, Prince et al. [92] showed that pooled plasma from HIV-1-infected patients failed to protect chimpanzees against intravenous challenge with nonpathogenic HIV-IIIB. In 1991, passive transfer of serum containing high titers of SIV antibody protected cynomolgus macaques against low-dose challenge with SIVsm [93]. The source of antibody in this study was heat-treated, pooled sera from macaques infected chronically with SIVsm. Conversely, in two similar studies, purified immunoglobulin from SIVmac251 infected rhesus macaques failed to protect naive macaques from low-dose SIVmac251 infection [52, 69]. In another study using SIV in macaques, newborns were protected from oral transmission of SIV by passive transfer of high-titer anti-SIV serum [115].

With the availability of SHIVs, which can infect macaques, researchers have recently focused on this virus. After passive transfer of plasma from chimpanzees infected with HIVDH12, two pigtailed macaques were completely protected from intravenous homologous challenge with pathogenic SHIVDH12 [104]. Statistical interpretation of this small study is not possible. Baba et al. [13] administered a triple combination of monoclonal antibodies, which have strong HIV-1-neutralizing activity, to the pregnant dams and then to their 4 newborn macaques. All 4 newborn macaques were protected against oral challenge with a nonpathogenic SHIV. In a follow-up study, 2 newborn macaques were only given the antibody cocktail after birth (no maternal immunization). Both newborn macaques were again protected against oral SHIV challenge. In a similar study, infusion of hyperimmune anti-HIV serum with two of the same monoclonal antibodies used by Baba et al. [13] protected 4 of 5 female rhesus macaques against vaginal transmission of pathogenic SHIV89.6PD [80]. Then again, a passive immunization strategy, which had protected 3 of 6 macaques against subcutaneous challenge with pathogenic SHIV-Ku, failed to protect animals, which were inoculated orally with SHIV-Ku [45, 64]. Finally, in Parren et al. [91], macaques required high doses of the HIV-1-neutralizing antibody, b12, to resist infection with pathogenic R5-tropic SHIV162P4 after intravaginal challenge. Infection of macaques, which received the lower doses of b12, occurred despite the presence of serum titers 16–80 times the in vitro (90%) neutralization titers.

Taken together, the data strongly suggest that HIV infection can be abrogated by high titers of neutralizing antibody and they support the possibility for a prophylactic vaccine, which induces neutralizing antibodies. Nonetheless, the need for high titers of neutralizing monoclonal antibodies confirms that development of a vaccine, which induces such antibodies at high levels, will be difficult.

Attenuated Viruses

In 1992, Daniel et al. [35] showed that rhesus macaques infected with SIVmac239Δnef were resistant to superinfection with SIVmac251, a closely related biologic clone. This observation opened the area of attenuated viruses, similar to the measles vaccine, to be used as SIV and HIV-1 vaccines. Other groups confirmed that SIVs without nef could induce protective immunity against closely related pathogenic SIVs with nef [8, 33, 110]. Soon thereafter, researchers in Australia reported a cohort of blood transfusion recipients (Sydney Blood Bank Cohort), infected with a strain of HIV, which had deletions in the U3 LTR and nef-coding region [38]. These individuals showed no signs of progression and had stable CD4+ T cell counts over 10–14 years. Initial enthusiasm was high.

The development of viral load assays allowed better characterization of SIV pathogenesis in macaques [58, 106]. Many animals infected with SIVmac239Δnef remain viremic, and over time, develop CD4+ T cell lymphopenia [33]. Also with time, three of the blood bank cohort were found to have decreasing CD4+ T cell counts and appreciable HIV viral loads [74]. Efforts were made to increase the safety of live-attenuated SIVmac239, called SIVmacΔ3, through additional disruptions of the vpr reading frame and part of the U3 region [124] (fig. 1). However, the more attenuated virus conferred protection less quickly and in a lower percentage of vaccinated animals [66, 124]. Additionally, despite multiple deletions, SIVmac239Δ3 was found to cause disease readily in neonatal macaques and gradually in adult macaques [12, 14]. Arguments can be made that newborn humans would not be exposed to an attenuated HIV in the absence of maternal immunity [125]. Still, SIVs attenuated by gene deletion appear to replicate chronically in most animals. Although protection induced by SIVs attenuated by gene deletions is strong, the uncertainty about long-term side effects has prevented human trials with a similarly constructed, attenuated HIV-1.

The mechanism(s) of protection conferred by attenuated SIVs has not been fully elucidated [65]. Although SIVΔnef and SIVmacΔ3 can confer sterilizing immunity against homologous challenge, these vaccines do not provide such protection against heterologous challenge [77, 114, 123]. The protection is not clearly mediated by neutralizing antibodies [33, 72]. SIV-specific cytotoxic T lymphocytes (CTLs) also do not appear to be the major mechanism [88]. Although encouraged by the ability to potently protect animals against homologous virus, researchers felt the risk of the use of attenuated lentiviruses in humans outweighed the potential benefits. Further, since investigations into the mechanisms of protection did not suggest which type of immune response plays a role, a marker for protective immunity, such as antisurface antigen antibody levels for hepatitis B virus protection [118–120], was not found. Consequently, studies involving other vaccine vectors must empirically test for protection.

Others and we have focused on additional ways to improve attenuated vaccine safety [26, 79, 108, 109, 116]. Through a gain-of-function approach, we hope to gain control of viral replication via an exogenous agent. With one approach, cells infected with the vaccine strain would be eliminated by drug administration [26, 109]. In another approach, the virus's replication is dependent upon a drug's presence [108, 116]. With either approach, the attenuated vaccine is given to a host. After the immune response has developed, the drug is discontinued (or administered depending on the approach employed) and viral replication ceases. Alternatively, Giavedoni et al. [54] have studied the inclusion of the interferon gamma gene within the attenuated viral genome to reduce replication. The same group has studied using protein-based vaccines before administering attenuated viruses [68]. However, both studies were too small for a conclusion to be reached. Researchers hope these approaches will improve the safety profile of attenuated lentiviral vaccines, while maintaining their ability to induce potent, protective immunity.

Envelope Approach

In a manner analogous to the hepatitis B virus vaccine, many researchers have examined the role of viral proteins, especially envelope, as a vaccine. Early reports with the NHP model showed success of gp120 in preventing infection in both macaques and chimpanzees [5, 11, 20, 61, 62]. Unfortunately, it soon became clear that envelope protein did not induce neutralizing antibodies and did not confer protection against pathogenic strains of SIV in macaques [57]. In these early studies, monovalent gp120 was used as the immunogen. It is now known that HIV and SIV Env exists on the virion as a heavily glycosylated, trimeric structure [reviewed in 24]. Recent attempts have focused on developing an Env immunogen, which retains a more native structure. Earl et al. [41] immunized rhesus macaques with oligomeric HIV gp140 (gp120 plus the ectodomain of gp41). The macaques were challenged with nonpathogenic SHIV-HXB2. Three of the 4 vaccinated animals were protected against infection, while both control animals became infected. This challenge virus does not replicate acutely to high titers in vivo and does not

replicate appreciably after 8 weeks in vivo. Additionally, the sera from the vaccinated animals failed to neutralize HIV primary isolates in vitro. Others have pointed out the difficulty in making a stable, trimeric form of gp120 [99]. It appears technical hurdles must be overcome before this hypothesis can be fully tested.

Investigators have also examined the role of Env glycosylation in protecting epitopes. Reitter et al. [94] infected rhesus macaques with mutants of SIVmac239, which lack certain Env glycosylation sites in the V1 loop. Macaques infected with this mutant strain develop antibodies, which neutralize the infecting strain and also neutralize wild-type SIVmac239, which is fully glycosylated. In a similar approach, Stamatatos and colleagues [15, 28] have examined the effect of deleting the central region of the heavily glycosylated V2 loop of an HIV Env. Immunization of rhesus macaques with oligomeric forms of this ΔV2 Env stimulates the production of antibodies, which neutralize some HIV-1 primary isolates. In the vaccine setting, ΔV2 Env immunization did not confer complete protection to CD8+ T-cell-depleted macaques, challenged with pathogenic SHIV162P4. However, the vaccinated animals controlled viremia in both the acute and chronic settings. In two similarly designed studies, rhesus macaques were immunized with gp120 of the same HIV-1 isolate (W6.1D) [82, 113]. In one study, the animals were challenged with a homologous SHIV strain and were protected from infection [82]. In the other, the animals were challenged with a heterologous SHIV [113]; all became infected and had viral loads similar to the controls.

Letvin et al. [76] used V3 loop peptides to induce V3-specific antibodies in rhesus macaques. The vaccinated animals had reduced viral load after challenge with nonpathogenic SHIV89.6. Nevertheless, the same vaccine strategy failed to reduce viral loads in animals challenged with pathogenic SHIV89.6P. In another attempt to elicit neutralizing antibodies, Cho et al. [29] tested a polyvalent Env-based vaccine. Pigtailed macaques immunized with this vaccine became infected with a nonpathogenic SHIV, but showed modest control of viremia in the acute period. Of note, the challenge SHIV did not replicate to high levels in the control monkeys after 8 weeks of infection. Kumar et al. [71] challenged rhesus macaques with two related pathogenic SHIV strains after gp120 protein vaccination. The vaccine had no effect on infection or viral replication.

Overall, HIV envelope has proven to be a difficult target for antibodies. No Env-based vaccine has consistently induced a protective response in the NHP model. However, the vaccines based on deglycosylated forms of Env show the most promise.

Pox Virus Approach

With the failure of many protein-based vaccines to generate protective humoral immunity, many researchers began to pursue vectors, which induce cellular immune responses. Recombinant poxviruses, including vaccinia, canarypox and fowlpox, are good vectors for intracellular expression of antigens and stimulate strong immune responses [90]. Daniel et al. [36] used recombinant vaccinia virus, which expressed SIV gag, pol and/or env, to immunize rhesus macaques. However, 17 of 18 animals became infected with low-dose intravenous challenge with SIVmac251. Disease course was similar in the control and vaccinated groups, despite strong immune responses in the vaccinated animals. Another group, after initially showing good protection in an early study, found that neither recombinant vaccinia nor recombinant canarypox protected rhesus macaques from infection with a macaque-adapted strain of HIV-2 [3, 46, 86]. Hirsch et al. [58] vaccinated rhesus macaques with trivalent modified vaccinia virus Ankara (MVA). Despite strong immune responses, all vaccinated animals became infected with SIVsmE660. The vaccinated animals had lower viral loads and progressed more slowly. In a follow-up study, the same group used an improved recombinant MVA vector, which had increased expression of SIV antigens [89]. Vaccination with MVA expressing SIV gag-pol, env, or gag-pol-env reduced viral replication by >2 $\log_{10}$ at 12 weeks and increased survival significantly. Yet, many vaccinated animals did develop low CD4+ T cell counts. In a separate study, Seth et al. [101] examined the CD8+ T cell response to MVA, which expressed SIV gag-pol, in rhesus macaques. MVA induced some SIV-specific CTL in all animals. As before, all vaccinated animals became infected. The set point viral load correlated with prechallenge SIV-specific CTL levels. Still, the average viral load was only reduced by 1.5 $\log_{10}$ at week 7 in the vaccinated animals. In a large study involving rhesus macaques, Benson et al. [19] vaccinated the animals with the NYVAC strain of vaccinia, which expressed SIV Env. The animals were challenged with SIVmac251 intravenously or intrarectally. Five of the 11 animals challenged intrarectally appeared to clear the infection, although all became infected. Similarly, 4 of the 12 intravenously challenged, vaccinated animals suppressed viral replication, although the difference was not statistically significant. In this study, the ability to control virus replication was not associated with any measured immune response. Overall, the vaccinia-based protocols achieved limited protection against pathogenic SIV. Further, there is the concern that

the utility of repeated vaccination with vaccinia could be limited by the induction of antivaccinia immunity, which has developed in some macaques [102].

DNA Vectors

In recent years, naked DNA has been used as a vector for delivering antigens [100]. DNA vectors induce strong immune responses to a variety of antigens in mice. DNA vectors primarily induce cellular immunity, while protein-based vaccines induce primarily humoral responses. With growing evidence that cellular immunity is important in controlling HIV-1 replication in vivo, investigators began using DNA vectors to improve the cellular immune response against HIV/SIV/SHIV. In a small study, chimpanzees were protected from nonpathogenic HIV-1 infection through DNA vaccination [21]. Mossman et al. [84] used DNA vectors with and without protein boosts in pigtailed macaques. The DNAs expressed many SIV genes, including, gag-pol and env. The animals were intrarectally challenged with pathogenic SIVmne. All animals became infected, but the animals receiving DNA alone controlled virus replication better than did the DNA plus protein-vaccinated animals and the control animals. Gorelick et al. [56] vaccinated pigtailed macaques with DNA, which expressed noninfectious virion. The SIV encoded in the DNA had nucleocapsid mutation, which results in a failure of the virus to package genomic viral RNA. The animals were challenged intravenously with pathogenic SIVmne. Approximately 50% of the vaccinated animals were able to control viremia 40 weeks after infection. Wang et al. [121] vaccinated rhesus macaques with DNA, which expressed noninfectious viral particles, through several routes, including the rectal mucosa. Although the animals developed high levels of anti-SIV IgA in their rectal secretions, CTL responses were limited. Two of 9 vaccinated animals were protected from intrarectal challenge with SIVmac239. The 7 infected vaccinated animals had moderately lower viral loads on average compared to control animals. Akahata et al. [6] also used DNA, expressing a nucleocapsid mutant to vaccinate rhesus macaques. The animals were challenged with a nonpathogenic SHIV. The vaccinated animals had lower viral loads in the acute period. Since the challenge SHIV used in this experiment does not replicate past 12 weeks in unvaccinated animals, the effect of vaccination on chronic viremia cannot be assessed.

Egan et al. [42] vaccinated rhesus macaques with DNA expressing SIV gag. The animals developed strong SIV-specific CTL responses. Yet, the vaccinated animals all became infected after intravenous challenge with pathogenic SIVsm E660. Further, the viral loads did not differ significantly between the vaccinated and control groups in the acute setting. The vaccinated animals did appear to have lower viral loads 1 month after infection. The same group, however, achieved significant viral load suppression in rhesus macaques challenged with pathogenic SHIV89.6P, when DNA was coadministered with interleukin-2 (IL-2) [17]. In this study, IL-2 was given as a long-lasting protein or as a separate DNA vector. In either case, animals receiving some form of IL-2 had increased SIV-specific CTL responses and dramatic postacute control of viral replication [16]. Interestingly, the animals vaccinated with DNA alone developed weak CTL responses and had poor control of viral replication.

Amara et al. [9] vaccinated rhesus macaques with multigenic DNA vector plus MVA boost. This combination stimulated the strong SIV-specific CTL production. Although the delay was not by initial design, the animals were intrarectally challenged with SHIV89.6P 29 weeks after the last immunization. Despite the long time period between the last vaccination and the challenge, the vaccinated animals strongly suppressed viral replication after 5 weeks. The controls had robust viral replication and developed disease. By 25 weeks, none of the 24 vaccinated animals had died, compared to 75% of the controls. Of interest, in an extension of this study, macaques were given the same DNA vaccine plus a gp120 protein boost [97]. Surprisingly, 3 of 8 macaques, which received this protein boost regimen, developed high viral loads after challenge. Additionally, macaques vaccinated with SIV gag-pol DNA vectors alone did not control viral replication well.

In summary, certain DNA vectors can elicit strong SIV-specific CTL responses in macaques. In some settings these CTL appear to participate in the control of virus replication, while in others, they do not. Reasons for these differences are not clear. Also, it is not clear which viral proteins must be targeted by CTLs in order to contain viral replication. In addition, the data from Mossman et al. [84] and Amara et al. [9] suggest that protein vaccination may negatively affect the ability of the animals to control virus replication. Perhaps, the protein boost converts the animal's antiviral immune response from Th1 to Th2, which may be ineffective in controlling HIV replication. Hopefully, this effect will not be seen in the participants of clinical HIV-1 vaccine trials, which use protein boosts.

Tat Approach

A growing body of evidence suggests that extracellular Tat is important in causing disease [51]. Tat is found in the supernatant of in vitro HIV-1 cultures and the serum of infected individuals [27, 43, 126]. Extracellular Tat can mediate effects on uninfected cells [44, 48, 122]. Based on the hypothesis that extracellular Tat is an important component in HIV and SIV pathogenesis and replication, Cafaro et al. [25] studied a Tat-based vaccine. The investigators hypothesized that if extracellular Tat is important for viral replication, a preexisting immune response against Tat may suppress viral replication. Seven cynomolgus macaques were immunized with the biologically active HIV-1 Tat protein, although the route, dose and adjuvant varied. Five of the 7 Tat-vaccinated macaques had undetectable viral loads in both the acute and chronic periods after challenge with pathogenic SHIV89.6P and no loss of CD4+ T cells. The control animals had high viral loads and quickly developed CD4+ T cell lymphopenia. The protection from disease did not correlate with anti-Tat antibodies, which developed in all seven vaccinated animals. The Tat-specific CTL response did correlate loosely with protection. In an effort to increase the anti-Tat cellular immune response, the same group used a DNA vector, which expressed HIV-1 Tat. Four of the 5 cynomolgus macaques given that Tat DNA vector potently suppressed viremia after challenge with SHIV89.6P. However, the 1 control animal vaccinated with the control DNA vector (which contained no tat sequence) also suppressed SHIV89.6P replication. As seen in the first study, protection against disease was associated with a strong Tat-specific CTL response.

In another protein-based vaccine study, Silvera et al. [105] immunized rhesus macaques with HIV-1 Tat or Tat toxoid. Despite the induction of anti-Tat humoral and cellular responses, vaccination provided no benefit against SHIV89.6P challenge. Viremia was similar in immunized and control animals. On the other hand, two related studies used Semliki Forest virus (SFV) and vaccinia vectors to immunize rhesus or cynomolgus macaques against Tat and Rev [111, 117]. The animals were challenged with passaged SHIV-BX08, R5-tropic virus, or SIVmac32H, a pathogenic clone of SIVmac251. In both studies, the vaccinated animals had partial control of viremia, although the degree of control was not as complete as was seen in the prior studies.

In summary, Tat-specific cellular immune responses appear to contribute to suppression of viral replication in the vaccine setting. This finding is consistent with the observation that escape from Tat-specific CTL may be important in SIV pathogenesis [7]. The failure of anti-Tat antibodies to suppress viremia argues against a role for extracellular Tat in pathogenesis. Future studies will address the duration of this protective response and if other anti-SIV responses can act synergistically with the anti-Tat response. Additionally, since extracellular Tat selects against X4-tropic viruses in vitro, anti-Tat antibodies could in vivo accelerate the phenotypic switch from R5 viruses to the more pathogenic X4-tropic versions [126].

Other Vectors

In addition to those mentioned, other viral and bacterial vectors have been assessed in the SIV/macaque model. Alphaviruses, such as SFV and Venezuelan equine encephalitis virus (VEE), are easily converted to single cycle particles, which intracellularly express high levels of the recombinant transgene [reviewed in 107]. These particles have a broad host range and appear to be safe. SFV-expressing Env protected rhesus macaques against severe, acute disease associated with the challenge virus, SIVPBj [83]. This strain of SIV typically causes a lethal gastrointestinal illness in the first few weeks [50]. While vaccinated animals were protected against this acute process, they all became chronically infected. The atypical nature of SIVPBj disease makes comparison or interpretation of this study very difficult. In another study, cynomolgus macaques were vaccinated with an Env-expressing SFV and then challenged with SHIV-4, which has the HIV-IIIB env. Three of the 4 vaccinated animals controlled viremia compared with 1 of the 4 control animals. Davis et al. [37] used recombinant VEE vectors to immunize four rhesus macaques against SIV antigens. After challenge with SIVsm, the vaccinated animals partially controlled virus replication. In a follow-up study, 6 rhesus macaques were again immunized with VEE vectors, expressing Env and Gag. SIV-specific CTL developed in 4 of 6 animals and each vaccinated animal developed a strong humoral response [67]. One month after the third vaccination, the animals were challenged intrarectally with SIVsm-H4. Vaccinated animals again showed modest control of viremia compared to controls. Although alphaviruses appear to be ideal vectors for high-level intracellular expression of an antigen, early efforts have not provided dramatic protection against infection or disease in the SIV/macaque model.

Adenoviruses can also be genetically engineered to express high levels of foreign proteins. Additionally, ade-

noviruses infect macaques with similar efficiency as humans and through mucosal routes. Buge et al. [22, 23] first used recombinant adenovirus, expressing gp120, with gp120 protein boosts. After intravaginal SIVmac251 challenge, the vaccinated and control animals had similar rates of infection and of disease. In more recent studies supported by the pharmaceutical company Merck, recombinant SIV gag adenovirus has elicited strong cellular responses in rhesus macaques [49]. Additionally, the recombinant adenovirus-vaccinated animals significantly suppressed viral replication after intravenous challenge with pathogenic SHIV89.6P. The potent suppression of viral replication correlated well with the prechallenge SIV-specific cellular responses. Since adenoviruses frequently infect humans naturally, the existence of preexisting antiadenovirus immunity could reduce the efficacy of this approach [31].

Vesicular stomatitis virus has been used successfully as a vaccine vector in animals [95, 96]. Recombinant vesicular stomatitis virus, expressing SIV gag and HIV env, were used to immunize 5 rhesus macaques [98]. All of the vaccinated animals and 6 control macaques were infected after intravenous challenge with pathogenic SHIV89.6P. On average, the controls had higher viral loads than the vaccinated animals. Although the decrease was less than that seen in the controls, the CD4+ T cell count fell significantly in the vaccinated animals after infection. After 2 months, 3 of the 6 control animals were euthanized secondary to simian AIDS symptoms, while none of the vaccinated animals had become ill. The significance of this type of protection needs to be determined.

Attenuated strains of poliovirus can be engineered to express foreign genes. Seven cynomolgus macaques were orally administered a 'cocktail' of 20 transgenic polioviruses, expressing gag, pol, env, nef and tat in overlapping fragments [34]. A strong anti-SIV humoral response was seen in each animal, while cellular responses were limited. After intravaginal challenge with SIVmac251, all 12 control macaques became infected. Two of the 7 vaccinated animals did not become infected and 2 other vaccinated animals strongly suppressed virus replication. This novel approach certainly warrants further study. In another strategy designed to elicit mucosal immunity, Berzofsky and colleagues [18] used SIV gag and pol peptides to immunize rhesus macaques. Three animals were immunized intrarectally and 4 subcutaneously. After the animals were challenged intrarectally with pathogenic SHIV-Ku, the intrarectally immunized animals controlled viremia in the postacute period. The subcutaneously immunized animals had similar viral loads to the control animals. At autopsy, little virus was found in the colon and intestine of the intrarectally immunized animals, while the control and subcutaneously immunized animals had 1–2 $\log_{10}$ more virus. These data suggest that local, mucosal immunity may be important in containing HIV-1 at the site of infection.

Conclusions

As shown, the literature on vaccine development in the NHP model of AIDS is confusing. With the variety of challenge viruses and primates, comparison of one study to another is often not possible. Some general concepts do, however, emerge from all of the data. First, prophylactic vaccination against HIV-1 appears to be possible, although extremely difficult. Second, vaccination, which may not affect the infection rate, may prevent disease. Third, although virus-specific CTL appear to be more important than neutralizing antibodies in containing virus, much work on understanding 'protective' immune responses still needs to be done. Finally, while many recent studies have shown containment of pathogenic SHIV replication, the validity of these results needs to be established. Pathogenic SHIVs typically, if not invariably, cause a pronounced, acute disease. Protection of this acute process in primates may or may not correlate with disease protection in humans infected with HIV-1. Similar to HIV-1 in humans, pathogenic SIVs in macaques cause a more gradual disease than the pathogenic SHIVs. Vaccine approaches, which are successful in containing SHIV replication in the postacute period, should be tested in macaques challenged with pathogenic SIVs. This challenge may more accurately reflect HIV-1 infection of humans and may have more relevance for chronic, viral suppression.

Human Studies

While not the focus of this review, human studies are ongoing [87]. Currently, one vaccine type, a monomeric gp120 protein approach, is being tested in phase III trials in North America, Europe and Thailand. In the fall of 2001, the interim analysis will be performed; the final analysis will occur in 2002. The vaccine trial is designed to detect protective efficacies of 30% or greater. The company plans to apply for Federal Drug Administration approval if the efficacy is at least 30% [47]. Several other approaches, including vaccinia vectors, lipopeptides, env V3-based peptide, Salmonella vectors, and DNA vectors,

are in phase I trials [1]. Canarypox phase II studies are now being completed. While early studies with canarypox vectors have not shown great immunogenicity, two similar canarypox vectors are being considered for phase III testing [30]. However, each trial would cost over USD 35 million and each would enroll over 10,000 individuals. It is not clear if the phase II data warrant the economic cost and loss of valuable, future trial participants. Of course, the need for an effective HIV-1 vaccine has never been greater. Therefore, it becomes a moral issue of whether or not to wait for better vaccine candidates or to move ahead with the ones showing, at least, some promise.

Addendum

Published after this paper was written, an article in *Nature* [Barouch et al.: 2002;415:335–339] reports that one of the IL-2/DNA vaccinated macaques by Barouch et al. [17] developed significant viremia and simian AIDS. The authors believe that a mutation in a CTL epitope allowed the virus to 'escape'.

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