HIV VACCINE DEVELOPMENT

Mahender Singh [2]**, Kuan-Teh Jeang** [1]**, and Stephen M. Smith** [2,3]

[1]*Molecular Virology Section, Laboratory of Molecular Medicine, NIAID, NIH, Bethesda, Maryland, USA, and,* [2]*Saint Michael's Medical Center &* [3]*New Jersey Medical School, Newark, New Jersey, USA*

TABLE OF CONTENTS

1. ABSTRACT

For the past two decades, scientists have aggressively pursued the development of a vaccine against human immunodeficiency virus (HIV). The magnitude of this effort is unprecedented in the history of infectious diseases. However, difficulties in finding promising candidate vaccines have limited the number of clinical efficacy trials. The macaque model is well suited for the evaluation of potential vaccines, but comparison of results among studies is often complicated by the use of different macaque species and/or challenge viruses. This review discusses current results obtained in the macaque model and human vaccine trials.

2. INTRODUCTION

The development of an effective, prophylactic HIV-1 vaccine has proven to be extremely difficult. In the 21 years since HIV-1 was discovered, an extraordinary amount of time, effort and money has been devoted to this endeavor. Unfortunately, traditional and non-traditional vaccine approaches against HIV-1 have not been successful. In this review, we will summarize efforts made in the non-human primate (NHP) model of AIDS and the limited work conducted in human trials. The development of a therapeutic HIV-1 vaccine will not be discussed.

3. HUMAN TRIALS

Vaccines, like new therapeutics, are evaluated in three successive stages of clinical testing, referred to as Phase 1, 2, and 3 trials (1). In Phase 1 testing, the side effects and safety of a given vaccine are measured in a small number of healthy volunteers who are at low risk of infection. Typically, 10 to 30 individuals are inoculated with the vaccine at different doses and immunization schedules. Participants are closely monitored for the development of adverse reactions, and immune responses to the vaccine are evaluated secondarily. Phase 1 trials are usually completed in approximately one year. In Phase 2 trials, several hundred people are given the vaccine to collect additional short-term safety data and, more so, to measure the immunogenicity of the vaccine. Participants in Phase 2 trials are individuals who have varied rates of risk for acquiring the infection, and these trials typically take 1.5 to 2 years to complete. Once a vaccine has been determined to be safe and sufficiently immunogenic, it is tested in an efficacy or Phase 3 trial.

Several thousand volunteers must be enrolled in a Phase 3 vaccine trial (2). The study must be placebo controlled and double-blinded such that neither the researchers nor the volunteer knows if he/she is receiving placebo or vaccine. Further, Phase 3 studies must be
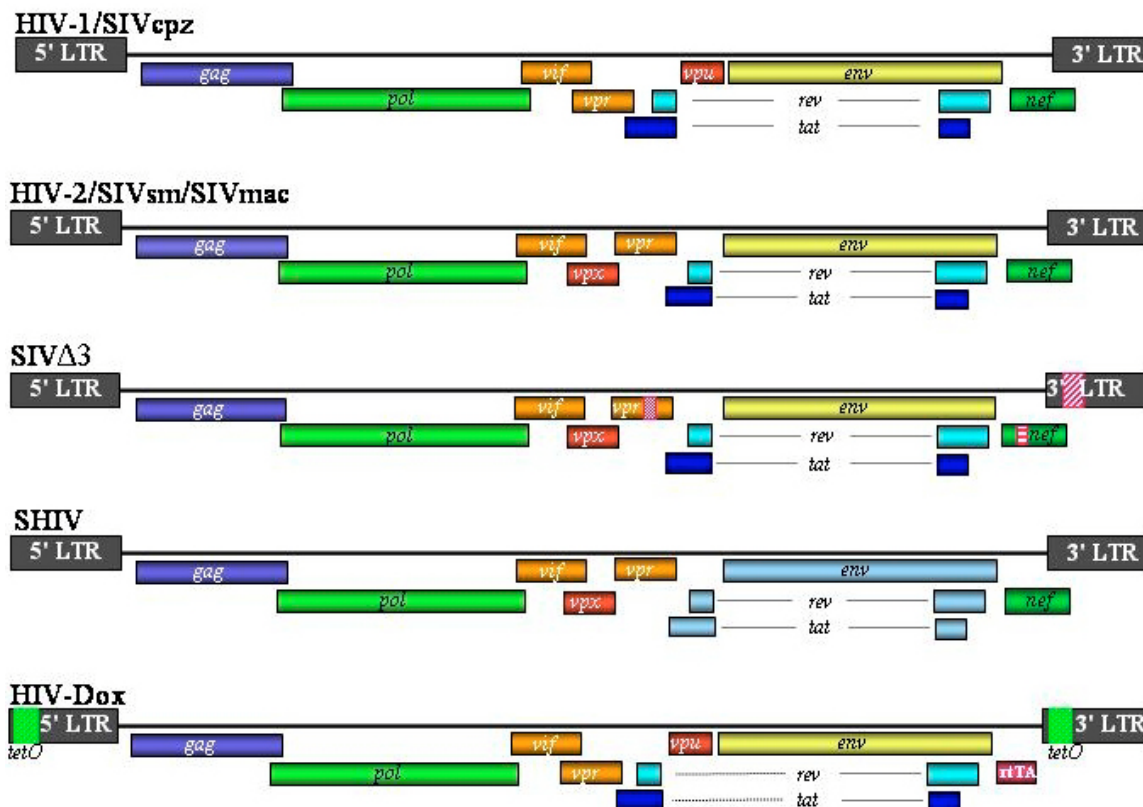
Figure 1. Genome organization of HIV-1, HIV-2 and SIV used in the NHP model of AIDS. Different open reading frames for genes are presented as labeled colored rectangles. Deletions in the genes of SIVΔ3 are shown as hatched rectangles. The chimeric virus, SHIV, has *tat*, *rev* and *env* of HIV-1 in the backbone of the SIVmac genome. The HIV-Dox contains the inducible tetracycline operator (*tetO*) sequences in the U3 region of both LTRs and rtTA gene in place of the *nef* gene.

conducted in a population with substantial risk of HIV-1 infection and can take three years to complete. Combined, Phase 1, 2, and 3 trials for a candidate HIV vaccine may require up to six years to yield the necessary safety, immunogenicity and efficacy data.

The National Institutes of Health in the United States has formed the HIV Vaccine Trials Network (HVTN) to help organize and sponsor vaccine research (3). The self-stated mandate of HVTN is to conduct "all phases of clinical trials, from evaluating candidate vaccines for safety and the ability to stimulate immune responses to testing vaccine efficacy. The HVTN mission is to develop and test preventive HIV vaccines. This research is done through multi-center clinical trials in a global network of domestic and international sites." Other countries, including Great Britain, Thailand, Kenya, Uganda, South Africa, Australia and Italy, actively support HIV vaccine trials (4). Several pharmaceutical companies have developed significant HIV vaccine projects, most notably Merck, Aventis and Glaxo Smith Kline (GSK). For several years, the International AIDS Vaccine Initiative (IAVI), a non-profit global organization, has been an advocacy group for HIV vaccine research and testing. Now, IAVI is directly supporting clinical testing. The HIV vaccine effort is not lacking for governmental, non-governmental or industrial support. Rather, the many institutions and foundations lack a clear immunologic target.

HIV-1 is a retrovirus of the lentivirus genus (5). The HIV-1 RNA genome is ~9 kilobases and encodes 9 polypeptides (Figure 1). Post-translation proteolytic digestion results in the formation of 15 discrete proteins. The major structural proteins are encoded by three genes, *gag* (group-specific antigen), *pol* (polymerase), and *env* (envelope), while the accessory proteins, Vif, Vpu, Vpr and Nef, and the regulatory proteins, Tat and Rev, are the primary translation products of multiply-spliced mRNA. Various vaccine strategies have used both individual and combined HIV proteins as potential immunogens in efforts to define more clearly the correlates of protective immunity.

To date, only one HIV-1 vaccine has been tested in Phase 3 clinical trials. This vaccine, AIDSVAX, is a monomeric version of the normally trimeric HIV-1 envelope glycoprotein, gp120. The vaccine was found to induce strong antibody responses against linear epitopes on gp120 and CD4$^+$ T-cell proliferative responses. However, in two independent trials, which tested slight variations of the same approach, the vaccine failed to confer protection against HIV-1 infection and did not significantly alter virus replication, as measured by plasma viral load (6-8).

The first of two Phase 3 trials of AIDSVAX was conducted in the United States, Puerto Rico, Canada and the Netherlands and enrolled 5,109 men and 309 women at high risk for acquiring HIV-1 infection (8). The volunteers were randomized to either the vaccine (AIDSVAX B/B) or placebo arm of the study at a ratio of 2:1. Those in the vaccine arm received AIDSVAX B/B, which contains two forms of gp120 derived from subtype B virus; the most common subtype found throughout North America and Europe. Of those enrolled, 5,009 participants completed the three-year study. While adverse reactions were minimal, the infection rates for the vaccine and placebo groups were 5.7% and 5.8%, respectively, and were not statistically different. The second efficacy trial was conducted in Thailand and enrolled 2,546 injection drug users (9, 10). For this study, the vaccine was changed to AIDSVAX B/E to reflect the subtypes of HIV more commonly found in Asia (subtypes B and E). This trial again showed no protection against HIV-1 infection providing convincing evidence that immunization with monomeric gp120 is ineffective as a vaccine strategy.

Despite its lack of success, AIDSVAX is being pursued in clinical trials, along with ALVAC, a recombinant form of canarypox. This two-vector strategy, supported in part by HVTN, is being studied in Thailand and is set to enroll 16,000 participants (enrollment began in late 2003) (9) at an estimated cost of $120 million. ALVAC has been studied extensively in Phase 1 and 2 trials, and has been shown to have an excellent safety profile. This is due to the fact that canarypox infects mammalian cells, where it completes a single cycle of replication but does not release infectious virions. In the current clinical trial (designated RV144), ALVAC is produced by Aventis and engineered to express HIV-1 subtype E env (gp120), subtype B env (gp41), gag and protease (designated ALVAC-HIV-vCP1521). Vaccination will consist of a prime-boost strategy in which ALVAC is administered as the priming agent followed by an AIDSVAX boost.

Pursuit of this large-scale, expensive trial is controversial. In a recent letter published in *Science*, several leading HIV researchers openly criticized the decision to continue this trial (11). Based on the weak immunogenicity of ALVAC, plans for a similar trial to be conducted in the United States were abandoned. The induction of cytotoxic T- lymphocyte (CTL) responses, thought to be a critical parameter for a successful prophylactic HIV vaccine, were limited when ALVAC was administered alone to healthy HIV-negative volunteers. ALVAC-HIV (vCP1521) induced CTL activity with a cumulative frequency of just 24% and sustained positive CTL responses were seen in only 9.8% of participants (12). Policy makers argue that the combination of soluble gp120 delivered by AIDSVAX plus intracellular antigen expression mediated by ALVAC might result in increased $CD4^+$ T-cell helper responses. However, in phase 1 and 2 testing of similar regimens, 21 individuals became infected with HIV-1 (13) with similar viral loads to seven placebo recipients who also became infected during the trials. Related prime-boost strategies when tested in NHP models

have not shown great promise (see below). While the canarypox vector has an excellent safety record, it is likely that safety has come at the expense of efficacy in this case. By comparison, vaccinia has a lower safety profile than ALVAC (based on historical data), yet induces strong CTL responses and is well-tolerated when used for smallpox vaccination (14).

Only three other Phase 2 trials are currently underway. One of these, HIVNET 026, is being conducted in Brazil, Haiti, Peru, and Trinidad and Tobago, and utilizes a similar approach to RV144 consisting of ALVAC priming plus a gp120 boost (3). Another trial, HVTN 050, is testing a defective adenovirus vector. Adenoviruses cause a variety of infectious syndromes in humans including conjunctivitis and pharyngitis. Merck has developed a replication-defective form of adenovirus, Ad-5, that expresses high levels of foreign proteins. Based on mixed results in macaques (Tables 2 and 3), Merck, with support from HVTN, is moving forward with Phase 2 testing in humans using Ad-5 expressing HIV-1 Gag. This study is being conducted at many different sites on several continents.

IAVI recently supported its own Phase 2 study, the first to employ DNA and modified vaccinia vectors in combination (4). Both vectors expressed consensus HIV-1 clade A Gag p24/p17 and 25 CTL epitopes derived from other HIV-1 proteins. In Phase 1 testing, the safety profile of the vaccine was good, and modest to moderate CTL responses were seen (15). However, the vaccine regimen failed to induce a strong antibody response, and overall immune responses were only observed in ~25% of volunteers. In light of these results, IAVI has decided not to pursue this approach in Phase 3 testing.

Several additional HIV-1 vaccine strategies are now being tested in Phase 1 trials. A comprehensive list can be found on the IAVI website (http://www.iavireport.org/trialsdb/).

4. NON-HUMAN PRIMATE MODELS

NHP from Africa harbor more than 30 lentiviruses, many of which are related to HIV-1 and HIV-2 (see review on HIV evolution in (16)). None of these viruses typically causes disease in its natural host; however, some isolates can cause an acquired immunodeficiency syndrome (AIDS) when inoculated into Asian macaques. Infection in captive macaques is used as a disease model to study simian AIDS. The simian immunodeficiency viruses (SIVs) are related to HIV in their genomic organization and sequence homology, and are divided into two subgroups: one represented by SIVsm from sooty mangabeys (*Cercocebus atys*) and the other by SIVcpz from chimpanzees (*Pan troglodytes*). SIVcpz has the same genetic organization as HIV-1, while SIVsm/SIVmac/SIVmne are more similar to HIV-2 (see Figure 1). Several strains of pathogenic SIV have been used as challenge viruses in vaccine studies (Table 1), the most common being SIVmac251, a biologic clone, and the closely related, SIVmac239, a molecular clone. Other SIV

Table 1. Simian immunodeficiency viruses used in vaccine development and their associated disease course in macaques

| Groups | Uncloned virus stocks | | Molecular cloned virus | |
	Name	Disease	Clones	Disease
SIVsm/SIVsmm	SIVsm/F236	AIDS, slow (R,P)	SIVsmH3, smH4	AIDS, slow (R,P)
	SIVsm/B670	AIDS, rapid (R)	n.a.	n.a.
	SIVsmm9	AIDS, rapid (R,P)	n.a.	n.a.
	SIVsm/E660	AIDS, intermed (P)	n.a.	n.a.
	SIVsm/E543	AIDS, rapid (P)	SIVsm/E543-3	AIDS (R,P)
	SIVsmm/PBjbcl3	Acutely lethal (P)	PBj4.41	Acutely lethal (P)
			PBj6.6	Acutely lethal (P)
SIVmac/SIVmm	SIVmac251	AIDS, rapid (R), intermed (C)	SIVmac251	None (R)
			SIVmac/BK28	Minimal (R)
			SIVmac/1A11	None (R)
	SIVmac239	AIDS, rapid (R), intermed (C)	SIVmac239	AIDS, rapid (R)
SIVmne	SIVmne	AIDS, intermed. (P,R)	SIVmne/E11S	AIDS, slow (R,P)

n.a. = not applicable; C = cynomolgus macaque; P = pigtailed macaque; R = rhesus macaque; rapid is defined as 25% of infected animals dead by 3 months, and two thirds by 1 year post inoculation; slow is defined as no deaths before 1 year; intermediate indicates that no deaths by 3 months but one third dead by 1 year; none indicates no evidence for AIDS in inoculated animals; minimal indicates rare deaths from AIDS occurring > 1 year post inoculation (Adapted from Hirsch, (19)).

strains used in vaccine challenge experiments include SIVsm, SIVmne, and SIV/Delta670.

Laboratory-generated chimeras between HIV-1 and SIV, called simian-human immunodeficiency viruses (SHIV), have also been created for use in pathogenesis and vaccine studies in macaques. SHIVs typically have gag, pol, vif, vpx, vpr and the LTR of SIV, while tat, rev and env are derived from HIV-1 (Figure 1)(17). While not all SHIVs are pathogenic in macaques, a subset of pathogenic SHIVs have been identified and shown to cause rapid immunodeficiency and disease progression. As a result, several pathogenic SHIVs including SHIV89.6P (derived from SHIV89.6) have been utilized as challenge viruses in vaccine studies. In the past few years, nine other SHIV strains have also been evaluated for infection and replication in macaques (Table 2).

In macaques, pathogenic SIV causes gradual CD4$^+$ T-cell depletion, while pathogenic SHIV tends to cause a profound, acute CD4$^+$ T-cell loss. As a result, some have argued that SIV disease more closely resembles that caused by HIV-1 in humans, and thus, is a better model for vaccine development. Others contend that SHIV contains the envelope of HIV-1 and is, therefore, better able to model vaccines that target the viral envelope glycoproteins. Moreover, while the chronic disease caused by pathogenic SHIV is clearly different from that of HIV-1 in humans, SIVmac and SIVsm may be too virulent. HIV-1 infection in humans causes AIDS within 8 to 10 years and has an average plasma viral load of 10^4-10^5 copies/ml (18) (Figure 2). Infection of macaques with SIVmac251 and SIVsmE660 causes severe immunodeficiency within 3 to 18 months and produces steady state viral loads ranging from 10^5-10^7 copies/ml (19, 20). Nearly 25-30% of animals infected with either SIVmac251 or SIVsmE660 fail to seroconvert and rapidly progress; this pattern of disease is rarely, if ever, seen in HIV-1 infection of humans (20-22). By studying these SIVs in macaques, researchers have voiced concerns that we may be raising the bar too high; an issue that remains unresolved.

Within the SIV model, there are also major differences in disease course and viral load among macaque species. In rhesus macaques of Indian origin, SIVmac251 and SIVmac239 cause peak viral loads between 10^7-10^8 copies/ml and steady-state viral loads between 10^5-10^6 copies/ml. In rhesus macaques of Chinese origin, the viral loads are 1 to 2 $\log_{10}$ lower at each time point and CD4$^+$ T-cell loss is more gradual (23). SIVmne infection results in lower viral loads than SIVmac or SIVsm infection (24). Cynomolgus macaques infected with SIVmac251 have lower viral loads (1 to 3 $\log_{10}$) during acute and chronic disease when compared with rhesus macaques (25). Rhesus macaques that express the MHC Class I allele, Mamu-A*01, naturally control SIVmac251/239 replication and have set point viral loads 1 to 2 $\log_{10}$ lower than Mamu-A*01 negative animals (26-28). Belated recognition of this fact has clouded the interpretation of studies where Mamu-A*01 animals are over-represented in the vaccinated groups (26, 28). The many nuances of the macaque model often make comparison of one study to another very difficult.

Among the different species of macaques, rhesus macaques (*Macaca mulatta*), cynomolgus macaques (*Macaca fascicularis*), and pigtailed macaques (*Macaca nemestrina*) are most commonly used for pathogenesis and vaccine studies (reviewed in (19)). Early studies focused on chimpanzees as the only NHP susceptible to infection with HIV-1; however, these animals are infrequently used today because of their failure to develop significant viremia and disease following HIV-1 inoculation. Moreover, legal and ethical issues have created mounting pressure for laboratories in the United States to discontinue research using chimpanzees. Consequently, SIV infection of macaques has become the most frequently used model to evaluate candidate vaccines (29). Despite the ability to establish SIV infection, this model is not without drawbacks. Due to the high cost of macaques and limited supply, vaccine studies are often constrained to the use of a

Table 2. Vaccine trials in macaques using SHIV as challenge virus

Author Reference	Macaque Species	SHIV challenge virus strain	Rt	Prime (Vector- Viral Antigens)	Boost (Vector- Viral Antigens)	Acute Viral Load	Chronic Viral Load	Disease/Comment
Akahata [107]	Rhesus	NM-3rN	iv	Zn-finger mutant, full-length proviral DNA	Same	2-3 $\log_{10}$ decrease	Earlier suppression of viremia than controls.	SHIV strain not potent. Control animals also suppressed viremia, but later than vaccinees.
Amara [108]	Rhesus	89.6P	iv	MVA vs. MVA+DNA Gag, Pol, Env	Same	2 $\log_{10}$ decrease	VL undetectable in most vaccinees	Study showed MVA alone worked well, despite much lower CTL response than DNA/MVA.
Amara [87]	Rhesus	89.6P	ir	DNA Gag, Pol, Env vs. Gag, Pol	MVA Gag, Pol, Env vs. Gag, Pol	No change	Gag-Pol animals had increased VL c/w Gag, Pol, Env animals	Inclusion of Env in vaccines is important in containing SHIV89.6P.
Bertley [104]	Rhesus	89.6P	ir	DNA Gag, Pol, Env with IL-2 or IL-12	MVA Gag, Pol, Env	No change	1-2 $\log_{10}$ decrease in all vaccine groups	Vaccine given intra-nasally. Immune responses weak. Limited efficacy.
Bogers [109]	Rhesus	89.6P	ivag	HSP70 linked gp120-p27-CCR5 peptides	Same	No change	Slight Decrease	Poorly controlled study. Role of anti-CCR5 anitbodies not clear.
Buckner [110]	Rhesus	SF162P4	iv	DNA gp140deltaV2	gp140	No Change	No discernible change.	CD8$^+$ T-cells depleted before challenge. Chronic viremia lower in some animals. Not enough controls.
Buge [111]	Rhesus	89.6P	ir	MVA+DNA Gag, Pol, Env	+/- gp120	No Change	Animals given gp120 had increased VL.	gp120 alum vaccination reduced efficacy of DNA/MVA vaccination.
Chackerian [112]	Pigtailed	SF162P3	iv	Papillomavirus like particles conjugated to CCR5 peptides	Same	No Change	No Change	Attempted to test if anti-CCR5 antibodies could block infection. Controls suppressed viremia too.
Dale [113]	Pigtailed	229(mn)	ir	DNA Gag, Tat, Rev	Papilloma Virus with Gag, Tat, Rev	No change	No Change	Inefficient system for inducing protection. Weak immune response.
Doria-Rose [114]	Pigtailed	89.6Pmn	ir	DNA/ Vaccinia Multigene combinations	DNA/ Vaccinia Multigene combinations	1-2 $\log_{10}$ decrease	3/5 controls, 15/24 animals had low VL.	Vaccinia-DNA or DNA-Vaccinia better than other combinations. Controls had low VLs as well.
Izumi [115]	Cynomolgus	SHIV-C2/1	iv	Vaccinia DIs Gag	Same	1-2 $\log_{10}$ decrease	1-2 $\log_{10}$ decrease, but still VL 10^4-10^5	Vaccinia strain DIs does not replicate in mammalian cells and confers limited control of SHIV.
Letvin [89]	Rhesus (excluding Mamu-A*01 animals)	89.6P	iv	DNA Gag, Pol, Nef +/-Env	Adenovirus Gag, Pol +/-Env	1-2 $\log_{10}$ decrease	2 $\log_{10}$ decrease in all three vaccine groups	Study designed to assess role of Env. However, Mock Env group still controlled viremia.
Makitalo [116]	Cynomolgus	SHIV-4	iv	DNA Gag, Pol, Tat-Rev, Env, Nef	MVA Gag, Pol, Tat-Rev, Env, Nef	1-2 $\log_{10}$ decrease	All animals, including controls, had undetectable VL at 8 weeks.	Small groups. Attempted to compare different immunization routes. Weak challenge stock.
Mascola [103]	Rhesus	89.6P	ivag	DNA Gag, Env plus IL-2 plasmid	Passive Transfer of Neutralizing Antibodies	DNA group same as Sham DNA with Neutralizing Antibodies	No Change	DNA vaccination did not enhance ability of neutralizing antibodies to clear infection.
Mooij [98]	Rhesus	89.6P	iv	DNA Gag, Env, Tat vs. Tat	Proteins Gag, Env, Tat vs. Tat	No change	>3 $\log_{10}$ reduction in 3/4 Gag, Env, Tat animals	Tat alone animals not protected. VL suppression correlated w/ Th2 response to Env & Th1 to Gag.
Ramsburg [117]	Rhesus	89.6P	iv	VSV Gag, Pol, Env	VSV vs. MVA Gag, Pol, Env	1 $\log_{10}$ decrease	2 $\log_{10}$ decrease in animals boosted w/ MVA	VSV better w/ MVA boost. Protection not from antibody, but not clear which CMI response important.
Santra [102]	Rhesus	89.6P	iv	DNA Gag, Env & IL-2	DNA or Poxvirus Gag, Env	1 $\log_{10}$ decrease	3 $\log_{10}$ decrease in all vaccine groups	Three different Poxviruses used w/ similar IR. However, DNA/IL-2 alone effectively controlled viremia.
Shiver [118]	Rhesus	89.6P	iv	Adenovirus Gag	Adenovirus Gag	1-2 $\log_{10}$ decrease	3 $\log_{10}$ decrease	Many vectors tested. Adeno-adeno prime/boost gave best control of viremia.
Slivera [97]	Rhesus	89.6P	iv	Tat or Tat toxoid from HXB or SHIV89.6	Same	No Change	No Change	Neither Tat nor Tat toxoid vaccination leads to control of viremia.
Takeda [119]	Rhesus	89.6PD	iv	DNA Proviral DNA w/ Env, Nef deletions	Sendai virus *Gag*	1 $\log_{10}$ decrease in 2/3 animals.	VL undetectable in all 3 vaccinees.	Study used historical controls. Small vaccine group. CTL data limited.
Voss [120]	Rhesus	89.6P	iv	gp120, HIV-1 NefTat Fusion protein, SIV Nef protein	Repeated vaccination	No Change	Low VL in animals receiving all three proteins.	Animals receiving NefTat & Nef did NOT control viremia. Protection varied w/ type of adjuvant.
Willey [121]	Rhesus	DH12R-PS1	iv	Vaccinia Gag, Pol, Env	AT-2 inactivated HIV-1 DH12 & SIVmac239	1 $\log_{10}$ decrease in 3/4 animals.	Partial control of viremia up to 60 weeks.	Vaccine regimen led to partial control. Viremia >10^3 in all 4 vaccinated animals during chronic phase.

c/w – compared with; ir – intra-rectal; iv – intravenous; ivag – intra-vaginal; MVA – Modified Vaccinia Ankara; Rt – route of challenge; VL – plasma viral load; VSV – vesicular stomatitis virus; w/ - with; IR- immune response.

small number of animals (N<20). Often, in the interest of economy, this initial group of animals is further divided into sub-groups of 4 to 5 macaques to simultaneously evaluate several related strategies. An unfortunate consequence of such small group sizes is the loss of statistical power and the potential for misinterpretation of results due to inapparent biases. However, in the absence of an alternative, research in the SIV-macaque model has provided valuable information on the induction of immune responses and the efficacy against pathogenic virus challenge for many candidate vaccines. In the following sections, we will review data generated in this model encompassing several different vaccine strategies.
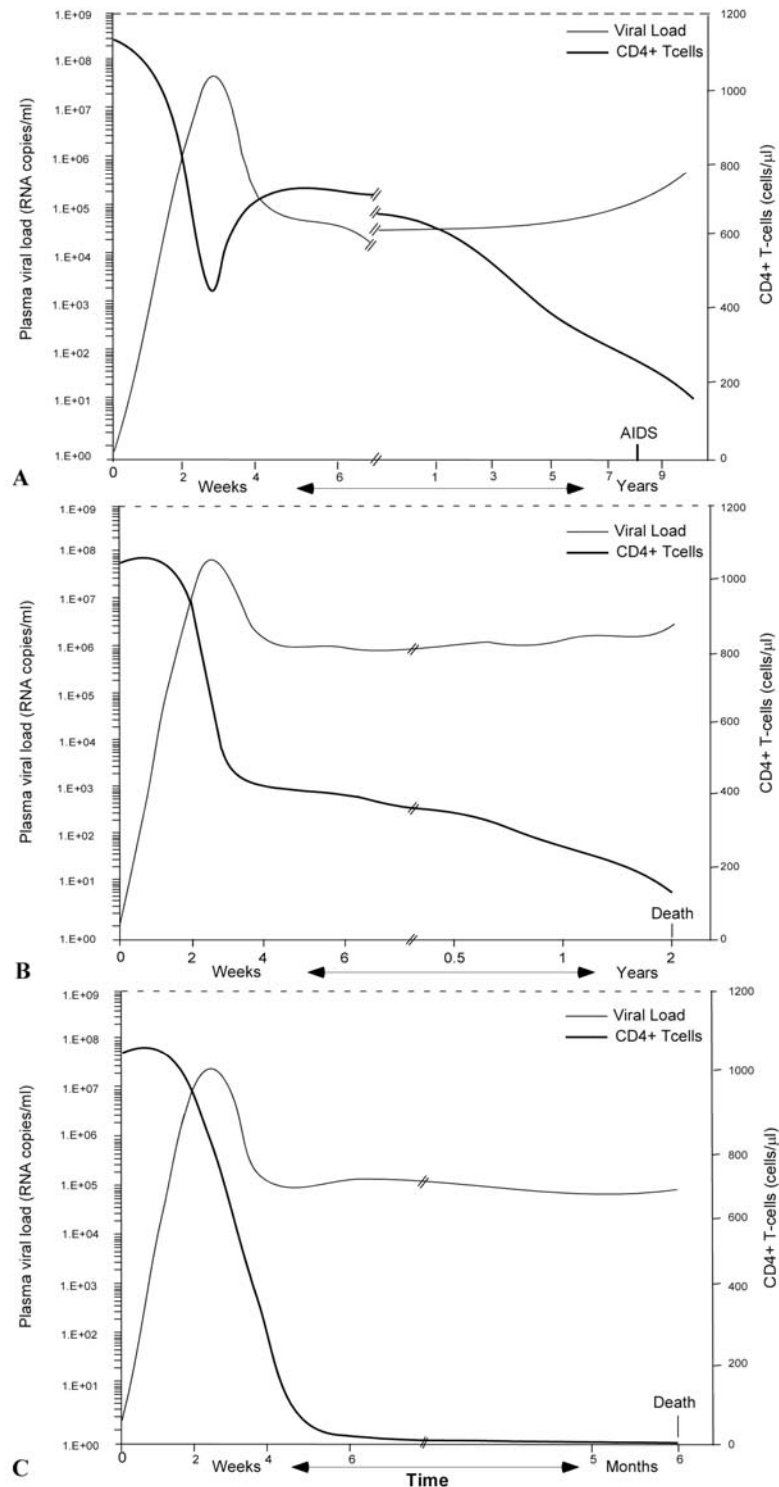
Figure 2. Contrast of HIV-1, SIV and SHIV infection. Schematic representation of typical course of each infection is shown. HIV-1 infection of humans results in highly variable rates of disease progression. **(A)** Typical HIV-1 infection is characterized by acute phase viremia followed by chronic intermediate levels of plasma viremia, a steady loss of peripheral CD4$^+$ T-cells and the development of AIDS after 7 to 10 years. **(B)** SIV infection of macaques follows a course similar to that of HIV-1 in humans with higher set-point viremia and steady loss of CD4$^+$ T-cells resulting in death within 2 years of infection. **(C)** In contrast, SHIV-89.6P infection of macaques results in near-complete destruction of the peripheral CD4$^+$ T-cell population within the first weeks of infection, uncontrolled high-level virus replication and rapid progression to death. (Adapted, in parts, from Feinberg and Moore, (106)).

4.1. Inactivated Whole Virus Vaccines

Using whole inactivated virus as an HIV vaccine was among the first strategies tested. Historically, this approach has been largely successful in affording protection against several other viral infections including polio (Salk vaccine) and hepatitis A. Such an approach worked well in macaques in initial experiments (30, 31). In the following years, whole-inactivated vaccines were shown to protect macaques from pathogenic SIV infection by intravenous (32), intra-rectal (33) and intravaginal routes (34). The initial optimism of these results turned out to be based on false hope. SIV used for vaccine preparations was grown in human cells and the human cellular proteins were incorporated into the vaccine virus particles. Such vaccine preparations induced antibody responses in vaccinated animals against the human cellular proteins and afforded protection against the challenge viruses, which were also propagated in human cells. High levels of antibodies against human cellular proteins were detected in the protected animals and the degree of protection correlated with the level of these antibody titers (35, 36). This observation of xeno-antigen-induced protection was further confirmed by experiments where macaques immunized with human cells alone (37), HLA-DR (38) or HLA class I proteins (39) were protected against SIV grown in human cells, but not against SIV grown in macaque cells. Several researchers tried to exploit the finding of xeno-antigen responses by designing vaccine strategies based on alloimmunization (40-42), but concerns over inducing autoimmune responses rendered this approach unattractive. In a recent attempt, Lifson *et al.* (24) reported that SIVmne infection could be significantly controlled after vaccination with chemically inactivated, homologous virus particles. However, the same animals were not protected from heterologous challenge with the more virulent SIVsmE660.

4.2. Passive Immunization

Passive immunization as a concept for evaluating the protective effect of humoral immunity has been studied by many groups, although this strategy is generally considered unfeasible as a way to protect large populations against HIV infection. Instead, passive immunization studies with antibodies or sera provide proof of concept for vaccines that induce a predominantly humoral response. Initial studies used polyclonal sera from vaccinated animals or animals that had controlled infection naturally (reviewed in (29)). While some studies using weakly pathogenic viruses, such as HIV-2 in pig-tailed macaques, showed efficacy, other trials using pathogenic viruses, like SIVmac, did not (43, 44).

The failure of pooled polyclonal sera in passive immunization against SIVmac led investigators to use potent neutralizing antibodies (NAbs) in similar studies. Several NAbs against HIV have been discovered. These NAbs are monoclonal and can be produced in large amounts *in vitro*. In passive transfer studies, synergistic combinations of such NAbs (2G12, 2F5, 4E10, F105, IgG1b12 or b12) conferred protection in macaques against intravenous (45), vaginal (46, 47) or oral (48) challenge with different SHIVs. In subsequent studies, neonatal macaques receiving post-exposure infusion of NAbs (b12, 2G12, 2F5 and 4E10) were protected from oral challenge with SHIV89.6P (49-52). Intravaginal application of one NAb (b12) at a high dose has been shown to protect macaques from vaginal challenge with SHIV 15 minutes after application of the NAb (53). These passive immunization studies confirm that potent antibodies at high levels can block infection with SHIV. However, despite many attempts, researchers have been unable to develop an immunogen that induces comparable NAbs *de novo*. Further, in these experiments, the serum titer needed to protect animals against infection is much higher (16 to 80 fold) than that needed to block infection *in vitro* (46).

4.3. Live-attenuated Virus Vaccines

Live-attenuated virus vaccines against several infectious viruses (e.g. polio and measles) have been successful due to their ability to replicate in the host and, thus, to induce a full range of durable immune responses. In the case of HIV-1 infection, safety concerns have prevented the development and testing of a live-attenuated HIV-1 vaccine in healthy human populations. As the virus establishes a persistent infection by incorporating its genome in the host genome, the safety profile for an attenuated HIV-1 vaccine would not be known for years to decades after the initial study in an experimental group. However, to date, this approach has been the most consistently successful in preventing infection with pathogenic SIV in macaques.

Research into a live-attenuated SIV vaccine in the macaque model began serendipitously. In a study that addressed the *in vivo* importance of the SIV *nef* gene, a cloned SIVmac239 carrying a premature stop signal at the 93rd codon of *nef* was found to quickly mutate *in vivo* and open up the *nef* reading frame (54). In a follow-up study, rhesus macaques were infected with a version of SIVmac239 that had a 182 base pair deletion in *nef*, SIVmac239Δnef (55). These animals were observed for one year and none developed disease. After this period of observation and chronic infection with SIVmac239Δnef, the same macaques were challenged with pathogenic SIVmac251; none became infected. This observation sparked interest in the development of a live-attenuated vaccine by gene deletion. SIV lacking *nef* was shown to induce protective immunity against closely related pathogenic SIV containing intact *nef* genes (56, 57), and against high doses of highly pathogenic strains of SIV (58), heterologous strains (59) or cell-associated virus (60) and challenge via the intravenous (55, 60), mucosal (61) or oral routes (62). Initial success with the attenuated SIV-macaque model raised expectations of an attenuated *nef*-deleted HIV vaccine. This hope was supported by observations in the Sydney Blood Bank Cohort (SBBC), a group of individuals who became infected with naturally attenuated HIV-1 through blood transfusion from the same donor (63). Similar to some attenuated SIV vaccines, this HIV-1 variant had deletions in *nef* and the U3 region of the LTR. These individuals remained clinically asymptomatic for 10 to 14 years and had stable CD4$^+$ T-cell counts. In further studies, triple-deletion mutant viruses with deletions in *nef*, *vpr* and upstream sequences of the LTR

(SIVmac239Δ3; Figure 1) induced protection against wild-type SIVmac, but the protection appeared to be time dependent: either full, partial or no protection was observed in macaques challenged at 79, 20 or 8 weeks after primary SIVmac infection, respectively (58).

Unfortunately, SIVmac239Δ3 was found to cause disease in neonatal macaques (64) and gradually in adult macaques as well (65), raising concerns about the safety of an attenuated vaccine approach. Further, members of the SBBC began to experience CD4$^+$ T-cell loss and appreciable increases in viral load (66). These and other observations increased safety concerns over the potential use of attenuated HIV vaccines in humans. In other macaque studies, the degree of protection was found to be inversely related to the level of attenuation (67, 68). Attenuated SIV vaccines only confer protection if the vaccine replicates at low, but consistent levels. Viruses that do not replicate appreciably do not confer protection. However, this need for low-level replication over long periods affords the virus time to mutate to a more pathogenic form (69-71). Consequently, work in this area has slowed and no attenuated form of HIV has been tested in humans.

In an attempt to improve the safety of attenuated vaccines, we and others have focused on the "gain-of-function" approach (72-75). This strategy attempts to gain control over virus replication by inserting exogenous genes (i.e. HSV-1 thymidine kinase) or gene expression systems (tetracycline dependent operon) into the *nef* reading frame. The first approach aims at elimination of infected cells by rendering them susceptible to drugs, while the second approach controls virus replication through drug dependency. With either approach, the attenuated vaccine is given to a host and, after an immune response has developed, the drug is discontinued (or administered depending on the approach employed) and virus replication ceases. In the tetracycline dependent system, the Tat/TAR axis of HIV is substituted with the inducible tetracycline operator (*tetO*) in the U3 region of the LTR and *nef* is replaced with a codon-optimized gene for the tetracycline-inducible repressor protein (Figure 1). Although the extent of the virus replication can be controlled by this system (73), the genetic stability of such viruses over time has been called into question based on the leakiness of the system (75) and the constant evolution of the virus over several passages *in vitro* (76). In another approach called "gain-of-repression", the virus is severely attenuated by expressing an NF-kappaB inhibitor (IkappaB-alphaS32/36A) in the *nef* gene with the expectation of decreased Tat-mediated transactivation of the virus using the NF-kappaB, Sp-1 *cis* acting sequences in the LTR (77). In this study, macaques immunized with SIVIkappaB-alphaS32/36A (an SIVmac239-based virus) showed virus-specific antibodies and T-cell responses, but the importance of these immune responses in protection remains unknown as no challenge studies were performed.

The mechanisms of protection conferred by attenuated SIV have not been fully elucidated (78). The potential protective mechanisms currently investigated are:

NAbs, antiviral cellular immune responses, beta-chemokine production and viral interference. NAb responses do not seem to be the dominant defense mechanism induced by live-attenuated SIV, since attempts to induce protection by passive transfer of antibodies from animals vaccinated with attenuated viruses have been generally unsuccessful (79). The results of *in vitro* neutralization assays vary widely depending on the specific virus and cell line used. For example, in macaques vaccinated with SIVmac239Δnef, high titers of NAbs against a T-cell line adapted strain of SIVmac251 developed. However, even in animals protected against SIVmac251 infection, little to no neutralizing activity against the primary SIVmac251 challenge stock was seen (57). It is, therefore, unlikely that attenuated SIV vaccines protect against pathogenic SIV challenge via NAbs.

The role of cell-mediated immunity in protection induced by live-attenuated SIV has also been questioned (80, 81). Sharpe *et al.* (80) followed the SIV-specific cellular responses in macaques infected with SIVmacGX2, which is attenuated via a 66 base-pair deletion in *nef*. The CTL responses against dominant Gag and Tat epitopes waned over time, yet macaques were protected against challenge with pathogenic SIVmac220, despite these declining epitope-specific CTL responses. Further, cytokine profiles (gamma interferon, INFgamma; IL2) remained unchanged in the immunized, protected macaques (81). At present it is not known whether the protection induced by live-attenuated SIV is due to competition for scarce target cells (82) or innate rather than adaptive immune responses. Vaccination of cynomolgus macaques with live, non-pathogenic SHIV-4 showed a correlation between spontaneous beta-chemokine production and IFNgamma-secreting cells, with complete protection against SIVsm challenge (83). In another study, rhesus macaques were infected with non-pathogenic SHIV89.6, and subsequently protected from SIVmac239 challenge. The protected animals had higher frequencies of SIV Gag-specific CTL and IFNalpha mRNA in PBMC. This finding suggests the involvement of both adaptive and innate immune responses in attenuated virus-induced protection (84). Elucidation of the protective mechanisms could be crucial for developing a vaccine that does not produce a chronic infection but does induce a similarly effective immune response.

4.4. Prime-Boost Strategies

The HIV envelope protein (Env) is heavily glycosylated and is produced as a single protein, gp160, which is subsequently cleaved to form gp120 (or surface unit, SU) and gp41 (or transmembrane, TM). Both HIV and SIV Env exist on the virion as homo-trimers of gp120/41, in which gp120 is bound to gp41 through non-covalent forces. This trimeric arrangement is present on the membrane of productively infected cells as well as virions (85). Binding of gp120 to the CD4 receptor and co-receptors (either CXCR4 or CCR5) on the cell surface triggers a series of conformational changes leading to gp41-mediated fusion of the viral and host cell membranes. Antibodies directed against either gp120 or gp41 may inhibit binding or fusion of HIV and, in principle,

Table 3. Vaccine trials in macaques using SIV as challenge virus

Author Reference	Macaque Species	SIV challenge virus strain	Rt	Prime	Boost	Acute Viral Load	Chronic Viral Load	Disease/Comment
Allen [99]	Rhesus (Mamu-A*A01)	mac239	ir	DNA Full-length Tat or Tat peptide (SL8)	MVA Tat	No Change	No Change	Despite strong Tat-specific CTL, SIVmac239 viremia was not controlled. Escape mutants not seen at 2 weeks.
Evans [122]	Rhesus	mac239	ir	Salmonella Gag	MVA Gag	No Change	No Change	Salmonella weakly immunogenic.
Fuller [123]	Rhesus	DeltaB670	ir	DNA Gag, Pol, & Env	Same	4/7 Animals had undetectable VL.	4 animals controlled VL with intermittent blips	Vaccine induced control of viremia in some animals. No correlation with immune response.
Hel [124]	Rhesus	mac251	ir	NYVAC Gag, Pol, & Env	DNA Gag, Env	No Change	VL < 10^4 copies/ml in 4/8 vaccinated animals vs. 1/8 controls	Partial protection at 24 weeks. Long-term efficacy unknown.
Horton [88]	Rhesus	mac239	ir	DNA Multiple genes	MVA Multiple genes	1 $\log_{10}$ decrease	No Change	Approach that worked against SHIV-89.6P failed against SIVmac239.
Kuate [125]	Rhesus	mac239	iv	Single cycle SIV particles	Same	1 $\log_{10}$ decrease	No Change	No effect on long term VL.
Lena [105]	Rhesus	mac251	oral	DNA Gag, Env +/- GM-CSF & IFNγ	Same	No Change	No Change	GM-CSF/IFNγ adjuvancy had no effect on SIVmac251 VL. Cytokines did increase immune responses.
Matano [126]	Rhesus	mac239	iv	DNA Multiple genes	Sendai virus Gag	1 $\log_{10}$ decrease	5/8 vaccinees had undetectable VL c/w 0/4 controls.	Impressive control of SIVmac239 associated with Gag CTL escape and less fit virus.
Mossman [127]	Cynomolgus	mne	ir	DNA All viral genes	gp120 & Gag particles	1 $\log_{10}$ decrease	No Change	Limited protection against a weakly pathogenic strain of SIV.
Muthumani [128]	Rhesus	mac251	ir	DNA Gag, Pol, Env, & Rev	Same	2 $\log_{10}$ decrease	2-3 $\log_{10}$ decrease	Immune correlate not established. VL still detectable and CD4$^+$ T-cells still dropped in vaccinees.
Negri [129]	Cynomolgus	mac251	ir	DNA Gag, Pol, Tat, Rev, Env, & Nef	SFV & then MVA	2-3 $\log_{10}$ decrease	3/4 vaccinees had undetectable VL c/w 2/8 controls.	Control of viremia correlated w/ post-challenge CTL. Controls had lower VLs than in Rhesus macaques.
Nilsson [130]	Cynomolgus	sm	ir	MVA Gag, Pol, Env	gp148 & p27	No Change	No Change c/w MVA vector alone	MVA alone animals had lower VLs than naïve animals. Too few animals per group.
Nilsson [131]	Cynomolgus	sm	ir	MVA or SFV Gag, Pol, Tat, Rev, Env, & Nef	MVA Gag, Pol, Tat, Rev, Env, & Nef	No Change	No Change	Vaccine made with SIVmac genes. Increased CTL in SFV/MVA group, but no control of SIVsm.
Pal [25]	Rhesus	mac251	ir	Canary pox Gag, Pol, & Env	gp120	Slight decrease	No Change	Analysis of results confused by recognition that Mamu-A*01 animals control SIVmac251 naturally.
Patterson [90]	Rhesus	mac251	ir	Adenovirus Env, Rev, Gag, & Nef	gp120	1-2 $\log_{10}$ decrease	No statistical difference.	Adenovirus-based vaccine did not provide substantial protection against mac251.
Vogel [132]	Rhesus (Mamu-A01)	mac251-32H	iv	Lipopeptides with Gag, Env, and Nef epitopes	Same	No Change	No Change	Vaccine induced little CTL response & no effect on VL.
Vogel [100]	Rhesus	mac239	ir	DNA Tat, Rev, Nef, & Gag-epitopes	MVA (iv) Tat, Rev, Nef, & Gag-epitopes	1 $\log_{10}$ decrease	No Change	Despite strong mucosal CTL response, only a small decrease in acute VL and no effect on chronic VL.
Zhao [26]	Rhesus	mac251	ir	Adenovirus Env, Rev, & Gag	gp120	1 $\log_{10}$ decrease	No Change	Adenovirus approach did not work against SIVmac251.

c/w – compared with; ir – intra-rectal; iv – intravenous; ivag – intra-vaginal; MVA – Modified Vaccinia Ankara; Rt – route of challenge; SFV – Semliki Forest Virus; VL – plasma viral load; w/ - with

neutralize infectivity. Efforts to induce the production of NAbs against the HIV envelope glycoproteins through immunization have not been successful. This is due, in part, to the relative neutralization resistance of primary isolates of HIV-1, compared to laboratory-adapted strains of the virus.

While efforts to induce NAb production through immunization continue, researchers are now focusing on the development of vaccines aimed at stimulating cell-mediated immunity. The goal of these vaccines is not to prevent infection, but rather to control or suppress viremia after infection has occurred. This is a novel approach

considering past experience with human viral vaccines designed to either block infection from occurring (e.g. hepatitis B virus) or enhance clearance of infection (e.g. rabies virus). A summary of recent vaccine efforts in this area follows for both SHIV (Table 2) and SIV (Table 3).

The studies are ordered alphabetically by first author. Table 2 contains studies, which use a pathogenic SHIV as the challenge virus; Table 3 details studies, which use a SIV strain as the challenge virus. Variables shown include species of macaque, strain of challenge virus, route of challenge, prime/boost summary, and effect of vaccination on acute and chronic viral loads. Finally, a comment or interpretation of the data is given. For both tables, the dose of the challenge virus has been purposely left out. Often the challenge routes vary and the challenge stocks are made independently of each other, making it difficult to present dosing information in a uniform manner. Other omitted variables include the number of immunizations and time between the last immunization and challenge. Many studies tested more than one strategy simultaneously, and only the approach that showed the most protection is detailed. It is also worth noting that vaccine-induced protection against SHIV-associated acute immunosuppression is more easily achieved than against SIV-associated chronic immunosuppression. As shown in Tables 2 and 3, 15 of the 22 SHIV studies used an approach that resulted in a significant reduction of chronic viremia. In contrast, only 4 of 19 SIV studies had some effect on chronic viral load, despite the use of similar or identical strategies.

In evaluating prime-boost vaccination strategies, two frequently used vectors are DNA and poxviruses. Direct inoculation of purified preparations of DNA as a means of immunization has become an exciting area of research, stemming from an unexpected observation in mice (86). DNA, encoding antigens or even adjuvant cytokines, can be delivered either intradermally, intramuscularly or mucosally, where it is taken up by host cells. Host cell production of the encoded proteins may enhance presentation of antigenic peptides on MHC Class I molecules, and facilitate the development of antigen-specific CTL responses. Cytokine delivery by DNA allows for localized delivery to the same tissue where antigen is being expressed and processed.

Several forms of recombinant poxviruses have also been developed as vaccine vectors, with modified vaccinia Ankara (MVA) being perhaps the most widely studied. Other forms include vaccinia DIs, recombinant vaccinia, canarypox and fowlpox. Recombinant poxviruses are used as vectors to encode foreign proteins that are expressed intracellularly. In theory, intracellular expression of the foreign protein should lead to the induction of enhanced cellular immune responses compared with exogenous introduction of a soluble protein. Poxviruses have differing abilities to replicate in primate cells, which may in turn influence their safety profiles. For example, canarypox, fowlpox and vaccinia DIs do not replicate in primate cells and may have better safety profiles than replication-competent MVA and recombinant vaccinia.

Recent Phase 2 clinical trials have shown that canarypox is, indeed, safe. However, it is also poorly immunogenic, underscoring the need to consider the balance of risk to benefit when developing an efficacious HIV vaccine.

A DNA prime/MVA boost strategy has been consistently effective against challenge with pathogenic SHIV89.6P in macaques. In early studies, both vectors encoded the viral Gag-Pol and Env proteins, while a recent study has shown that Gag-Pol without Env is less effective as an immunogen (87). Repeated immunization with MVA alone, expressing Gag-Pol and Env, produced control of SHIV89.6P (87). Interestingly, when gp120 protein boosting was added to the DNA/MVA Gag-Pol/Env regimen, the protective efficacy against SHIV89.6P decreased. Animals that received the gp120 boost were less able to control viremia compared to those immunized only with DNA/MVA. Using a similar vaccination approach, Horton et al. (88) failed to show any long-term benefit against SIVmac239 infection. However, immunization with NYVAC, a form of vaccinia, in combination with a DNA boost conferred partial protection to rhesus macaques challenged with SIVmac251. In this study, 50% of the NYVAC/DNA animals controlled viremia up to 24 weeks (long term viral load data were not provided) compared with 12.5% of control animals.

Several other recombinant viruses have been used as vectors to deliver HIV genes, including replication-deficient adenovirus. Merck Research Laboratories have demonstrated that a DNA prime/adenovirus boost vaccination strategy led to good control of SHIV89.6P. Letvin et al. (89) recently tested the role of Env in the DNA/adenovirus approach against SHIV89.6P. Unlike the DNA/MVA results, animals vaccinated with DNA/adenovirus vectors encoding Gag-Pol and Nef, but not Env, still controlled viremia; however, the CD4$^+$ T-cell count was better preserved in macaques immunized with Env-containing vectors. Adenoviral vectors expressing Gag-Pol/Env were used to boost immune responses primed by recombinant vesicular stomatitis virus (VSV) that also expressed Gag-Pol/Env. Again, SHIV89.6P viremia was reduced, and the animals boosted with the adenoviral vector controlled viremia better than those boosted with the VSV vector. In contrast, two recent studies tested recombinant adenoviral vectors against challenge with SIVmac251 (26, 90). While potent cellular immune responses were induced in the vaccinated animals (91, 92), they failed to control viremia to any great extent. Although not formally published, Merck Research Laboratories have generated similar findings using a DNA prime/adenovirus boost that was effective against SHIV89.6P, but failed to confer significant protection against SIVmac239 (93).

Most vaccine strategies target Gag-Pol and Env as the main viral antigens. However, some experiments have studied Tat, which potently transactivates the HIV and SIV promoters. Tat is also believed to contribute to HIV pathogenesis in other ways, including the induction of apoptosis and the induction of cytokine production. Early studies used Tat as the sole immunogen, and demonstrated that SHIV viremia could be controlled by anti-Tat

immunity (94-96) when Tat in the SHIV challenge was derived from HIV-1. Unfortunately, recent studies have failed to confirm this observation. Silvera *et al.* (97) tested Tat as either a pure soluble protein or as a chemically inactivated toxoid from HIV-1 IIIB or SHIV89.6P. Rhesus macaques immunized with these preparations developed strong humoral and Tat-specific CD4$^+$ T-cell helper responses. Yet, after challenge with SHIV89.6P, the immunized animals had viral loads equivalent to that of non-immunized controls. In further studies, Mooij *et al.* (98) used a DNA prime/protein boost strategy to compare immunization of rhesus macaques with Tat alone versus Tat/Env and Gag. Animals that received Tat alone did not control SHIV89.6P viremia and failed to develop a strong Tat-specific CTL response, while those that received Tat/Env/Gag achieved partial control of viremia. Using a DNA prime/MVA boost approach, strong Tat-specific CTL responses were generated in rhesus macaques; however, the animals developed significant viremia after challenge with SIVmac239 (99). Vogel *et al.* (100) had similarly disappointing results after SIVmac239 challenge using a multiple antigen approach that included Tat. Taken together, these data suggest that immunization with Tat-based vaccines alone is insufficient to generate broadly protective immunity. The conflicting studies using SHIV, and the negative studies with SIVmac239, do not currently support continued development of Tat-based strategies unless a new approach is conceived.

In the past several years, investigators have used cytokines as adjuvants for SHIV vaccines. Barouch *et al.* (101) first demonstrated that IL-2, when fused to the Fc portion of IgG, increased the efficacy of a DNA-based vaccine against SHIV89.6P. The IL-2/Ig fusion protein was delivered either as a soluble protein or as a plasmid encoding IL-2/Ig. Macaques given either form of IL-2/Ig had improved virologic control compared to animals that received the DNA vaccine alone. Co-administration of DNA with IL-2/Ig was associated with increased CTL responses against Gag and Env epitopes. These investigators have continued to use an IL-2/Ig expression plasmid as an adjuvant in SHIV vaccine studies (102, 103). In other studies, administration of either IL-2 or IL-12 plasmids (104) to macaques in conjunction with intra-nasal DNA prime/MVA boost had little effect on the ability of the immunized animals to control SHIV89.6P viral load. Lena *et al.* (105) studied the combined effect of GM-CSF and IFNgamma as plasmid adjuvants to DNA vaccination against SIVmac251. Co-administration of the cytokines was associated with increased cellular and humoral immune responses; however, SIVmac251 viremia was not affected by immunization. To date, data on the comparative efficacy of cytokines and their adjuvant effects are limited.

5. CONCLUSIONS AND FUTURE DIRECTIONS

In the history of medicine, no vaccination effort has been as large or as extensive as that against HIV-1. Many different approaches and multiple attempts at each have not yielded a clear avenue to follow. From these efforts we have learned that prevention of infection through vaccination (i.e. attenuated SIV) is possible. However, we also know that it is difficult to induce NAbs by vaccination, despite the recognition that this approach offers the most promise for a truly preventive vaccine. CTL-based vaccines can protect against disease in some NHP models of HIV, but not in others, and escape from CTL can lead to vaccine failure (133). HIV-1 vaccine trials are expensive and heavily politicized, and the current scientific landscape suggests that a successful HIV-1 vaccine may still be years away. Until that time, widespread use of antiretroviral drugs offers a tangible hope to slow the epidemic (134).

What we have not discussed here are aspects of human innate and intrinsic immunity against HIV. Studies in these areas have lagged behind the current focus on anti-HIV adaptive (humoral and cell mediated) immunity. Recent findings, however, raise exciting prospects that human cells may contain protein factors such as TRIM5 and APOBEC3 that serve to attenuate HIV replication (135). Moreover, there is emerging evidence that the nucleic-acid based immunity directed against foreign pathogens found in plant and invertebrate cells (i.e. siRNA and miRNA; 136, 137) are also physiologically conserved defenses used by human cells against viruses. In data to be presented, we find that HIV-1 indeed contains siRNA (Bennasser *et al.*, unpublished) and miRNA (138) sequences. These sequence motifs can and do provoke HIV-specific restriction in human cells. Going forward, vaccination approaches that collectively elicit innate, intrinsic, and adaptive immunity may be needed in order for the human host to ward off HIV.

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Send correspondence to: Stephen M. Smith, Saint Michael's Medical Center, Newark, New Jersey, 07102, USA, Tel: 973-877-2711, Fax: 973-877-2661, E-mail: ssmith1824@aol.com

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