Lessons in Nonhuman Primate Models for AIDS Vaccine Research: From Minefields to Milestones

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Nonhuman primate (NHP) disease models for AIDS have made important contributions to the search for effective vaccines for AIDS. Viral diversity, persistence, capacity for immune evasion, and safety considerations have limited development of conventional approaches using killed or attenuated vaccines, necessitating the development of novel approaches. Here we highlight the knowledge gained and lessons learned in testing vaccine concepts in different virus/NHP host combinations.

In the early years of the AIDS pandemic, the search for an animal model for HIV-1 infection focused on experimental infection of chimpanzees (Pan troglodytes), resulting in productive infections and new information about transmission (Fultz et al. 1986a,b). However, disease in chimpanzees occurred rarely and only after >10 years of infection (Novembre et al. 1997). During the early 1980s, outbreaks of immunodeficiency-associated diseases occurred in Asian macaques (Macaca species; Figs. 1 and 2) at multiple primate centers. The animals succumbed to neoplasms and opportunistic infections, paralleling the newly described human disease now known as AIDS (Gottlieb et al. 1981; Masur et al. 1981; Siegal et al. 1981). Whereas some cases were associated with a D-type retrovirus (Daniel et al. 1984; Marx et al. 1984; Stromberg et al. 1984), others were linked to novel simian lentiviruses (Daniel et al. 1985; Letvin et al. 1985; Benveniste et al. 1986; Murphey-Corb et al. 1986) related to the newly discovered etiologic agent for human AIDS (Barre-Sinoussi et al. 1983; Gallo et al. 1984; Popovic et al. 1984). The source of the lentiviral infections in Asian macaques was cross-species transmission via documented or presumed exposure in captivity to African nonhuman primates (NHPs) infected in the wild (Apetrei et al. 2005).

Disease in Asian macaques—M. mulatta (rhesus), M. nemestrina (pigtailed), and M. fascicularis (cynomolgus)—had striking similarities with human AIDS, including acute and then progressive loss of CD4+ T cells followed by clinical immunodeficiency, opportunistic infections, and neoplasms (reviewed in Hirsch and Lifson 2000). The causative viruses—simian
immunodeficiency viruses (SIVs)—were shown to transmit the infection and reproduce the characteristic disease course. The molecular cloning of these SIVs allowed the development of chimeric viral constructs with HIV genes spliced into SIV backbones, termed simian-human immunodeficiency viruses (SHIVs; Li et al. 1992) intended to address certain questions in NHP models. SHIVs bearing HIV Envelope protein (Env) allowed testing of HIV Env-based vaccines or other Env-targeted interventions. The ability to infect NHP with a known amount of a characterized SIV or SHIV stock, via a defined route, and to obtain blood and tissue samples at specific times after inoculation represented powerful experimental advantages. The recapitulation of key aspects of the pathogenesis of human HIV-1 infection, combined with the compressed time course of disease progression, and the ability to perform interventions make experimental SIV or SHIV infection a valuable tool for addressing many important questions in AIDS research. This work focuses on the AIDS vaccine research using NHP models that has guided vaccine development. NHP models allow analyses of (1) the transmission of viruses across mucosal barriers, (2) the definition of relevant challenges via different routes, (3) the timing of viral dissemination, (4) the establishment

Figure 1. Many monkeys, more viruses; right choice brings truth—wrong choice ... delusion. (Original artwork by Joel Ito, Oregon National Primate Research Center.)

Figure 2. Representative image of *Macaca mulatta*. (Original artwork courtesy of Joel Ito, Oregon National Primate Research Center.)
of viral reservoirs in the newly infected host, (5) the quality and magnitude of innate and adaptive immune responses, and (6) demonstration of the protective effects of passive antibody. Comparative immunogenicity and challenge studies have allowed comparisons of vaccines. The outcomes of selected vaccine trials with NHP are summarized in Table 1. NHP models have provided invaluable information for HIV vaccine development; this work aims to provide an understanding of the utility and the limits of this information, critical to interpretation of the results and effective utilization of the models.

NHP Models

The first described SIVs were isolated from infected rhesus or pigtailed macaques (Daniel et al. 1985; Benveniste et al. 1986; Murphy-Corb et al. 1986). Following these studies, additional SIVs were identified and transmitted to other macaque species (Apetrei et al. 2004; Gautam et al. 2009; Souquiere et al. 2009). In addition, molecular chimeras of these viruses have been engineered, allowing multiple viruses with diverse properties to be used in different species and subspecies of macaque. Each combination of virus and route of administration in a different macaque species arguably constitutes a distinct model. Some viruses are molecular clones, some are “swarms” or complex quasispecies mixtures of related viruses, the exact composition and diversity of which may vary with the passage history and production method used, even for different virus preparations designated by the same isolate nomenclature. Each of these individual models has different strengths and limitations. One of the key lessons from the experience of using NHP models for AIDS research is the importance of choosing a model that is appropriate to the question posed. This issue has been at the heart of controversies surrounding the interpretation of vaccine experiments in NHP. This work highlights the key features of some different experimental models and points out areas in which there is room for continued development.

Advances in Understanding Mucosal Transmission in NHP

Most HIV infections involve sexual transmission via mucosal routes. Whereas initial experiments in macaques and chimpanzees used intravenous inoculation, subsequent studies modeled mucosal transmission via rectal (Keele et al. 2009), vaginal (Stone et al. 2010), and penile routes (Ma et al. 2011) or oropharyngeal transmission in neonates (Abel et al. 2006). In the absence of overt mucosal lesions, attempted mucosal transmission with infected cells rather than cell-free virus has been unsuccessful (Sodora et al. 1998), although a recent report claimed successful vaginal transmission using infected cells (Salle et al. 2010). Mechanistic details of mucosal transmission, including how virus applied to a mucosal surface reaches initial target cells, the identity of these initial target cells, and the mechanisms of local amplification at mucosal sites and pathways of dissemination leading to systemic infection remain only partially understood and somewhat controversial, but NHP models allow experimental investigation (Haase 2010). Whereas Langerhans cells can take up virus from vaginal inocula (Miller and Hu 1999), CD4⁺ T cells are the first cells productively infected at mucosal sites (Li et al. 2009a). Far from being simply a physical barrier, the female genital mucosa is a complex, dynamic tissue in which mere exposure to viral inocula can trigger complex responses, including innate immune responses with elements that both hinder and facilitate the establishment of infection, in part by recruiting additional target cells (Li et al. 2009a). Infection can occur across either the vaginal or cervical mucosae, influenced by the stage of menstrual cycle and vaginal epithelial thickness, with sites of preexisting inflammation predisposing to local infection. Whereas local innate and adaptive immune responses influence mucosal infection, it appears that in both naive and suboptimally vaccinated hosts, the virus-specific mucosal T-cell responses are too little, and too late, to significantly alter the course of infection (Reynolds et al. 2005). Understanding how deposition of
Table 1. Selected vaccine trials in NHPs and their outcomes

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Species</th>
<th>Challenge virus</th>
<th>Routea</th>
<th>Outcome</th>
<th>Reference(s)</th>
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<tr>
<td><strong>A. Protein only vaccines</strong></td>
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<tr>
<td>Gp120 protein (HIV-IIIB)</td>
<td>Pan troglodytes</td>
<td>HIV-1 HXB2 (IIIB)</td>
<td>IV</td>
<td>2/2 protected</td>
<td>Berman et al. 1990</td>
</tr>
<tr>
<td>Gp120 protein (HIV-SF2)</td>
<td><em>P. troglodytes</em></td>
<td>HIV-SF2</td>
<td>IV</td>
<td>1/2 infected virus control in 1/2?</td>
<td>el-Amad et al. 1995</td>
</tr>
<tr>
<td>Gp130 protein (SIVmac1A11)</td>
<td><em>Macaca mulatta</em></td>
<td>SIVmac251</td>
<td>IV</td>
<td>0/4 protected</td>
<td>Giavedoni et al. 1993</td>
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<tr>
<td><strong>B. Vaccinia (Vac) prime, subunit boost vaccines</strong></td>
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<tr>
<td>Vac-Env gp160 (SIVmne) prime + gp160 protein (SIVmne) boost</td>
<td><em>Macaca nesmestina</em></td>
<td>SIVmne clone or swarm</td>
<td>IV</td>
<td>4/4 protected from infection</td>
<td>Hu et al. 1992</td>
</tr>
<tr>
<td>Vac-Env gp160 (SIVmne) prime + gp160 protein (SIVmne) boost</td>
<td><em>M. nesmestina</em></td>
<td>SIVmne clone or swarm</td>
<td>IR</td>
<td>4/4 protected from swarm by IR</td>
<td>Polacino et al. 1999a</td>
</tr>
<tr>
<td>Vac-Env + Vac-Gag/Pol + Gag/Pol/Env particles; Vac-Gag/Pol + Gag/Pol particles</td>
<td><em>M. nesmestina</em></td>
<td>SIVmne clone or swarm</td>
<td>IV</td>
<td>Only Gag/Pol/Env (gp160) was fully protective</td>
<td>Polacino et al. 1999b</td>
</tr>
<tr>
<td>Vac-Env gp160 or gp130 (SIVmac239) + gp160 or gp130 protein (SIVmac239)</td>
<td><em>M. mulatta</em></td>
<td>SIVmac251</td>
<td>IV</td>
<td>0/4 protected; virus measured by p27 lower in virus primed</td>
<td>Giavedoni et al. 1993; Ahmad et al. 1994</td>
</tr>
<tr>
<td>[Vac-Env gp160 (SIVmne) + vac-Gag (SIVmne) ] prime + [VLPs (SIVmne) boost or DNA boost with all SHIV genes]</td>
<td><em>M. nesmestina</em></td>
<td>SHIV-89.6P</td>
<td>IR</td>
<td>Lower PVL and protection from CD4 loss in DNA +Vaccinia groups</td>
<td>Doria-Rose et al. 2003</td>
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<tr>
<td><strong>C. Live attenuated SIV</strong></td>
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<tr>
<td>SIVmac239Δnef</td>
<td><em>M. mulatta</em></td>
<td>SIVmac251</td>
<td>IV</td>
<td>4/4 protected</td>
<td>Daniel et al. 1992</td>
</tr>
<tr>
<td>SIVmac239Δnef</td>
<td><em>M. mulatta</em></td>
<td>SIVsmE660</td>
<td>IV</td>
<td>10/10 infected; 2 log10 difference in PVL</td>
<td>Reynolds et al. 2005</td>
</tr>
<tr>
<td>SIVmac239Δnef, -vpr, NRE</td>
<td><em>M. mulatta</em></td>
<td>SIVmac251</td>
<td>None</td>
<td>All rapidly developed AIDS</td>
<td>Baba et al. 1995</td>
</tr>
<tr>
<td><strong>D. Adenovirus vaccines with and without boosting</strong></td>
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<tr>
<td>Ad5 Gag (SIVmac239) (replication defective) alone or primed with DNA</td>
<td><em>M. mulatta</em></td>
<td>SHIV-89.6P</td>
<td>IR</td>
<td>Reduction in viremia (2 log10) and protection from CD4 loss</td>
<td>Shiver et al. 2002</td>
</tr>
<tr>
<td>DNA prime + Ad5 Gag (SIVmac239) (replication incompetent)</td>
<td><em>M. mulatta</em></td>
<td>SIVmac239</td>
<td>IR</td>
<td>Temporary reduction in early viremia (0.5 log10)</td>
<td>Casimiro et al. 2010</td>
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</table>

*aIV, intravenous inoculation; IR, atraumatic intrarectal inoculation.*
a viral inoculum on a mucosal surface leads to a rampant, disseminated, systemic infection of lymphoid tissues within 10–14 days is a critical area of active research.

Early NHP mucosal transmission studies used large inocula to maximize the chances of infection. More recently, researchers have challenged using repeated exposure with lower titered inocula, either through a specified number of challenges, or until all control animals become infected. Such studies are more complex and resource intensive but better represent typical human mucosal exposures. In the majority of human heterosexual mucosal HIV infections, the initial disseminated systemic infection is established by a single viral variant 80% of the time, indicative of infection with a single viral particle, with only a few variants typically involved in the remaining cases (Keele et al. 2008). This information stimulated efforts to improve macaque mucosal transmission models by characterizing the genetic diversity of challenge stocks, the identity of variants present in challenge stocks, and the number of variants involved in establishing new infections in naive hosts by different routes and doses to best mimic HIV mucosal transmission (Keele et al. 2009; Liu et al. 2010; Stone et al. 2010; Ma et al. 2011).

A Concise History of NHP Vaccine Approaches

Following the cloning of the HIV-1 genome (Alizon et al. 1984; Hahn et al. 1984; Luciw et al. 1984), investigators were quick to design recombinant protein vaccines intended to elicit responses to Env, since it was shown that neutralizing antibodies (NAbs), which can block virus infection in vitro, are directed exclusively to Env. Recombinant Envs were produced in diverse expression systems and shown to elicit NAbs against laboratory-adapted HIV-1 isolates (Lasky et al. 1986; Arthur et al. 1987; Palker et al. 1988). Broader responses were associated with Envs produced in mammalian cells (Haigwood et al. 1992), and the glycoprotein was shown to bind antibodies from HIV-positive serum that could neutralize divergent strains of HIV-1 (Steimer et al. 1991). However, the discovery that primary HIV-1 isolates were far more difficult to neutralize than laboratory-adapted stocks caused significant concern (Berman et al. 1992; Matthews 1994). Mammalian cell-produced monomeric gp120 protected a handful of chimpanzees from infection with one lab-adapted HIV-1 strain (Berman et al. 1990), although only partially protecting against another such strain (el-Amad et al. 1995). A SIV Env gp130 monomer provided only a hint of control and no protection against SIVmac251 infection in rhesus macaques (Ahmad et al. 1994), and did not protect in another, heterologous SIV challenge system (Stott et al. 1998).

We now know that Env is a highly variable glycosylated protein trimer that efficiently disguises its conserved domains, which are only transiently exposed during binding to CD4 and the chemokine coreceptors in the process of membrane fusion. It is thus not surprising that even mammalian cell-produced recombinant Env proteins do not readily display the determinants required for the generation of NAbs that can bind native Env trimers on virions to block infection effectively. Approaches to modifying Env for better presentation of key neutralization determinants have been reviewed (Haynes and Montefiori 2006). Recombinant protein vaccines typically show partial protection from SHIV challenge (Earl et al. 2001), but not from SIV challenge, thus fueling the debate about the relative merits of SHIV versus SIV challenge models. A major conclusion from the early Env vaccine work was that clinically relevant NAbs, although likely to be important in a protective vaccine, were difficult to generate and maintain, likely requiring more authentic trimeric forms of Env.

Because most licensed viral vaccines are attenuated or inactivated versions of the pathogen, efforts subsequently focused on these approaches for HIV-1, recognizing the safety issues inherent in an attenuated HIV. Live attenuated vaccines (LAV) were pursued in SIV models, with impressive protection against high-dose, highly pathogenic, intravenous challenge (Daniel et al. 1992). Progression to AIDS
in some LAV-immunized newborn macaques underscored safety concerns (Baba et al. 1995) even as other studies demonstrated an inverse relationship between extent of attenuation and protective efficacy (Wyand et al. 1996). However, the underlying mechanism(s) accounting for LAV protection remain unclear and a topic of continuing investigation (Wyand et al. 1999; Koff et al. 2006). The contribution of persistent low-level viral replication and associated ongoing immune stimulation to the protection afforded by live attenuated SIV vaccines is an important question that remains to be clarified.

The other traditional approach to vaccination, using inactivated virions or infected cells as immunogens, protected macaques against challenge with homologous SIV (Desrosiers et al. 1989; Murphey-Corb et al. 1989) or heterologous SIV (Johnson et al. 1992). However, follow-up experiments showed the mechanism for protection in these early studies was xenoreactivity to HLA antigens in the immunogens produced from human cells. Protection was seen only when matched HLA antigens were present on the SIV challenge virus. Indeed, immunization with purified HLA antigens alone conferred such protection, when the challenge virus was grown in HLA matched cells (Arthur et al. 1995). Studies controlling for confounding effects of host cell proteins incorporated into virions demonstrated that conventional inactivation methods can destroy the conformation of envelope glycoproteins, resulting in inferior immunogens (Cranage et al. 1995). Novel approaches that inactivate retroviral infectivity by preferential covalent modification of internal virion proteins required for infection while not affecting envelope glycoproteins on the virion surface (Arthur et al. 1998; Rossio et al. 1998) provided noninfectious virions with native envelope glycoproteins for use as immunogens, alone or in prime boost regimens, conferring partial protection against SIV or SHIV challenge (Lifson et al. 2002, 2004). Further developments in this “native whole virion immunogen” strategy include vaccination with proviral genomes that can produce such particles in vivo (Wang et al. 2000) and “single cycle” virus vaccines that infected target cells, but produce noninfectious progeny virions and can reduce virus loads after SIV challenge (Evans et al. 2005; Jia et al. 2009). Although not fully effective alone against high-dose SIV challenge, these one-round vaccines showed significant improvement against repeated low-dose challenge (Alpert et al. 2010). Vaccines that present authentic virion structures in vivo may be useful as boosting agents and continue to be explored (Poon et al. 2005).

Subsequent efforts aimed at developing recombinant viral vectors expressing HIV or SIV genes that are targets of cytotoxic T cells, in an approach aimed at controlling rather than preventing infection. Prime boost or combination approaches employed more than one means of antigen presentation and showed better protection in macaques after SIV challenge compared with recombinant protein-only vaccines. Vaccinia emerged early as a preferred recombinant viral vector, eliciting strong T-cell responses to Env in humans. Responses were higher in vaccinia-naive individuals (Cooney et al. 1991) and were boosted by the addition of recombinant protein to the regimen (Cooney et al. 1993). Because of safety concerns with replication competent vaccinia strains, increased emphasis was placed on more attenuated variants such as Modified Vaccinia Ankara (MVA), which were immunogenic in macaques (Barouch et al. 2001b). Fowlpox vectors such as ALVAC were used to evade preexisting immunity to vaccinia, but by themselves showed only modest activity in macaques (Pal et al. 2002). Once again, there were conflicting data with nominally the same approach tested in different SIV/macaque models. Env gp160-based vaccinia virus prime and subunit boost immunization provided “sterilizing” immunity in M. nemestrina challenged intravenously with SIVmne (Hu et al. 1992). However, whereas similar vaccines based on SIVmac239 were immunogenic, they failed to protect in M. mulatta challenged with the related virus swarm SIVmac251 (Ahmad et al. 1994; Table 1). Differential outcomes like these were some of the factors that led some investigators to attempt to standardize studies by utilizing a
common species, the rhesus macaque, and a common SIV challenge virus, SIVmac239, and to develop a common SHIV challenge, SHIV-89.6P (Uberla 2005). This approach facilitated comparisons between studies, but adoption of a limited number of standardized models, without a compelling rationale for their superiority or relevance also carried significant risk.

In 1993, DNAvaccination emerged as a promising new tool, utilizing mammalian expression vectors as vaccines with impressive results in mice (Ulmer et al. 1993). However, such vaccines proved poorly immunogenic in macaques (Barouch et al. 2001a). The addition of cytokine genes to the DNA vaccines increased responses (Barouch et al. 2000, 2002), and the use of electroporation to enhance DNA uptake dramatically improved immunogenicity (Otten et al. 2004). A multitude of combination, or prime boost, experiments were performed with SIV or SHIV challenge to explore the DNA vaccines in combination with poxvirus vectors and proteins (Pal et al. 2006), with varying degrees of success (Doria-Rose et al. 2003; Dale et al. 2004; Mossman et al. 2004; Rosati et al. 2005; summarized in Table 1).

Interest in cellular immunity increased following the negative clinical trial results with the antibody targeting VaxGen vaccine and findings suggesting that T cells might be responsible for protection in multiply exposed yet uninfected sex workers in Africa (Rowland-Jones et al. 1998; Kaul et al. 2001). The daunting challenges in developing immunogens capable of inducing broadly neutralizing antibodies also contributed to the shift in emphasis. The development of recombinant adenovirus vectors to induce/enhance T-cell responses, when used alone or in combination with DNA or proteins, was vigorously pursued by a number of groups due to their impressive immunogenicity in model systems including NHP (Barouch and Nabel 2005; Robert-Guroff 2007).

Significant viral control after intravenous challenge with SHIV-89.6P was observed following vaccination with Ad5-Gag(SIV) when used alone or as a boost to a DNA prime (Shiver et al. 2002). These findings were used to support the approach of the STEP clinical trial, although the vaccine components and immunization regimens were not an exact match. Subsequently, a similar vaccine experiment using DNA prime/Ad5-Gag(SIV) boost was performed in NHP using SIVmac239 as the challenge virus. The effects on viremia in this experiment were modest and transient, and limited to the subset of animals expressing the MHC Class I allele MamuA*01, with evidence for viral escape at 6 months postchallenge (Casimiro et al. 2005).

A very recent NHP study explicitly designed to simulate the STEP trial as closely as possible yielded negative results, matching the clinical results (D Watkins, pers. comm.).

The results of the STEP trial forced the field to take a hard look at the T-cell-only vaccine hypothesis, adenovirus vectors, and the NHP results (Watkins et al. 2008). Replication of competent adenovirus vectors had been developed in parallel with the Ad5 vectors. Although DNA/MVA vaccines had shown robust virus control after SHIV-89.6P challenge (Amara et al. 2001), a vaccine based on DNA priming (with or without cytokines IL-12 or IL-15), replicating Ad5-SIV boost, and a recombinant gp140 protein plus SIV Nef, showed essentially no virus control after SIVmac251 challenge (Demberg et al. 2008). These results suggest that comparative studies in different models are not only important, but that they remain critical until one or more of the models is validated as convincingly replicating vaccine efficacy demonstrated in human trials. Importantly, different NHP models may be better suited for evaluation of different vaccine approaches.

Despite the generation of adenovirus-specific immunity, which limited the use of the vector to one or two immunizations, results using Ad5 were encouraging with respect to the ability to generate strong T-cell responses (Santra et al. 2005), and in combination with DNA, strong B- and T-cell responses (Seaman et al. 2005) in macaques. The vectors were also immunogenic in humans in Phase I trials, although human responses were not as robust as those in macaque, and limited by preexisting antivector immunity (Koup et al. 2010). Combination vaccine experiments to test Ad vectors with other vectors continue, although the sobering lack
of efficacy in the STEP trial and relatively widespread preexisting immunity to Ad5 (O’Brien et al. 2009) have diminished enthusiasm for this vector. To circumvent preexisting antivector immunity issues, efforts are currently directed to developing vaccine vectors based on rare human (Ko et al. 2009; McVey et al. 2010) or chimpanzee adenovirus serotypes.

The conventional anamnestic expansion-dependent central memory T-cell type of vaccine responses induced by these vaccines typically were unable to provide truly effective, sustained control of viral replication (Reynolds et al. 2005). To test the hypothesis that a qualitatively different type of immune response might be more effective, persistent rhesus cytomegalovirus-based vectors expressing most of the SIV genome were explored and elicited strong, broad, persistent CD4+ and CD8+ effector memory T-cell responses that were associated with stringent control of replication following repeated titered intrarectal challenge with SIV-mac239 (Hansen et al. 2009, 2011). If these results can be translated by developing safe human cytomegalovirus vectors, or other vectors that generate persistent effector T-cell responses, this could be a major advance toward developing an effective vaccine.

**IMMUNE RESPONSES**

Antiviral immune responses operative in primate lentivirus infection can be considered as (1) intrinsic (Malim and Bieniasz 2011), (2) innate (Carrington and Alter 2011), or (3) adaptive, comprising both humoral (Overbaugh and Morris 2011) and cellular (Walker and McMichael 2011) immunity. For each response, there is evidence that lentiviruses evolve countermeasures to overcome host mechanisms. These responses in NHP are discussed in turn below.

**“Intrinsic” Immune Responses**

The intrinsic immune responses that may restrict lentiviral infection include the interferon inducible APOBEC3 protein system (Simon et al. 2005; Goila-Gaur and Strebel 2008) and tetherin (CD317, BST-2 [Neil et al. 2008; Jia et al. 2009; McNatt et al. 2009; Zhang et al. 2009]), which are covered by Malim and Bieniasz (2011). Another intrinsic immune mechanism particularly relevant to vaccines studies is the TRIM5α protein, which interferes with primate lentiviral replication after entry (Stremlau et al. 2004). Importantly, genetic polymorphisms have recently been described in the TRIM5α sequences of rhesus macaques that represent a key new variable to control in future NHP studies (Newman et al. 2006; Newman and Johnson 2007; Kirmaier et al. 2010; Lim et al. 2010). Depending on the sequence of the target SIV capsid protein, TRIM5α polymorphisms can have profound influence on permissiveness for viral replication. The sequence of SIVmac239 appears to be refractory to these effects, whereas those of SIVsmE543 and SIVsmE040 appear to be quite sensitive. Other viruses and viral swarms, including SIVmac251 and particularly SIVsmE660, may show an intermediate range of sensitivities, perhaps reflecting a mixture of sensitive and resistant capsid sequences in the viral stocks. Because these polymorphisms can exert profound effects on viral replication for susceptible viruses, studies conducted with such viruses must be stringently controlled for TRIM5α genotypes, much like controlling for MHC I alleles associated with spontaneous control of viral replication (Goulder and Watkins 2008). If not, control of viral replication could be erroneously attributed to an experimental intervention rather than the uneven distribution of the TRIM5α genotypes among groups.

**Innate Immune Responses**

In NHP models, innate immune responses can be broadly considered as those associated with (1) cells that secrete soluble immune active mediators or (2) cells that are either dedicated components of the innate immune system (e.g., NK cells) or that bridge the innate and adaptive immune systems (dendritic cells). The complexity of these interactions is exemplified by the response to vaginal inoculation of rhesus macaques with SIV. In response to such inoculations, vaginal epithelial cells produce...
chemokines, including CCL20, that attract plasmacytoid dendritic cells (PDC) and T cells (Li et al. 2009a). The PDCs produce abundant amounts of the antiviral cytokine interferon-α. However, the net effect of the cytokine responses is to facilitate viral replication by recruiting activated CD4⁺ T cells to the site of local inflammation, providing additional targets for infection. This local amplification at mucosal portals of viral entry may facilitate systemic dissemination and is a potential target for intervention (Li et al. 2009a; Haase 2010). There are important differences in NK cell populations between humans and different NHP species (for NK cell function, see Reeves et al. 2010; Siliciano and Greene 2011 and references). There are also differences in the numbers and apparent trafficking of these NK populations during primary and chronic SIV infection. A role for NK cells in controlling viral replication in SIV infection remains to be convincingly demonstrated, although this is well established for HIV in humans by genetic association studies demonstrating that pairing of certain KIR genotypes and MHC I alleles can affect disease progression (Martin et al. 2002, 2007; Martin and Carrington 2005; Qi et al. 2006). Current efforts to apply pyrosequencing methods to characterize these loci in experimentally important NHP species should help to determine whether such associations exist for infected NHP (Wiseman et al. 2009).

Adaptive Immune Responses

The MHC loci of monkeys and humans differ. Among the NHP species commonly used for AIDS research, the MHC of the Indian rhesus macaque has been most extensively studied (Wiseman et al. 2009). In contrast to humans, rhesus macaques lack a MHC I C locus, but have greater numbers of alleles for their A and B loci than do humans (Bontrop and Watkins 2005; Goulder and Watkins 2008). The full impact of this difference is incompletely understood.

B-CELL RESPONSES

The daunting diversity of HIV presents a challenge for humoral immune protection. For highly variable pathogens like HIV, broadly neutralizing antibodies directed to native Env oligomers on the virion surface block infection in vitro and can protect macaques from infection in passive transfer studies but are difficult to raise by vaccination. Depletion of B cells using anti-CD20 antibodies in NHP models has been pivotal in demonstrating the role of antibodies in limiting infection in vivo. Even when antibodies failed to fully block infection, some clinical benefits accrued (summarized below). When given prophylactically, high doses of both polyclonal and monoclonal NAbs can prevent or limit infection (Haigwood and Statonatos 2003; Mascola 2003). The effectiveness of prophylactic passive IgG or mAbs was observed in several primate HIV, SIV, and SHIV studies (Prince et al. 1991; Putkonen et al. 1991; Conley et al. 1996; Shibata et al. 1999) and SCID-hu mouse models (Gauduin et al. 1995, 1997). These animal models have allowed assessment of the importance of dose, timing, and specificity of the antibody preparations. NAbs are effective in preventing the establishment of infection in vivo when present at high concentrations at the time of viral challenge or a few hours later, including challenge at mucosal surfaces (Baba et al. 2000; Mascola et al. 2000). Protection of newborn macaques with passively transferred serum containing SIV NAbs was fully effective in blocking oral infection (Van Rompay et al. 1998). In newborn macaques exposed orally to SHIV-SF162p3, substerilizing levels of IgG matched to the challenge virus resulted in better virus control and rapid development of de novo NAbs (Ng et al. 2010). Vaccinated macaque dams transferred anti-SIV antibodies to their infants, which were subsequently protected from oral/conjunctival challenge (Van Rompay et al. 2003). Whereas HIV immune globulin (HIVIG) IgG can directly limit the infectivity of HIV-1 and SHIV in vivo (Igarashi et al. 1999), non-neutralizing IgG was not effective, implicating NAbs as the active component in polyclonal HIVIG. Challenge studies in macaques with the R5-tropic SHIVDH12, using HIVIG purified from chimpanzee-derived IgG, were matched for HIVDH12 and protected only at doses of HIVIG that neutralized virus
to 100% in vitro, in the range of 200 mg/kg (Shibata et al. 1999).

A summary of many of the passive immunization studies using SHIV challenge is shown (Table 2), with testing of both polyclonal serum (HIVIG from HIV+ sera and CHIVIG from the sera of infected chimpanzees) as well as human neutralizing mAbs. All of these Ab preparations targeted native Env. At least four lessons have been learned: (1) Antibodies protect in a dose-dependent manner, with higher doses protecting more animals; (2) relatively high doses of mAbs, or mAbs plus polyclonal HIVIG or CHIVIG, are needed for protection against viral challenge; (3) lower levels of antibodies are better able to protect when the challenge dose is reduced; and (4) intravenously administered IgG can protect against mucosal challenge, presumably through transudation. In SIV models, NAbS delivered within the first 24 hours of infection can block infection or, at lower doses, affect control of viral replication and the development of de novo responses (Haigwood et al. 2004). Passive immunization with NAbS has progressed to proof of concept studies in which a NAb is expressed from a transgene delivered by recombinant adenovirus-associated virus (AAV), resulting in sustained high plasma NAb levels in vivo and protection from infection (Johnson et al. 2009). Related approaches are under consideration for feasibility studies in humans.

Although NAb function has correlated with protection in these studies, other antibody-mediated effector functions, such as antibody-dependent cellular cytotoxicity (ADCC) or antibody-dependent cell-mediated viral inhibition (ADCVI), might also be critical in vivo. ADCC, measuring the ability of antibody and Fcγ receptor (FcγR)-bearing effector cells to kill target cells expressing HIV antigens, correlated inversely with disease progression in the Multicenter AIDS Cohort Study (Baum et al. 1996). Non-neutralizing IgG with ADCC activity was ineffective in blocking SIV infection in newborn macaques (Florese et al. 2009). ADCVI, which occurs when antibody forms a bridge between an infected target cell and FcγR-bearing effector cell, thereby limiting viral

production, can also limit HIV, SIV, and SHIV infection by direct killing. During acute HIV infection, ADCVI antibodies develop weeks to months earlier than do NAbS and can inhibit both autologous and heterologous strains of HIV-1. Moreover, there is an inverse correlation between ADCVI antibody activity and plasma viremia during the acute phase of HIV infection (Forthal et al. 2001). Serum from rhesus macaques that does not neutralize PBMC-passaged, uncloned SIVmac251 can have potent ADCVI activity (Forthal et al. 2006). Such non-neutralizing serum protected newborn macaques from oral challenge with SIVmac251 (Van Rompay et al. 1998). Engineered antibodies have provided a direct demonstration of a role for FcγR-mediated antibody functions in preventing lentivirus infection (Hessell et al. 2007). Different forms of the neutralizing mAb IgG1b12, with equivalent ELISA and neutralizing activity, provided different levels of protection against vaginal challenge with SHIV162p3 depending on whether they had a functional FcγR-binding domain. Thus, ADCVI, ADCC, or related FcγR-mediated activities might be involved in both modulating established infection and in preventing new infection.

**T-Cell Responses**

Responses by SIV-specific CD8+ T cells are believed to contribute to control of viral replication, although the extent and precise mechanisms remain controversial. SIV-specific CD8+ T-cell responses become measurable around the time of the decline from peak viremia in primary infection (Veazey et al. 2001). Depletion of CD8+ cells by administration of a monoclonal antibody during chronic infection results in a transient increase in plasma viremia, coinciding with the period of depletion of CD8+ cells (Schmitz et al. 1999). However, the available mAbs directed against CD8a also deplete some NK cells, and depletion of CD8+ lymphocytes can result in proliferation of CD4+ T cells, potentially providing additional targets for viral infection and increasing viremia via this mechanism (Okoye et al. 2009). Perhaps the clearest evidence of CD8+ T-cell control of viral replication is provided by evidence of immune escape
Table 2. Effects of dose and route of challenge on passive protection in SHIV infection of rhesus macaques.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Dose</th>
<th>Route</th>
<th>Treatment</th>
<th>Protection</th>
<th>Lessons learned</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHIV-89.6</td>
<td>40 (AID₅₀)</td>
<td>IV</td>
<td>HIVIG (400); mAb 2F5 (15); mAb 2G12 (15)</td>
<td>3/6</td>
<td>High doses of HIVIG plus NmAbs partially protect from IV challenge.</td>
<td>Mascola et al. 1999</td>
</tr>
<tr>
<td>SHIV-89.6</td>
<td>100 (TCID₅₀)</td>
<td>IVag</td>
<td>HIVIG (400); mAb 2F5 (15); mAb 2G12 (15)</td>
<td>4/5</td>
<td>The same combination and dose partially protects vs. high-dose mucosal challenge.</td>
<td>Mascola et al. 2000</td>
</tr>
<tr>
<td>SHIV-DH12</td>
<td>100 (TCID₅₀)</td>
<td>IV</td>
<td>CHIVIG (230)</td>
<td>1/1</td>
<td>High levels of polyclonal IgG protect vs. high-dose IV challenge.</td>
<td>Shibata et al. 1999</td>
</tr>
<tr>
<td>SHIV-DH12</td>
<td>100 (TCID₅₀)</td>
<td>IV</td>
<td>CHIVIG (25)</td>
<td>0/2</td>
<td>10-fold lower levels failed to protect, demonstrating a dose response.</td>
<td></td>
</tr>
<tr>
<td>SHIV-DH12</td>
<td>10 (TCID₅₀)</td>
<td>IV</td>
<td>CHIVIG (25)</td>
<td>1/1</td>
<td>This 10-fold lower dose could protect against a 10-fold lower IV challenge.</td>
<td></td>
</tr>
<tr>
<td>SHIV-vpu CCR5</td>
<td>10 (AID₅₀)</td>
<td>IV</td>
<td>mAb F105 (10); mAb 2F5 (10); mAb 2G12 (10)</td>
<td>4/4 adults</td>
<td>Neutralizing mAbs alone protects vs. low-dose IV challenge.</td>
<td>Baba et al. 2000</td>
</tr>
<tr>
<td>SHIV-vpu CCR5</td>
<td>10 (AID₅₀)</td>
<td>oral</td>
<td>mAb F105 (10); mAb 2F5 (10); mAb 2G12 (10)</td>
<td>4/4 infants</td>
<td>NmAbs—IgGs—can fully protect infants vs. oral challenge.</td>
<td>Baba et al. 2000</td>
</tr>
<tr>
<td>SHIV-SF162P3</td>
<td>500 (TCID₅₀)</td>
<td>IVag</td>
<td>mAb 2G12 (40)</td>
<td>3/5</td>
<td>A single NmAb partially protects vs. high-dose mucosal challenge.</td>
<td>Hessel et al. 2009</td>
</tr>
<tr>
<td>SHIV-SF162P4</td>
<td>300 (TCID₅₀)</td>
<td>IVag</td>
<td>mAb b12 (25)</td>
<td>4/4</td>
<td>NmAb directed to the CD4bs protects vs. a lower dose mucosal challenge.</td>
<td>Parren et al. 2001</td>
</tr>
<tr>
<td>SHIV-SF162P4</td>
<td>300 (TCID₅₀)</td>
<td>IVag</td>
<td>mAb b12 (5)</td>
<td>2/4</td>
<td>A fivefold lower dose is less effective in protection, same dose and mucosal route.</td>
<td>Parren et al. 2001</td>
</tr>
</tbody>
</table>

Continued
by mutation of viral sequences affecting CTL epitopes. Examples have been reported for both early escape for epitopes that are not structurally constrained, such as the MamuA*01 restricted epitope tat SL8, and later escape of structurally constrained epitopes such as the MamuA*01 restricted epitope gag CM9, which typically emerges later in infection, in combination with compensatory mutations outside the epitope itself, partially restoring replicative fitness of the mutant virus (Goulder and Watkins 2004). Similar evidence based on in vivo selection implicating MHC-I restricted activity of CD8+ T cells comes from a study of a SIV-mac239 variant engineered to be incapable of the MHC-I downregulation via Nef, but intact for other Nef functions. The SIVmac239 variant underwent extensive mutation in vivo in infected macaques to restore the capacity to down-regulate MHC-I, associated with a relative loss of control of viral replication, supporting the importance of MHC-I restricted killing by CD8+ T cells (Swigut et al. 2004). CD8+ T cells could potentially contribute to control of viral replication via both cytolytic and noncytolytic mechanisms, including production of antiviral cytokines or chemokines, including MIP-1B (Cocchi et al. 1995; Gauduin 1998). The ability of CD8+ T cells to efficiently load lytic granules and up-regulate granzyme B in response to stimulation by HIV-1 infected autologous CD4+ T cells, and to eliminate infected cells in co-cultures, correlated with elite controller status in HIV-infected humans (Migueles et al. 2008). Preliminary observations in SIV-infected rhesus macaques suggest a similar correlation between these activities and the extent of control of viral replication (SA Migueles, in prep.). Recent progress with systems for adoptive transfer of ex vivo selected and expanded autologous cells (Berger et al. 2008; Bolton et al. 2010; Minang et al. 2010) and characterization of Mauritian cynomolgus macaques with limited MHC heterogeneity (Burwitz et al. 2009) show promise for enabling adoptive transfer studies to address the in vivo antiviral activities of T-cell populations with different functional properties. Studies in Mauritian cynomolgus have also demonstrated an improved capacity of responses from MHC heterozygotes to control SIV replication in vivo (O’Connor et al. 2010), paralleling similar observations in humans (Carrington et al. 1999).

The role of virus-specific CD4+ T cells in antiviral immune responses is incompletely understood. In addition to the “helper” function

<table>
<thead>
<tr>
<th>Virus</th>
<th>Dosea (TCID50)</th>
<th>Routeb</th>
<th>Treatment (mg/kg)</th>
<th>Protection</th>
<th>Lessons learnedc</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHIV-SF162P4</td>
<td>300</td>
<td>IVag</td>
<td>mAb b12 (1)</td>
<td>0/4</td>
<td>A fivefold still lower dose is ineffective against the same dose and mucosal route.</td>
<td>Hessell et al. 2010</td>
</tr>
<tr>
<td>SHIV-BaL</td>
<td>2000 (TCID50)</td>
<td>IR</td>
<td>mAb 2F5 (50)</td>
<td>5/6</td>
<td>Partially protects vs. IR challenge with gp41 NmAb 2F5.</td>
<td>Hessell et al. 2010</td>
</tr>
<tr>
<td>SHIV-BaL</td>
<td>2000 (TCID50)</td>
<td>IR</td>
<td>mAb 4E10 (50)</td>
<td>5/6</td>
<td>Partially protects vs. the same IR challenge with gp41 NmAb 4E10.</td>
<td>Hessell et al. 2010</td>
</tr>
</tbody>
</table>

aAID50, animal infectious dose that infects 50% of test animals in a titration experiment; TCID50, tissue-culture infectious dose that infects 50% of test wells using Reed–Muench analysis.
bIV, intravenous inoculation; IVag, atraumatic intravaginal inoculation; IR, atraumatic intrarectal inoculation; HIVIG, HIV immune globulin, purified IgG; CHIVIG, purified IgG from HIV-infected chimpanzee.
cNmAbs, neutralizing monoclonal antibodies; CD4bs, CD4 binding site.
traditionally ascribed to CD4+ T cells, they may mediate other functions. Animals chronically infected with live attenuated SIVmac239Δ nef virus were found to develop abundant populations of SIV antigen-specific effector memory CD4+ T cells, capable of up-regulating perforin expression and degranulation upon stimulation with SIV antigens (Gauduin et al. 2006), although any direct role such cells play in the robust protection mediated by SIVmac239Δ nef immunization remains to be demonstrated. The extent to which the prominent responses by SIV-specific CD4+ T cells contribute to the impressive protection afforded by vaccines based on recombinant rhesus CMV vectors (Hansen et al. 2009), and the mechanisms responsible, remain tantalizing areas for future research.

CONCLUSIONS

NHP models have been successfully used to evaluate the safety, immunogenicity, and protective efficacy of different candidate AIDS vaccine approaches and to conduct proof of concept studies for novel vaccine concepts. The available data suggest the potential predictive value of certain NHP models for some vaccines. In some models, most notably those involving X4-tropic SHIV challenges, lack of predictive value has been demonstrated. In others, NHP models represent arguably the only feasible, relevant approach to rigorously investigate mechanistic questions related to vaccine efficacy, such as the early events that lead to a rampant systemic infection. Despite their many unique advantages, NHP models have been controversial, in part because of a lack of understanding about the differences in their biology and the extent to which they mimic human disease. Recognition of the range of different models available, along with better informed selection of appropriate models for particular experiments, coupled with appropriately informed interpretation of the results of these studies, should not only improve the utility of the studies but also increase our understanding of the immunopathology of disease. Continuing development and refinement of NHP models will be critical for developing an effective vaccine for the prevention of HIV infection and AIDS.

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