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Non-Human Primate Models for AIDS Vaccine Research

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Abstract

Since the discovery of simian immunodeficiency viruses (SIV) causing AIDS-like diseases in Asian macaques, non-human primates (NHP) have played an important role in AIDS vaccine research. A multitude of vaccines and immunization approaches have been evaluated, including live attenuated viruses, DNA vaccines, viral and bacterial vectors, subunit proteins, and combinations thereof. Depending on the particular vaccine and model used, varying degrees of protection have been achieved, including prevention of infection, reduction of viral load, and amelioration of disease. In a few instances, potential safety concerns and vaccine-enhanced pathogenicity have also been noted. In the past decade, sophisticated methodologies have been developed to define the mechanisms of protective immunity. However, a clear road map for HIV vaccine development has yet to emerge. This is in part because of the intrinsic nature of the surrogate model and in part because of the improbability of any single model to fully capture the complex interactions of natural HIV infection in humans. The lack of standardization, the limited models available, and the incomplete understanding of the immunobiology of NHP contribute to the difficulty to extrapolate findings from such models to HIV vaccine development. Until efficacy data become available from studies of parallel vaccine concepts in humans and macaques, the predictive value of any NHP model remains unknown. Towards this end, greater appreciation of the utility and limitations of the NHP model and further developments to better mimic HIV infection in humans will likely help inform future AIDS vaccine efforts.

Keywords

Non-human primates; live attenuated virus; prime-boost; SHIV; HIV; SIV

INTRODUCTION

Successful vaccines made to date are primarily against pathogens that can induce protective immunity as a result of natural exposure. Well known examples include smallpox, polio, and measles. Survivors of natural infections develop life-long immunity against disease upon re-exposure. In fact, the observation that immunity can be acquired as a result of natural exposure formed the basis for the practice of active immunization, beginning with variolation in centuries past and continuing with vaccinations in modern history [1]. In the case of HIV infection, evidence for protective immunity acquired from natural infection is far from clear. Cytotoxic T-lymphocyte (CTL) responses have been implicated in the control of virus replication in the acute phase of HIV infection [2,3]. Preservation of T-helper cell functions correlates with better clinical outcome [4,5]. Substantial HIV-specific antibody and CTL responses can be generated by infected individuals, but they are ineffective in controlling infection, as escape variants eventually take over [6-8]. Significantly, CTL and proliferative and mucosal IgA responses have been detected in rare cases of uninfected partners of infected individuals and multiply-

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exposed seronegative individuals [9-12]. Whether these responses account for the control of infection remains unclear. On the other hand, there is mounting evidence for superinfection in HIV-positive individuals [13-16], indicating the absence of protective immunity from natural exposure. In short, data available to date do not support the notion of naturally acquired immunity against HIV infection and diseases, as has been observed in many vaccine-preventable diseases.

So far, the only direct evidence supporting the feasibility of inducing protective immunity against primate lentiviruses has come from non-human primate (NHP) models. A number of vaccine strategies and immunization approaches have shown protection against infection or diseases. Recent studies have shed important insights on the potential correlates of protection, but also on the significant obstacles yet to be overcome. However, because of the complexity and limitations of the NHP models, it remains difficult to extrapolate data from these models to inform the development of HIV vaccines. As a result, the utility of NHP models in HIV vaccine development has been debated. This article reviews some of the underlying issues and proposes potential directions that may result in more effective use of NHP models for HIV vaccine research. The reader is referred to a number of excellent articles that provide a more in-depth review of the NHP models, summation of vaccine trials in NHP, and discussions on the pros and cons of specific vaccine approaches [17-24; <http://hiv-web.lanl.gov/cgi-bin/vaccine/public/index.cgi>].

NON-HUMAN PRIMATE MODELS FOR AIDS

HIV-1 and HIV-2

The search for an animal model for AIDS started soon after the discovery of HIV-1 as the etiologic agent. Alter *et al.* [25] reported seroconversion and transient lymphadenopathy in chimpanzees inoculated with plasma from HIV-infected patients. However, with the exception of a few isolated cases resulting from serial passages [26,27], HIV infection in chimpanzees generally does not lead to AIDS. Evidence indicating chimpanzees being the natural host of an endemic virus SIVcpz, a likely predecessor of HIV-1 [28], may explain the lack of pathogenic responses. In any case, the endangered species status of chimpanzees coupled with restricted availability and high costs prohibit the general use of this animal model for AIDS research.

HIV-1 infection in pig-tailed macaques was attempted in the early 1990's [29]. However, infection was transient and sporadic. Even though serial passages in neonates resulted in enhanced replication and durable antibody responses, no evidence of CD4⁺ T-cell depletion was observed [30]. Recently, several host restriction factors for HIV-1 replication have been identified. Macaque TRIM-5 α , a component of cytoplasmic bodies, blocks HIV-1 replication at a step after viral entry, prior to reverse transcription [31]. The action of APOBEC3G, a single strand DNA-editing enzyme inducing hypermutation and DNA degradation, can be counteracted by the viral *vif* gene product [32]. Further understanding of species-specific restriction factors and their interactions with viral protein targets may point to new approaches to adapt HIV-1 for more efficient replication in macaques.

HIV-2 is believed to have evolved as a result of cross-species transmission of SIVsmm, a lentivirus endemic to some sooty mangabey populations in Western Africa [33]. Because of its close relatedness to SIVsmm, HIV-2 infection of NHP was explored as a model for AIDS. Early efforts resulted in mostly transient infections [34,35]. Upon repeated passages, several HIV-2 strains have been adapted in baboons [36,37] and pig-tailed macaques [38,39] that are capable of inducing persistent viremia, rapid CD4⁺ T-cell decline, and AIDS. However, HIV-2 models have not been widely used for HIV vaccine research, perhaps in part because of their similarity to SIV and in part because of the focus on HIV-1. Nevertheless, it should be noted

that HIV-2 infection of baboons or pig-tailed macaques provides the only models for AIDS pathogenesis based on a virus of human origin, rather than SIV or SIV/HIV chimera, SHIV (see below). HIV-2 isolates, including HIV-2/287, can utilize CXCR4 as the co-receptor [40-42], a feature shared with HIV-1, but not with SIV. The basis for this difference is not known, but may be related to adaptation in humans. In this sense, HIV-2 models may provide unique advantages for vaccine and pathogenesis studies not previously appreciated.

SIV and SIV/HIV Chimera

SIV was isolated in the early 1980's from monkeys with AIDS-like diseases or lymphoma [21,43-45]. According to the species from which it was first isolated, it has been designated SIVmac (from rhesus macaques), SIVsmm (from sooty mangabeys), or SIVmne (from pig-tailed macaques, *Macaca nemestrina*). These isolates share a common ancestor, SIVsmm, a virus that is endemic and generally non-pathogenic in its natural hosts, sooty mangabeys [33]. Experimental inoculation of SIV into a number of Asian macaque species, including rhesus, pig-tailed and cynomolgus monkeys, results in a spectrum of pathological responses similar to AIDS in humans. Because of its ability to cause AIDS-like diseases in relatively accessible primate species, SIV infection of macaques has been the animal model of choice for AIDS vaccine research.

Several key findings establish the similarities between SIV infection of macaques and HIV-1 infection of humans. Like most HIV-1 isolated from early infection [46,47], the majority of SIV isolates examined to date utilize the CCR5 coreceptor for viral entry [41,48-50]. Infection by SIV is characterized by massive, rapid, and selective depletion of memory T cells in gut-associated lymphoid tissues, a finding later confirmed in HIV infection [51-56]. Both viruses replicate not only in activated and proliferating T cells, but also resting T cells [57]. Acute infection in HIV-1 and SIV models resolves with the onset of antigen-specific immune responses [2,3,58-61]. Both viruses utilize similar evasion tactics to escape from host immune responses, including modification of glycosylation patterns in viral envelope protein [62-65] and mutations in neutralization and CTL determinants [6,7,66]. Importantly, in both HIV and SIV infections, plasma viral load after the acute phase ("viral setpoint") predicts the rapidity of disease progression [67-69]. Peripheral blood CD4⁺ T-cell depletion often precedes the onset of AIDS-defining events (e.g., opportunistic infections, neoplastic diseases; hematological and neurological disorders), although the duration of disease-free periods differs significantly between HIV-1-infected humans and SIV-infected macaques (an average of 8-10 years for humans vs. 0.5-3 years for macaques infected with the majority of pathogenic SIV strains). These features common between HIV and SIV infections define the unique advantage of the SIV model for the study of HIV pathogenesis.

On the other hand, the SIV model also has a number of shortcomings. First, by its very nature, SIV infection of macaques only provides a "surrogate" model for HIV infection. SIV shares approximately 80% genomic sequence homology with HIV-2, but only 40-50% with HIV-1 [70]. Serological cross-reactivity between SIV and HIV-1 is limited [71]. Efficacy of HIV-1-based vaccines, therefore, cannot be directly evaluated in the SIV model. Second, most of the commonly used SIV isolates have been multiply passaged in macaques to select for increased virulence and rapid disease progression [23,72]. The basis for the increased virulence is not clear, but is likely related to the accumulation of mutations in multiple regions of the viral genome (e.g., *gag* and *env*) and the acquisition of CTL-escape and neutralization-resistance phenotypes [7,66,73]. Viruses with enhanced virulence may allow for a more rapid and uniform determination of challenge outcome in vaccine studies with few animals. However, the relevance of these viruses to HIV infection is not clear, and the reliance on these models for challenge studies may underestimate vaccine efficacy. Third, the choice of macaque species or genotype also needs to be considered. Using animals with defined genotypes, such as rhesus

macaques with Mamu-^{*}01 and Mamu-B^{*}17 major histocompatibility complex (MHC) I alleles, may provide a more uniform outcome than non-MHC-matched animals, but may also bias the result because the allele has been linked to better disease outcome after SIVmac239 infection [74,75]. Infection in rhesus macaques of Chinese origin is characterized by lower viral load and less pronounced CD4⁺ T cell depletion than those of Indian origin [76-79]. Similarly, infection in cynomolgus macaques (*Macaca fascicularis*) appears to be less virulent than infection in Indian rhesus, with plasma viral loads more compatible with typical HIV infection in humans [80]. Finally, differences between experimental inoculation of animals and natural transmission in humans also need to be considered. Most, if not all, current models rely on the use of cell-free virus as inoculum. It is not clear to what extent this provides an adequate model for natural transmission, which likely involves both cell-free and “cell-associated” viruses. The commonly used intravenous route of inoculation is highly reproducible and is a reasonable mimic of blood-borne HIV transmission. However, other than experimental inoculation at mucosal sites (intrarectal, intravaginal, oral), there is currently no established macaque model for sexual transmission. Since vaccine studies are usually limited by the availability of animals, mucosal inoculations generally employ relatively high doses of cell-free virus inoculum to achieve uniform infection. The relevance of such models has been debated, since natural sexual transmission through intact mucosa appears to be a low probability event [81]. In this context, a low-dose, repeated mucosal exposure model may offer a useful alternative [82].

To address the need for direct testing of HIV vaccines in an animal model, chimeric viruses were developed, in which the *tat*, *rev*, *vpu* and *env* genes of HIV-1 were inserted into the genome of the pathogenic molecular clone of SIVmac239 [83-87]. Inoculation of macaques with these chimera resulted in persistent infection [85,87-89] and, upon serial *in vivo* passages, rapid CD4⁺ T-cell depletion, followed by AIDS-like diseases [90,91]. SHIV shares many of the advantages of SIV macaque models. In addition, it allows direct testing of Env-based HIV-1 vaccines. However, there are also significant differences between commonly used SIV and the SHIV strains. For example, infection of SHIV89.6P results in rapid depletion of peripheral blood CD4⁺ T-cells (generally within 2-4 weeks) [91], in contrast to the gradual decline observed in most SIV and HIV-1 infections. SHIV89.6P utilizes CXCR4 for infection, unlike SIV and most HIV-1 early isolates, which utilize CCR5 [41]. The difference in co-receptor usage is reflected in target cell populations after infection and the disease course that follows. Harouse *et al.* [92] observed that a CCR5-tropic virus, SHIV162P, caused a profound loss of CD4⁺ T-cells in the intestine, not in the periphery, whereas the opposite was observed for a CXCR4-tropic virus, SHIV33A. Nishimura *et al.* [93] reported that a CXCR4-using SHIV, DH12R, targets naïve T-cells, resulting in rapid CD4⁺ T-cell loss in the periphery, whereas SIVmac239 primarily targets CCR5-expressing memory CD4⁺ T-cells. SHIV89.6P is also relatively sensitive to neutralizing antibodies [91,94], whereas SIVmac239 is highly resistant [95]. Proper interpretation of vaccine efficacy data will require in-depth understanding of the biological properties of the challenge models used [96]. Currently, there is only one established SHIV challenge model, SHIV162P, that is based on a CCR5-using virus. However, the significant variations in setpoint viral load, and the gradual and variable decline of CD4⁺ T-cells in the periphery [97] make it difficult to rely on these parameters as indicators of vaccine protection. Obviously, further development and refinement of SHIV models are needed.

PROTECTIVE IMMUNITY AGAINST HIV/AIDS: INSIGHT FROM NHP STUDIES

NHP models have been used to evaluate the safety, immunogenicity and protective efficacy of multiple vaccine approaches. Perhaps one of the most important insights gained from these studies is the feasibility of immune protection against primate lentivirus infection and disease. As it is beyond the scope of this article to review all the vaccine approaches tested in NHP models, the discussion below will focus on those that have shown general applicability and protective immunity in multiple models.

Live attenuated vaccine, as exemplified by *nef*-deleted mutant SIVmac239 Δ *nef*, has been shown to protect against challenge by highly pathogenic cloned virus SIVmac239, or uncloned SIVmac251 in rhesus macaques [98]. Maximal protection was reached 6-10 months after vaccination, possibly due to the need for immune responses to mature [99-101]. On the other hand, protection has also been observed in macaques as early as 21 days after vaccination [102]. In this case, protection did not correlate with any specific T-cell or antibody responses measured [103]. The potential role of viral interference or competition for target sites needs to be examined. Efficacy of live attenuated vaccine appears to depend on the replicative capacity of the vaccine virus, as multiply deleted virus SIVmac239 Δ 3 [101,104], or tissue culture-passaged virus SIVmac1A11 [105], afforded only partial or little protection. It is also important to note that protection induced by live attenuated virus vaccine was primarily effective against the homologous virus and was significantly reduced against a heterologous pathogenic virus, SIVsmE660 [106]. Furthermore, the live attenuated virus approach has been associated with significant safety concerns that are likely to preclude the development of similar HIV vaccines for the general population in the foreseeable future. SIVmac239 Δ *nef* showed no attenuation in newborn macaques [107]. Disease progression in adult macaques was delayed, but not abrogated [108-110]. There are also theoretical risks associated with the ability of retroviruses to integrate into the host chromosome [111]. Finally, without an appropriate animal model for HIV-1 pathogenicity, it is difficult to assess the safety of candidate live vaccines. Nevertheless, live attenuated vaccines may serve as an excellent model to study HIV pathogenesis and correlates of protection against primate lentiviruses.

The use of different vaccination approaches for priming and boosting (“prime-boost”) was originally explored as a means to overcome anti-vector immunity elicited against the priming immunogen and to augment antigen-specific responses by subunit protein boost [112,113]. This approach was found to enhance antigen-specific responses in mice, macaques, and humans primed with a recombinant vaccinia virus and boosted with recombinant HIV-1 envelope protein [112,114-117]. Protective efficacy of this “prime-boost” approach was first demonstrated in a moderately pathogenic SIVmne model against both intravenous and mucosal infection [114,117-119]. Inclusion of multiple antigenic targets (e.g., envelope and core antigens) in the vaccine design augmented the breadth of protection against uncloned virus challenge [120]. A poxvirus and protein prime-boost regimen also protected against SHIV IIIB challenge in pig-tailed macaques [121]. On the other hand, Giavedoni *et al.* [122] and Daniel *et al.* [123] reported that immunization with a similar prime-boost regimen resulted only in reduction of viral load in a minority of animals challenged with a highly pathogenic virus, SIVmac251, with no apparent benefit in disease outcome. Abimiku *et al.* [124] showed that macaques immunized with recombinant canarypox vaccines and boosted with subunit HIV-1 proteins were partially protected against infection by a divergent but non-pathogenic HIV-2. Hirsch *et al.* [125] showed that immunization with a modified vaccinia Ankara (MVA) expressing multiple SIV antigens followed with inactivated SIV failed to protect against infection by a more pathogenic challenge virus, SIVsmE660, but was able to reduce virus load resulting in prolonged disease-free survival. It therefore appears that immune responses elicited by these early attempts at virus vector priming and protein boosting were suboptimal, sufficient to protect against challenge virus of low pathogenicity, but failed to contain more robust ones. It is noteworthy that Patterson *et al.* [126] achieved protection against mucosal challenge by a highly pathogenic virus, SIVmac251, using replication-competent adenovirus for priming and subunit proteins for boosting. This result lends further support for the continued investigation of the vector-protein “prime-boost” strategy for immunization.

Other “prime-boost” strategies have also been explored. In particular, DNA priming with recombinant virus boosting was found to elicit strong T-cell responses [127,128]. Significant and sustained reduction of viral load has been achieved by DNA/MVA prime-boost against CXCR4-using SHIV [129,130]. But its efficacy against SIVmac251 or SIVmac239 challenge

was much less impressive [131-133]. Similarly, replication-defective adenovirus vector, alone or as a booster to DNA priming, elicited robust T-cell responses and significant reduction of viral load after SHIV89.6P challenge [134,135]. DNA prime with recombinant Sendai virus boost has protected cynomolgus macaques against SHIV89.6PD challenge [136,137]. Protection by DNA prime and recombinant attenuated *Listeria monocytogenes* boost was recently reported [138]. The order of DNA versus recombinant vector for priming or boosting was examined. Contrary to earlier observations of McMichael and colleagues [127,128], priming by recombinant poxvirus followed by DNA boost is at least as effective as the reverse order for eliciting protective immunity [139]. Whether this difference relates to the properties of replication-competent vs. non-replicative poxvirus vectors remains unclear. Although immunity elicited by DNA alone is relatively weak, it potentiates responses to booster immunization by recombinant vectors [130,131,134,139,140]. In this sense, current methods of measuring immune responses may not be sufficient to fully reveal the action of priming. In part because of the disappointing results obtained to date with DNA vaccines in humans and in part because of the need to circumvent anti-vector immunity elicited by priming vectors, increasing efforts have been focused on heterologous vectors for prime-boost. Ramsberg *et al.* [141] reported that prime-boost with attenuated recombinant vesicular stomatitis virus (VSV) and recombinant MVA elicited substantially better responses and protective immunity against SHIV89.6P challenge than repeated immunizations with recombinant VSV of different serotypes. Triple combination prime-boost with DNA, recombinant Semliki Forest virus and MVA vectors has also shown protective immunity in cynomolgus macaques against SIVmac251 [142]. Other heterologous vector prime-boost strategies are sure to follow [e.g., 143].

DNA priming followed by protein boosting has been found to be effective to induce antibody responses [144-147]. With the reemerging emphasis on vaccines that can elicit neutralizing antibodies, DNA-protein prime-boost is increasingly being used as a platform to evaluate novel antigen designs. Because protection against primate lentiviruses most likely will require both the humoral and cellular arms of host immune responses, systematic evaluation of various prime-boost approaches appears to be necessary. So far, ample evidence has been accumulated supporting the notion that heterologous prime-boost approaches can elicit greater immune responses than single immunization modalities. However, the mechanism underlying such enhanced response is not well understood [131,148,149]. Detailed analysis of the role of innate immunity and the development of adaptive responses by systematic and comparative prime-boost studies may help identify optimal approaches to enhance protective immunity. Finally, even though prime-boost approaches have shown promise, they also have significant shortcomings, including the need to manufacture multiple vaccine components (usually on diverse technical platforms) and to comply with complex immunization schedules. As in all combination approaches, the potential for increased side effects also needs to be considered.

Studies in NHP models have also helped define the correlates of protection against primate lentiviruses. The most definitive information has been obtained from passive transfer of neutralizing antibodies. Early studies by Emini *et al.* [150] showed that neutralizing antibody directed to the V3 loop of HIV-1 protects chimpanzees against infection by a T-cell line-adapted (TCLA) virus, HIV-1 IIIB. However, the implication of this finding for vaccine development has been debated because of the discovery that primary isolates of HIV-1 differ significantly from TCLA viruses in their neutralization sensitivity [151,152] and the observation that the V3 loop sequence is highly variable. Nevertheless, results from a number of studies have firmly established that passively transferred neutralizing antibodies, monoclonal or polyclonal, when present in sufficient quantity, can protect macaques against both CXCR4- and CCR5-using SHIV [153-158]. So far, none of the vaccine approaches tested can elicit neutralizing antibody responses comparable with those needed to achieve protection in passive transfer studies [157,159]. Therefore it remains a major goal in AIDS vaccine

research to design immunogens that elicit robust and broadly neutralizing antibody responses. It is intriguing to note that passively transferred neutralizing antibodies given within 24h post-infection can delay disease significantly [160]. It appears that the presence of neutralizing antibodies during acute viremia can accelerate the development of an effective humoral response. Several challenge studies have shown that neutralizing antibody detection is accelerated in vaccinated macaques [139,161]. Therefore, it is also important to examine if sub-optimal neutralizing antibodies, together with recall responses and cell-mediated immunity elicited by active immunization, will suffice to afford protection.

Although the outbred nature of macaques limits the use of passive transfer experiments to demonstrate directly the role of cell-mediated immunity in protection, it is clear that such responses are of critical importance. Selective depletion of T-cell subsets and correlative studies have established the importance of antigen-specific CD4⁺ and CD8⁺ T cell responses in control of virus replication [2,3,58-61,162]. However, there is as yet no consensus on any single or combination of parameter(s) to measure T cell responses that are predictive of vaccine protection in NHP. Multiparametric analysis that measures multiple phenotypic markers and functional responses [163] may be necessary. Furthermore, studies in NHP have also revealed the possible importance of balanced immune responses. Induction of antigen-specific CD4⁺ responses in the absence of functional CD8⁺ responses has been suggested as the possible reason for the apparent enhancement of infection in immunized macaques after challenge [164,165]. CD8-mediated antiviral factors have been identified in HIV-1-infected individuals and have been shown to be highly effective in blocking infection by primary virus isolates [166]. However, current knowledge is still insufficient to fully define the nature of the anti-viral activity and to determine if and how such responses can be elicited by vaccination. In this regard, studies of innate responses in the context of vaccination and challenge infection should receive greater attention.

LIMITATIONS AND FUTURE DIRECTIONS

Although substantial knowledge has been gained from NHP models, it is not necessarily straightforward to extend these findings to inform HIV vaccine development. The controversy surrounding the failed efficacy trial of gp120 subunit protein vaccines may serve to illustrate this point. Since this vaccine has been shown to elicit neutralizing antibodies and protect chimpanzees against HIV-1 IIIB challenge [167], the failure of this vaccine in human trials [168,169] has been viewed as evidence to invalidate NHP models. While this view may be justified as far as the HIV-1 IIIB challenge model in chimpanzees is concerned, key findings from NHP models as a whole are remarkably consistent with the results from human trials. First, although gp120-elicited antibodies neutralized TCLA viruses and other highly sensitive isolates (e.g., HIV-1 SF2), sera from immunized chimpanzees and humans failed to neutralize typical primary HIV-1 isolates [151,152]. In this sense, chimpanzees are suitable for immunogenicity assessment, but not for challenge studies. Second, a similar SIV envelope protein vaccine failed to elicit neutralization antibodies and to protect macaques against SIVmac251 infection [122]. Thus, available data from both NHP models are consistent with the outcome of human efficacy trials. In other words, for a vaccine that bases its mode of action primarily on neutralizing antibodies, protection can be achieved if sufficient neutralizing antibodies are present (as in HIV-1 IIIB infection of chimpanzees), but not when they are lacking (as in SIV models and in humans). Proper interpretation of findings from NHP models therefore requires better understanding of the characteristics and the limitations of the models used.

As discussed in previous sections, a key limitation of the NHP model is its intrinsic nature as a surrogate model for HIV infection. SIV models do not allow direct testing of HIV vaccines. Currently available SHIV models do not adequately represent the spectrum of HIV genotypes

and phenotypes. In particular, very few CCR5-using and non-subtype B SHIV are available as challenge stocks. Selection for increased virulence by serial passage in macaques may be useful for rapid and reliable read-out of challenge outcome, but may also result in misjudgment of vaccine efficacy. Recently, several host restriction factors for HIV-1 replication in macaque cells have been identified [31,32]. If the nature of host restriction and the target sites on the virus can be identified, it may be possible to introduce limited and specific alterations in HIV-1, enabling it to replicate more efficiently in macaque cells and establish persistent infection *in vivo*. The availability of such challenge viruses may allow direct testing of HIV-1 vaccines in a more relevant model. Until then, currently available surrogate models are best suited for understanding the basic biology of immune protection and testing of vaccine concepts, not necessarily vaccine products *per se*.

As illustrated by the example of gp120 trials discussed above, another difficulty to extract information from NHP models is the seemingly contradictory findings from different models. Several factors may contribute to this. At the most basic level, there is a lack of comparability and standardization of reagents, methods and challenge stocks, making it difficult to compare data from different vaccine studies. Better standardization of reagents and comparability of experimental design is urgently needed and is only possible through a concerted effort. On another level, the apparent discordance could be a reflection of the different properties of the challenge model used. For instance, immune responses required for protection against a neutralization-sensitive virus, such as SHIV89.6P, will most likely be different from that for a neutralization-resistant one, such as SIVmac239. Since HIV-1 infection in humans results in a wide spectrum of responses and outcomes, it is doubtful any single model will adequately recapitulate such complexity. Future efforts will most likely rely on models that reflect the range of HIV-1 infection, in terms of viral genotype and phenotype, as well as the mode of transmission. Finally, intrinsic differences between NHP species and humans may also contribute to discordant findings. In addition to their varying susceptibility to virus infection, different species may recognize immunogens and respond to adjuvants differently. For example, species specificity of the adjuvant activities of bacterial lipopolysaccharides and CpG oligonucleotides has been well recognized [170-172]. Proper interpretation of discordant results from different species will require better understanding of the mechanisms of action of the immunogens and adjuvants involved.

The predictive value for any animal model depends on validating data from human trials. The lack of efficacy data from human vaccine trials to date makes it risky to select of any single NHP model to “rank-order” candidate vaccines for clinical development. On the other hand, it is not feasible and is scientifically unsound to screen all experimental vaccines in early phase human trials. Judicious use of appropriate NHP models will greatly accelerate AIDS vaccine development. Towards this end, better understanding of the basic biology of NHP models, development of models that better reflect HIV in natural transmission, and greater emphasis on comparative and parallel-track studies in humans and NHP are critically needed.

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