

Preclinical challenge studies of AIDS vaccines in non-human primates have an important role in the AIDS vaccine development effort. However, to be most useful, challenge models need to be improved to more closely reflect the actual biological circumstances of HIV-1 infection and transmission in humans.

## AIDS vaccine models: Challenging challenge viruses

Animal models are essential in the search for a vaccine to prevent HIV-1 infection. Of these, rhesus macaque models are, by far, the most important.

Rhesus monkeys can be experimentally infected with various simian immunodeficiency virus (SIV) strains of differing virulence, many of which cause simian AIDS (refs. 1,2). However, because HIV-1 does not productively infect macaques, it cannot be used as a challenge virus to assess whether a given vaccine can prevent or ameliorate infection<sup>1,2</sup>. Hence, preclinical AIDS vaccine models rarely test the identical vaccine constructs that are planned for human use. Instead, studies in rhesus macaques explore the potential protective efficacy of vaccine concepts, not the actual vaccines being developed for human trials. To increase the relevance of the macaque model to human vaccine trials, hybrid viruses that contain elements of both the SIV and HIV-1 genomes, designated SHIVs (simian/human immunodeficiency viruses), were first engineered several years ago for use as challenge viruses in pre-clinical AIDS vaccine studies<sup>3–6</sup>. In these chimeric viruses, several HIV-1 genes, most commonly *tat*, *rev* and *env*, are substituted for their SIV homologs. One highly virulent SHIV challenge virus, SHIV-89.6P, has been widely used in vaccine experiments, and is usually now considered the ‘virus-of-choice’<sup>6,7</sup>.

Infection of naive macaques with SHIV-89.6P leads, almost invariably, to a rapid and virtually complete loss of CD4<sup>+</sup> T-cells from the peripheral blood and lymphatic tissues<sup>6,7</sup> (Fig. 1). Virus replication proceeds at a rapid and highly reproducible rate, and infected macaques progress very rapidly to AIDS and death. The consistency and rapidity of the virological and immunological outcomes of SHIV-89.6P challenge is useful in vaccine challenge studies, as a statistically significant conclusion can be drawn from a limited number of animals in an acceptably short time-frame<sup>7</sup>. Furthermore, the extreme virulence of SHIV-89.6P infection has been thought to provide a rigorous challenge model for the comparative evaluation of different vaccine concepts. However, SHIV-89.6P differs in important ways from the HIV-1 strains that most commonly infect and are transmitted between humans. In addition, accumulating evidence suggests that despite its exceptional virulence in macaques, SHIV-89.6P may be a paradoxically easy virus for the immune system to control. In other words, SHIV-89.6P may be a sheep in wolf’s clothing, with the potential to lead the AIDS vaccine development effort down the wrong path.

The first SHIVs were constructed when the goal of vaccine development was the outright prevention of HIV-1 infection (‘sterilizing immunity’) via vaccine-induced neutralizing antibodies against HIV-1 Env. Although vaccines able to induce effective neutralizing antibody responses have not yet been developed, *env* is still the primary HIV-1 gene included in SHIVs (refs. 1–6). Perhaps by force of habit, this

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has remained so even when the vaccine approaches being evaluated do not include HIV-1 Env, and are often predicated on the induction of antiviral CD8<sup>+</sup> T-cell responses rather than

neutralizing antibodies. The belief that sterilizing immunity to HIV-1 is an attainable goal has waned recently, and the goal of vaccine development has shifted to the containment of virus replication post-infection (primarily by vaccination-induced antiviral CD8<sup>+</sup> T-cell responses)<sup>8,9</sup>. A vaccine that allows a substantial containment of viremia in vaccinated individuals who become infected would represent a significant advance, as diminished virus replication is associated with substantially decreased rates of disease progression and HIV-1 transmission<sup>10,11</sup>.

The utility of the SHIV models is obvious, yet their limitations must also be considered. Some limitations are inherent to all animal models of human infections; there may be species-specific, overt or subtle differences in how retroviruses are transmitted, or in the way they cause disease<sup>1,2</sup>. However, other limitations can be specific to certain challenge viruses. For instance, a virus that replicates very poorly in a host may be misleadingly easy to protect against by vaccination, and is of little value for studies of viral pathogenesis. Conversely, vaccine studies that employ particularly virulent viruses or experimental challenges delivered at high doses via intravenous inoculation may underestimate the potential protective efficacy of some vaccine strategies.

The prototypes of genetically engineered SHIVs usually replicated poorly when first inoculated into macaques, and were non-pathogenic<sup>1–6</sup>. Substantially higher replication and greatly enhanced pathogenicity were then achieved by serial passage of the SHIVs through naive macaques<sup>1,2,6,7</sup>. The resulting SHIV variants, exemplified by SHIV-89.6P, mutated to cause a rapid, nearly complete depletion of the total CD4<sup>+</sup> T-cell population in newly infected macaques. The acquired ability to extensively destroy CD4<sup>+</sup> T cells is associated with an increased membrane-fusing capacity of the envelope glycoproteins<sup>12</sup>.

Many vaccine immunogens, alone or in combination, have now been evaluated in the SHIV-89.6P model, including naked DNA, recombinant viruses, subunit proteins and peptides<sup>8,9,13–19</sup>. Despite the diversity of immunogens, a surprisingly consistent outcome has been seen in multiple studies: the vaccinated animals become persistently infected with SHIV-89.6P, but the rapid and complete decline in peripheral CD4<sup>+</sup> T cells does not occur<sup>8,9,13–19</sup>. Instead, the animals maintain near-normal CD4<sup>+</sup> T-cell counts, and have greatly reduced viremia compared to unvaccinated controls. These results have been greeted with substantial enthusiasm, because the ‘successfully’ vaccinated animals can control virus replication at levels that are, in HIV-1-infected

humans, associated with significantly lowered disease progression and transmission rates—probably the most attainable near-term goals of AIDS vaccine research<sup>8–11</sup>.

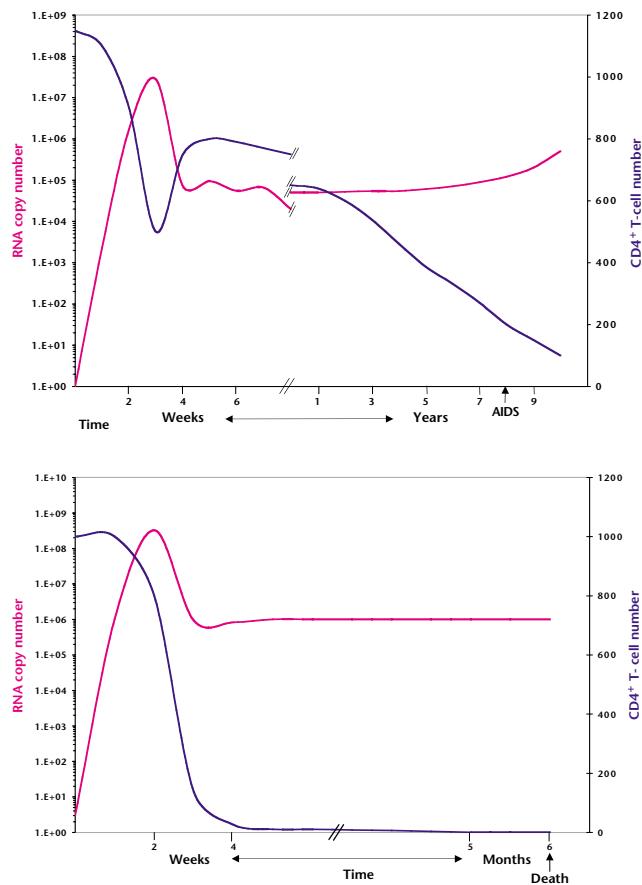
In HIV-1-infected humans, a disease course like that following SHIV-89.6P challenge of macaques is not unprecedented, but is extremely rare<sup>20,21</sup> (Fig. 1). A fear, therefore, is that because the rapid destruction of CD4<sup>+</sup> T-cells caused by SHIV-89.6P is abnormal, so might be the nature of the protection achieved against this decline induced by such a variety of vaccine antigens.

We have three interrelated concerns about whether SHIV-89.6P, despite its logistical advantages, should be considered as the virus-of-choice in pre-clinical AIDS vaccine studies. First, although the HIV-1 *env* included in SHIV-89.6P was derived from a ‘dual-tropic’ virus that used both the CCR5 and CXCR4 coreceptors for entry into macaque CD4<sup>+</sup> T-cells, sequential passage in macaques has selected for a SHIV that uses only CXCR4; SHIV-89.6P is a ‘pure X4 virus’<sup>22</sup>. Primary infection of humans with viruses that use CXCR4, either alone or as well as CCR5, is rare, but when this occurs, a rapid, irreversible loss of peripheral CD4<sup>+</sup> T-cells is observed<sup>23</sup>. An accelerated loss of CD4<sup>+</sup> T cells also ensues whenever CXCR4-using viruses emerge during chronic HIV-1 infection, probably due to their enhanced membrane-fusing capacity and their tropism expansion to target naive CD4<sup>+</sup> T-cells in the thymus and periphery<sup>23,24</sup>. However, most HIV-1 strains transmitted between humans use only CCR5 as their coreceptor (R5 viruses), and they do not cause a rapid, irreversible loss of peripheral CD4<sup>+</sup> T-cells during primary infection<sup>23–25</sup>. Nor does the latter happen

when macaques are infected with an R5 SHIV such as SHIV-162P (ref. 26). Overall, X4 viruses and R5 viruses interact with the primate immune system in significantly different ways, yet it is against the transmission and local, early amplification of R5 viruses that an effective HIV-1 vaccine must protect<sup>9,25,26</sup>.

Our second concern relates to the central role of CD4<sup>+</sup> T-helper cells in determining the outcome of the initial battle between HIV-1 replication and the immune system<sup>27</sup>. Studies in both humans and macaques show that allowing CD4<sup>+</sup> T-cell help to mature by suppressing viral replication during primary infection can be highly beneficial to the subsequent course of disease<sup>27–30</sup>. Conversely, a virus that replicates with unusual rapidity may destroy too many T-helper cells before they can coordinate both antiviral B cell and CD8<sup>+</sup> T cell-mediated immunity. Such an outcome has a catastrophic effect on the immune response. Indeed, the loss of T-cell help is so striking that humans and macaques with extremely rapidly progressing HIV-1 or SHIV-89.6P infection fail to seroconvert to viral antigens<sup>6,7,12,20,21</sup>. The temporal development of partially effective adaptive immunity and the destruction of the helper T cells that coordinate these responses can be finely balanced<sup>30,31</sup>. A very early, vaccine-elicited memory response to viral antigens might restrict the initial replication of SHIV-89.6P just sufficiently to preserve enough of the CD4<sup>+</sup> T-helper pool during the early, critical generative stages of the antiviral response. Protection might arise by priming of CD4<sup>+</sup> T cells to provide help for early production of neutralizing antibodies by B cells, and/or via activation of antiviral CD8<sup>+</sup> T cells to limit early expansion in the number of virus-infected cells. Whatever the actual nature of the ‘protective’ immune effectors raised by various vaccine approaches, their magnitude would be amplified after infection via exposure to viral antigens produced during challenge virus replication. The surviving CD4<sup>+</sup> T-helper cells could then coordinate effector CD8<sup>+</sup> T-cell and B-cell memory responses that contain virus replication.

Thirdly, and perhaps most importantly, SHIV-89.6P is unusually sensitive to autologous neutralizing antibodies<sup>6,32</sup>. If prior vaccination enables a partial preservation of CD4<sup>+</sup> T cells following challenge, sufficient help would then be available for B cells to mount an anamnestic neutralizing antibody response directed against the SHIV-89.6P Env. Given the sensitivity of SHIV-89.6P to autologous neutralizing antibodies, it may be relatively easy to suppress virus replication, and thereby protect against the rapid decline in CD4<sup>+</sup> T cells and rapid disease progression that typically follows SHIV-89.6P infection. However, this dynamic is not



**Fig. 1** Contrasting natural histories of AIDS virus infections. HIV-1 infection of humans results in highly variable rates of disease progression that are associated with set-point levels of virus replication and rates of decline of peripheral CD4<sup>+</sup> T cells. A schematized typical course of progression to AIDS following initial HIV-1 infection is shown, characterized by chronic intermediate levels of plasma viremia, a steady loss of peripheral CD4<sup>+</sup> T cells, and the development of AIDS after 7–10 years (above). In contrast, SHIV-89.6P infection of rhesus macaques results in near-complete destruction of the peripheral CD4<sup>+</sup> T-cell population within the first weeks of infection, uncontrolled high-level virus replication, and rapid progression to AIDS and death (below).

characteristic of typical HIV-1 infection and the subsequent immune response in humans. In infected humans, HIV-1-specific CD4<sup>+</sup> T cells are rarely acutely depleted to the extent that antiviral immune responses cannot be generated<sup>20,21,24</sup>. Moreover, most primary HIV-1 isolates resist antibody neutralization, and only rarely can the combined activity of antiviral CD4<sup>+</sup> and CD8<sup>+</sup> T-cell responses durably keep virus replication at low levels<sup>10,24,27</sup>.

We believe some of the vaccines that have protected macaques against SHIV-89.6P infection represent important concepts that should be expedited into clinical trials in humans. But the protection elicited by other vaccines may be artifacts of the SHIV-89.6P model, and irrelevant to protecting humans against HIV-1. Few vaccine strategies have been evaluated for protective efficacy using both SHIV-89.6P and alternative pathogenic SIV challenge models. For those that have, the results obtained in the SHIV-89.6P model have always been the more impressive<sup>13,16,33,34</sup> (and D. Watkins, pers. comm.). Indeed, marked and durable control of a pathogenic SIV has never been achieved by vaccination, other than by a live-attenuated vaccine<sup>1,2,8,9</sup>. We therefore suggest that vaccine concepts that have given positive results in the SHIV-89.6P model should be retested against an R5 virus that does not cause an abnormally rapid and profound loss of CD4<sup>+</sup> T-cells (such as SHIV-162P, SIVsmE660, SIVmac251 or SIVmac239). Furthermore, given the tremendous genetic diversity of HIV-1 worldwide, it is essential that the ability of a given vaccine strategy to protect against genetically distinct virus challenges be rigorously investigated in macaque models. So far, all of the experimental SHIV challenge studies have employed vaccine immunogens that are genetically identical (or nearly so) to the challenge viruses<sup>13–18</sup>. The breadth of protective efficacy that can be engendered by vaccination has yet to be addressed experimentally in macaques, yet a single amino-acid change in Gag is reported to be sufficient to enable SHIV-89.6P to escape from immune containment<sup>35</sup>. Given that all of the SHIVs currently used in vaccine studies employ a ‘backbone’ of SIV genes derived from a single molecular clone (SIVmac239), it will be necessary to develop immunogens that differ from the challenge virus across the viral genome, and not just in the *tat*, *rev* and *env* genes that represent the HIV-1 component of the SHIV.

Animal models are an essential resource for evaluating the safety and comparative immunogenicity of candidate AIDS vaccine strategies in preparation for Phase I human studies. Some vaccine concepts should be rejected before Phase I human studies begin, if a similar concept is so obviously superior in a macaque model. However, if protection from the acutely pathogenic effects of SHIV-89.6P is the only requirement to move a vaccine concept into Phase I human trials, the evaluation pipeline for AIDS vaccines may be overburdened by vaccines of widely varying immunogenicity and plausibility.

Animal models cannot determine whether a vaccine will be effective against HIV-1 infection of humans; only Phase III trials in humans can do so. However, challenge experiments in the macaque models can potentially add important insights to those gained in Phase I and II studies in humans, and they should meaningfully inform decisions that will impact many thousands of volunteers and involve many millions of dollars. To be most informative and help-

ful, challenge studies in macaques should now seek to resolve the most difficult issues in AIDS vaccine development, and, depending on the nature of the scientific question under evaluation, studies may need to use more than a single challenge virus. Within this context, it is essential that macaque models be improved so as to mimic, as closely as possible, the actual circumstances of HIV-1 infection and transmission in humans.

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