

## Mini Review

# Nonhuman Primate Models for Empirical Research of Acquired Immunodeficiency Syndrome

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To develop preventive or therapeutic interventions for acquired immunodeficiency syndrome (AIDS), experimental infection model systems are needed for studying human immunodeficiency virus type 1 (HIV-1), the causative agent of AIDS. However, since HIV-1 infect only human and chimpanzee, it is difficult to conduct the infected animal experiments with HIV-1 itself. Simian immunodeficiency virus (SIV) infects rhesus monkey and causes AIDS-like symptoms. SIV/macaque model has revealed extremely important information in AIDS research. While detailed analysis of deep tissue using timely necropsy is informative in SIV-infected animals, such experiments would be impossible in humans. To overcome the narrow host range of HIV-1 and to develop a dependable animal model for AIDS, a chimeric simian-human immunodeficiency virus (SHIV) between SIV and HIV-1 was engineered. SHIV research revealed that the target cell affinity and pathogenesis of the virus were greatly affected by the interaction between cellular receptors (CCR5 and/or CXCR4) and the env gene of HIV-1, and that the CCR5-tropic virus was important for pathogenesis. Other experimental models have also been developed, such as HIV-1mt, in which > 90% of the genome is derived from HIV-1. This review presents the recent progress in SIV/SHIV/HIV-1mt research regarding the pathogenesis, prevention and cure of HIV infection.

**INTRODUCTION**

In the thirty five years since acquired immunodeficiency syndrome (AIDS) was first reported in 1981, human immunodeficiency virus (HIV) infection has spread rapidly worldwide. According to the World Health Organization (WHO) and United Nations Program on AIDS (UNAIDS), there were 36.9 million HIV-positive individuals in 2014, with 2 million newly infected individuals and 1.2 million HIV-related deaths. Thus, AIDS remains a significant global public health issue. Combined antiretroviral therapy (cART) has contributed to the dramatic reduction in mortality; however, several unresolved concerns remain such as drug-resistance, side effects and medical costs. While cART has shown some significant advances, it has failed to completely eradicate latent virus in the host, and fundamental therapeutic interventions for AIDS have not been established. In addition, effective HIV vaccines remain elusive.

**PHYLOGENETIC RELATIONSHIP BETWEEN HIV AND SIV**

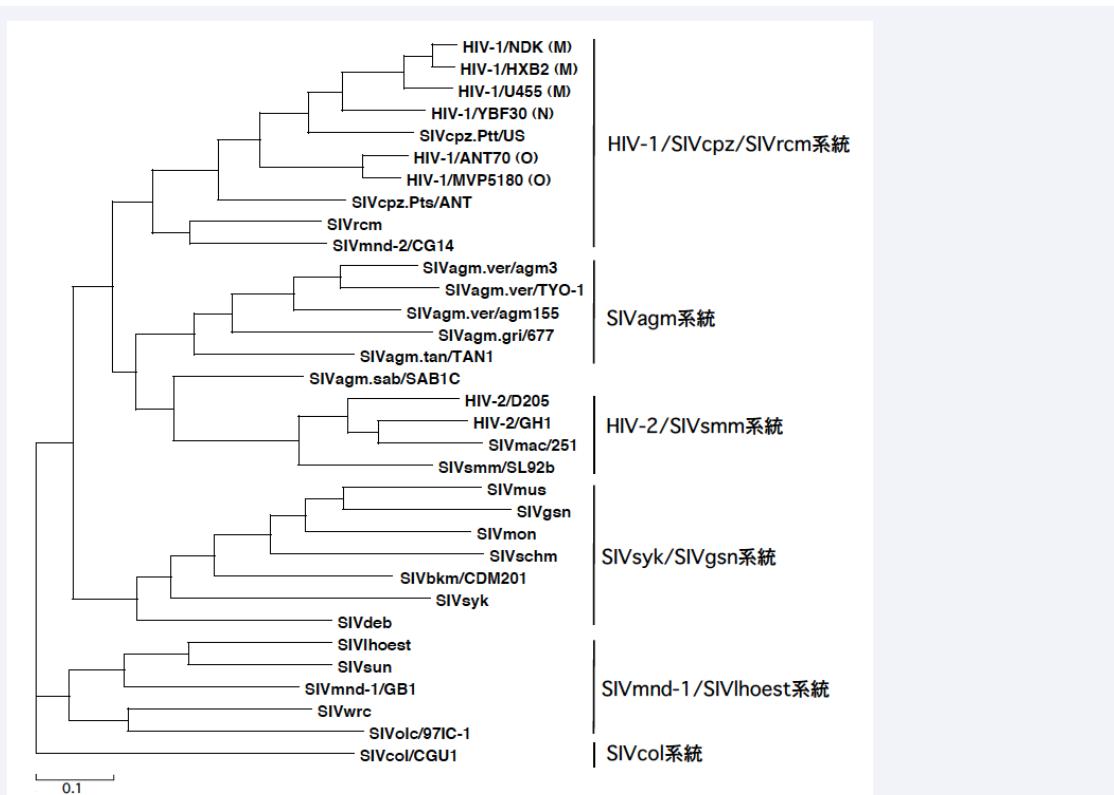
Soon after HIV was recognized, research efforts focused on where and how the AIDS virus emerged. SIV, which is similar to HIV, was isolated from rhesus macaques that died of AIDS-like symptoms in a United States primate center. A number of African-origin monkeys including African Green Monkey, (AGM)

retained antibodies against SIV, whereas Asian-origin macaques, including rhesus macaques in the wild, showed no SIV infection. Therefore, it was hypothesized that the AIDS virus was derived from African-origin monkeys. However, genetic analysis of SIV from AGMs refuted the simple scenario of infectious transmission from AGM to human [1]. Although the monkey-derived viruses tend to be discussed collectively, there are many types of SIV in fact, and the phylogenetic relationship is complicated.

We clarified the whole genome sequence of SIVagm, (SIV isolated from AGM) and showed that HIV is not derived directly from SIVagm [1]. In addition, we reported the whole genome sequence of mandrill-derived SIV (SIVmnd), contributing to clarify the full picture of phylogenetic relationship of primate lentivirus [2]. At almost the same time, a virus isolated from Sooty Mangabey in West Africa (SIVsmm) was found to be similar to HIV-2, which was prevalent in West Africa [3]. SIVcpz derived from chimpanzee was found to be similar to HIV-1 [4]. Thus, phylogenetic analysis revealed relationships between HIV-1 and HIV-2 and the primate lentiviruses including various SIVs (Figure 1).

**SIV MODEL**

Although the HIV research at the molecular/cellular level advanced significantly after the initial reports of HIV-1



**Figure 1** Molecularly evolutionary phylogenetic tree of lentivirus in primate. The phylogenetic tree is generated by maximum-likelihood method on the basis of pol gene sequences using ClustalW and TreeView software. HIV-1 is derived from SIVcpz that chimpanzees residing in Central Africa were naturally infected. HIV-2 is derived from SIVsmm that sooty mangabees residing in West Africa were naturally infected. The most frequently used SIV as macaque AIDS model is SIVmac isolated from rhesus macaques with AIDS-like symptom under the captivity situation in the U.S. primate center. SIVmac is considered to be derived from SIVsmm.

identification, many of them were partial analytical research, and their relationship to the virulence of the infected individual level could not be confirmed. Thus, the fundamental mechanisms of HIV-1 progression to AIDS remain unclear. Although important knowledge could be obtained from human clinical samples, such samples have limitations such as unidentified infection times, analysis with only peripheral blood, and multiple effects of antiretroviral drugs. Therefore, an experimental animal model is essential for analyzing the disease progression mechanism and to develop prophylactic and therapeutic interventions against AIDS. Viral and host factors require analysis from a wide viewpoint. However, the narrow host range of HIV-1 is a significant limitation, and *in vivo* HIV-1-infection animal experiments are difficult to perform. On the other hands, SIV, which is genetically similar to HIV-2, infects Asian-origin macaques including rhesus monkeys and cynomolgus monkeys and causes AIDS symptoms.

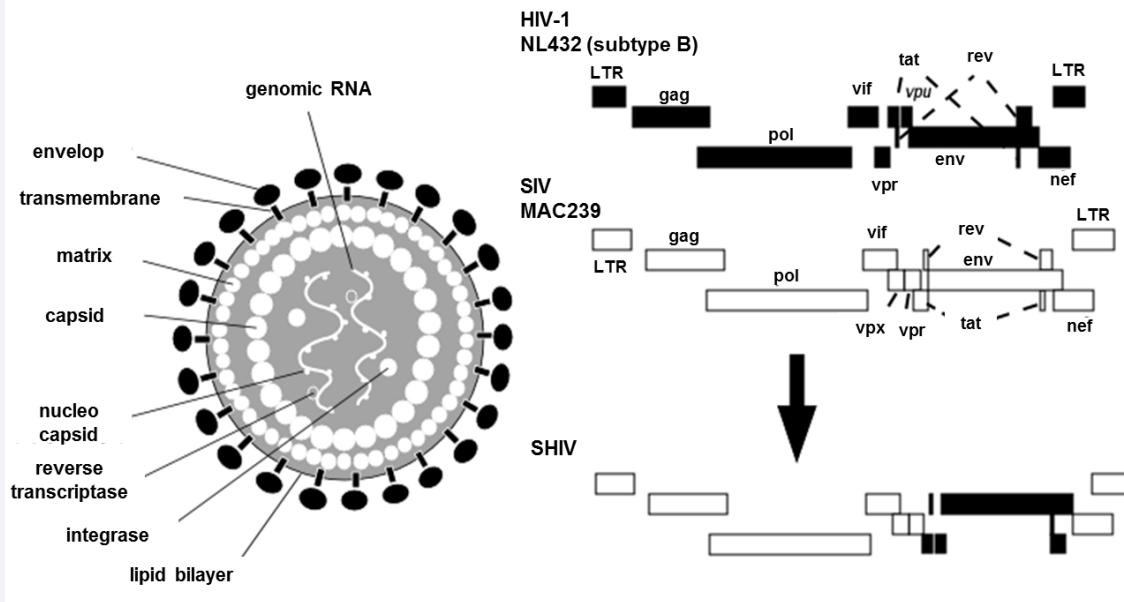
After the discovery of SIV, the importance of HIV research using monkeys has been recognized and studies using the SIV have been actively carried out especially in the United States primate centers. The strain of SIV described in this review was mainly SIVmac, which was isolated from rhesus monkey that died with AIDS-like symptoms in the United States primate centers in the 1980s. Phylogenetic analysis indicated that SIVmac of rhesus monkey was transmitted from sooty mangabey (SIVsmm) in captivity situation in the United States primate center. SIV causes AIDS in inoculated rhesus macaques within 1 or 2 years. Since the

pathological condition of the SIV/macaque model is comparable to HIV-1-infected human patients, the SIV/macaque model is an excellent study model for analyzing the pathogenesis of HIV infection in [5]. The SIVmac/macaque model is currently the most widely used animal model in the field of AIDS research.

By SIV/macaque model, extremely important findings in the research of AIDS pathogenesis have been revealed. In these studies, detailed analysis of deep tissues by continuous biopsy and timely necropsy, which is impossible in humans, was quite useful. The major contributions of SIV research in AIDS pathogenesis are as follows: 1) SIV is the causative agent of simian AIDS, and the pathological condition of SIV-infected monkeys is very similar to that of HIV-infected human patients; 2) SIV/HIV disease is zoonotic; 3) Cellular immunity was important to suppress viral replication in infected individuals; 4) SIV-attenuated vaccine could prevent SIV infection; and 5) the primary anatomical compartment targeted by SIV was the intestine, which is one of the most common sites of HIV infection [5-7].

We established an *in vivo* experimental system with combined antiretroviral therapy (cART). Briefly, SIV-infected macaques were fed anti-HIV drug-kneaded biscuits over time [8]. We reported that the SIV/macaque model treated with cART over 1 year showed no accumulation of SIV mutations in SIV-infected monkeys during cART, that is, no transmission to uninfected cells in SIV-infected monkeys during cART [9]. This cART-treated

## particulate structure and genome organization of viral RNA



**Figure 2** Particle structure and genome organization of SHIV. The black color represents HIV-1-derived genes. The white color represents SIV-derived genes. SHIV is the chimeric virus encoding HIV-1-derived genes including env and some accessory genes on the basis of SIV backbone.

primate model is expected to promote the development of new therapeutic ways in combination with the CART.

### SHIV MODEL

The SIV/macaque model is an excellent model for analyzing the pathogenesis in HIV infection in infected individual level. However, this model is inappropriate for analyzing the role of neutralizing antibody in protective immunity due to the low homology of viral envelope proteins (Env) between SIV and HIV, which results in different Env structures and antigenicities. To address the issue, we generated the first chimeric simian and human immunodeficiency virus (SHIV), which substitutes partial HIV-1 genome including *env* gene into the corresponding region in the SIV genome (Figure 2) [10,11]. After that, a pathogenic SHIV strain was obtained by monkey adaptation [12]. Pathogenic SHIV strains were used to study the functional significance of HIV Env in infected individual level and neutralizing antibodies for protection against infection. For instance, the secondary receptor tropism (tropism for CCR5 and CXCR4) determined by the HIV-1 *env* gene resulted in the identification of significant differences in the targeted tissues and pathogenesis in infected individuals [13]. In other instances, the importance of HIV Nef, which is known to downregulate CD4 receptor, and Vpu, which has been known to antagonize the viral restriction factor like tetherin, for *in vivo* pathogenesis were demonstrated [14,15].

We performed a comparative analysis of highly pathogenic SHIV-KS661 and chronic SHIV -# 64 and showed that SHIV -# 64 infection exhibited the reduction of CD4+ T lymphocytes in small intestine even though SHIV -# 64 was suppressed easily by the host immune system [16]. We also showed that the pathological condition in small intestine remained active even though the

host immunity controlled viral infection in peripheral blood [17]. Previously, a goal of prophylactic and therapeutic interventions for AIDS was the reduction of plasma viral load and recovery of circulating CD4+ T cells. The current focus is now on the reduction of CD4+ T lymphocytes in the intestine as the primary pathological target tissue [6]. Because it was demonstrated that the small intestine was vulnerable to SHIV infection in the SHIV/primate model, the analysis of the condition of small intestine in SHIV-infected monkeys is thought to be useful for elucidating AIDS pathogenesis and developing prophylactic and therapeutic interventions.

We performed fundamental research for AIDS vaccine development using the SHIV/macaque model. Although the biodegradable nano particle enhanced the Env-specific immune response in mouse and rhesus macaque, unfortunately, instead of finding a protective effect, we observed an infectious enhancement [18]. The enhancement of an immune response is not always connected to that of protective effect in some viruses that replicate in immune cells. Therefore, it is critical to use appropriate animal models to evaluate the effectiveness of an immune mechanism for the protective effect in AIDS vaccine development.

The clinical trial of HIV vaccine conducted by Merck was suddenly discontinued in 2007, as the vaccine candidate having a protective effect in a SHIV/macaque model showed no protective effect in HIV infection in the human clinical trial. In addition, the vaccinated group showed a tendency of higher viremia than the placebo group. Thus, the SHIV/macaque model could not properly evaluate the effect of vaccine candidate. This fact indicated that infections with HIV and SIV were more difficult to control than SHIV infection. Such differences could be due to the fact that

most circulating highly pathogenic SHIV and HIV/SIV viruses differed with regard to which co-receptors the viruses used and, consequently, the target cells, tissues and pathogenesis patterns differed [19]. Highly pathogenic SHIV preferentially infects naïve T cells with high affinity to CXCR4, and peripheral CD4+ T cells exhibit rapid depletion. On the other hand, SIV and HIV have high affinity to CCR5 and preferentially infect memory T cells residing in the effector site such as the intestinal mucosa [20]. Although many studies used highly pathogenic SHIV that were CXCR4-tropic, CCR5-tropic virus was thought to be important in human AIDS pathogenesis, prompting development of CCR5-tropic SHIV model.

Intravenous inoculation with highly pathogenic CXCR4-tropic SHIV-KS661 caused dramatic AIDS with persistent infection of high viremia and the lack of an antibody response against SHIV due to the rapid deletion of systemic CD4+ T cells. However, intrarectal inoculation with the same virus showed that controlled viremia below the detection limit after transient viremia peak with only transient CD4+ T cell decrease and the antibody response [16]. In contrast, SIV caused persistent infection with stably high viremia despite the antibody response (Figure 3).

To construct a SHIV/macaque model that closely resembled HIV-infected human patients, five amino acids in Env of the highly pathogenic SHIV-KS661 were changed to switch the second receptor tropism from CXCR4-tropic to CCR5-tropic, and the virus was acclimated to rhesus monkey by animal-to-animal passage [21]. The monkey-adapted virus was named as SHIV-MK38, and showed the similar neutralization resistance against neutralizing antibody as HIV-1 clinical isolates [22]. Genetic analysis of the *env* gene showed most mutations were associated with potential N-linked glycosylation sites and net charge, suggesting that a

steric shielding structure may block the access of neutralizing antibody. This neutralization-resistant CCR5-tropic SHIV-MK38 will be evaluated as a challenge virus.

## HIV-1MT MODEL

When the virus replicates, it interacts with a variety of molecules in the infected host cell. Thus, the host range is determined by the interaction with host factors that promote or suppress the viral replication process. The virus introduces mutations, which result in reduced interactions that might protect the host against infection. It is thought that the virus consequently acquires positive changes, used to newly infect the host, thereby enhancing viral infection. In general, the interaction with the receptor molecule of the host cell, the first step of the infection process, is thought to be important.

In the case of HIV, the interaction between CD4 and CCR5/CXCR4 molecules expressed on the host cell surface and the *env* product of the virus is the first step. This initial interaction is associated with the virus' affinity to the target cell and tissue in infected individuals.

As HIV-1 cannot infect rhesus monkeys, SHIV was generated during the development of an AIDS model in primates. Surprisingly, the generated SHIV maintained the *env* gene derived from HIV-1. This result indicated that the reason HIV-1 did not infect rhesus monkeys had little to do with the interaction between *env* and the cell surface receptor.

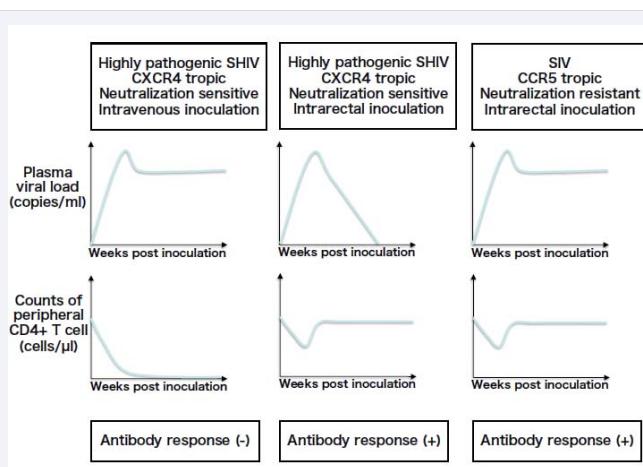
The virus developed a mechanism to counteract host restriction factors. The host restriction factors, APOBEC3G [23] and TRIM5alpha [24], interact with several viral components - *vif* gene (the counterpart of APOBEC3G) and *gag* gene (the counterpart of TRIM5 alpha)—and are related to HIV-1-resistance in rhesus monkeys. These findings led generation of the new recombinant virus, composed of >90% HIV-1 including only partial *gag* and *vif* genes derived from SIV infectious to macaques [25,26]. Such studies would offer the development of an effective AIDS animal model. For instance, drug resistance for viral protein like integrase with mutations in their gene coding sequences in the animals treated with integrase inhibitor [27]. In other instance, there is evidence of association of treatment with anti-CD8 antibodies have rapid progression of HIV infection as measured by viral load, whereas untreated controls are the elite controllers infected with macaque-tropic HIV-1 [28]. Moreover, it would be useful for understanding how cross-species transmission occurs.

## CONCLUSION

Studies using pathogenic and non-pathogenic SIV/SHIV/HIV-1mt have contributed to our understanding of AIDS pathogenesis by connecting accumulated knowledge at the *in vitro* cellular level with an understanding of how pathogenesis emerges in infected individuals. Sampling, experience, skills and information through SIV/SHIV/HIV-1mt research will provide effective infectious and pathogenic animal models, which will be useful for developing prophylactic and therapeutic interventions against AIDS.

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**Figure 3** The comparison between pathogenesis of highly pathogenic SHIV and SIV. When highly pathogenic CXCR4-tropic SHIV is intravenously inoculated to rhesus macaques, it depleted peripheral CD4+ T cells in the short period and exhibited persistent infection with high viremia without an antibody response against the virus in the host. However, when highly pathogenic SHIV is intrarectally inoculated, viremia is suppressed below the detection limit because the recovery of peripheral CD4+ T cells after the transient reduction renders the antibody response active. In contrast, when SIV is intrarectally inoculated, it exhibited the persistent infection with high viremia despite the antibody response that occurs in the host.

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