Short Communication: Comparative Susceptibility of Rhesus Macaques of Indian and Chinese Origin to Vaginal Simian–Human Immunodeficiency Virus Transmission as Models for HIV Prevention Research

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Abstract

Historically, Indian rhesus macaques (iRMs) have been preferred for simian immunodeficiency virus (SIV)/HIV prevention, pathogenesis, and treatment studies, yet their supply is limited. Chinese rhesus macaques (cRMs) are currently more available, yet little is known regarding the relative susceptibility of this subspecies to vaginal transmission of SIV or simian–human immunodeficiency virus (SHIV). In this study, we compared the susceptibility of 40 cRMs and 21 iRMs with a single vaginal challenge with SHIVsf162P. Our results showed that cRMs have comparable primary SHIV infection as iRMs, underscoring their equal importance in studies of HIV transmission and prevention.

Keywords: HIV, SIV, rhesus macaques, transmission, prevention, treatment

EVEN IN THE era of combination antiretroviral therapy, there were ~2.1 million new HIV infections in 2015 (UNAIDS data), and only half of infected individuals have access to antiretroviral therapy. While the major routes of HIV transmission are clear, the mechanisms of how HIV transmission occurs remain largely unknown. A better understanding of HIV transmission is critical for developing new strategies for prevention. However, studies of the early stages of HIV transmission in humans are at best challenging to perform, thus nonhuman primate (NHP) models are essential for transmission and prevention studies.

Currently, there are various types of NHP models. NHPs in the macaque genus (*Macaca*) are susceptible to experimental simian immunodeficiency virus (SIV) or chimeric simian–human immunodeficiency virus (SHIV) infection, which include cynomolgus macaques (*Macaca fascicularis*), pig-tailed macaques (*Macaca nemestrina*), and rhesus macaques (*Macaca Mulatta*). Among the rhesus, the subspecies of Indian rhesus macaque (iRM) has historically been the most commonly used NHP model for academic research as the National Primate Centers were originally stocked with animals imported from India. Moreover, the most pathogenic SIV strains in use today were developed through serial pas-

sage of weaker viruses in iRMs, suggesting that there may have been selective pressures in this subspecies.¹ Further studies of SIV in iRMs and Chinese rhesus macaques (cRMs) suggested that cRMs are less susceptible to disease progression and thus not as good a model for pathogenesis and vaccine studies.² Nonetheless, we and others have developed the cRM model of SIV infection for pathogenesis^{3,4} and antiretroviral therapy studies. In some ways, the cRM model more closely mimics HIV-1 infection in humans than iRMs, with a relatively low viral set point in chronic SIV infection and slower rate of disease progression. In fact, due to this slower clinical course, and to determine whether serial passage may increase viral virulence in cRMs, efforts were made to increase virus pathogenicity by serial passage of the virus in cRMs to try and mimic the evolution of the SIV model in the iRM model.⁵ Nonetheless, it is clear that a larger proportion (~30%) of cRMs spontaneously control infection to low or even undetectable levels in plasma, making them less useful for treatment studies or studies that require sustained viremia for interpretation (therapeutic vaccines etc.), which somewhat hinders the use of this model. However, peak viral loads in SIV-infected cRMs parallel those of iRMs,^{4,6} suggesting there is little difference in early control of viremia.

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The discrepancy of viral load levels is found only during chronic infection between the two subspecies as ~30% begin to have lower viremia after 12 weeks of infection and some spontaneously control infection to undetectable levels in plasma.⁷ Thus, this rhesus subspecies may be especially useful for determining both early transmission events and the immunologic mechanisms of viral control post-SIV/SHIV infection.

Despite showing identical peak plasma viremia after SIV infection, it is unclear, however, whether there are actual differences in the susceptibility of SHIV infection in cRMs and iRMs. To address this, we compared the vaginal transmission rates of 40 female naïve cRMs with 21 iRMs vaginally inoculated with a single high dose of SHIVsf162P3 to determine whether there were inherent differences in the rates of infection of these subspecies to identical vaginal challenges.

All experimental procedures described in this study were approved by the Tulane Institutional Animal Care and Use Committee. All animals were housed indoors at the Tulane National Primate Research Center in accordance with standard husbandry practices following the Guide for the Care and Use of Laboratory Animals (NIH). For physical examinations and blood sampling, 10 mg/kg ketamine was used and sodium pentobarbital was used for euthanasia.

All macaques were used as placebo controls on vaginal microbicide prevention studies and were intramuscularly treated with Depo-Provera 28–35 days before challenge. All macaques were vaginally sham inoculated with either 3 mL sterile phosphate buffered saline or hydroxyl methyl cellulose gel 30 min before vaginal challenge with 300 TCID₅₀ of SHIVsf162P3. The virus was obtained from the NIH AIDS Reagent Program, NIAID, NIH: SHIVsf162P3 virus was obtained from Drs. Janet Harouse, Cecilia Cheng-Mayer, and Ranajit Pal and the DAIDS, NIAID.⁸ Infection was assessed by weekly monitoring of viremia for the first 28 days and through 77 days thereafter. Fisher's exact test was used to test frequencies of detectable plasma viral loads (pVL) at day 7 postinfection (p.i.) and resistance of first SHIV challenge between cRMs and iRMs. The Mann–Whitney test was used

to compare median pVL between cRMs and iRMs at each time point postinfection.

pVL were quantified longitudinally post-SHIV infection. Interestingly, 20% (8/40) of cRMs, but 4.8% (1/21) of iRMs, had detectable pVL as early as 7 days p.i. (Fig. 1A) ($p = .1455$), with comparable median levels at this time point (Fig. 1B) ($p = .9907$). Furthermore, only 10% (4/40) of cRMs failed to become infected for the first-attempt inoculation, whereas almost twice as many iRMs at 19% (4/21) resisted the first challenge, although the difference was not significant ($p = .4291$). In all studies, control animals that did not get infected in this single challenge were recycled as controls in later experiments, and all cRMs and iRMs that did not get infected the first time became infected as controls on the second study, indicating that none were inherently resistant to challenge. Both groups reached peak viremia by day 21 p.i. with comparable peak levels, indicating that there were no significant differences in early viral replication once transmission occurred ($p = .6978$). Overall, the pVL were comparable from day 7 p.i. through day 49 p.i., but then pVL decreased in some cRMs to undetectable levels, whereas iRMs maintained at least 10^3 copies/ml for ~2 weeks longer before being reduced to undetectable levels ($p = .1806$). A few iRMs had sustained detectable levels of virus through 77 days (Fig. 1A) ($p = .4826$). These observations were consistent with previous studies in iRMs, demonstrating that SHIVsf162P3 infection often becomes undetectable in plasma by 70–90 days.⁹ In summary, these data clearly show that cRMs are at least as susceptible to a single vaginal SIV challenge as iRMs, if not more so. Postinfection control of viremia may be slightly greater in cRMs, but there does not appear to be any inherent resistance to vaginal infection of this subspecies.

To mimic HIV infection, various routes of viral inoculation in experimental NHPs have been used, such as oral, intravenous, intravaginal, intrarectal, and penile transmission. This study indicates that these types of experiments can reliably be performed in the cRM model as the rate of transmission is identical if not slightly higher than that of iRMs. Thus, cRM

FIG. 1. Comparison of SHIV plasma viral loads (pVL) between Indian rhesus macaques (iRMs) and Chinese rhesus macaques (cRMs). The Mann–Whitney test was used to compare median pVL between iRMs and cRMs at each time point post-SHIV infection. Two-tailed tests with $p < .05$ were considered as significant; however, no significant differences were found between iRMs and cRMs at each time point ($p > .05$). (A) Individual SHIV loads of iRMs and cRMs. (B) Median SHIV loads of iRMs and cRMs at each time point post-SHIV infection (median $\pm$ error (interquartile range)). Dashed line shows the limit of detection (30 copies/ml). SHIV, simian–human immunodeficiency virus.

animals are equally suitable for testing prevention methods, including microbicides, pre-exposure prophylaxis (PrEP), or even vaccines, assuming that protection from infection is the endpoint. They may also be useful in studying early events in the pathogenesis of infection, including investigations into the bottleneck of viral variants that enter mucosal tissues and early viral diversity in transmitted/founder viruses.¹⁰ These studies demonstrate that cRMs and iRMs have identical susceptibilities to vaginal SHIV transmission and thus cRMs are highly useful for addressing many fundamental questions in HIV transmission, the earliest events in viral pathogenesis, and for various HIV prevention strategies, including vaccines, microbicides, and PrEP.

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Author Disclosure Statement

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