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Efficient mucosal transmissibility but limited pathogenicity of R5 $SHIV_{SF162P3N}$ in Chinese origin rhesus macaques

Alexandra Mumbauer, BS, **Agegenhu Gettie, BS**, **James Blanchard, DVM, PhD**[#], and **Cecilia Cheng-Mayer, PhD**

Aaron Diamond AIDS Research Center, New York, NY10065

[#]Tulane National Primate Research Center, Tulane University Medical Center, Covington, LA 70433

Abstract

Background—Infection of rhesus macaques (RMs) of Indian origin with SIV or SHIV provided powerful tools to study HIV-1 transmission and disease, and for testing the efficacy of novel drugs, vaccines and prevention strategies. In developing alternative nonhuman primate AIDS models for the CCR5 (R5)-tropic $SHIV_{SF162P3N}$, we characterized virus transmission and infection in Chinese origin RMs.

Methods—Virologic, immunologic and pathogenic evaluations of R5 $SHIV_{SF162P3N}$ infection in Chinese RMs challenged intrarectally (ir) or intravaginally (ivg) were performed and compared to those previously observed in Indian origin rhesus exposed to the same inoculum dose and *via* similar route.

Results—R5 $SHIV_{SF162P3N}$ transmits efficiently across mucosal surfaces in Chinese RMs. The magnitude and kinetics of early virus dissemination following intrarectal inoculation in the Chinese macaques were similar to those observed in Indian rhesus, but a trend towards increased $SHIV_{SF162P3N}$ vaginal infectivity and rapid virus spread was seen in the Chinese macaques compared to the Indian origin animals. Once infected, however, set-point viremia in the ir- and ivg-infected Chinese rhesus was significantly lower and the animals survived longer compared with infected Indian rhesus.

Conclusions—The R5 $SHIV_{SF162P3N}$/Chinese rhesus macaque infection model is suitable for studies of mucosal HIV-1 transmission and protection, but the high frequency of spontaneous control of chronic viremia and reduced virulence with $SHIV_{SF162P3N}$ in this macaque subspecies may limit its utility in studying HIV-1 pathogenesis and in evaluating vaccines and antiretrovirals that rely on reduction in chronic viral load or AIDS development as an experimental endpoint.

Keywords

SHIV; rhesus macaque subspecies; mucosal transmission; AIDS

Correspondence to: Cecilia Cheng-Mayer, PhD, Aaron Diamond AIDS Research Center, 455 First Avenue, 7th Floor, New York, NY 10065. Phone:212-448-5080. Fax:212-448-5028. cmayer@adarc.org.

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Introduction

Studies in nonhuman primates are recognized as playing a critical role in advancing our understanding of HIV-1 transmission, pathogenesis, as well as basic vaccine, prevention and treatment concepts[1–4]. Experimental infection of Asian macaques with simian or simian-human immunodeficiency viruses (SIV and SHIV, respectively) have provided important information on the early host events and kinetics of virus transmission and replication, the dynamics of CD4+ T cell homeostasis during virus infection, the mechanisms of disease induction and host immune responses[5–9]. In particular, we have used infection of Indian rhesus macaques (*Macaca mulatta*) with pathogenic CXCR4 (X4) and CCR5 (R5) tropic SHIVs to study the impact of tropism on AIDS pathogenesis[10–14], and have evaluated the ability of topical microbicides used alone or in combination with vaccines to prevent virus transmission using this model[15,16]. Because the majority of HIV-1 transmitted/founder viruses are CCR5-tropic, with neutralization susceptibility profiles that are typical of primary viruses[17,18], we focused on developing an R5 SHIV infection model that recapitulates key features of HIV-1 infection in humans. We showed that infection of Indian origin RMs via the intravenous (iv), intrarectal (ir) or intravaginal (ivg) route with R5 $SHIV_{SF162P3N}$ resulted in acute CD4+ T cell depletion in the gut, uncontrolled replication and progression to AIDS, with switch in coreceptor preference towards CXCR4 in ~50% of iv- and ir-infected animals[19–21]. This model therefore mimics the type of transmission route in the majority of HIV-1 infected patients, allowing studies of viral selection through mucosal transmission, and late stage coreceptor switch that is also seen in chronically infected HIV individuals not on treatment. Furthermore, development of giant cell encephalitis (SIVE) was observed in ~30% of monkeys with symptoms of AIDS, with neuropathology mirroring that of HIV-1 associated encephalitis (HIVE) in infected patients (unpublished observations), providing an important model to study neuropathogenesis.

Several features of infection in Indian RMs however differ from that of HIV-1 infected individuals. These include a faster rate of progression to AIDS[22], high plasma virus levels and a rapid and sustained loss of circulating and mucosal CD4+ CCR5+ memory T cells that is not seen in HIV-1 infected patients[23–25]. This raises the concern that the Indian RM infection models may not fully reproduce the immunopathogenic events occurring during HIV-1 infection. Accordingly, and because Indian RMs were often in short supply, alternative macaque species such as cynomolgous, pig-tailed or rhesus macaques from different geographic origin were used as models of HIV infection and AIDS[26]. Several groups have reported that the slower course of infection and reduced risk of progression to AIDS in SIV/SHIV-infected Chinese origin RMs are closer to HIV-1 infections in untreated adult humans than infection of Indian RMs[27–31]. Moreover, similar to HIV-1 infected patients, the levels of immune activation in SIV-infected Chinese RMs are markers of disease progression[32,33]. These findings led to the suggestion that SIV/SHIV infection of Chinese origin rhesus is a more relevant model of AIDS outcomes than Indian RMs[28,34].

In the present study, we evaluated mucosal transmissibility and pathogenesis of R5 $SHIV_{SF162P3N}$ in Chinese RMs and compared infection outcomes with those observed in Indian RMs. We found that the transmission efficiency, magnitude and kinetics of early virus dissemination were similar in the two macaque subspecies following intrarectal inoculation, but transmission and early virus spread with intravaginal challenge appeared to be more efficient in the Chinese than Indian RMs. Consistent with findings for SIV, mucosal infection of Chinese RMs with R5 $SHIV_{SF162P3N}$ resulted in attenuated pathogenicity when compared to infection in Indian RMs, as evidenced by lower levels of set-point viremia and prolonged survival. Understanding these subspecies differences in response to R5 $SHIV_{SF162P3N}$ should guide the use of this virus in NHP studies of HIV-1 transmission and prevention, resistance to infection and progression to pathology.

Methods

Animal inoculation and clinical assessments

All intrarectal (ir) or intravaginal (ivg) inoculations were carried out in adult rhesus monkeys (*Macaca mulatta*) of Chinese origin housed at the Tulane National Primate Research Center (TNRPC) in compliance with the *Guide for the Care and use of Laboratory Animals*. Chinese origin rhesus were 5 – 10 years old and were confirmed to be serologically and virus negative for simian type D retrovirus, and serologically negative for SIV and simian T-cell lymphotropic virus prior to infection. The males were born and raised at TNRPC whereas the females were purchased from an outside vendor, the latter were used without Depo-provera treatment and randomized with regard to the stage of the menstrual cycle at the time of ivg challenge. Macaques received a single 10^4 50% tissue culture infectious dose ($TCID_{50}$) of the cell free challenge stock $SHIV_{SF162P3N}$[10]. Whole blood from the inoculated animals was collected weekly for the first eight to eleven weeks, biweekly for another 16 weeks, and monthly thereafter. Surgery was performed during acute [2–3 weeks post-infection (wpi)] and chronic (12–18 wpi) phase of infection for tissue collection. These include the colonic, mesenteric, iliac and/or inguinal lymph nodes for lymphoid cell isolation and immunohistological examination, as well as ~20cm of the jejunum for processing of laminar propria lymphocytes (LPL), with ileum and colon wedge biopsies for immunohistological analysis. Animals were euthanized at end of study period (~50 weeks for ivg and >60 weeks for ir) by intramuscular administration of telazol and buprenorphine followed by an overdose of sodium pentobarbital. Euthanasia was considered to be AIDS related if the animal exhibited peripheral blood CD4+ T-cell depletion (<200/mm^3), greater than 25% loss of body weight, or combinations of the following conditions: diarrhea unresponsive to treatment, opportunistic infections, peripheral lymph node atrophy, and abnormal hematology. Plasma viremia was quantified by branched DNA analysis (Siemens Medical Solutions Diagnostic Clinical Lab, Emeryville, CA) and absolute CD4+ and CD8+ cell counts were monitored in TruCount tubes (BD Biosciences, Palo Alto, CA). The percentages of CD4+ T cells in the tissue cells were analyzed by flow cytometry (FACScalibur) using CD3-fluorescein isothiocyanate (FITC), CD4-phycoerythrin (PE) and CD8-peridinin chlorophyll protein (PerCP) antibodies. Except for CD3-FITC (BioSource, Camarillo, CA), all antibodies were obtained from BD Biosciences. Comparative data from Indian-origin rhesus monkeys were obtained from other pathogenesis studies in which the animals received the same virus inoculum dose[21]. The cohort of Indian female rhesus monkeys used was similarly randomized with respect to the menstrual cycle.

Statistical Analyses

Plasma virus loads were transformed to $\log_{10}$ copies/ml before all analysis. Peak viral load was the highest recorded value (2–4wpi), and baseline CD4+ T cell count was measured at pre-infection. Plateau (set-point) viremia and CD4+ T cell counts were calculated as the median of all values between days 56 and 168 post-infection, with differences examined using Mann-Whitney *U*-tests. Changes in the percentage of tissue CD4+ T lymphocytes over time were also determined using Mann-Whitney *U*-tests. Disease-free survival curves for the intrarectal and intravaginal infected macaques were estimated using the Kaplan-Meier method, and statistical significance of the differences in the survival curves was determined by log-rank test. A *P* value less than 0.05 was considered statistically significant.

Results

Efficient mucosal transmission of R5 $SHIV_{SF162P3N}$ in Chinese RMs

To determine mucosal transmissibility and pathogenicity of R5 $SHIV_{SF162P3N}$ in rhesus macaques of Chinese origin, we inoculated six males intrarectally and six females

intravaginally with 10,000 $TCID_{50}$ virus. All animals in the ir- and ivg-inoculated groups became infected, with seroconversion at 4–5 weeks post-infection (wpi). Peak viremia of 6–8 log_{10} RNA copies/ml plasma was detected in both ir- and ivg-infected macaques (Figures 1A and B), with no significant difference in magnitude between the two groups (Figure 1C). The kinetics of virus spread was also comparable among the two inoculation groups, with plasma viremia detected at 1 wpi in six of six ir- and four of six ivg-infected RMs, reaching peak one week later in all except GL26, an ir-inoculated animal that peaked at 3 wpi (Figure 1D). Viral load subsequently declined, with greater control in the ivg- than the ir-infected macaques. Virus replication reached undetectable levels (< 165 RNA copies/ml plasma) in all six ivg-infected Chinese RMs between 8–16wpi, with partial rebound to <4 log_{10} RNA copies/ml plasma in three of the six animals. In comparison, only one ir-infected Chinese RM (FV03) suppressed plasma viremia below the level of detection. Accordingly, viral set-point was significantly higher in the ir- than the ivg-infected Chinese origin RMs (Figure 1C, p=0.015). The difference in viral control between the two inoculation groups suggests a route-dependent effect on $SHIV_{SF162P3N}$ replication in Chinese RMs, and is consistent with our previous findings of a route-dependency in infection outcome of Indian origin rhesus monkeys with this virus[21].

The infected animals all experienced transient peripheral CD4+ T cell loss with the onset of viremia. The absolute number of this T-cell subset decreased by approximately 15% during the first two weeks of infection in the ir-infected animals (from a median baseline value of 1037 at d0 to 877 CD4+cells/μl blood) and by approximately 30% in the ivg-infected monkeys (from a median baseline value of 1409 at d0 to 998 CD4+cells/μl blood) (Figures 1A and B). Peripheral CD4+ T cell count stabilized or fluctuated thereafter in six of the six ivg- and three of the six ir-infected animals. The exceptions were the ir-infected rhesus GL26, GB30 and GP22 where a gradual decline in CD4+ T lymphocytes was seen despite a viral load that is $<10^4$ RNA copies/ml plasma in the latter two animals. We concluded therefore that R5 $SHIV_{SF162P3N}$ transmits efficiently across mucosal surfaces in Chinese RMs, but infection is frequently controlled. Because the level of set-point viremia is a strong predictor for disease progression in HIV-1 infected humans and SIV-infected Indian RMs[35,36], the two ir-infected Chinese macaques with sustained viremia > 10^4 RNA copies/ml plasma for over 40 weeks of infection (GL26, GB40) were followed for AIDS development.

Disease progression in ir-infected Chinese RM

GB40 was euthanized at 97 wpi for AIDS-unrelated causes. Viral load in this animal at the time of euthanasia was ~3 log_{10} copies/ml plasma, with a peripheral CD4+ T cell count of 578 cells/ul blood. In contrast, GL26 developed clinical symptoms consistent with AIDS, including chronic diarrhea and weight loss, and was euthanized at 99 wpi with a CD4+ T cell count of 226 cells/ul blood. Histological examination revealed secondary and mycobacterial (*M. Avium*) infection. Peak viremia in this macaque reached 6–7 log_{10} RNA copies/ml plasma but dropped ~2 log thereafter, reaching a plateau of 4–5 log_{10} RNA copies/ml (Figure 1E). A rise in viremia to near peak plasma viral load level however was seen toward end-stage disease. Examination of mucosal and lymph node CD4+ T cells during the course of infection showed severe acute depletion of gut CD4+ T cells (90%; 2wpi), with minimal loss in the lymph node compartments. Gut CD4+ T cell loss was sustained during chronic infection (17wpi) and at the time of necropsy (99wpi), with ~50% preservation of this lymphocyte subset in the lymph node compartments at end-stage disease. R5 $SHIV_{SF162P3N}$, therefore, can induce AIDS in Chinese origin rhesus in a manner similar to HIV-1 infection in humans.

R5 $SHIV_{SF162P3N}$ infection is attenuated in mucosally infected Chinese RMs in comparison to Indian RMs

SIV infection in Chinese RMs had been reported to be more attenuated compared to RMs of Indian origin[27–31]. Since an objective of this study is to assess the utility of R5 $SHIV_{SF162P3N}$ infection of Chinese origin RMs in studies of HIV-1 transmission and pathogenesis, we compared the virologic and immunologic parameters in ir- and ivg-infected Chinese RMs to those observed previously in Indian RMs[21]. We found that the peak viremia was of similar magnitude in the Chinese (n=6) and Indian (n=11) ir-infected RMs (Figures 2A and B, $p>0.05$). However, while a range in set-point viremia was seen in both subspecies hosts, the median steady-state viremia was significantly lower in the Chinese rhesus than in the Indian origin monkeys (Figure 2B, $p=.0075$). This result is consistent with findings with SIV that the differences between the subspecies appear in the chronic phase.

There was no significant difference in baseline peripheral CD4+ T cell counts between the Chinese and Indian origin ir-infected animals ($p>0.05$), but the Indian origin ir-infected RMs displayed greater loss of peripheral CD4+ T cells during acute infection than the Chinese origin ir-inoculated monkeys (Figure 2A). The Indian ir-infected animals suffered a 33% drop in median peripheral CD4+ T cell count (from 635 to 427 CD4+cells/μl blood at 2 wpi), while the ir-infected Chinese rhesus only lost 15% of this lymphocyte subset (from 1037 to 877 CD4+cells/μl blood) at the corresponding time post-infection. The peripheral CD4+ T cell loss of the two subspecies remained divergent during the chronic phase of infection, consistent with the differences in viral load. CD4+ T lymphocyte count declined steadily in the ir-infected Indian but varied widely among the ir-infected Chinese RMs. Severe depletion of CD4+ T lymphocytes in the lamina propria (LPL) of the gut occurred in both Indian and Chinese origin ir-infected RMs (Figure 2B). However, the dynamics of this loss varied between the two macaque subspecies. CD4+ T cells constituted less than 10% of total gut CD3+ T lymphocytes in ir-infected Chinese RMs as compared to 20% in the ir-infected Indian RMs at 2 wpi, despite similar peak viremia. Accordingly, the depletion of CD4+ T lymphocytes in the LPL was highly significant during peak viremia ($p=0.0009$) in the Chinese and only approached significance in the Indian RMs ($p=0.062$). Further analysis of the percentage of CD4+ T lymphocytes in the LPL during the chronic phase of infection (12 –18wpi) revealed continued diminution of CD4+ T cells in the Indian RMs, resulting in a loss that is now significant. The percentage of CD4+ T lymphocytes in the LPL of the Chinese ir-infected RMs rose during the chronic phase, suggestive of gut CD4+ T cell reconstitution[37], but is still significantly lower than the uninfected controls.

As in the ir-inoculated RMs, peak viremia was of similar magnitude in the Chinese (n=6) and Indian (n=8) origin RMs exposed intravaginally to the same inoculum dose (Figure 3A, $p>0.05$). But while a greater range in set-point plasma viral load was found among the Indian origin ivg-infected animals, the median set-point viremia in this subspecies host was significantly higher compared to animals of Chinese origin (Figure 3B, $p=0.02$). The subspecies difference in $SHIV_{SF162P3N}$ chronic phase viremia therefore is route independent. With the onset of viremia, peripheral CD4+ T counts declined substantially in both Indian and Chinese origin ivg-inoculated RMs (Figure 3A). The median peripheral CD4+ T cell count dropped by 30% in the Chinese ivg-infected RMs (from 1409 to 998 CD4+cells/μl blood) and by 47% in the ivg-infected Indian rhesus monkeys (from 1097 to 576 CD4+cells/μl blood) during peak viremia. However, five of the six ivg-infected Chinese RMs were able to recover their blood CD4+ T cell loss post-peak, while only four of eight ivg-inoculated Indian RMs showed a rebound in peripheral CD4+ T cell levels. Unlike the ir-infected RMs, both macaque subspecies infected by the intravaginal route experienced only moderate gut CD4+ T cell loss during acute infection (30–40%; Figure 3B), and only

during the chronic phase (12 –18wpi) did the percentages of CD4+ T cells plummet, with more severe depletion in the Indian (>90%) than the Chinese origin RMs (>75%).

R5 $SHIV_{SF162P3N}$ is minimally pathogenic in Chinese rhesus monkeys

Ten of the eleven ir-infected Indian origin RMs (91%) progressed to disease over a 1–1.5 year infection period as compared to one of six ir-infected Chinese RMs (16.7 %), with a RP phenotype in four of the eleven ir-infected Indian RMs (36.4%) and none of the ir-infected macaques of Chinese origin (Table 1). Kaplan-Meier analysis of disease progression showed statistically significant difference in the rate of disease progression between the two macaque subspecies (Figure 4A, p= 0.0009; log-rank test). The percentage of animals AIDS-free at 60wpi was 27.3% for the ir-infected Indian origin macaques and 100% for the ir-infected macaques of Chinese origin. In comparison, there was no statistical significant difference in the rate of disease progression between the ivg-infected Chinese and Indian RMs (p>0.05), with 75% and 100% of animals AIDS-free at 40wpi in the ivg-infected Indian and Chinese origin rhesus respectively. However, whereas all six of the Chinese origin RMs were infected following a single high-dose ivg exposure, only 8 of the 12 Indian RMs challenged intravaginally with the same inoculum dose established systemic infection (Table 1). Furthermore, the observation that the kinetics of early virus spread in the ivg-and ir- infected Chinese RMs was similar (Figure 1D) contrasts with our findings with this virus in the Indian RMs[21], prompting us to compare early $SHIV_{SF162P3N}$ dissemination in the two subspecies hosts. We found that the time to peak 1viremia overlapped in the ir-infected Indian and Chinese RMs, but varied by geographical origin for the ivg-infected animals (Figure 4B). Peak virema was w2 in all six ivg-infected Chinese rhesus, but it took 3–4 weeks to reach peak viremia in 2 of the 8 ivg-infected Indian origin macaques, with one showing no evidence of systemic infection until 3 wpi. These results of ivg inoculation suggest that the Chinese origin monkeys may be more susceptible to $SHIV_{SF162P3N}$ vaginal infection than Indian origin animals.

Discussion

In developing AIDS models of HIV-1 infection using different nonhuman primate species or rhesus macaques of different geographic origin with SIVs or SHIVs, it is important to understand the characteristics of infection with each virus in the various animal hosts to guide the rational design and optimal use of the models. In this study, we established that R5 $SHIV_{SF162P3N}$ transmits efficiently across the rectal and vaginal/cervical mucosa of Chinese origin rhesus macaques, modeling the type of transmission route in the majority of HIV-1 patients. This model therefore allows for studies of viral selection though mucosal transmission as well as evaluation of the effectiveness of vaccines and pharmacological agents to block virus acquisition. However, a large number of the $SHIV_{SF162P3N}$ ir- and ivg-infected Chinese origin animals controlled their infection, with undetectable plasma viremia found more frequently in the ivg- than the ir-infected animals, and AIDS development in only one of six ir-infected Chinese rhesus after 99 weeks of infection. The variability in chronic viremia and limited pathogenicity of $SHIV_{SF162P3N}$ in Chinese origin rhesus may restrict the utility of this model in understanding the mechanisms of HIV-1 disease development, in dissecting cellular and humoral immune responses over the course of infection from the time of inoculation to the development of fatal immunodeficiency, and in evaluating vaccines and antiretrovirals that aim at reducing viral loads post-infection and delaying AIDS progression.

Consistent with reports with SIV[27–31], peak viremia was similar but viral load post-infection was lower and survival was longer in the $SHIV_{SF162P3N}$-infected Chinese RMs compared to Indian origin macaques. The lack of a rapid progressor phenotype and sustained peripheral CD4+ T cell levels in the infected Chinese origin monkeys provided further evidence that

the clinical course of $SHIV_{SF162P3N}$ infection differs in the two macaque subspecies. Several hypotheses have been proposed to explain the divergent outcome of SIV/SHIV chronic infection in rhesus macaques of Chinese and Indian origin. It has been suggested that because the inoculating virus was passaged and recovered from Indian RMs, it is better adapted and replicate more successfully in the Indian RM immune environment than in the "foreign" Chinese RM immune milieu[28,30]. $SHIV_{SF162P3N}$ was also passaged and recovered from Indian RMs, providing a plausible explanation for the differences in pathogenesis we observed between the two macaque subpopulations. Genetic differences including factors governing CCR5 expression, cellular molecules that restrict viral replication and adaptive immunity could also affect the biological consequences of viral infections in the two monkey subspecies[34,38,39,40]. The animals used in this study were genotyped for TRIM5α, with results showing that genetic polymorphism at this locus cannot explain the difference in R5 $SHIV_{SF162P3N}$ infection of Indian and Chinese RMs. 33.3% of the male (2 of 6) as well as female (2 of 6) Chinese origin macaques used in the current study expressed the restrictive homozygous TFP/TFP allele compared to 72.2% of the male (8 of 11) and 37.5% of the female (3 of 8) Indian rhesus monkeys (data not shown). CD8 T cell mediated immunity has been suggested to play a role in the spontaneous control of chronic viremia in Chinese origin rhesus[37]. Very little is known about the degree to which class I alleles in the Chinese rhesus population confer protection for SIV/SHIV challenges. Recent advances in identifying major histocompatibility complex (MHC) alleles for Chinese-origin macaques[41–45] will be important for studying cellular immunology in this monkey subpopulation and its impact on SIV/SHIV replication.

All six Chinese origin macaques not treated with progesterone were infected with one high dose $SHIV_{SF162P3N}$ intravaginal challenge, a rate of infection that is similar to that achieved following ir inoculation (Figures 1A and B). This finding contrasts early studies in Indian RMs using nonphysiological high doses of SIVs and SHIVs, including $SHIV_{SF162P3N}$, showing that the infection rate was lower after inoculation by the ivg than by ir route[21,46–50]. Moreover, the kinetics of early virus spread was similar in Chinese RMs infected by the ir and ivg routes (Figure 1D), differing from observations of slower kinetics of virus dissemination and greater variability in RNA levels in Indian origin monkeys infected by the ivg route than those infected by the ir route[48,51,52]. Baseline peripheral CD4+ T cell counts were significantly higher in the ivg-inoculated Chinese RMs than the Indian RMs ($p=0.0080$). If this observation in peripheral blood translates into the genital mucosa, the greater number of CD4+ T cells and targets could provide a possible explanation for the successful ivg transmission and rapid spread of virus in the Chinese origin animals. Alternatively, and because the differences in the rate of infection and kinetics of early dissemination in the two macaque subspecies were only seen with ivg and not ir inoculation, differential host response to exposure at the genital mucosa with an Indian RM-adapted virus may have favored vaginal transmissibility and the initial wave of viral replication in the Chinese RMs. Studies in a larger number of animals will be required to confirm and understand the differences in $SHIV_{SF162P3N}$ vaginal transmission in Chinese and Indian RMs.

In summary, our findings with R5 $SHIV_{SF162P3N}$ in Chinese and Indian origin rhesus macaques further illustrate that the subspecies differences in pathogenicity after SIV/SHIV infection is independent of the route of inoculation and the virus strain used. The ease of mucosal transmission with $SHIV_{SF162P3N}$ in Chinese RMs suggests that this model will be suitable for prevention, early host events and kinetics of virus replication studies. In particular, it will be of interest to determine if transmission of different viral variants and/or local host responses influenced the observed subspecies differences in $SHIV_{SF162P3N}$ genital mucosal infection. However, the finding that a significant fraction of the $SHIV_{SF162P3N}$-infected Chinese origin animals, especially those infected intravaginally controlled virus

replication to levels that are intermittent or below conventional detection highlights the limitation of this model for vaccine and therapeutic trials aimed at reducing set-point viremia and HIV-1 disease progression.

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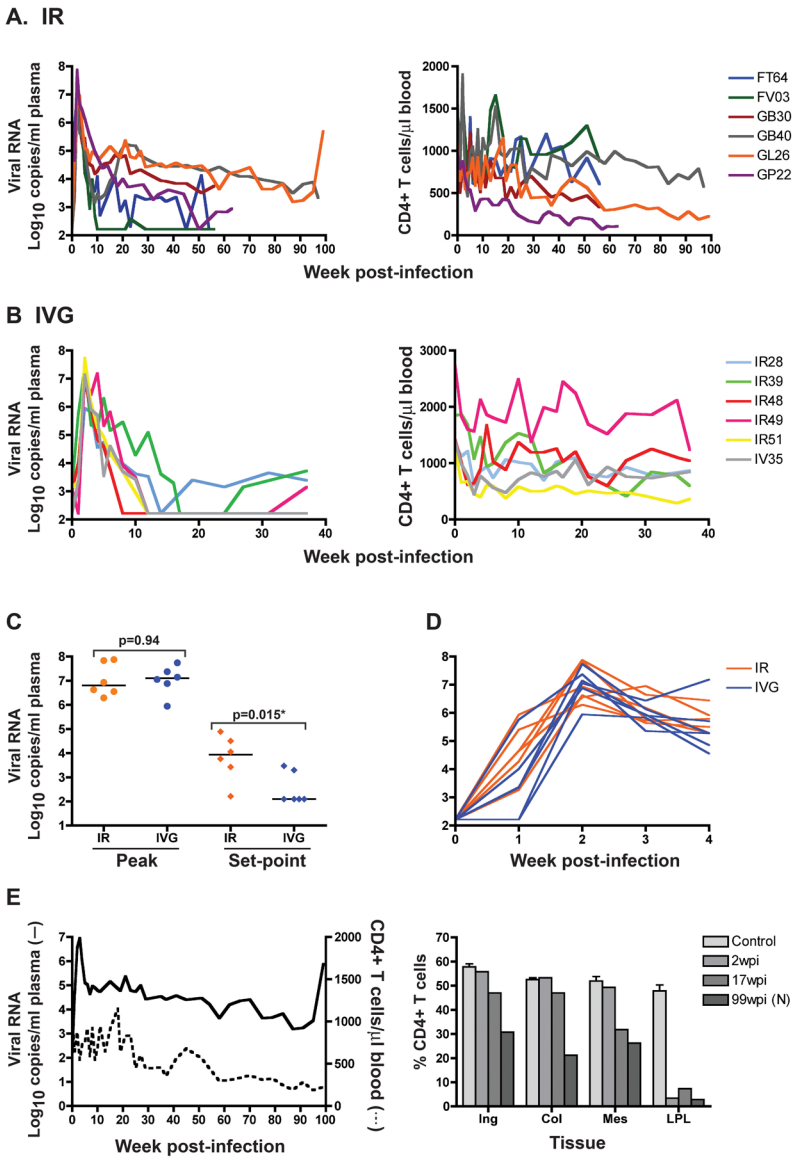

Figure 1.

Viral load and peripheral CD4+ T cell count in ir- (**A**, n=6) and ivg- (**B**, n=6) inoculated Chinese RMs. Comparison of peak and set-point viral load **(C),** and kinetics of virus dissemination within the first four weeks of infection **(D)** in the ir- and ivg-infected Chinese RMs. **(E)** Plasma viremia, peripheral CD4+ T count during the course of infection, and tissue CD4 T cells at time of necropsy in macaque GL26. Horizontal bars in (C) indicate median values, and baseline tissue CD4+ T cell count shown for reference in (E) were generated from 3–4 macaques (control). N, necropsy.

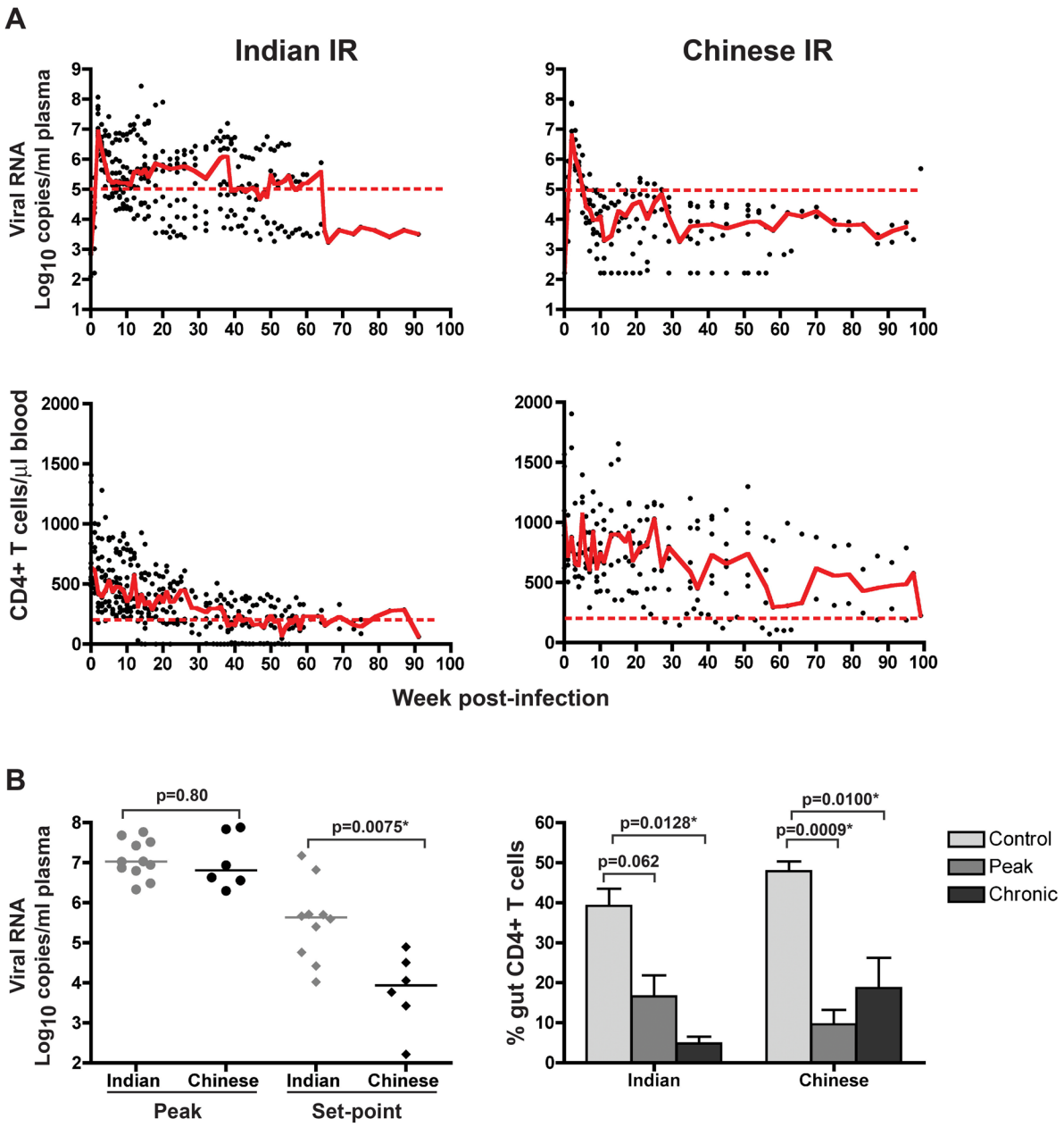

Figure 2.

Comparison of the changes in viral load (VL) and CD4+ T cell levels over time **(A),** and peak, set-point and gut CD4+ T cell depletion **(B)** in Indian and Chinese RMs infected intrarectally with R5 $SHIV_{SF162P3N}$. Trend lines in **(A)** were created using the median VL or CD4 count values at each time point, and the dashed lines marked a set-point of 5 log_{10} RNA copies/ml plasma or a CD4+ T cell count of 200 cells/ul blood. The horizontal bars in (B) represent median values, and the control gut CD4+ T cell counts were generated from 3 and 12 uninfected Indian and Chinese origin rhesus respectively, with error bars indicating mean and standard deviations.

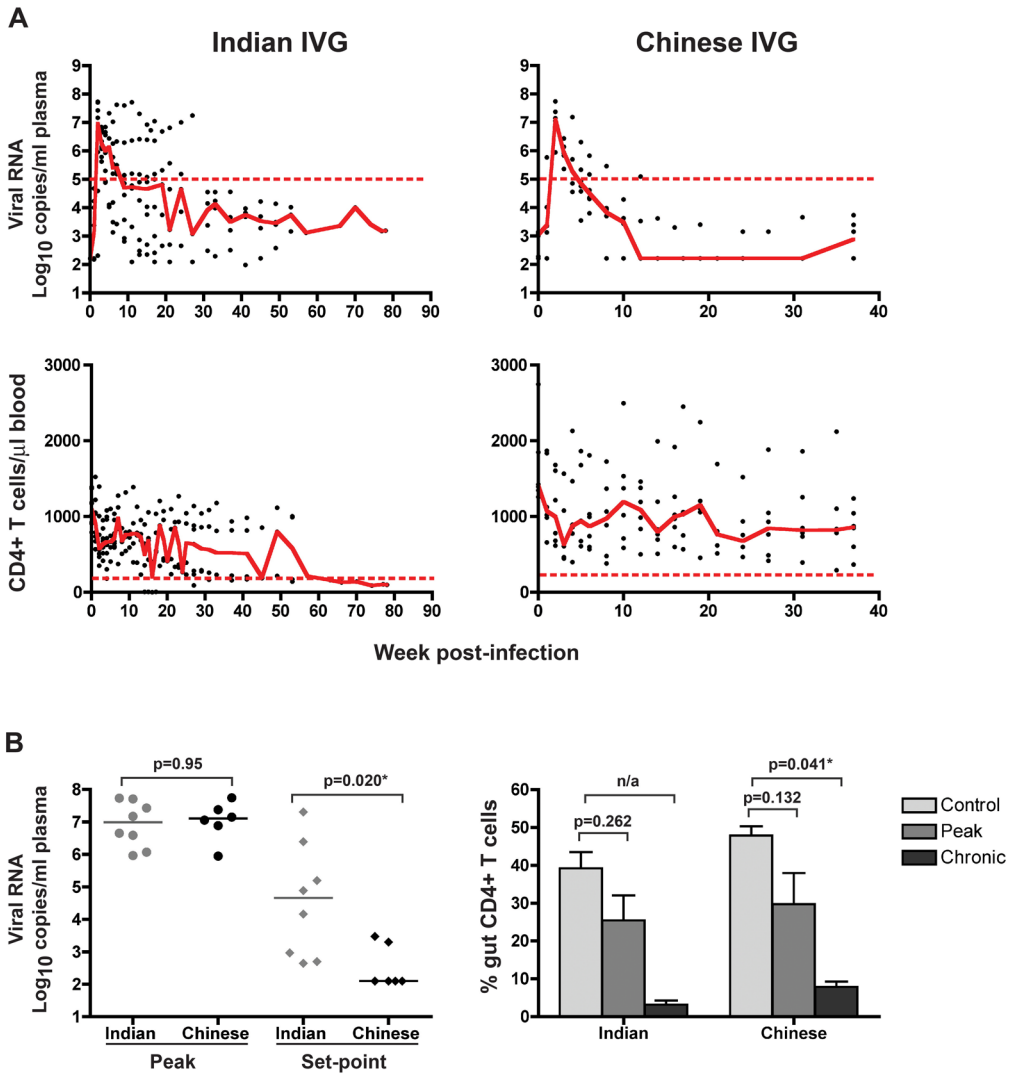

Figure 3.

Comparison of the changes in viral load (VL) and CD4+ T cell levels over time **(A),** and peak, set-point and gut CD4+ T cell depletion **(B)** in Indian and Chinese RMs infected intravaginally with R5 $SHIV_{SF162P3N}$. Trend lines in **(A)** were created using the median VL or CD4 count values at each time point, and the dashed lines marked a set-point of 5 log_{10} RNA copies/ml plasma or a CD4+ T cell count of 200 cells/ul blood. The horizontal bars in (B) represent median values, and the control gut CD4+ T cell counts were generated from 3 and 12 uninfected Indian and Chinese origin rhesus respectively, with error bars indicating mean and standard deviations. n/a, statistics analysis for the ivg-infected Indian RMs at set-point viremia could not be determined because data from only two animals were available.

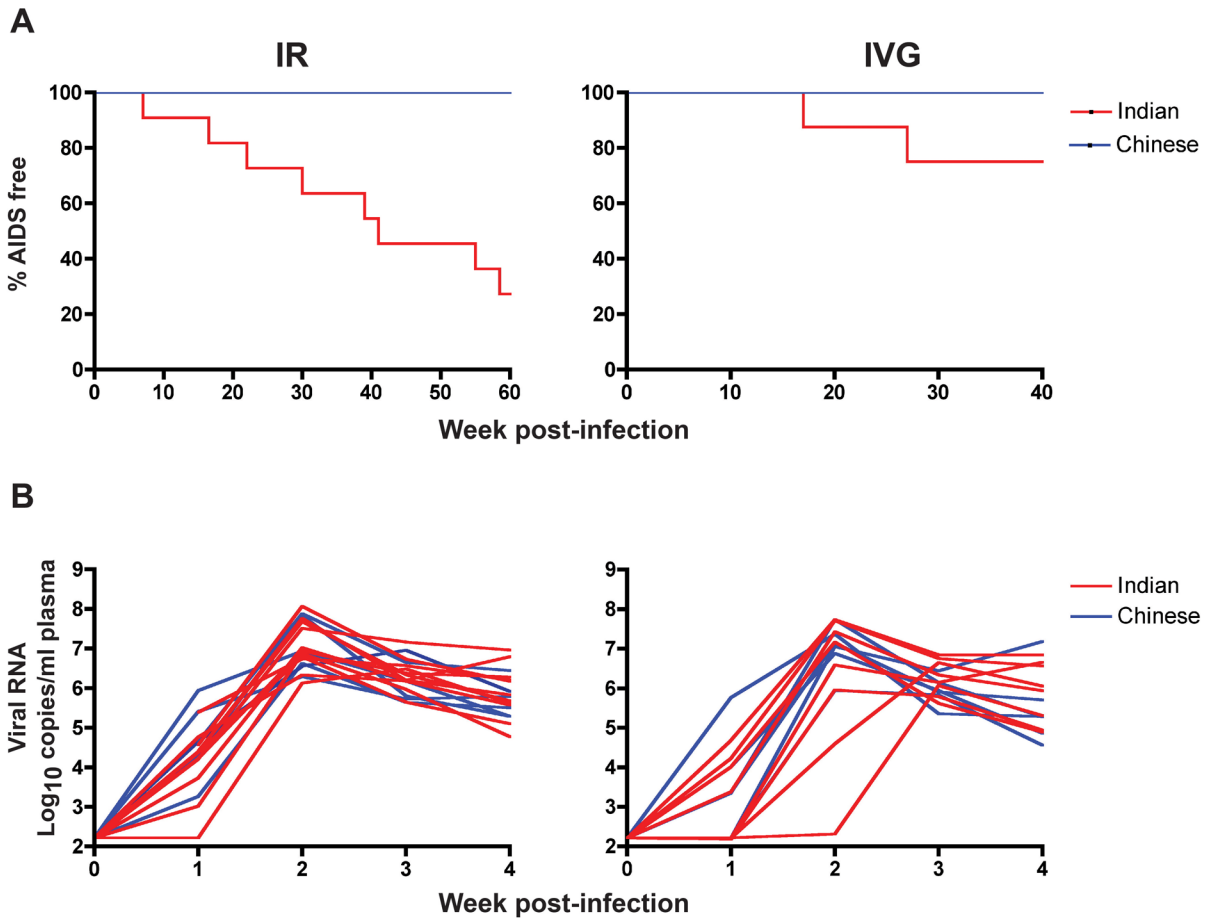

Figure 4.

(A) Kaplan-Meier disease-free survival curves for ir- and ivg-infected Chinese and Indian RMs. AIDS development within a 60 and 40 week infection period in the ir- and ivg-infected macaques respectively is shown. (**B**) Kinetics of early virus spread in the ir- and ivg-infected Chinese and Indian RMs. Plasma RNA levels within the first four weeks of infection in the ir-infected Indian (n=11) and Chinese (n=6), and ivg-infected Indian (n=8) and Chinese (n=6) rhesus macaques are shown.

Table 1

Infection outcome in Chinese and Indian rhesus macaques inoculated intrarectally (ir) or intravaginally (ivg) with R5 $SHIV_{SF162P3N}$.

	IR-inoculated		IVG-inoculated	
	Indian (n=11)	**Chinese (n=6)**	**Indian (n=12)**	**Chinese (n=6)**
% Infected	100	100	66.7	100
% infected with AIDS	91	16.7	25	0
% infected with RP phenotype	36.4	0	25	0