Published in final edited form as:

J Med Primatol. 2008 December ; 37(Suppl 2): 13–23. doi:10.1111/j.1600-0684.2008.00325.x.

Differential Pathogenicity of SHIV_{SF162 P4} Infection in Pig-tailed and Rhesus Macaques

Patricia Polacino¹, Kay Larsen¹, Lindsey Galmin², John Suschak², Zane Kraft³, Leonides Stamatatos^{3,4}, David Anderson¹, Susan W. Barnett⁵, Ranajit Pal², Kristen Bost⁶, A. H. Bandivdekar⁷, Christopher J. Miller⁶, and Shiu-Lok Hu^{1,8,*}

¹Washington National Primate Research Center, University of Washington, Seattle, WA 98121

²Advanced BioScience Laboratories, Inc., Kensington, MD 20895

³Seattle Biomedical Research Institute, Seattle, WA 98109

⁴Department of Global Health, University of Washington, Seattle, WA 98195

⁵Novartis Vaccines and Diagnostics, Emeryville, CA 94608

⁶California National Primate Research Center, University of California, Davis, CA 95616

⁷National Institute for Research in Reproductive Health, Mumbai, INDIA

⁸Department of Pharmaceutics, University of Washington, Seattle, WA 98195

Abstract

Background—Differential pathogenicity has been observed in cynomolgus and rhesus macaques following primate lentivirus infection. However, little is known about the comparative susceptibility of pig-tailed macaques to lentivirus infection and diseases.

Methods—We compared the *in vivo* infectivity and pathogenicity of a CCR5-tropic SHIV_{SF162 P4} after intravenous, intravaginal or intrarectal inoculation in rhesus and pig-tailed macaques. Plasma viral load, peripheral blood CD4⁺ T cell counts and clinical signs were monitored.

Results—Both rhesus and pig-tailed macaques are similarly susceptible to SHIV_{SF162 P4} infection by intravenous and mucosal routes. However, SHIV replication was significantly more robust in pig-tailed macaques than in rhesus, resulting in persistent viremia in 9/21 pig-tails vs. 2/24 rhesus ($p < 0.013$) and severe CD4⁺ T-cell depletion in 2/21 pig-tails (vs. none in rhesus).

Conclusions—Together with earlier observations, our findings underscore the importance of considering host genetic and immunological factors when comparing vaccine efficacy in different macaque species.

Keywords

Plasma viremia; AIDS vaccine; intravenous; intrarectal; intravaginal

Introduction

Due to their propensity to develop AIDS-like diseases resulting from primate lentivirus infection, Asian macaques have been widely used for HIV vaccines and pathogenesis studies [24,27,32,52]. Among macaque species, rhesus (*Macaca mulatta*), cynomolgus (*M.*

*Corresponding Author: Dr. Shiu-Lok Hu, 3000 Western Ave, Seattle, WA 98121, USA; Tel: 1-206-616-9764; Fax: 1-206-543-1589.

fascicularis) and pig-tailed (*M. nemestrina*) macaques are most commonly used. However, in part because of the considerable logistics required, only a limited number of studies have been undertaken to examine the relative infectivity and pathogenicity of primate lentiviruses among these macaque species. The paucity of comparative data, coupled with the variability of challenge stocks prepared from different laboratories, makes it difficult to interpret divergent findings from even similar vaccine approaches evaluated in different macaque models.

Substantial differences in susceptibility to primate lentivirus-induced diseases have been observed between different species, or subspecies, of macaques. ten Haaf et al. [55] compared early plasma viral load in cynomolgus and rhesus macaques infected with different strains of SIVmac or SHIV89.6P inoculated via intravenous or various mucosal routes. After SIV infection, cynomolgus macaques had lower setpoint viral load than rhesus macaques. However, no such difference was observed in animals infected with SHIV89.6P, or among animals inoculated via different routes. Rhesus macaques of Chinese origin infected intravenously with SIVmac239 [33], or intravaginally with SIVmac251 [35], have significantly lower plasma viral load than rhesus macaques of Indian origin [33,35]. Among the IV inoculated animals, disease progression was more rapid in rhesus monkeys of Chinese origin than those of Indian origin. Reimann et al. [48] compared pathogenicity of intravenous SHIV89.6P or SIVmac251 infection in cynomolgus macaques, and in rhesus macaques of Chinese or Indian origins. In contrast to the findings reported by ten Haaf et al. [55], they observed lower plasma viral load following either SIVmac251 or SHIV89.6P infection in cynomolgus and Chinese rhesus, as compared to Indian rhesus. The attenuated pathogenicity in cynomolgus macaques was associated with higher frequency of viral antigen specific cellular and neutralizing antibody responses.

On the other hand, little is known about lentivirus infection in pig-tailed macaques relative to these other species. Pig-tailed macaques are of interest, not only because they are more distantly related to rhesus and cynomolgus macaques [20,56], but also because they may be more susceptible to primate lentivirus-induced diseases than rhesus macaques. Rosenberg et al. [50] demonstrated increased susceptibility of pig-tailed as compared to rhesus macaques to the early gastrointestinal distress and death resulting from SIVPBj-14 infection. Persistent infection and/or AIDS-like disease progression have also been observed in pig-tailed macaques inoculated with primary isolates of HIV-2 [37,43], in contrast to the common observation of transient infection and lack of pathogenicity in HIV-2 infected rhesus macaques [11,16]. Hirsch et al. [26] passaged SIVagm in pig-tailed macaques and observed species-specific variations in pathogenicity correlating with the extent of *in vivo* replication. Chen et al. [12] observed enhanced infectivity, but no pathogenicity, after serial passages of an R5-tropic subtype C SHIV in pig-tailed macaques. Despite these early indications, information is still lacking on the comparative susceptibility of pig-tailed and rhesus macaques to a pathogenic primate lentivirus commonly used for vaccine studies.

SHIV_{SF162P} is one of the few existing CCR5-tropic chimeric viruses capable of establishing persistent infection in rhesus macaques [58]. Infection causes an early and dramatic loss of CD4⁺ T cells in the gut-associated lymphoid tissue compartment, followed by a gradual decline of CD4⁺ T cells in peripheral blood [25]. However, post-acute phase plasma viral load varies significantly after SHIV_{SF162P} infection in rhesus macaques and AIDS-like diseases progression remains rare [30]. Different passages of SHIV_{SF162P} have been established and used in a number of vaccine challenge studies, primarily with rhesus macaques [2,8,9,13,14, 59]. In an attempt to develop a more robust model for the evaluation of vaccine efficacy in a macaque model, we compared the *in vivo* infectivity and pathogenicity of a standard challenge stock of SHIV_{SF162P4} after intravenous (IV), intrarectal (IR) or intravaginal (IVag) inoculation in rhesus and pig-tailed macaques.

Materials and Methods

Animals

A total of 27 rhesus macaques (of Indian, Chinese, or hybrid origin) and 22 pig-tailed macaques, all tested negative for simian type D retrovirus by serology and polymerase chain reaction (PCR), were used in this study. Animals were inoculated with the same challenge virus (see below) via different routes as summarized in Table 1 and described in further details under Results. Procedures for IV, IR and IVag inoculations were as previously described [2,31,34, 38]. Studies with pig-tailed macaques were performed at the Washington National Primate Research Center (WaNPRC), the IV and IR studies with rhesus macaques at the California National Primate Research Center (CNPRC), and the IVag studies with rhesus at Advanced BioScience Laboratories, Inc. (ABL). Peripheral blood was collected by venipuncture and analyzed for viral load and lymphocyte subsets as described below. Animals were monitored for general health, body weight and temperature by routine physical examinations. All animals were cared for in accordance with established guidelines and the experimental procedures performed under approval from the respective Institutional Animal Care and Use Committees.

Virus stock

A macaque-passaged stock SHIV_{SF162 P3}, was originally established in Dr. Cecilia Cheng-Mayer's laboratory [25]. Lymph node cells and PBMC from a macaque infected with SHIV_{SF162 P3} collected at 2 weeks after infection were amplified in human PBMC to generate a SHIV_{SF162 P4} stock. This virus shares the same *env* gp120 sequences as HIV-1_{SF162} and similar neutralization susceptibility (data not shown). A standardized stock of SHIV_{SF162 P4} was prepared in rhesus peripheral blood mononuclear cells (PBMC) at ABL for vaccine challenge studies. Briefly, 0.5 ml of plasma from a SHIV162P4-infected macaque (from Dr. L. Stamatos) was injected intravenously into a naïve Indian rhesus macaque. Following infection, blood (10 ml) and bone marrow (5 ml) of this macaque were injected into a second naïve animal. At the peak level of infection as evident from plasma viremia, PBMCs of the second macaque were collected, depleted of CD8⁺ T cells, activated with phytohemagglutinin (PHA) and a virus stock was isolated by co-culturing with PHA-activated PBMCs from naïve macaques. The *in vitro* infectivity of this stock as determined by 50% tissue culture infectious dose (TCID₅₀) was 3.6×10^3 /ml on rhesus PBMC.

Plasma viral load

Viral load was determined by one of the following methods: real-time reverse transcription (RT)-PCR [15,46,54] was used for samples from all pig-tailed macaques at WaNPRC; branched-chain DNA (bDNA) [39] for samples from rhesus at CNPRC, and nucleic acid sequence based amplification (NASBA) [49] for samples from rhesus at ABL.

Hematology and immunophenotype analysis

Absolute numbers of peripheral blood CD3⁺/4⁺ cells were determined by flow cytometry according to regulations from the Centers for Disease Control and Prevention and described in further details in by Polacino et al. [46].

Results

Study design

Twenty-seven rhesus macaques and 22 pig-tailed macaques were enrolled in this study at three performance sites (Table 1). All animals were inoculated with a single dose of the same challenge stock of SHIV_{SF162 P4} virus prepared in rhesus macaque PBMC. Ten pigtailed macaques were inoculated by intravenous inoculation, with 2 animals each at 10-fold serial

dilutions from 360 to 0.036 TCID₅₀. Similarly, two rhesus macaques each were inoculated at 36 or 3.6 TCID₅₀. For IR inoculations, six rhesus and six pig-tailed macaques each were inoculated at 1,800 or 360 TCID₅₀. In addition, seven rhesus macaques were inoculated intravaginally with 3,600 TCID₅₀ and four at 1,800 TCID₅₀. Plasma viral load, CD4⁺ T cell counts and disease progression were monitored for up to 24 weeks. Plasma RNA load data of four of the seven macaques inoculated intravaginally with 3,600 TCID₅₀ were presented in an earlier study [2].

Similar *in vivo* infectivity of SHIV_{SF162 P4} in rhesus and pig-tailed macaques

All rhesus and pigtailed macaques inoculated intravenously were infected, including those that received only 0.036 TCID₅₀ dose of SHIV_{SF162 P4}, indicating the *in vivo* infectivity of this stock was >30-fold greater than its *in vitro* titers measured in rhesus PBMC. The mean peak viral load for intravenously infected pigtailed and rhesus macaques was 3.2×10^7 and 4.2×10^6 copies/ml ($p \leq 0.036$) of plasma at wk 2 after infection (Fig. 1A-B). There was no correlation between peak viral load, or the time to peak viremia, with the amount of virus inoculum.

Similar infectivity was also observed in rhesus and pigtailed macaques inoculated via mucosal routes. Regardless of the species tested, all six animals inoculated IR with 1,800 TCID₅₀ and five out of six macaques inoculated with 360 TCID₅₀ became infected. In a parallel study, six of seven rhesus macaques inoculated IVag at 3,600 TCID₅₀ and three of four inoculated at 1,800 TCID₅₀ became infected (Table 1). One of the animals (M632) initially inoculated with 1,800 TCID₅₀ resisted infection as evident from the lack of plasma viremia, tissue proviral DNA and anti-SIV antibodies in serum (data not shown). Following re-inoculation with 3,600 TCID₅₀, this macaque was clearly infected (Fig 1E). The mean peak viral load in IR or IVag infected rhesus (1.7×10^7 and 1.2×10^7 copies/ml of plasma, respectively; Fig. 1C and E) was not significantly different from that in IV or IR inoculated pigtailed macaques (3.2×10^7 and 2.1×10^7 copies/ml of plasma, respectively; Fig. 1B and D). There was also no discernable difference in the time to reach peak viremia (two weeks after inoculation) in IV or IR inoculated pig-tailed macaques (Fig. 1B and D). However, we did observe a difference in the proportion of animals that showed a delay in the time to reach peak viremia among IVag infected rhesus macaques as compared to IV inoculated pig-tailed macaques. Among rhesus macaques, viral load peaked at three weeks, rather than two, after IVag inoculation in seven of nine infected animals (Fig. 1E), as compared to 1/10 IV ($p < 0.0055$), or 2/11 IR ($p < 0.0216$) infected pig-tailed macaques (Fig. 1B and D). Unfortunately, samples were not available from IV or IR inoculated rhesus to ascertain whether this delay was shared among rhesus macaques, or only in IVag inoculated animals.

Differential control of plasma viremia in rhesus and pig-tailed macaques infected with SHIV_{SF162 P4}

Despite the similarity in infectivity and peak plasma viral load between rhesus and pig-tailed macaques inoculated with SHIV_{SF162 P4}, significant differences were observed in the persistence of viremia in the post-acute phase of infection. Plasma viremia remained $>10^3$ copies/ml in five of 10 IV inoculated pig-tailed macaques (Fig. 1B). The mean plasma viral load was $>5.3 \times 10^4$ copies/ml between wks 16-24. In contrast, none of the four similarly inoculated Indian rhesus macaques showed persistent viremia beyond the acute phase (Fig. 1A).

Among IR inoculated animals, four of the 11 infected pig-tailed macaques showed persistent viremia with a mean plasma viral load of $1.5\text{--}5.2 \times 10^5$ /ml between wks 16-24 after infection (Fig. 1D). In contrast, none of the 11 similarly inoculated rhesus macaques showed persistent viremia (Fig. 1C). Among IVag inoculated rhesus macaques, two of the nine infected animals showed persistent plasma viral load of $10^5\text{--}10^6$ /ml between wks 14-24 after infection (Fig. 1E).

However, differences in the proportion of IR or IVag infected rhesus with persistent viremia (0/11 vs. 2/9, respectively) did not reach statistical significance.

Taken together, SHIV_{SF162 P4}-infected pig-tailed macaques showed significantly higher peak ($p < 0.025$) and setpoint ($p < 0.003$) plasma viral load than similarly infected Indian rhesus macaques (Fig. 2A). Using a value of $< 10^3$ copies/ml of mean plasma viral load between weeks 12-24 as an indication of control of infection, a significantly higher proportion of infected pig-tailed macaques showed persistent viremia after SHIV_{SF162 P4} infection, as compared to similarly infected rhesus macaques ($p < 0.013$) (Fig. 2B).

Peripheral blood CD4⁺ T-cell depletion in SHIV_{SF162 P4}-infected pig-tailed macaques

Peripheral blood CD4⁺ T cell level was monitored for all animals. Two of the 21 infected pig-tailed macaques (K03388 inoculated IV and J04009 inoculated IR) showed precipitous and irreversible CD4⁺ T-cell depletion beginning at 12 weeks after infection (Fig. 3G). In addition, one animal (K02305 inoculated IR, Fig. 3D) showed CD4⁺ T cell level at approx. 200/microliter from week 12 to 24 after infection. Animal J04009 also developed anemia and failed to gain weight. All animals were euthanized at the end of the study period of 24 weeks.

In contrast, none of the 15 similarly infected rhesus macaques showed persistent viremia or CD4⁺ T-cell depletion in peripheral blood within the study period of 20 to 24 weeks (Fig. 3F). One of the nine IVag infected rhesus macaques did show significant decline in peripheral blood CD4⁺ T cell level (M367, Fig. 3E), which remained at approx. 200/microliter from week 6 to week 14 after infection. However, differences between rhesus and pig-tailed macaques in the proportion of animals showing severe CD4⁺ T-cell depletion did not reach statistical significance. None of the infected rhesus showed any other clinical sign throughout the study period of 24 weeks.

Discussion

This study documents the similar infectivity, but differential control of primate lentivirus replication in pig-tailed versus rhesus macaques. To our knowledge, this is the first report of a comparative study with different macaque species inoculated with a CCR5-tropic SHIV developed for the evaluation of HIV vaccine candidates.

Results from this study allow us to draw several major conclusions. First, SHIV_{SF162 P4} showed similar infectivity in pig-tailed and rhesus macaques following IV and IR inoculations. Second, inoculum dose had no effect on the peak or setpoint plasma viral load in infected animals, in agreement with previous observations [17,2151]. Third, the route of inoculation did not significantly affect the peak or setpoint viral load among infected pig-tailed or rhesus macaques. Fourth, the time to peak viral load was delayed by a week in vaginally infected animals as compared to IV infected ones, similar to previously reported observations [1,21, 34]. Finally and importantly, our results showed significantly higher setpoint plasma viremia and lower proportion of animals controlling infection in SHIV_{SF162 P4}-infected pig-tailed macaques as compared to similarly infected rhesus macaques. Unfortunately, the study duration was too short to fully document disease progression among infected animals. Nevertheless, about 10% (2/21) of infected pig-tailed showed severe and irreversible peripheral CD4⁺ T cell depletion within the first three months after infection, which is rarely observed in similarly infected rhesus macaques [2,30]. In a parallel vaccine study, among 11 naïve pig-tailed macaque infected with the same stock of SHIV_{SF162 P4}, three showed peripheral blood CD4⁺ T cell depletion (one at week 8 and two at week 28 after inoculation), two of which developed AIDS-like clinical signs and were euthanized at weeks 22 and 42 (data not shown). Taken together, these findings indicate increased susceptibility of pig-tailed macaques to lentiviral induced disease as compared to the commonly used Indian rhesus macaques. The

proportion of SHIV_{SF162 P4}-infected pig-tailed macaques developing CD4⁺ T cell depletion (5/32 in 7 months from two studies combined) and the rapid onset of AIDS-like disease (2/32 within 7 months) are comparable to SIVmac251-infected Indian rhesus macaques [48], which represents one of the more virulent models of lentivirus infection in macaque species.

Findings from this study are in line with earlier observations suggesting an increased susceptibility of pig-tailed macaques to lentivirus induced diseases. Rosenberg et al. [50] showed that pig-tailed are more susceptible than rhesus macaques to death resulting from gastrointestinal distress following SIVPBj-14 infection. Buch et al. [7] demonstrated innate differences between rhesus and pig-tailed macaques infected with SHIV KU-2 in the development of neurological diseases. Persistent and pathogenic infection of primary isolates of HIV-2 has been observed in pig-tailed macaques [37,43]. Infection of pig-tailed macaques with SHIV HXBc2 (IIIB) vpu⁺, a CXCR4-tropic virus generally considered as non-pathogenic in rhesus macaques, lead to CD4⁺ T cell depletion in 20% of infected animals [28]. Passage of SHIV HXBc2 (IIIB) in pig-tailed macaques resulted in a highly pathogenic virus, SHIV 229, which induces rapid and irreversible peripheral CD⁺ T cell depletion and AIDS [19]. Findings here provide a direct comparison between pig-tailed and rhesus macaques for their susceptibility and ability to control primate lentivirus infections.

The basis for the differences observed between these two macaque species remains not clear. Pig-tailed macaques have been found to be defective in the host restriction factor TRIM5 α [6], used by rhesus macaques to restrict replication by certain retroviruses, such as HIV-1 [53]. However, this is not likely to explain our observations here, because SHIV_{SF162 P4} has the same capsid as SIVmac, which is not susceptible to rhesus TRIM5 α restriction. Although target cell abundance and replenishment may play a role [29,42,44,45], the similar levels of plasma viral load in pig-tailed and rhesus macaques in the acute phase infection indicate that SHIV_{SF162 P4} replicates and disseminates to establish systemic infection equally well in the two macaque species. The fact that the major difference observed between these two species is their ability to control viral replication in chronic infection (and possibly the resulting disease progression) indicates that differences in host antiviral immune responses, innate or adaptive, may be involved.

Comparative studies of pathogenic SIV infection in rhesus versus non-pathogenic infection their natural host sooty mangabeys have revealed the important role of inflammatory responses and cellular activation in lentivirus induced diseases [3,5,18,22;29;32,36,40,42,47,57]. Pathogenic SIVmac infection in rhesus results in inflammatory cytokine responses and CD4⁺ T cell activation, leading to increased target cell availability, decreased repopulation of central memory T cells and apoptosis. While comparative studies between susceptible macaque species, such as rhesus and pig-tailed macaques, have yet to be performed on these parameters, early studies of Rosenberg et al. [50] have indicated that PBMC from pig-tailed macaques, unlike those from rhesus, have reduced CD4⁺/CD8⁺ T-cell ratios and a skewing of T cells towards CD45RA^{hi} expression. Increased level of pre-activated CD4⁺ T-cells thus could lead to enhanced viral replication. Whether and how increased cellular activation contributes to the persistence of plasma viremia in pig-tailed macaques will need to be addressed by future studies.

Adaptive responses may also contribute to the differential control of viral replication. Reimann et al. [48] reported that the reduced pathogenicity in SIVmac251- or SHIV89.6P-infected cynomolgus macaques, as compared to Indian rhesus, was in part due to the higher frequency of Gag- and Env-specific gamma interferon-positive T cells and virus neutralizing antibody responses. Results from our studies indicate that pig-tailed macaques are fully capable of mounting gamma interferon-positive T cell and antibody responses, including virus neutralizing antibodies against the challenge virus, SHIV_{SF162 P4} (unpublished data). This

notion is also supported by the fact that about 50% of the infected pig-tailed macaques were able to control acute infection and reduce the mean peak viremia from $>10^7$ copies/ml to the setpoint viral load of $<10^4$ copies/ml. However, considerable differences in post-acute viral load indicate that intra-species variation in host factors, such as relative effectiveness of various major histocompatibility complex genes alleles to present viral antigens, may play a significant role in the uncontrolled lentivirus replication found in pig-tailed macaques, as has been demonstrated in humans and other macaque species [4,10,23,41]. Further comparative studies of host factors contributing to the innate and adaptive responses of macaque species to lentivirus infection will be needed.

Non-human primates provide the only experimental models available to-date for the assessment of the effectiveness of candidate AIDS vaccines. However, in part because of the lack of efficacy data from human vaccine trials, the predictive value of any particular animal model cannot be taken for granted. It is therefore risky to rely on any single non-human primate model to “rank-order” candidate vaccines for clinical development. Results from the present study indicate that efficacy in any vaccine trial may depend not only on the immunogen, vaccination regimen and the challenge virus, but also the species of macaques used. Comparative infectivity and pathogenicity data from different macaque species and better understanding of the factors contributing to these differences may result in more informed use of macaque models for HIV vaccine development.

Acknowledgments

We thank Dr. Nancy Miller (DAIDS, NIAID) for her support and coordination, Heather Mack, Valerie Teske, Ryan Wallerstedt, Ding Lu, Roxanna Colon, and Lara Compton for expert technical assistance, Dr. Steve Kelly and staff at WaNPRC and Dr. Deborah Weiss at ABL for veterinary care of animals housed at the respective sites. This study is supported in part by NIH grants and contracts N01 AI060005 and N01 A130057 (ABL), P51 RR0165 (CNPRC), P01 AI066314 and U19 AI51596 (CJM), N01 AI060006 and P51 RR0166 (WaNPRC), and P01 AI05456 (SLH).

This work was supported in part by NIH grants and contracts N01 AI060005 and N01 A130057 (ABL), P51 RR0165 (CNPRC), P01 AI066314 and U19 AI51596 (CJM), N01 AI060006 and P51 RR0166 (WaNPRC), and P01 AI05456 (SLH).

References

1. Ambrose Z, Larsen K, Thompson J, Stevens Y, Finn E, Hu SL, Bosch ML. Evidence for Early Local Viral Replication and Local Production of Antiviral Immunity upon Mucosal Simian-Human Immunodeficiency Virus SHIV89.6 Infection in *Macaca nemestrina*. *J Virol* 2001;75:8589–8596. [PubMed: 11507204]
2. Barnett SW, Srivastava IK, Kan E, Zhou F, Goodsell A, Cristillo AD, Ferrai MG, Weiss DE, Letvin NL, Montefiori D, Pal R, Vajdy M. Protection of macaques against vaginal SHIV challenge by systemic or mucosal and systemic vaccinations with HIV-envelope. *AIDS* 2008;22:339–348. [PubMed: 18195560]
3. Barry AP, Silvestri G, Safrin JT, Sumpter B, Kozyr N, McClure HM, Staprans SI, Feinberg MB. Depletion of CD8+ Cells in Sooty Mangabey Monkeys Naturally Infected with Simian Immunodeficiency Virus Reveals Limited Role for Immune Control of Virus Replication in a Natural Host Species. *J Immunol* 2007;178:8002–8012. [PubMed: 17548637]
4. Bontrop RE, Watkins DI. MHC polymorphism: AIDS susceptibility in non-human primates. *Trends in Immunology* 2005;26:227–233. [PubMed: 15797514]
5. Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, Kazzaz Z, Bornstein E, Lambotte O, Altmann D, Blazar BR, Rodriguez B, Teixeira-Johnson L, Landay A, Martin JN, Hecht FM, Picker LJ, Lederman MM, Deeks SG, Douek DC. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med* 2007;12:1365–1371. [PubMed: 17115046]
6. Brennan G, Kozyrev Y, Kodama T, Hu SL. Novel TRIM5 Isoforms Expressed by *Macaca nemestrina*. *J Virol* 2007;81:12210–12217. [PubMed: 17804491]

7. Buch SJ, Villinger F, Pinson D, Hou Y, Adany I, Li Z, Dalal R, Raghavan R, Kumar A, Narayan O. Innate Differences between Simian-Human Immunodeficiency Virus (SHIV)KU-2-Infected Rhesus and Pig-Tailed Macaques in Development of Neurological Disease. *Virology* 2002;295:54–62. [PubMed: 12033765]
8. Buckner C, Gines LG, Saunders CJ, Vojtech L, Srivastava I, Gettie A, Bohm R, Blanchard J, Barnett SW, Safrit JT, Stamatatos L. Priming B cell-mediated anti-HIV envelope responses by vaccination allows for the long-term control of infection in macaques exposed to a R5-tropic SHIV. *Virology* 2004;320:167–180. [PubMed: 15003872]
9. Burke B, Derby NR, Kraft Z, Saunders CJ, Dai C, Llewellyn N, Zharkikh I, Vojtech L, Zhu T, Srivastava IK. Viral evolution in macaques coinfecting with CCR5- and CXCR4-tropic SHIVs in the presence or absence of vaccine-elicited anti-CCR5 SHIV neutralizing antibodies. *Virology* 2006;355:138–151. [PubMed: 16920175]
10. Carrington M, O'Brien SJ. The Influence of HLA Genotype on AIDS. *Annual Review of Medicine* 2003;54:535–551.
11. Castro BA, Nepomuceno M, Lerche NW, Eichberg J, Levy JA. Persistent infection of baboons and rhesus monkeys with different strains of HIV-2. *Virology* 1991;184:219–226. [PubMed: 1678564]
12. Chen Z, Huang Y, Zhao X, Skulsky E, Lin D, Ip J, Gettie A, Ho DD. Enhanced Infectivity of an R5-Tropic Simian/Human Immunodeficiency Virus Carrying Human Immunodeficiency Virus Type 1 Subtype C Envelope after Serial Passages in Pig-Tailed Macaques (*Macaca nemestrina*). *J Virol* 2000;74:6501–6510. [PubMed: 10864663]
13. Cherpelis S, Jin X, Gettie A, Ho DD, Barnett SW, Srivastava I, Stamatatos L. DNA-immunization with a V2 deleted HIV-1 envelope elicits protective antibodies in macaques. *Immunol Lett* 2001;79:47–55. [PubMed: 11595289]
14. Derby NR, Kraft Z, Kan E, Crooks ET, Barnett SW, Srivastava IK, Binley JM, Stamatatos L. Antibody Responses Elicited in Macaques Immunized with Human Immunodeficiency Virus Type 1 (HIV-1) SF162-Derived gp140 Envelope Immunogens: Comparison with Those Elicited during Homologous Simian/Human Immunodeficiency Virus SHIVSF162P4 and Heterologous HIV-1 Infection. *J Virol* 2006;80:8745–8762. [PubMed: 16912322]
15. Doria-Rose NA, Ohlen C, Polacino P, Pierce CC, Hensel MT, Kuller L, Mulvania T, Anderson D, Greenberg PD, Hu SL, Haigwood NL. Multigene DNA priming-boosting vaccines protect macaques from acute CD4⁺-T-cell depletion after simian-human immunodeficiency virus SHIV89.6P mucosal challenge. *J Virol* 2003;77:11563–11577. [PubMed: 14557642]
16. Dormont D, Livartowski J, Chamaret S, Guetard D, Henin D, Levagueresse R, van de Moortelle PF, Larke B, Gourmelon P, Vazeux R. HIV-2 in rhesus monkeys: serological, virological and clinical results. *Intervirology* 1989;30:59–65. [PubMed: 2708010]
17. Endo Y, Igarashi T, Nishimura Y, Buckler C, Buckler-White A, Plishka R, Dimitrov DS, Martin MA. Short- and Long-Term Clinical Outcomes in Rhesus Monkeys Inoculated with a Highly Pathogenic Chimeric Simian/Human Immunodeficiency Virus. *J Virol* 2000;74:6935–6945. [PubMed: 10888632]
18. Estes JD, Gordon SN, Zeng M, Chahroudi AM, Dunham RM, Staprans SI, Reilly CS, Silvestri G, Haase AT. Early Resolution of Acute Immune Activation and Induction of PD-1 in SIV-Infected Sooty Mangabeys Distinguishes Nonpathogenic from Pathogenic Infection in Rhesus Macaques. *J Immunol* 2008;180:6798–6807. [PubMed: 18453600]
19. Fernandez CS, Stratov I, De Rose R, Walsh K, Dale CJ, Smith MZ, Agy MB, Hu SL, Krebs K, Watkins DI, O'Connor DH, Davenport MP, Kent SJ. Rapid Viral Escape at an Immunodominant Simian-Human Immunodeficiency Virus Cytotoxic T-Lymphocyte Epitope Exacts a Dramatic Fitness Cost. *J Virol* 2005;79:5721–5731. [PubMed: 15827187]
20. Footen, J. Classification and distribution of living macaques. In: Lindburg, DG., editor. *The Macaques: Studies in Ecology, Behavior, and Evolution*. Van Nostrand-Reinhold; New York: 1980. p. 1-9.
21. Greenier JL, Miller CJ, Lu D, Dailey PJ, Lu FX, Kunstman KJ, Wolinsky SM, Marthas ML. Route of simian immunodeficiency virus inoculation determines the complexity but not the identity of viral variant populations that infect rhesus macaques. *J Virol* 2001;75:3753–3765. [PubMed: 11264364]
22. Gordon SN, Klatt NR, Bosinger SE, Brenchley JM, Milush JM, Engram JC, Dunham RM, Paiardini M, Klucking S, Danesh A, Strobert EA, Apetrei C, Pandrea IV, Kelvin D, Douek DC, Staprans SI,

- Sodora DL, Silvestri G. Severe Depletion of Mucosal CD4⁺ T Cells in AIDS-Free Simian Immunodeficiency Virus-Infected Sooty Mangabeys. *J Immunol* 2007;179:3026–3034. [PubMed: 17709517]
23. Goulder PJR, Watkins DI. Impact of MHC class I diversity on immune control of immunodeficiency virus replication. *Nat Rev Immunol* 2008;8:619–630. [PubMed: 18617886]
 24. Haigwood NL. Predictive value of primate models for AIDS. *AIDS Rev* 2004;6:187–198. [PubMed: 15700617]
 25. Harouse JM, Gettie A, Tan RC, Blanchard J, Cheng-Mayer C. Distinct pathogenic sequela in rhesus macaques infected with CCR5 or CXCR4 utilizing SHIVs. *Science* 1999;284:816–819. [PubMed: 10221916]
 26. Hirsch VM, Dapolito G, Johnson PR, Elkins WR, London WT, Montali RJ, Goldstein S, Brown C. Induction of AIDS by simian immunodeficiency virus from an African green monkey: species-specific variation in pathogenicity correlates with the extent of in vivo replication. *J Virol* 1995;69:955–967. [PubMed: 7815563]
 27. Hu SL. Non-human primate models for AIDS vaccine research. *Curr Drug Targets Infect Disord* 2005;5:193–201. [PubMed: 15975024]
 28. Hu, SL.; Klaniecki, JE.; Travis, BM.; Wrey, T.; Pennathur, S.; Montefiori, DC.; Thompson, JL.; Agy, MB.; Kuller, L.; Morton, WR. Immunization with HIV-1 gp160 by the “prime and boost” regimen protects macaques against SHIV HXBc2 challenge. In: Brown, F.; Burton, D.; Doherty, P.; Mekalanos, J.; Norrby, E., editors. *Vaccines 97*. Cold Spring Harbor Laboratory; Cold Spring Harbor, New York: 1997. p. 291-298.
 29. Kaur A, Di Mascio M, Barabasz A, Rosenzweig M, McClure HM, Perelson AS, Ribeiro RM, Johnson RP. Dynamics of T- and B-Lymphocyte Turnover in a Natural Host of Simian Immunodeficiency Virus. *J Virol* 2008;82:1084–1093. [PubMed: 18032490]
 30. Kraft Z, Derby NR, McCaffrey RA, Niec R, Blay WM, Haigwood NL, Moysi E, Saunders CJ, Wrin T, Petropoulos CJ, McElrath MJ, Stamatatos L. Macaques Infected with a CCR5-Tropic Simian/Human Immunodeficiency Virus (SHIV) Develop Broadly Reactive Anti-HIV Neutralizing Antibodies. *J Virol* 2007;81:6402–6411. [PubMed: 17392364]
 31. Kuller L, Benveniste RE, Tsai CC, Clark EA, Polacino P, Watanabe R, Overbaugh J, Katze MG, Morton WR. Intrarectal inoculation of macaques by the simian immunodeficiency virus, SIVmne E11S: CD4⁺ depletion and AIDS. *J Med Primatol* 1994;23:397–409. [PubMed: 7731030]
 32. Lackner AA, Veazey RS. Current Concepts in AIDS Pathogenesis: Insights from the SIV/Macaque Model. *Annual Review of Medicine* 2007;58:461–476.
 33. Ling B, Veazey RS, Luckay A, Penedo C, Xu K, Lifson JD, Marx PA. SIV(mac) pathogenesis in rhesus macaques of Chinese and Indian origin compared with primary HIV infections in humans. *AIDS* 2002;16:1489–1496. [PubMed: 12131186]
 34. Lu Y, Pauza CD, Lu X, Montefiori DC, Miller CJ. Rhesus macaques that become systemically infected with pathogenic SHIV 89.6-PD after intravenous, rectal, or vaginal inoculation and fail to make an antiviral antibody response rapidly develop AIDS. *J Acquir Immune Defic Syndr Hum Retrovirol* 1998;19:6–18. [PubMed: 9732063]
 35. Marthas ML, Lu D, Penedo MC, Hendrickx AG, Miller CJ. Titration of an SIVmac251 stock by vaginal inoculation of Indian and Chinese origin rhesus macaques: transmission efficiency, viral loads, and antibody responses. *AIDS Res Hum Retroviruses* 2001;17(15):1455–1466. [PubMed: 11679158]
 36. Mattapallil JJ, Douek DC, Hill B, Nishimura Y, Martin M, Roederer M. Massive infection and loss of memory CD4⁺ T cells in multiple tissues during acute SIV infection. *Nature* 2005;434:1093–1097. [PubMed: 15793563]
 37. McClure J, Schmidt A, Rey-Cuille MA, Bannink J, Mishner L, Tsai CC, Anderson D, Morton WR, Hu SL. Derivation and characterization of a highly pathogenic isolate of human immunodeficiency virus type 2 that causes rapid CD4⁺ cell depletion in *Macaca nemestrina*. *J Med Primatol* 2000;29:114–126. [PubMed: 11085573]
 38. Miller CJ, McChesney MB, Lu X, Dailey PJ, Chutkowski C, Lu D, Brosio P, Roberts B, Lu Y. Rhesus macaques previously infected with simian/human immunodeficiency virus are protected from vaginal challenge with pathogenic SIVmac239. *J Virol* 1997;71:1911–1921. [PubMed: 9032322]

39. Miller CJ, Marthas M, Greenier J, Lu D, Dailey PJ, Lu Y. In Vivo Replication Capacity Rather Than In Vitro Macrophage Tropism Predicts Efficiency of Vaginal Transmission of Simian Immunodeficiency Virus or Simian/Human Immunodeficiency Virus in Rhesus Macaques. *J Virol* 1998;72:3248–3258. [PubMed: 9525652]
40. Milush JM, Reeves JD, Gordon SN, Zhou D, Muthukumar A, Kosub DA, Chacko E, Giavedoni LD, Ibegbu CC, Cole KS, Miamidian JL, Paiardini M, Barry AP, Staprans SI, Silvestri G, Sodora DL. Virally Induced CD4+ T Cell Depletion Is Not Sufficient to Induce AIDS in a Natural Host. *J Immunol* 2007;179:3047–3056. [PubMed: 17709519]
41. O'Connor DH, Mothe BR, Weinfurter JT, Fuenger S, Rehauer WM, Jing P, Rudersdorf RR, Liebl ME, Krebs K, Vasquez J, Dodds E, Loffredo J, Martin S, McDermott AB, Allen TM, Wang C, Doxiadis GG, Montefiori DC, Hughes A, Burton DR, Allison DB, Wolinsky SM, Bontrop R, Picker LJ, Watkins DI. Major Histocompatibility Complex Class I Alleles Associated with Slow Simian Immunodeficiency Virus Disease Progression Bind Epitopes Recognized by Dominant Acute-Phase Cytotoxic-T-Lymphocyte Responses. *J Virol* 2003;77:9029–9040. [PubMed: 12885919]
42. Okoye A, Meier-Schellersheim M, Brenchley JM, Hagen SI, Walker JM, Rohankhedkar M, Lum R, Edgar JB, Planer SL, Legasse A, Sylwester AW, Piatak M Jr, Lifson JD, Maino VC, Sodora DL, Douek DC, Axthelm MK, Grossman Z, Picker LJ. Progressive CD4+ central memory T cell decline results in CD4+ effector memory insufficiency and overt disease in chronic SIV infection. *J Exp Med* 2007;204:2171–2185. [PubMed: 17724130]
43. Otten RA, Brown BG, Simon M, Lupo LD, Parekh BS, Lairmore MD, Schable CA, Schochetman G, Rayfield MA. Differential replication and pathogenic effects of HIV-1 and HIV-2 in *Macaca nemestrina*. *AIDS* 1994;8:297–306. [PubMed: 8031510]
44. Pandrea I, Apetrei C, Gordon S, Barbercheck J, Dufour J, Bohm R, Sumpter B, Roques P, Marx PA, Hirsch VM, Kaur A, Lackner AA, Veazey RS, Silvestri G. Paucity of CD4+CCR5+ T cells is a typical feature of natural SIV hosts. *Blood* 2007;109:1069–1076. [PubMed: 17003371]
45. Pandrea I, Onanga R, Souquiere S, Mouinga-Ondeme A, Bourry O, Makuwa M, Rouquet P, Silvestri G, Simon F, Roques P, Apetrei C. Paucity of CD4+ CCR5+ T Cells May Prevent Transmission of Simian Immunodeficiency Virus in Natural Nonhuman Primate Hosts by Breast-Feeding. *J Virol* 2008;82:5501–5509. [PubMed: 18385229]
46. Polacino P, Cleveland B, Zhu YD, Kimata JT, Overbaugh J, Anderson D, Hu SL. Immunogenicity and protective efficacy of Gag/Pol/Env vaccines derived from temporal isolates of SIV_{mn} against cognate virus challenge. *J Med Primatol* 2007;36:254–265. [PubMed: 17669214]
47. Raffatellu M, Santos RL, Verhoeven DE, George MD, Wilson RP, Winter SE, Godinez I, Sankaran S, Paixao TA, Gordon MA, Kolls JK, Dandekar S, Baumler AJ. Simian immunodeficiency virus-induced mucosal interleukin-17 deficiency promotes *Salmonella* dissemination from the gut. *Nat Med* 2008;14:421–428. [PubMed: 18376406]
48. Reimann KA, Parker RA, Seaman MS, Beaudry K, Beddall M, Peterson L, Williams KC, Veazey RS, Montefiori DC, Mascola JR, Nabel GJ, Letvin NL. Pathogenicity of simian-human immunodeficiency virus SHIV-89.6P and SIV_{mac} is attenuated in cynomolgus macaques and associated with early T-lymphocyte responses. *J Virol* 2005;79:8878–8885. [PubMed: 15994781]
49. Romano JW, Shurtleff RN, Dobratz E, Gibson A, Hickman K, Markham PD, Pal R. Quantitative evaluation of simian immunodeficiency virus infection using NASBA technology. *Journal of Virological Methods* 2000;86:61–70. [PubMed: 10713377]
50. Rosenberg YJ, White BD, Papermaster SF, Zack P, Jahrling PB, Eddy GA, Burke DS, Lewis MG. Variation in T-lymphocyte activation and susceptibility to SIV_{pbj}-14-induced acute death in macaques. *J Med Primatol* 1991;20:206–210. [PubMed: 1682498]
51. Ruprecht RM, Baba TW, Rasmussen R, Hu Y, Sharma PL. Murine and simian retrovirus models: the threshold hypothesis. *AIDS* 1996;10:S33–40. [PubMed: 8883607]
52. Staprans SI, Feinberg MB. The roles of nonhuman primates in the preclinical evaluation of candidate AIDS vaccines. *Expert Rev Vaccines* 2004;3:S5–32. [PubMed: 15285703]
53. Stremlau M, Owens CM, Perron MJ, Kiessling M, Autissier P, Sodroski J. The cytoplasmic body component TRIM5 α restricts HIV-1 infection in Old World monkeys. *Nature* 2004;427:848–853. [PubMed: 14985764]

54. Suryanarayana K, Wiltout TA, Vasquez GM, Hirsch VM, Lifson JD. Plasma SIV RNA viral load determination by real-time quantification of product generation in reverse transcriptase-polymerase chain reaction. *AIDS Res Hum Retroviruses* 1998;14:183–189. [PubMed: 9462929]
55. ten Haaf P, Almond N, Biberfeld G, Cafaro A, Cranage MP, Ensoli B, Hunsmann G, Polyanskaya N, Stahl-Hennig C, Thorstensson R, Titti F, Heeney J. Comparison of early plasma RNA loads in different macaque species and the impact of different routes of exposure on SIV/SHIV infection. *Journal of Medical Primatology* 2001;30:207–214. [PubMed: 11555139]
56. Tosi AJ, Morales JC, Melnick DJ. Comparison of Y Chromosome and mtDNA Phylogenies Leads to Unique Inferences of Macaque Evolutionary History. *Molecular Phylogenetics and Evolution* 2000;17:133–144. [PubMed: 11083929]
57. Veazey RS, DeMaria M, Chalifoux LV, Shvetz DE, Pauley DR, Knight HL, Rosenzweig M, Johnson RP, Desrosiers RC, Lackner AA. Gastrointestinal Tract as a Major Site of CD4+ T Cell Depletion and Viral Replication in SIV Infection. *Science* 1998;280:427–431. [PubMed: 9545219]
58. Vlasak J, Ruprecht RM. AIDS vaccine development and challenge viruses: getting real. *AIDS* 2006;20:2135–2140. [PubMed: 17086052]
59. Xu R, Srivastava IK, Kuller L, Zarkikh I, Kraft Z, Fagrouch Z, Letvin NL, Heeney JL, Barnett SW, Stamatatos L. Immunization with HIV-1 SF162-derived Envelope gp140 proteins does not protect macaques from heterologous simian-human immunodeficiency virus SHIV89.6P infection. *Virology* 2006;349:276–289. [PubMed: 16527321]

Fig. 1. Plasma viral load in rhesus (left panels) and pig-tailed (right panels) macaques after SHIV_{SF162 P4} inoculation. Panels A and B: Numbers in parenthesis refer to the IV inoculum dose expressed in TCID₅₀. Panels C and D: Animals in the top line of the legend received an inoculum of 1,800 TCID₅₀ via the IR route and those in the bottom line received 360 TCID₅₀. Panel E: Open symbols indicate animals that received an inoculum of 1,800 TCID₅₀ via IVag route and the rest received 3,600 TCID₅₀. RNA load of macaque M632 following challenge with 1,800 TCID₅₀ and rechallenge with 3,600 TCID₅₀ was shown respectively as M632 and M632R. Panel F and G: Summary of all viral load data from all infected animals. Please note that different symbols are used in these panels: open symbols

denote viral load in animals inoculated by mucosal (IR or IVag) route and closed symbol, those inoculated by IV route. Solid lines represent the geometric mean values of viral load from all infected animals.

Fig. 2. Differential control of SHIV_{SF162} p4 replication in rhesus and pig-tailed macaques. Panel A: Horizontal lines indicate the mean plasma viral load at peak (week 2 or 3) or setpoint (between week 16 and 24) after infection. Individual values for all infected animals, regardless of the route of inoculation, are represented. P-value was determined by two-sided non-parametric Mann-Whitney U test. Panel B: Proportion of animals controlling infection, as defined by setpoint viremia $<10^3$ copies/ml (indicated by the shaded area in Panel A) is indicated in the solid bars. P-value was determined by two-sided Fisher's exact test.

Fig. 3. Peripheral blood CD4⁺ T cells numbers in rhesus (left panels) and pig-tailed (right panels) macaques after SHIV_{SF162} P4 inoculation. Symbols are the same as described in the legend for Fig. 1. CD4⁺ T cell counts of macaque M632 following challenge with 1,800 TCID₅₀ (M632) and 3,600 TCID₅₀ (M632 R) are shown separately in Fig. 3E. Solid lines in Panels F and G show animals that developed CD4⁺ T cell decline.

Table 1

In vivo infectivity of SHIV_{SF162 P4} in rhesus and pig-tailed macaques:

Route	Rhesus Macaques		Pig-tailed Macaques	
	TCID ₅₀	Infected/total	TCID ₅₀	Infected/total
IV	36 – 3.6	4/4	360-0.036	10/10
IR	1,800	6/6	1,800	6/6
	360	5/6	360	5/6
IVag	3,600	6/7	ND	ND
	1,800	3/4		