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Comparative Evaluation of Simian, Simian–Human, and Human Immunodeficiency Virus Infections in the Pigtail Macaque (*Macaca nemestrina*) Model

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ABSTRACT

The global impact of HIV/AIDS intensifies the need for a preventive vaccine and nonhuman primate models can help provide critical insights into effective immunity. Pigtail macaques (*Macaca nemestrina*) are increasingly studied as a nonhuman primate model for AIDS. We compared the virologic and immunologic characteristics of HIV-1, SIV, and SHIV infection of naive pigtail macaques across a series of preclinical HIV vaccine studies. SIV_{mac251} and SIV_{mac239} infection of naive pigtail macaques resulted in a gradual decline in peripheral $CD4^+$ T cells in the setting of high levels of viremia, approximating most closely human infection of HIV-1. In contrast, the CXCR4-utilizing $SHIV_{mn229}$ virus resulted in rapid depletion of $CD4^+$ T cells and minimal generation of humoral or cellular immune responses, similar to that observed with $SHIV_{89.6P}$ infection of rhesus macaques. Infection with the CCR5-utilizing, rhesus macaque passaged, $SHIV_{SF162P3}$ resulted in some overall $CD4^+$ T cell decline, however, three of eight macaques naturally control $SHIV_{SF162P3}$ viremia to very low levels in the setting of robust adaptive immunity. Despite attempts at infecting pigtail macaques with HIV-1 strains passaged in juvenile pigtail macaques *in vivo* or in PBMC isolated from pigtail macaques *in vitro*, only lower nonsustained levels of viral replication were observed. Our results provide a series of virologic models with which to evaluate potential AIDS vaccines in pigtail macaques.

INTRODUCTION

MACAQUE MODELS OF HIV/AIDS have provided many insights into HIV immunopathogenesis. The Indian rhesus macaque (*Macaca mulatta*) is the best characterized and most commonly studied model, however, this species has become increasingly difficult to study in recent years and there has been renewed interest in alternate pathogenic models.[1,2] Alternate models include Chinese rhesus macaques, cynomolgus macaques (*M. fascicularis*), and pigtail macaques (*M. nemestrina*). Chinese rhesus and cynomolgus macaques have, however, a somewhat less pathogenic course following infection with SIV_{mac251} and $SHIV_{89.6P}$ compared to Indian rhesus macaques.[3] Several groups now primarily study pigtail macaques as an animal model for HIV and there are growing data on the common MHC molecules expressed by pigtail macaques and the simian immunodeficiency virus (SIV) epitopes they present.[4] This now allows the detailed study of immunopathogenesis issues in pigtail macaques originally pioneered with Indian rhesus macaques.

In addition to the various Asian macaque species studied for HIV vaccine and pathogenesis analyses, several different types of challenge viruses have been used across the different macaque species. SIV_{mac251} has become the "gold standard" of vaccine challenge viruses studied, initially derived from and primarily evaluated in rhesus macaques.[5] This virus primarily utilizes the CCR5 coreceptor *in vivo*, targeting memory $CD4^+$ T cells and being more difficult to neutralize with antibodies, similar to most infecting HIV-1 strains.[6] Several chimeric SHIV viruses have also been studied, expressing HIV-1 Env primarily utilizing either the CXCR4 coreceptor *in vivo* such as $SHIV_{89.6P}$ or the CCR5 coreceptor such as $SHIV_{SF162P3}$.[7–9] The

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CXCR4-utilizing SHIVs typically cause very rapid $CD4^+$ T cell depletion, initially targeting naive $CD4^+$ T cells. If the initial infection is sufficiently blunted by vaccination, the X4-utilizing SHIVs are more readily susceptible to neutralizing antibodies.[10,11] Other SIV and SHIV strains, as well as HIV-2, have also been evaluated with success in pigtail macaque models.[12–15] Pigtail macaques have been evaluated for infection with HIV-1_{LAI} although no persistent infections resulted.[16]

Several studies have evaluated mucosal infection of macaques through the oral, vaginal, and rectal routes in an effort to more closely mimic the common modes of transmission of HIV-1 in humans compared to intravenous administration.[7,10] Although each of the many virus/macaque model systems has provided some insights into HIV-1 pathogenesis, no comparison has been made across various virus models in pigtail macaques. We have performed a series of infection and vaccine studies with SIVs, SHIVs, and HIV-1 in pigtail macaques and here we analyze this experience and compare outcomes and immunologic responses across these different virus infection models of pigtail macaques.

MATERIALS AND METHODS

Monkeys

Juvenile macaques (*Macaca nemestrina*) were free from HIV-1/SIV/simian retrovirus (SRV) infection, housed under physical containment level 3 (PC3) conditions and anaesthetized with ketamine (10 mg/kg intramuscular, IM) prior to procedures. All experiments were performed according to National Institutes of Health guidelines on the care and use of laboratory animals, and were approved by the University of Melbourne Animal Experimentation and Ethics Committee.

Derivation of infectious virus isolates

SIV_{mac251}. A challenge stock of SIV_{mac251} was kindly provided by R. Pal (Advanced BioScience Laboratories, Kensington, MD[17]). The SIV_{mac251} (561L) challenge stock 50% tissue culture infectious dose ($TCID_{50}$/ml) was 8100 on the CEM × 174 human T cell line, and 5120 $TCID_{50}$ on rhesus (*Macaca mulatta*) peripheral blood mononuclear cells (PBMC) (R. Pal, personal communication). Macaques were inoculated with SIV_{mac251} intrarectally (IR) or intravenously (IV). The IR dose was delivered over 2 days, using 0.25 ml of the supplied stock diluted to 1 ml with normal saline for each day[18] (total dose: 8100 $TCID_{50}$ on CEM × 174 T cells). The IV dose was given as 5×10^{-3} ml of the stock dose (total dose: 40 $TCID_{50}$ on CEM × 174 cells), diluted to 1 ml with normal saline, and delivered via the femoral vein.[4]

SIV_{mac239}. Plasmid pSIV239 encoding SIV_{mac239} was constructed from two molecular clones: p239SpSp5′ and p239SpE3′ encoding the 5′ and 3′ SIV halves, respectively (National Institute of Health AIDS reagent repository, contributed by R. Desrosiers) as described.[18] Proviral DNA was inoculated either IM (300 μg) or into the epidermis via a gene gun (15 μg; Helios, Bio-Rad Laboratories).[18]

$SHIV_{mn229}$. This virus was derived from serial *in vivo* passage in *M. nemestrina* of a $SHIV_{HXB2}$ clone.[19] $SHIV_{mn229}$ was expanded on phytohemagglutinin (PHA)-activated *M. nemestrina* PBMC.[20] Macaques were inoculated atraumatically IR with $SHIV_{mn229}$ in 0.5 ml doses over 2 days. The stock generated was 1×10^5 $TCID_{50}$/ml on the CEM × 174 T cell line. The doses delivered IR to macaques were 2×10^2 ($N = 2$), 2×10^3 ($N = 2$), 4×10^4 ($N = 2$), 1×10^5 ($N = 11$), and 2×10^5 ($N = 2$) $TCID_{50}$/ml. A 1×10^5 $TCID_{50}$ $SHIV_{mn229}$ IR dose represents a monkey infectious dose of ≥500.[21]

$SHIV_{SF162P3}$. Dr. Janet Harouse contributed $SHIV_{SF162P3}$ to the NIH AIDS Research and Reference Reagent Program, and the virus was expanded on CD8 T cell-depleted human PBMC prior to use.[7,8] Female macaques were inoculated atraumatically intravaginally with $SHIV_{SF162P3}$ (3×10^3 $TCID_{50}$/ml) in 0.5 ml doses over 2 days (total 6×10^3 $TCID_{50}$/ml).

HIV-1_{LAI}. Stocks of HIV-1_{LAI} were expanded in PHA-activated autologous *M. nemestrina* PBMC as described.[22] Macaques were inoculated with HIV_{LAI} (10^6 $TCID_{50}$) by intravenous injection. HIV-1_{LAI} isolate K98227/W35 was selected from an infected neonatal macaque with detectable HIV-1 RNA in the plasma 35 weeks postinfection.[23,24] HIV-1_{LAI} isolate K98227/W35 was amplified on PHA-stimulated human PBMC and maintained in RPMI-1640 media supplemented with fetal calf serum (10%; Commonwealth Serum Laboratories, Melbourne, Australia) rHuman interleukin-2 (IL-2) (50 U/ml; Hoffmann-LaRoche, Nutley, NJ). The $TCID_{50}$ (C8166 human T cell line) of 10^6/ml was administered via the femoral vein to six macaques.

Detection of viral infection

Plasma SIV and SHIV RNA were quantified by reverse transcriptase real-time polymerase chain reaction (RT-PCR) using an SIV Gag-specific molecular beacon and primers.[25] The SIV Gag reverse transcriptase real-time PCR has a limit of detection of 1500 RNA copies per ml of plasma. Peripheral CD4 T cells were quantified using human antibodies specific for T lymphocyte markers CD3 and CD4 and flow cytometry (FACScan, BD) as described.[20] Plasma HIV-1_{LAI} and HIV-1_{LAI} isolate K98227/W35 RNA were assessed by RT-PCR (Amplicor HIV Monitor , Roche Diagnostic Systems, Branchburg, NJ) according to the manufacturer's instructions for human plasma. The detection limit for HIV-1_{LAI} was 200 copies of RNA per ml of plasma, and for isolate K98227/W35 the detection limit was 20 RNA copies/ml of plasma.

T cell immunity assays

ELISpot. Antigen-specific interferon-γ (IFN-γ)-secreting cells were enumerated using a Monkey IFN-γ ELISpot commercial kit (U-CyTech, Utrecht, The Netherlands) as previously described.[20] PBMC were isolated and stimulated with SIV_{mac239} or HIV-1 consensus subtype B Gag overlapping 15-mer peptide pools (1 μg/ml/peptide; supplied by the NIH AIDS Research and Reference Reagent Program) for 18 h. Cells were washed and transferred to anti-IFN-γ monoclonal antibody-coated flat-bottomed 96-well plates containing antigen and incubated for a further 5 h. Cells were lysed and plates washed prior to incubation with biotinylated anti-IFN-γ polyclonal rabbit antibody, followed by incubation with gold-labeled anti-biotin IgG antibody. IFN-γ spots were developed and analyzed

Table 1. Source of Infectious Virus Isolates and Use in *M. nemestrina* Studies

Virus	*Origin (reference)*	*Prior* in vivo *passage history*	*Tropism*	*Dose used in pigtail macaques*	In vitro *amplification of stock isolates*	*Route*	*Number pigtail macaques infected* n *(%)*	*Peak VL*[a] *(SE)*	*Set point VL*[b] *(range)*	*Undetectable viremia by week 11* n *(%)*	*Set point CD4 T cells*[c]	*Number euthanased by week 20* n *(%)*	*Reference for current use in pigtail macaques*
SIV_{mac251}	Original isolate: R. Desrosiers[5] Source: R. Pal and N. Miller[17]	Stock derived from Rhesus macaque isolate	R5	8×10^3 $TCID_{50}$ (IR) 4×10^1 $TCID_{50}$ (IV)	*M. mulatta* PBMC[17]	IR or IV	11 (100%)	7.58 (0.18)	6.32 (3.77–7.82)	0 (0%)	75%	5 (45%)	4, 18
SIV_{mac239}	Infectious molecular clone constructed from fragments supplied by R. Desrosiers[18]	Molecular clone derived from Rhesus macaque isolate	R5	15 μg DNA ID 1 mg DNA IM	Plasmid DNA—molecular clone	ID via gene gun or IM	4 (100%)	8.0 (0.08)	6.69 (5.85–7.48)	0 (0%)	72%	3 (75%)	18
$SHIV_{mn229}$ ($SHIV_{HXB2}$-derived)	$SHIV_{mn229}$ from M. Agy[19,35] Original $SHIV_{HXB2}$ from J. Sodroski[36]	Pigtail macaques (2 passages)	X4	2×10^2–2×10^5 $TCID_{50}$	*M nemestrina* PBMC	IR	19 (100%)	8.09 (0.12)	6.13 (4.49–6.81)	0 (0%)	3%	19 (100%)	20, 21
$SHIV_{SF162P3}$ ($SHIV_{SF162}$-derived)	J. Harouse and C. Cheng-Meyer[7,8,31]	Rhesus macaques (3 passages)	R5	6×10^3 $TCID_{50}$	Human PBMC	Ivag	8 (100%)	7.52 (0.19)	5.19 (<3.81–6.08)	3 (38%)	77%	0	29
HIV 1_{LAI}	M. Agy[16]	None	X4	$TCID_{50}$ 10^6	*M. nemestrina* PBMC	IV	6 (100%)	2.92 (0.08)	2.58 (<2.3–2.8)	6 (100%)	100%	0	22, 28
HIV 1_{LAI} isolate K98227/W35	M. Agy and M. Bosch[24]	Neonatal pigtail macaque with high VL	X4	$TCID_{50}$ 10^6	Human PBMC	IV	6 (100%)	3.36 (0.14)	1.79 (<1.7)	6 (100%)	107%	0	23

[a]Log_{10} SIV or HIV-1 RNA copies/ml of plasma. Peak generally occurs at 2–3 weeks following viral inoculation.
[b]Set point VL: mean viral load for all macaques, taken from weeks 5–11 postchallenge. "Range" taken from week 11 or 12 postinfection.
< indicates where the result is below the lower limit of detection for the assay.
[c]CD4%: CD4 T cells expressed as percent of total CD4 T cells at baseline.

by an automated reader (AID, Strassberg, Germany). Results were normalized to antigen-specific IFN-γ-secreting precursor frequency per 10^6 PBMC.

Intracellular cytokine staining (ICS). Induction of antigen-specific intracellular IFN-γ expression in $CD3^+CD8^+$ or $CD3^+CD4^+$ T lymphocytes was assessed by flow cytometry as previously described.[21] Briefly, 200 μl of whole blood was incubated with 1 μg/ml overlapping 15-mer peptide pools in dimethyl sulfoxide (DMSO) and the costimulatory antibodies CD28 and CD49d (BD Biosciences Pharmingen, San Diego, CA) for 7 h. Brefeldin A (10 μg/ml; Sigma) was included during the last 5 h of the incubation. Anti-CD3-PE, anti-CD4-FITC, and anti-CD8-PerCP (BD) antibodies were added to each well and incubated for 30 min. Red blood cells were lysed (FACS lysing solution, BD), washed with phosphate-buffered saline (PBS), and the remaining cells permeabilized (FACS Permeabilizing Solution 2, BD). Permeabilized cells were then incubated with anti-human IFN-γ-APC antibody (BD) prior to fixing with formaldehyde and acquisition (FACScan, BD). Acquisition data were analyzed using CellQuest (BD). The percentage of antigen-specific gated lymphocytes expressing IFN-γ was assessed in both $CD3^+CD4^+$ and $CD3^+CD8^+$ lymphocyte subsets.

Western blot analysis

Antibodies to HIV-1, SIV, and SHIV were tested by Western blot using 200 μg standardized HIV-1 or HIV-2 viral lysate at the Australian National Serologic Reference Laboratories as described.[22,26]

TZM-bl luciferase reporter gene assay for SHIV neutralizing antibodies

Neutralization was measured as a function of reductions in luciferase reporter gene expression after a single round of virus infection in TZM-bl cells as described.[27] TZM-bl cells, which express CD4 and CCR5, were obtained from the NIH AIDS Research and Reference Reagent Program, as contributed by John Kappes and Xiaoyun Wu. Briefly, cell-free $SHIV_{mn229}$ or $SHIV_{SF162P3}$ (each 200 $TCID_{50}$, amplified on human PBMC) was incubated with serial dilutions of test samples in triplicate in a total volume of 150 μl for 1 h at 37°C in 96-well flat-bottom culture plates. Freshly trypsinized cells (10,000 cells in 100 μl of growth medium containing 75 μg/ml DEAE dextran and 2.5 μM indinavir) were added to each well. One set of control wells received cells plus virus (virus control) and another set received cells only (background control). After a 48 h incubation, 100 μl of cell lysate was transferred to a 96-well black solid plate (Costar) for measurements of luminescence using Bright Glo substrate solution as described by the supplier (Promega). Neutralization titers are the dilution at which relative luminescence units were reduced by 50% compared to virus control wells after subtraction of background.

RESULTS

Virology

Our laboratory has ready access to pigtail macaques, an increasingly studied nonhuman primate model for AIDS. To further analyze the potential for studying pigtail macaques as a macaque model for HIV, we compared the outcome infection of naive pigtail macaques with SIV_{mac251} and SIV_{mac239}, $SHIV_{SF162P3}$ (R5), and $SHIV_{mn229}$ (X4) and two strains of HIV-1_{LAI} in a total of 54 animals as summarized in Table 1.[4,18,20–23,28,29] Encouragingly, all naive pigtail macaques studied became infected with each virus isolate, although the outcome of infection was markedly different. The course of infection was followed for each of the viruses using assays to determine the plasma viral RNA (viral load) and the effect on CD4 T cell decline (Fig. 1).

Inoculation of pigtail macaques with SIV_{mac251} and SIV_{mac239}[4,18] resulted in persistent high levels of viral replication and a gradual persistent decline in $CD4^+$ T cells, with no animals studied completely controlling virus replication. SIV_{mac239} inoculated as proviral DNA (ID or IM) or with *in vitro*-cultured SIV_{mac251} (IR or IV) resulted in similar levels of

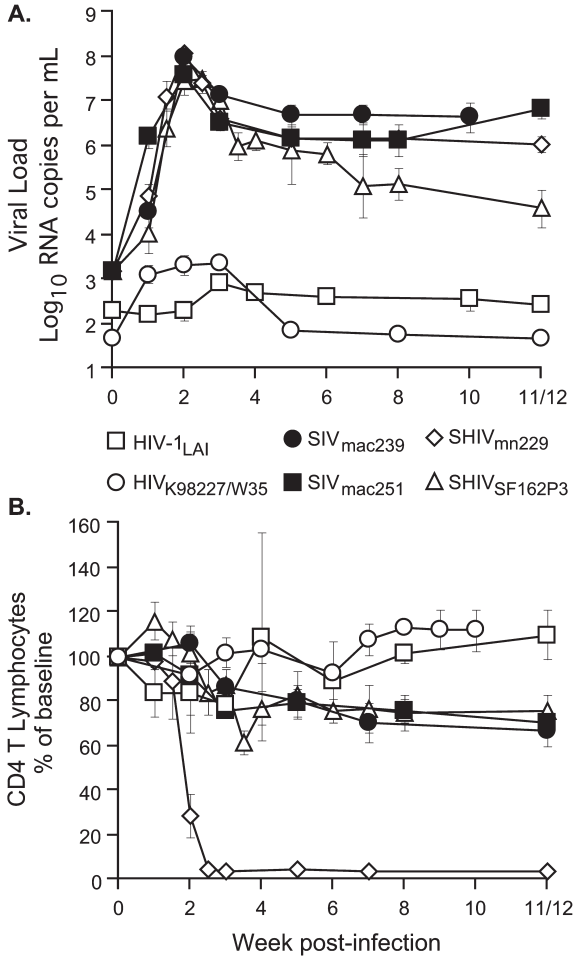

FIG. 1. Comparative course of infection for the immunodeficiency virus challenges in pigtail macaques. (**A**) Mean (± standard error of the mean) plasma viral RNA is shown following infection with each of the immunodeficiency viruses tested. (**B**) Mean (± standard error of the mean) %CD4 T lymphocytes for each of the viral challenge groups. CD4 T lymphocytes are normalized as a percent of the total number assessed preinfection.

viral replication, peaking 2 weeks postinfection with mean detectable viral loads of 8.0 and 7.6 $\log_{10}$ copies/ml, respectively (Table 1 and Fig. 1A). CD4 T cells declined to ~70% of the mean preinfection levels by weeks 11 to 12 postinoculation (Fig. 1B). Some SIV-infected macaques were euthanized as AIDS-related illnesses (respiratory symptoms, weight loss, lethargy) became evident (53%, Table 1).

Intrarectal infection with the X4-utilizing $SHIV_{mn229}$[20,21] resulted in an aggressive pathogenic infection with a complete loss of $CD4^+$ T lymphocytes within 3 weeks of infection. Mean peak and set point $SHIV_{mn229}$ viral load were similar to that seen using SIV_{mac239} or SIV_{mac251}. $SHIV_{mn229}$ was administered IR using doses ranging from 2×10^2 to 2×10^5. Each IR dose infected the macaques, sharing the same course of infection irrespective of dose: peak viral load at 2 weeks, diminishing CD4 T cells by 2–3 weeks postinfection (not shown). Macaques commonly suffered from diarrhea immediately following $SHIV_{mn229}$ infection and problems associated with immunodeficiency (weight loss, diarrhea, respiratory symptoms) were commonly detected by 11–12 weeks postinfection.

Nonhuman primate models of X4-utilizing SHIVs such as $SHIV_{89.6P}$ and $SHIV_{mn229}$ have the disadvantage of targeting a different $CD4^+$ T cell population than the R5-utilizing HIV-1 strains that are generally transmitted between humans.[6] We therefore evaluated $SHIV_{SF162P3}$, an R5-utilizing SHIV than had been used for vaginal infection of rhesus macaques.[7] $SHIV_{SF162P3}$ was propagated on mitogen-activated, CD8-depleted human PBMC *in vitro*, although the yield was lower compared to the X4 $SHIV_{mn229}$, with a $TCID_{50}$ of 3×10^3/ml. We administered the $SHIV_{SF162P3}$ intravaginally, with the dose delivered (6×10^3 administered over 2 days[29]) infecting all eight naive pigtail macaques studied. The mean peak viral load (VL) was 7.52 $\log_{10}$ copies/ml at 2 weeks postinfection (Fig. 1). $SHIV_{SF162P3}$ infection did not lead to an acute depletion of CD4 T lymphocytes, but a slow decline was observed in some animals (Figs. 1 and 2). Although the mean peak VL following $SHIV_{SF162P3}$ infection was similar to that observed with SIV_{mac} and $SHIV_{mn229}$ infection of pigtail macaques, the mean set point VL was $\geq$1 $\log_{10}$ lower and three of the eight macaques controlled plasma viral levels to below our detection threshold by 8 weeks postinfection (Fig. 2).

The HIV-1_{LAI} isolates infected the pigtail macaques,[21,22,28] but were poorly replicative following an *in vivo* passage. Low but detectable plasma viral RNA levels primarily occurred early following inoculation and no long-term effect on total peripheral CD4 T cells resulted. HIV-1 DNA isolated from macaque PBMC was persistently detected following infection, ensuring that the HIV-1 viral RNA detected early following infection was not due to the bolus of IV viral challenge used, however, we did not observe any macaque reactivating high levels of virus replication (data not shown[23]).

T cell immunity

The relative contributions of cellular and humoral immunity to the control of the primate lentiviral infection of pigtail macaques were then assessed. Gag-specific T cell immune responses were compared by IFN-γ ELISpot following challenge with HIV_{LAI} isolate K98227/W35, SIV_{mac251}, $SHIV_{mn229}$, and $SHIV_{SF162P3}$ using the same kit-based assay and overlapping Gag 15-mer peptides across all studies (Fig. 3A–D). Strong T cell immune responses were detected by 3 weeks following HIV_{LAI} challenge—mean response was 336.25 ($\pm$ SEM 109.7) spot-forming cells per million PBMC. Similarly, T cell responses were detected early (week 2.5) following $SHIV_{SF162P3}$ challenge (Fig. 3D). These responses declined, but remained readily detectable to week 11. SIV_{mac251} Gag-specific T cell responses were not analyzed by ELISpot very early postinfection, although variable responses were detectable from 8 weeks postchallenge, with a mean response of 280 ($\pm$ SEM 150.5) spot-forming cells per million PBMC by 21 weeks postinfection. The acutely pathogenic nature of the $SHIV_{mn229}$ infection

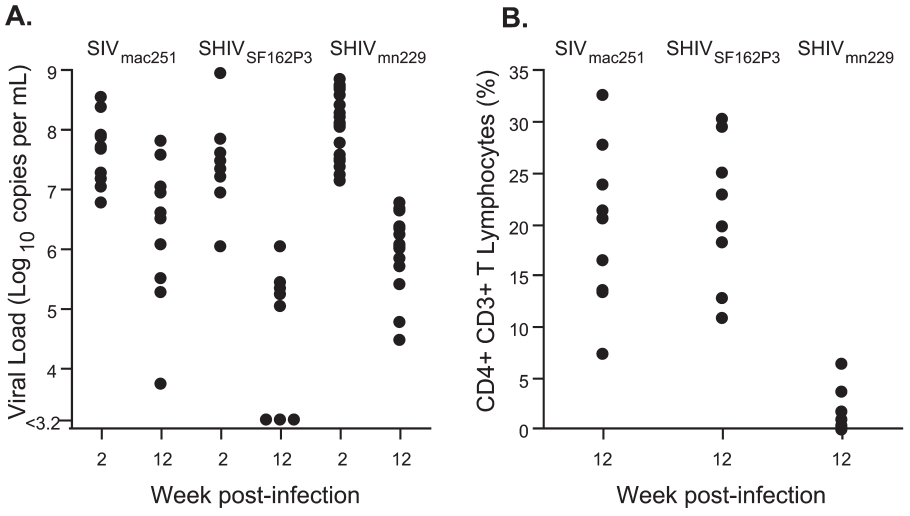

FIG. 2. Comparison of viral load (**A**) and %CD4 T cell (**B**) range between SIV_{mac251} ($n = 10$), $SHIV_{SF162P3}$ ($n = 8$), and $SHIV_{mn229}$ ($n = 15$). The range for peak (week 2) and set point (weeks 11–12 postinfection) viral load data is shown. The range of %CD4 T cells is shown at weeks 11–12 postinfection.

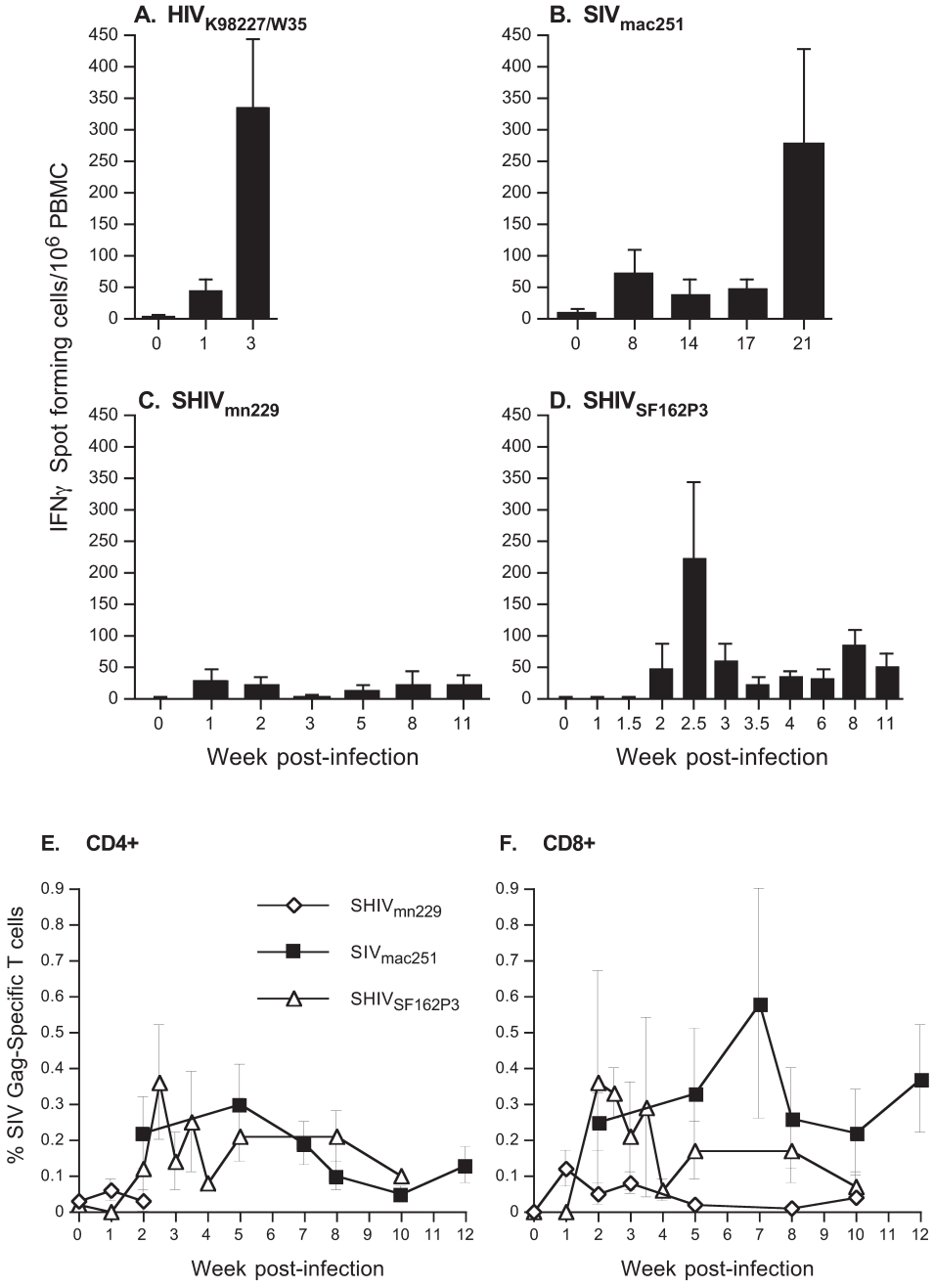

FIG. 3. Gag-specific T cell immune responses measured by IFN-γ ELISpot postchallenge and ICS. Mean ($\pm$ standard error of the mean) T cell responses detected following challenge with (**A**) HIV_{LAI} isolate K98227/W35, (**B**) SIV_{mac251}, (**C**) $SHIV_{mn229}$, and (**D**) $SHIV_{SF162P3}$. ELISpot responses are shown for each week they were analyzed postchallenge. (**E**) Mean ($\pm$standard error) $CD4^+$ and (**F**) $CD8^+$ T cell responses to SIV Gag during infection with SIV_{mac251}, $SHIV_{mn229}$, and $SHIV_{SF162P3}$ by IFN-γ ICS.

of naive pigtail macaques abrogated induction of T cell responses using the IFN-γ ELISpot assay, being consistently low over the 11-week infection course.

We also analyzed and phenotyped T cell immunity during acute SIV_{mac251}, $SHIV_{mn229}$, and $SHIV_{SF162P3}$ infection on fresh whole blood by ICS (Fig. 3E and 3F). We observed CD4 and CD8 T cell responses to a pool of SIV Gag overlapping peptides in SIV_{mac251} and $SHIV_{SF162P3}$ infected pigtail macaques, however, $SHIV_{mn229}$ inoculated animals rarely mounted Gag-specific T cell immune responses.

Humoral immunity

Western blot analysis was first used to show the profile of antibody recognition of HIV/SIV antigens by individual macaques early (week 3) and later (week 11) postinfection (Fig.

4A). Macaques infected with SIV_{mac239}, SIV_{mac251} (not shown), or $SHIV_{SF162P3}$, and, to a lesser extent, HIV-1_{LAI}, were able to mount broad and rapid specific antibody responses. In contrast, consistent with the rapid immunosuppression, antibody responses were weak or not detectable following infection with $SHIV_{mn229}$.

Neutralizing antibody responses against the infecting inoculum were also assessed for macaques challenged with $SHIV_{mn229}$ or $SHIV_{SF162P3}$. Figure 4B shows a time course of the mean NAb titer for the SHIV-infected macaques over the 11-week infection. NAb titers following $SHIV_{SF162P3}$ were detected earlier (week 4) and were greater than following $SHIV_{mn229}$ over the 11-week infection. Weaker NAb to $SHIV_{mn229}$ were not detected until 11 weeks postchallenge.

DISCUSSION

This work provides a comparative analysis of the virologic and immunologic outcomes following infection of pigtail macaques with a broad range of primate lentiviruses. SIV_{mac239} and SIV_{mac251} infection of pigtail macaques resulted in a slowly progressive infection similar to that seen in Indian rhesus macaques. Although all 15 pigtail macaques infected with SIV_{mac239} and SIV_{mac251} remained persistently viremic, significant variability is observed in set point viral loads. This is similar to human HIV-1 infection and can probably be explained in large part by MHC differences across the outbred population of pigtail macaques studied. We recently reported that expression of the pigtail macaque MHC class I molecule *ManeA*10*, which presents an immunodominant SIV Gag epitope, slows progression of SIV_{mac251} infection, similar to that observed with the *MamuA*01* molecule in Indian rhesus macaques.[17]

The X4-utilizing $SHIV_{mn229}$ infection resulted in a uniform acutely pathogenic infection similar to $SHIV_{89.6P}$ infection of Indian rhesus macaques and other macaque species including *M. nemestrina*. However, pathogenic X4-SHIV infection of pigtail or other macaque species does not mimic natural HIV-1 infection of humans particularly well, targeting a different $CD4^+$ T cell population—initially $CXCR4^+$ naive CD4 T cells and then central memory CD4 T cells[6]—than most human HIV-1 infections. Also, the acutely pathogenic nature of the X4-SHIV infection of pigtail macaques prevents the activation of detectable immune responses. Despite the rapidly progressive infection of naive macaques, even partial abrogation of acute infection with X4-utilizing SHIVs by vaccination allows generation of NAb to these strains, which are typically easier to neutralize than R5 viruses. This may result in enhanced protection being observed by vaccination following X4-utilizing SHIV challenge compared to an R5-utilizing SIV challenge.[10,30]

An R5-utilizing SHIV challenge stock in macaques, such as $SHIV_{SF162P3}$, could mimic human HIV-1 infection more closely and permit direct evaluation of HIV-1 Env-based vaccines. However, to date $SHIV_{SF162P3}$ has been variably pathogenic in rhesus macaques[7,8,31] and we observed a similar outcome following infection of pigtail macaques, with three of eight inoculated animals completely controlling virus replication to below detectable levels. Robust SHIV-specific cellular and humoral immune responses are observed in $SHIV_{SF162P3}$-infected pigtail macaques, which likely facilitate control of viremia in some animals. A variably pathogenic outcome is problematic when powering vaccine studies and we have had

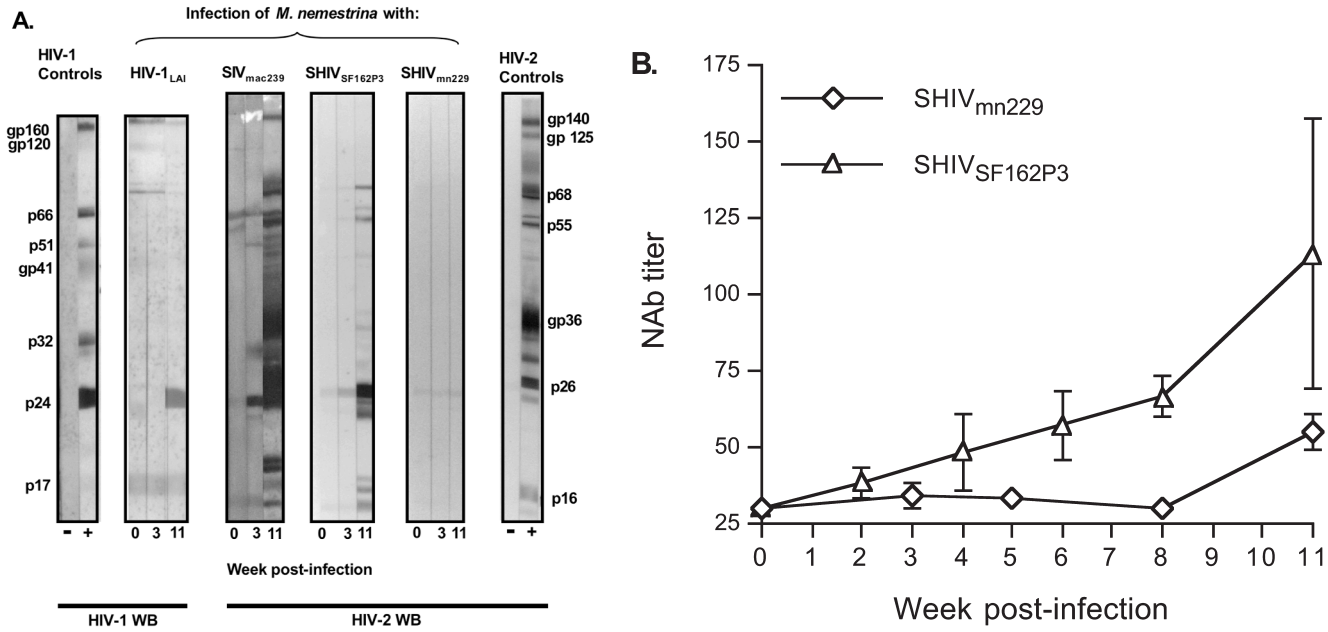

FIG. 4. Antiimmunodeficiency virus antibodies. (**A**) Western blot detection of antibodies following infection. An example of the Western blot profile following challenge with each of the immunodeficiency virus models used in pigtail macaques is shown. Human HIV-1 or HIV-2-positive and -negative sera were used as controls to help define the antigens detected. (**B**) Comparison of plasma neutralizing antibody titer following infection with $SHIV_{mn229}$ or $SHIV_{SF162P3}$.

to use acute $SHIV_{SF162P3}$ viral loads, which are less variable than set point viral load levels, as a primary virologic endpoint when using $SHIV_{SF162P3}$ as a vaccine challenge stock.[29]

It is likely that the number of *in vivo* passages and the macaque species used have an important bearing on the outcome of infection with various primate lentiviruses. For example, we recently observed that our $SHIV_{mn229}$ challenge stock has a critical T cell escape mutation at the KP9 SIV Gag epitope.[19] This occurred following passage through at least one pigtail macaque expressing the restricting *ManeA*10* molecule. Immune escape may lead to more pathogenic infections.[32] Rhesus-derived virus stocks, such as SIV_{mac239}, SIV_{mac251}, and $SHIV_{SF162P3}$, may contain T cell escape mutations restricted by Mamu MHC alleles following their *in vivo* passage in rhesus macaques, which could enhance pathogenicity in subsets of rhesus macaques sharing common MHC alleles.[33] Further passage of R5 viruses such as $SHIV_{SF162P3}$ through pigtail macaques could result in more reliably pathogenic outcomes following infection and assist robust AIDS vaccine evaluation studies in this important nonhuman primate model.

In our studies, viruses were generally given by a single route, and a comparison of our results is thus limited. Some differences have been reported in pathogenicity between IV and intravaginal routes of infection with SIVmac.[34] However, in general where high titers of challenge virus are used, the infection is uniform and the actual route may play a small role in the outcome of infection. Indeed, in our studies using SIV_{mac251}, which was given by both the IR and IV routes, no differences in outcome of infection were observed.[4,18] Further, the X4-using $SHIV_{mn229}$ resulted in a uniformly pathogenic outcome over a 500-fold difference in dose.[20–21]

Our studies have also been limited in that we have not been able to compare virus infections of pigtail macaques directly with the commonly used Indian rhesus macaque infection model. However, comparing across studies suggests SIV_{mac251} infection in pigtail macaques is comparable to similar infections of rhesus macaques. Reimann *et al*.[3] show that IV inoculation of Indian rhesus macaques with SIV_{mac251} leads to a median peak viremia of 7.49 $\log_{10}$ copies per ml and set point (days 35–77) levels of 5.69 $\log_{10}$ copies per ml, similar to our data in pigtail macaques (mean peak 7.58, mean set point 6.32 $\log_{10}$ copies per ml). Harouse *et al.*[7] have shown that intravaginal $SHIV_{SF162P3}$ infection of Indian rhesus macaques resulted in peak viremia ranging between 10^6 and 10^8 copies of RNA/ml, and the set point ranged from $<10^3$ (undetectable) to $\sim10^7$ copies of RNA/ml of plasma, again similar to our data in pigtail macaques (mean peak 7.52, mean set point 5.19 with range $<10^3$ to 10^6 $\log_{10}$ copies per ml). Similarly, our data on the X4-using $SHIV_{mn229}$ in pigtail macaques are very similar to the wide experience with X4-using $SHIV_{89.6P}$ in Indian rhesus macaques.[3]

In summary, our comparative virologic and immunologic data on six different primate lentivirus infections of pigtail macaques highlight the advantages and disadvantages of each system. We found that SIV_{mac} infection reliably results in a pathogenic infection most closely related to human HIV-1 infection. One caveat with pigtail macaque studies using SIV_{mac251}, and most likely other viruses, is that care must be taken in evenly allocating pigtail macaques with MHC alleles such as *ManeA*10* that assist in the partial control of viremia between control and active vaccine groups.[4] This is similar to the situation with Indian rhesus macaques.[17] Controlling this important nonvaccine variable should help progress pigtail macaque AIDS vaccine studies into clinical trials.

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