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Macaque species susceptibility to simian immunodeficiency virus: increased incidence of SIV central nervous system disease in pigtailed macaques versus rhesus macaques

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Abstract Immune pressure exerted by MHC class I-restricted cytotoxic T cells drives the development of viral escape mutations, thereby regulating HIV disease progression. Nonetheless, the relationship between host immunity and HIV central nervous system (CNS) disease remains poorly understood. The simian immunodeficiency virus (SIV) macaque model recapitulates key features of HIV infection including development of AIDS and CNS disease. To investigate cell-mediated immunity regulating SIV CNS disease progression, we compared the incidence of SIV encephalitis and the influence of MHC class I allele expression on the development of CNS disease in rhesus macaques (*Macaca mulatta*) versus pigtailed macaques (*Macaca nemestrina*). After inoculation with the immunosuppressive swarm SIV/DeltaB670 and the neurovirulent molecular clone SIV/17E-Fr, pigtailed macaques progressed more rapidly to AIDS, had higher plasma and cerebrospinal fluid (CSF) viral loads, and were more likely to progress to SIV-associated encephalitis (SIVE) compared to rhesus macaques. In addition, MHC class I alleles were neuroprotective in both species (*Mamu-A*001* in rhesus macaques and *Mane-A1*084:01:01* in pigtailed macaques); animals expressing these alleles were less likely to develop SIV encephalitis and correspondingly had lower viral replication in the brain. Species-specific differences in susceptibility to SIV disease demonstrated that cell mediated immune responses are critical to SIV CNS disease progression.

Keywords SIV · Macaque · CNS disease · MHC class I

Importance

Although advances in HIV treatment have reduced AIDS mortality and incidence of severe neurological complications including dementia, HIV-associated cognitive deficits still develop in patients receiving anti-retroviral therapy. The SIV macaque model recapitulates key features of HIV infection including development of AIDS and CNS disease. To investigate host factors regulating SIV CNS disease progression, we compared outcomes of SIV infection in two different species of Asian macaques commonly used to model HIV-associated diseases: rhesus macaques and pigtailed macaques. We found that pigtailed macaques had more rapid progression of disease and were more likely to progress to encephalitis. We also found that the expression of particular MHC class I alleles had a neuroprotective effect in each species. Differences in susceptibility to SIV disease development among species of Asian macaques demonstrate that host factors play critical roles in regulating SIV disease progression, including SIV CNS disease.

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Introduction

Given the marked similarities between simian immunodeficiency virus (SIV) and HIV, SIV/macaque models have been extremely valuable for elucidating the pathogenesis of HIV, discovering therapeutics, and developing vaccines. The most widely used species for SIV studies include rhesus macaques (*Macaca mulatta*) and pigtailed macaques (*Macaca nemestrina*). Although SIV inoculation of Indian-origin rhesus macaques is the best characterized primate model of HIV infection, variable disease progression after SIV inoculation and limited availability of these animals have promoted interest in the use of alternate macaque species for SIV studies (Cohen 2000). In particular, pigtailed macaques have gained popularity as a primate model to study HIV pathogenesis because of their larger size, tractable temperament, and susceptibility to SIV infection (Klatt et al. 2012). Previous studies have suggested that SIV infection in pigtailed macaques more closely recapitulates HIV infection in humans and accelerates progression to SIV-induced encephalitis compared to SIV-inoculated rhesus macaques (Batten et al. 2006; Zink et al. 1997). Recently, HIV-1 was adapted to successfully induce AIDS in a CD8+ T cell-depleted pigtailed macaque model (Hatziioannou et al. 2014).

A great deal of evidence has identified cytotoxic lymphocyte-mediated MHC class I dependent immune responses as a crucial mechanism for HIV control (Limou and Zagury 2013; Pereyra et al. 2010). In particular, *HLA-B*5701* and *HLA-B*27* MHC class I alleles are overrepresented in individuals with delayed progression to AIDS (Blankson 2010; Goulder et al. 1996; Kaslow et al. 1996; Migueles et al. 2000; Schneidewind et al. 2007). Similar relationships have been identified in SIV/macaque models. MHC class I alleles of rhesus macaques (*M. mulatta*) have been especially well characterized in the context of SIV (Andrade et al. 2009; Goulder and Watkins 2008; Nakamura et al. 2011; Reed et al. 2011). The rhesus macaque MHC class I alleles *Mamu-A1*001*, *Mamu-B*17*, and *Mamu-A*07* are associated with increased viral control and decreased progression to AIDS (Mothe et al. 2003; Muhl et al. 2002; O'Connor et al. 2003; Pal et al. 2002; Reed et al. 2011; Wu et al. 2011). Like humans and rhesus macaques, individual MHC class I alleles that present SIV peptides to cytotoxic T cells have also been identified in pigtailed macaques (Mankowski et al. 2008; Queen et al. 2011; Smith et al. 2005b). Of these, the pigtailed macaque MHC class I allele *Mane-A*084:01* (formerly named *Mane-A*10*) has been associated with lower peak plasma viral load and slower progression to AIDS in animals inoculated with SIVmac251 (Klatt et al. 2012). In our well-characterized model of HIV CNS disease using pigtailed macaques inoculated with both SIV/17E-Fr and SIV/DeltaB670, we found that *Mane-A*084:01* is a neuroprotective allele associated with decreased likelihood of progression to SIV CNS disease independent of influencing plasma viral load (Mankowski et al. 2008; Queen et al. 2011). Interestingly, *Mane-A*084:01* recognizes an immunodominant SIV Gag epitope KP9 that is homologous to the HIV Gag epitope KF11, which is restricted by *HLA-B*57:01* in humans (Smith et al. 2005a, b).

Few studies have directly compared SIV disease progression in rhesus and macaques and pigtailed macaques (Benveniste et al. 1988; Klatt et al. 2012; Polacino et al. 2008), and no studies have reported in detail whether the induction of SIV CNS disease varies between these two macaque species. Although advances in HIV treatment have dramatically reduced the incidence of HIV-associated dementia (HAD), neurocognitive defects in treated HIV-infected individuals persist, manifest as less severe HIV-associated neurocognitive deficits (HAND) (Spudich 2013). SIV/macaque models will be essential for establishing the causes of HAND and for testing compounds to effect virus eradication.

The accelerated consistent SIV pigtailed macaque model of CNS developed at Johns Hopkins recapitulates key features of HIV-associated neurologic disease, including high viral load in the cerebrospinal fluid (CSF) and encephalitis with abundant SIV replication in the brain (Clements et al. 2008; Zink et al. 1997; Zink et al. 1999), as well as PNS damage (Laast et al. 2007; Laast et al. 2011), and therefore serves as the basis for comparison with disease progression in rhesus macaques inoculated with the same SIV combination. To investigate whether host factors regulate SIV CNS disease progression, we compared the course of disease and the incidence of SIV encephalitis (SIVE) in rhesus and pigtailed macaques that were dual-inoculated with both SIV/DeltaB670, an immunosuppressive swarm, and SIV/17E-Fr, a neurovirulent molecular clone (Zink et al. 1997; Zink et al. 1999).

Methods

Animal studies

Forty-four pigtailed macaques (*M. nemestrina*) and 29 rhesus macaques (*M. mulatta*) were intravenously inoculated with both SIV/DeltaB670 (50 AID50) and SIV/17E-Fr (10,000 AID50) as previously described (Zink et al. 1997). Animals did not receive any treatment and were perfused with sterile saline at euthanasia to remove blood and circulating virus from brain. Animals were MHC class I typed by sequence-specific primer (SSP)-PCR (Mankowski et al. 2008). By design, pigtailed macaques were euthanized at approximately 84 days post-inoculation or at the onset of AIDS-defining illness (whichever came first) given consistent progression to AIDS at this time point (Zink et al. 1997; Zink et al.

1999). Initially, three rhesus macaques (RH25, RH26, and RH27) also were euthanized at day 84 post-inoculation to compare directly with pigtailed macaque study endpoints. As these three rhesus macaques did not develop either AIDS or CNS disease, the subsequent 26 rhesus macaques were euthanized at the onset of AIDS-defining illness, which ranged from 36 to 560 days post-inoculation. Animals were euthanized if any two of the following signs developed: weight loss greater than 15 % of baseline, clinical signs of organ-specific disease (i.e., CNS, lung, etc.), intractable diarrhea, or opportunistic infection. All animal procedures in this study were performed according to the principles set forth by the Institutional Animal Care and Use Committee at Johns Hopkins University and the National Research Council's Guide for the care and use of laboratory animals.

Quantitation of SIV RNA

To measure ongoing viral replication in infected animals, SIV RNA in plasma, CSF, and tissues was measured by qRT-PCR using primers in the SIV gag region as previously described (Clements et al. 2002; Queen et al. 2011). The primers used to detect unspliced viral RNA were: forward primer, SGAG03, 5′-CAGGGAAIIAAGCAGATGAATTAG-3′; reverse primer, SGAG04, 5′-GTTTCACTTTCTCTTCTGCGTG-3′; and probe, pSUS05, 5′-(6-carboxyfluorescein [FAM])ATTTGG A T T A G C A G A A A G C C T G T T G G A G (6-carboxytetramethylrhodamine [TAMRA])-3′ with cycle conditions of 50 °C for 30 min, 94 °C for 15 min to reverse transcribe RNA, which was followed by 45 cycles of PCR at 94 °C for 15 s, 55 °C for 15 s, and 60 °C for 30 s (detection limit 100 copies/mL) (Queen et al. 2011).

Quantitation of pro-inflammatory markers in the CSF

CSF samples were centrifuged to remove cells then stored at −80 C for subsequent analysis. CSF levels of IL-6 and CCL2 were measured by ELISA (R&D Systems, Minneapolis, MN) as previously described (Mankowski et al. 2004).

Histopathology

Sections of brain were scored for the presence of encephalitis as previously described (Mankowski et al. 2004). SIV-associated encephalitis was defined as multifocal perivascular accumulations of macrophages and multinucleated giant cells and glial nodules (Mankowski et al. 2004). Hematoxylin and eosin stained sections of frontal and parietal cortex, basal ganglia, thalamus, midbrain, and cerebellum were scored in a blinded fashion by two pathologists.

MHC class I allele genotyping

Expression of *Mane-A1*084:04:01* in pigtailed macaques was determined by sequence-specific PCR as previously described (Mankowski et al. 2008). MHC class I genotyping in rhesus macaques was determined by multiple allele-specific PCR by the MHC Typing Service provided by David Watkins as previously described (Kaizu et al. 2007).

Statistical analysis

Survival curves for pigtailed macaques and rhesus macaques up to day 84 post-inoculation were compared by the Gehan-Breslow-Wilcoxon test. Similarly, groupwise comparisons of plasma and CSF viral load, CD4+ T cell counts, and CSF IL-6 and CCL2, and terminal tissue viral load were performed using the two sample nonparametric Mann–Whitney test. The Fisher's exact test (one-sided) was used to identify associations between MHC class I allele expression versus development of SIV CNS disease.

Results

Accelerated progression to AIDS with a greater decline in CD4+ lymphocytes in pigtailed macaques compared to rhesus macaques

SIV-inoculated rhesus macaques survived significantly longer than pigtailed macaques (Fig. 1a). All pigtailed macaques developed AIDS-defining criteria and were euthanized by approximately three months p.i. (mean 78 days p.i.) with a steady decline in CD4+ T cells from day 45 post-inoculation onwards. In contrast, rapid progression to AIDS in rhesus macaques was much less common; although four rhesus macaques progressed rapidly (<60 days p.i.), most rhesus macaques developed AIDS and were euthanized between 120 and 350 days p.i. (mean 213 days p.i.; Table 1) Interestingly, rhesus macaques and pigtailed macaques had similar rates of progression to AIDS before 60 days p.i. (Fig. 1b); however, between 60 and 80 days p.i., pigtailed macaques progressed to AIDS at a more rapid rate than rhesus macaques. By design, all pigtailed macaque studies were terminated by 90 days p.i. due to uniform progression to AIDS defining criteria.

To compare kinetics of immunosuppression after SIV infection, we measured CD4+ T cell counts in blood longitudinally in all animals, revealing a greater decline from baseline levels of CD4+ T cells in pigtailed macaques compared to rhesus macaques throughout the course of infection (Fig. 1c). There was no significant difference between absolute CD4+ T cell counts at 84 p.i. in rhesus macaques compared to pigtailed macaques; however, rhesus macaques had a

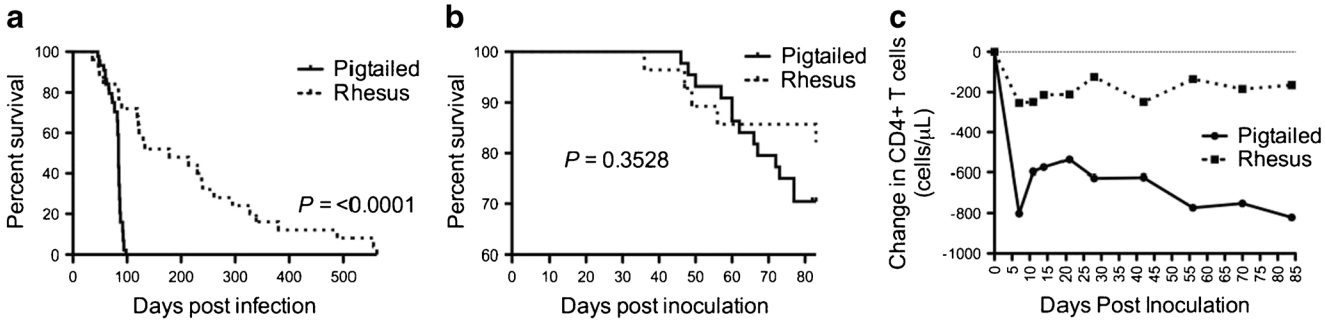

Fig. 1 Survival and CD4+ T cell loss in pigtailed macaques and rhesus macaques. SIV-inoculated rhesus macaques (*dotted line*) survived significantly longer than pigtailed macaques (*solid line*) (**a**), although up to approximately day 60 p.i. both species have similar progression to AIDS (**b**). After SIV-inoculation, pigtailed macaques had a greater drop in CD4 T cells (*solid line*) than rhesus macaques (*dotted line*) as measured in absolute change from prebleed baseline (**c**). *P* value for the survival curves were compared using the Gehan-Breslow-Wilcoxon Test

significantly lower terminal absolute CD4+ lymphocyte count (median 169 cells/μL) than pigtailed macaques (median 337 cells/μL; P=0.0003, Mann–Whitney, data not shown). The nadir median CD4+ cell count in rhesus was reached at >300 days p.i. consistent with a more protracted course of disease progression in rhesus macaques than pigtailed macaques.

Pigtailed macaques consistently developed higher viral loads in both plasma and cerebrospinal fluid than rhesus macaques

SIV RNA was measured in both the plasma and CSF of all animals throughout the course of infection (Fig. 2a, b). Pigtailed macaques had a significantly higher viral load than rhesus macaques at day 84 p.i. in both plasma (Fig. 2c; P=0.0002, Mann–Whitney) and CSF (Fig. 2d; P=0.0093, Mann–Whitney *t* test). Even with the longer duration of infection in rhesus macaques compared to pigtailed macaques, terminal viral loads were higher in pigtailed macaques in both the plasma (P=0.009, Mann–Whitney *t* test) and CSF (P=0.062, Mann–Whitney).

Pigtailed macaques developed SIV-induced CNS disease more frequently than rhesus macaques

To quantify the incidence and severity of SIV-induced CNS disease, the CNS of all animals were scored for the presence of SIV encephalitis, defined by presence of multifocal glial nodules and perivascular cuffs of macrophages and multinucleated giant cells. By these established criteria, pigtailed macaques developed SIV encephalitis more frequently than rhesus macaques. (Table 2; P=0.068, one-sided Fisher's exact test). Correspondingly, pigtailed macaques had higher levels of CCL2 (MCP-1) in the CSF than rhesus macaques during both acute (Fig. 3b; P=<0.0001 at d7 p.i, Mann–Whitney) and chronic phases of infection (P=0.046 at d84 p.i. Mann–Whitney). In addition, when terminal CSF CCL2 levels were compared, CCL2 was significantly higher (P=0.045, Mann–Whitney *t* test) in pigtailed macaques compared to rhesus macaques. Although CSF IL-6 levels in pigtailed macaques were slightly higher than rhesus macaques in acute and chronic stages of infection, these differences were not significant (P=0.13 and 0.22, respectively; Mann–Whitney). There was no species difference in terminal CSF IL-6 levels (P=0.92, Mann–Whitney).

Comparable terminal tissue SIV RNA levels in pigtailed macaques and rhesus macaques

To compare the extent of virus replication in peripheral tissues and the CNS at terminal time points, we measured SIV RNA levels in the spleen and basal ganglia (Fig. 4a, b) from rhesus macaques and pigtailed macaques. While viral RNA levels were higher in both the spleen and basal ganglia of pigtailed macaques compared to rhesus macaques, there was not a significant difference between the two groups.

MHC class I alleles play an important role in the development of SIV-encephalitis in both pigtailed macaques and rhesus macaques

To determine whether host genetics contribute to the progression of neurologic disease across macaque species, we genotyped all animals for expression of MHC class I alleles previously associated with lower plasma viral loads including *Mamu-A1*001* in rhesus macaques and *Mane-01*084:01:01* in pigtailed macaques. Five of 29 (17 %) of rhesus macaques expressed *Mamu-A1*001*; 14 of 44 (32 %) of the pigtailed cohort expressed *Mane-01*084:01:01* (Table 3). In pigtailed macaques, *Mane-01*084:01:01* expression was not associated with either altered plasma or CSF viral loads (Fig. 5a, c). In contrast, rhesus macaques that expressed *Mamu-A1*001* had significantly lower terminal plasma viral loads (Fig. 5b; P=0.0190, Mann–Whitney), but viral load in the CSF was not associated with this MHC class I allele (Fig. 5d; P=0.3710, Mann–Whitney). Neither allele was associated with altered levels of CSF CCL2 or IL-6 (data not shown). However, both pigtailed macaques expressing *Mane-01*084:01:01* and

Table 1 MHC I allele genotyping and presence of SIV-associated encephalitis at death/euthanasia

Pigtailed macaques				Rhesus macaques			
Animal ID	Days PI	SIVE	Mane-01*084:01:01	Animal ID	Days PI	SIVE	Mamu-A*001
PT20	46	+	−	RH01	36	+	−
PT43	48	+	+	RH02	47	+	−
PT33	50	+	−	RH03	49	+	−
PT18	57	+	−	RH04	56	+	−
PT42	60	+	−	RH05	84	−	+
PT41	60	+	−	RH06	85	+	−
PT37	62	+	−	RH25[a]	85	−	−
PT21	66	+	−	RH26[a]	86	−	−
PT27	67	+	−	RH27[a]	87	−	
PT11	72	+	−	RH07	90	+	−
PT30	73	+	−	RH19	119	+	−
PT32	77	+	+	RH08	121	+	−
PT17	77	+	+	RH20	122	+	−
PT26	82	+	−	RH28	130	−	
PT25	82	+	−	RH24	133	+	−
PT34	83	+	−	RH09	178	+	−
PT14	83	+	−	RH29	214	−	−
PT20	83	−	+	RH10	230	−	−
PT44	84	−	−	RH21	238	−	+
PT35	84	−	−	RH11	240	−	−
PT31	84	−	−	RH12	261	−	−
PT29	84	+	−	RH13	295	−	−
PT28	84	−	+	RH22	327	−	+
PT19	84	−	+	RH14	339	−	−
PT16	84	+	−	RH15	380	−	−
PT23	85	−	+	RH16	489	−	+
PT15	85	−	−	RH23	532	−	−
PT12	85	+	+	RH17	554	−	−
PT08	85	+	−	RH18	560	−	+
PT23	86	+	+				
PT22	86	−	+				
PT03	86	+	−				
PT39	87	−	−				
PT09	87	+	−				
PT02	87	+	−				
PT40	88	−	+				
PT04	88	+	−				
PT07	92	−	+				
PT01	92	+	−				
PT36	93	−	+				
PT05	93	−	−				
PT10	94	+	−				
PT06	94	−	−				
PT38	98	−	+				

A total of 44 pigtailed macaques and 29 rhesus macaques were dual inoculated with SIV/B670 and SIV/17E-Fr

[a] The initial three rhesus macaques that were dual-inoculated were euthanized at d84 prior to the development of AIDS

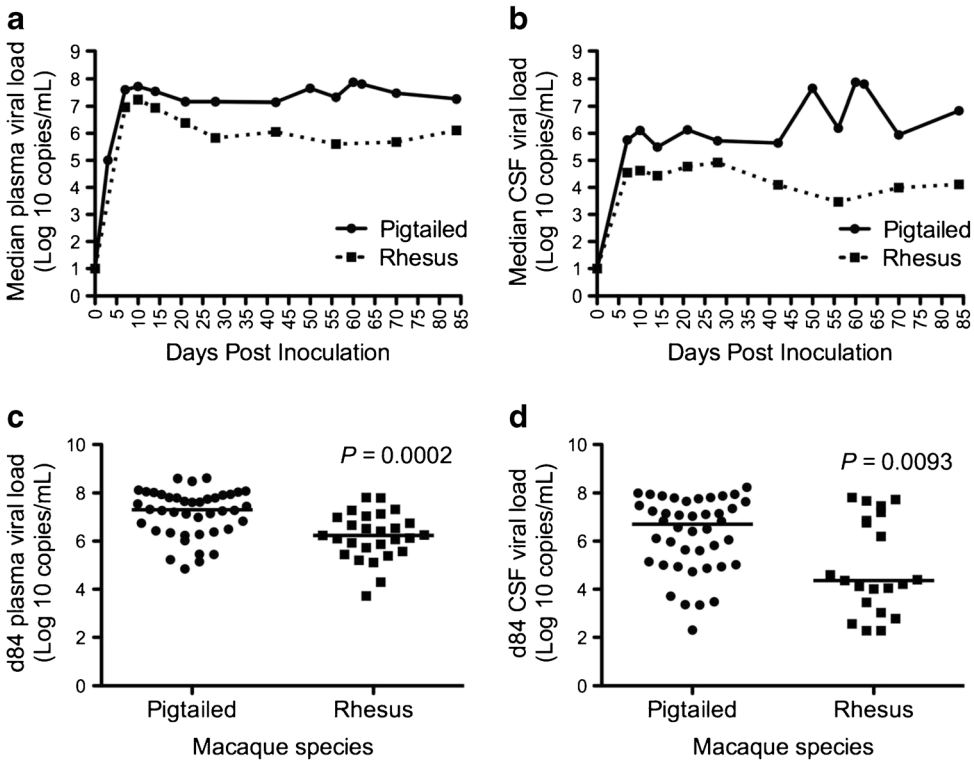

Fig. 2 Comparative viral replication between pigtailed and rhesus macaques in plasma, CSF, and tissues. The viral load in both plasma (**a**) and CSF (**b**) was higher in pigtailed macaques (*solid line*) compared to rhesus macaques (*dotted line*). By d84 p.i., viral load in the plasma (**c**) and CSF (**d**) were significantly lower in rhesus macaques (*boxes*) compared to pigtailed macaques (*circles*). *P* values for groupwise comparisons (**c**, **d**) were determined using the Mann–Whitney test. Horizontal lines represent the median

rhesus macaques expressing *Mamu-A1*001* had lower levels of SIV RNA (P=0.057 and P=0.011, respectively, Mann–Whitney) in the brain consistent with MHC class I-mediated neuroprotection (Fig. 5e and f).

Discussion

Rhesus macaques and pigtailed macaques have proven to be valuable animal models of HIV due to their susceptibility to SIV; however, few studies have directly compared SIV outcomes in these different macaque species. Over the last 20 years, we have studied a large number of both pigtailed macaques and rhesus macaques that were inoculated with the same SIV combination, allowing a large retrospective comparative study. Based on our observations and other reports (Klatt et al. 2012; Zink et al. 1997), we hypothesized that rhesus macaques would progress to AIDS more slowly and develop SIV CNS disease less frequently. In addition, we examined whether the MHC class I allele *Mamu-A1*001* would show similar neuroprotective effects in rhesus macaques as previously shown for the *Mane-A1*084:01:01* MHC class I allele in pigtailed macaques (Mankowski et al. 2008).

Table 2 The incidence of SIV-associated encephalitis in pigtailed macaques compared to rhesus macaques

Species	SIVE−	SIVE+	Total
Pigtailed	16	28	44
Rhesus	15	11	26
Totals	31	39	70

Pigtailed macaques are more likely to develop SIV-associated encephalitis (SIV-E) than rhesus macaques based on histopathologic scoring (P= 0.0686, one-sided Fisher's exact test)

SIV-infected pigtailed macaques progressed more rapidly than rhesus macaques, with shorter survival time post-inoculation, a greater decline in CD4+ cells, and higher longitudinal plasma and CSF viral load compared to rhesus macaques. In addition, at terminal time points, plasma and CSF viral loads as well as SIV RNA levels in the spleen and brain were higher in pigtailed macaques than rhesus macaques. Overall, pigtailed macaques died or were euthanized due to AIDS-defining illness much earlier than rhesus macaques. Although we initially suspected that rhesus macaques would exhibit a similar course of infection as pigtailed macaques, when the first three rhesus macaques were euthanized at three months post-infection (the time point by which all pigtailed macaques had progressed to AIDS), none of the rhesus macaques developed encephalitis or AIDS-defining illnesses. Thus, subsequent rhesus macaques were allowed to progress until the development of AIDS-defining illness, which in many animals did not occur until after 200 days post inoculation.

Consistent with previous findings in both rhesus and pigtailed macaques (Benveniste et al. 1988; Klatt et al. 2012;

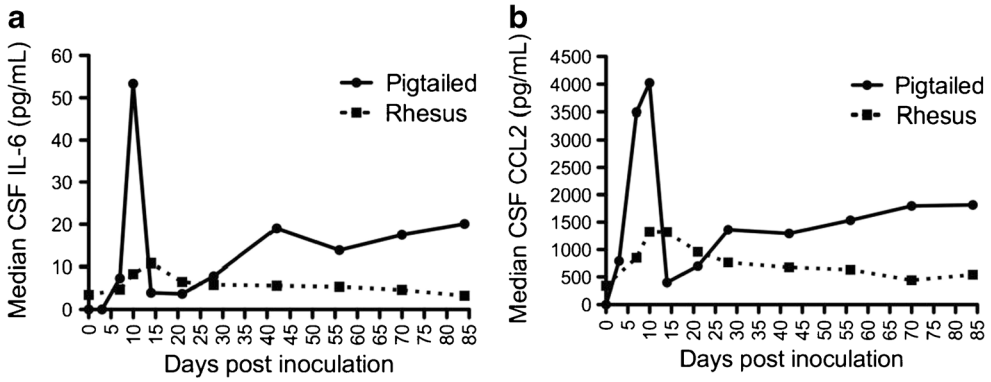

Fig. 3 Longitudinal pro-inflammatory markers in the CSF in pigtailed macaques versus rhesus macaques. CSF levels of IL-6 (**a**) and MCP-1 (CCL2) (**b**) were higher at acute, asymptomatic, and chronic/terminal phases of disease in pigtailed macaques (*solid line*) compared to rhesus macaques (*dotted line*)

Kneitz et al. 1993; Letvin and King 1990; McClure et al. 1989), we found decreases in CD4+ T cell levels in both species after SIV inoculation; however, there was a much greater decrease in CD4+ T cells from pre-inoculation baseline levels in pigtailed macaques. Pigtailed macaques showed a classic pattern of acute CD4+ T cell decrease followed by a slight rebound then a progressive loss of CD4+ T cells over time. Conversely, rhesus macaques had an initial drop that was sustained over 84-days post-inoculation. The median CD4+ T cell levels then continued to decline over time as animals progressed past 500 days, consistent with rhesus macaques progressing more slowly to end-stage disease than pigtailed macaques despite comparable acute viremia.

Rhesus macaques did not develop neurologic disease as frequently as pigtailed macaques. With the dual-inoculum of the neurovirulent molecular clone SIV/17E-Fr and the immunosuppressive swarm SIV/B670, 64 % of pigtailed macaques developed SIVE compared to 42 % of rhesus macaques, although the mean time to euthanasia for rhesus macaques (213 days) was substantially longer than pigtailed macaques (78 days). We also compared several well-defined biomarkers predictive of SIV CNS disease including CSF IL-6 and CCL2 (Mankowski et al. 2004). Pigtailed macaques had higher IL-6 and CCL2 in the CSF at acute (d10 p.i.) and chronic (d42 p.i. to terminal) time points. Terminal CSF IL-6 levels in rhesus macaques were similar to pigtailed macaque values, consistent with prolonged disease progression in rhesus macaques. In contrast, terminal CSF CCL2 levels were significantly higher in pigtailed macaques than rhesus macaques.

Recently, Klatt et al. performed a retrospective study that compared SIVmac239 infection in rhesus macaques versus pigtailed macaques. Although pigtailed macaques developed AIDS more rapidly than rhesus macaques, they did not have a correspondingly higher plasma viral load in contrast to our findings in this report (Klatt et al. 2012). Previously, Polacino et al. compared $SHIV_{SF162\ P4}$ infection in pigtailed macaques versus rhesus macaques and concluded that pigtailed macaques had significantly higher peak and set-point (100+ days p.i.) plasma viral loads as well as a significantly higher proportion of animals that showed persistent viremia (Polacino et al. 2008). Similar to the findings of Polacino et al. our group of SIV-inoculated pigtailed macaques had higher viral loads in

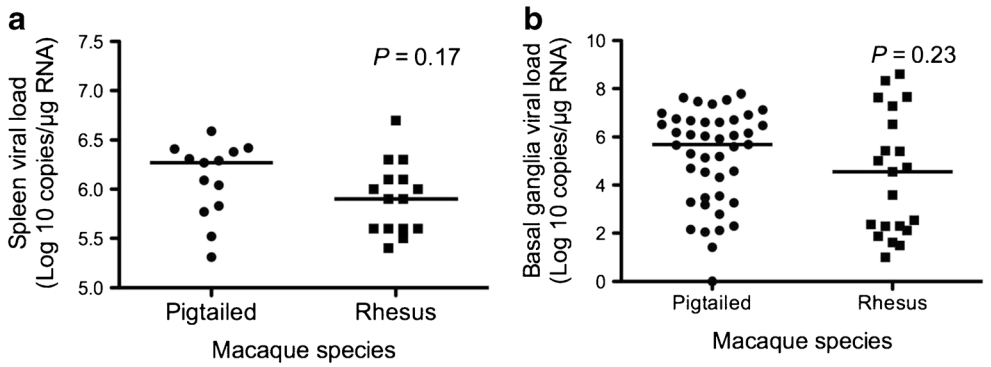

Fig. 4 Comparative terminal tissue viral loads in pigtailed and rhesus macaques. Viral load in the spleen (**a**) and basal ganglia (**b**) were higher in pigtailed macaques, although these differences are not statistically significant. *P* values for groupwise comparisons were determined using the Mann–Whitney test. *Horizontal lines* represent the median

Table 3 The effect of host genetics on development of SIVE in pigtailed and rhesus macaques

Pigtailed macaques				Rhesus macaques			
Mane-A1*084:01:01	SIVE−	SIVE+	Total	Mamu-A*001	SIVE−	SIVE+	Total
POS	9	5	14	POS	5	0	5
NEG	7	23	30	NEG	12	10	22
Total	16	28	44	Total	17	10	27

Both Mane-A1*084:01:01 in pigtailed macaques and Mamu-A*001 in rhesus macaques were neuroprotective, in that expression of these alleles was associated with failure to develop encephalitis. This association was stronger in pigtailed macaques (P=0.0113, one-sided Fisher's exact) compared to rhesus macaques (P=0.0377, one-sided Fisher's exact), although expression of the neuroprotective MHC class I alleles significantly decreased the risk of progression to SIV CNS disease in both species

both plasma and CSF than rhesus macaques. Additional important differences between our study and previous reports include persistent viremia with much higher plasma viral loads, and uniform progression to AIDS-defining illness in all pigtailed macaques in a relatively short time frame. This is likely due to the specific inoculum used in our cohort; all animals in our cohort were dual-inoculated with a macrophage-tropic, neurovirulent clone as well as an immunosuppressive swarm, as opposed to single inoculation with cloned SIVmac239 used in the aforementioned studies.

Another study compared the neuropathogenesis of simian-human immunodeficiency virus (SHIV)(KU-2) infection of rhesus macaques versus pigtailed macaques and found that 21 of 22 pigtailed macaques failed to develop SHIV-associated neurologic disease or productive CNS viral replication. While the virus was macrophage tropic in rhesus macaques, leading to effective CNS viral replication in most animals, the lack of neurologic disease in pigtailed macaques was attributed to the inherent failure of the SHIV to replicate in pigtailed macaque macrophages (Buch et al. 2002). Macrophage tropism has been well established as a prerequisite for SIV or SHIV strains to cause lentiviral-associated neurologic disease in both rhesus and pigtailed macaques (Buch et al. 2002; Czub et al. 1996; Mankowski et al. 1997; Stephens et al. 1997).

This is the first study to directly compare the pathogenesis of rhesus macaques and pigtailed macaques specifically challenged with a neurovirulent SIV inoculum. To examine the effects of host genetics on disease progression, we focused on the most well-characterized MHC class I allele in each macaque species: *Mamu-A1*001* in rhesus macaques and *Mane-A*084:01:01* in pigtailed macaques. Although there is a large amount of variation in *Mamu-A1*001* expression between different wild and captive populations of rhesus macaques, 31.8 % of the pigtailed macaques in this study expressed *Mane-A*084:01:01* which is similar to prevalence reported in other studies (Pratt et al. 2006). Of our rhesus, 18.5 % expressed *Mamu-A1*001* which falls within the range of reported frequency of *Mamu-A1*001* expression that varies from <1 to 33 % depending on the population and country of origin (Kanthaswamy et al. 2010; Kyes et al. 2006; Muhl et al. 2002).

In this study, expression of the *Mamu-A1*001* allele in rhesus macaques was associated with a lower plasma viral load, consistent with previously reported findings (Lim et al. 2010; Mothe et al. 2003; Muhl et al. 2002; Pal et al. 2002). Surprisingly, this difference seen in the peripheral virus did not translate to the CSF, a compartment in which levels of viral RNA were not associated with expression of this MHC class I allele. We nonetheless found lower levels of SIV RNA and less inflammation in the brains of rhesus macaques that expressed *Mamu-A1*001*, demonstrating that *Mamu-A1*001* is neuroprotective. This suggests that MHC class I-mediated viral control in the brain differs from control in the periphery, underscoring the unique nature of CTL efficacy in the central nervous system. While there was no difference in either plasma or CSF viral loads of pigtailed macaques associated with expression of *Mane-A*084:01:01*, both SIV RNA and inflammation in the brain were lower in pigtailed macaques that expressed *Mane-A*084:01:01*.

This study illustrates the marked differences in SIV disease progression that develop in genetically distinct species after inoculation with an identical neurovirulent inoculum. In addition, the lack of appreciable difference in plasma viral loads between pigtailed macaques and rhesus macaques at acute infection (d10 p.i. P=0.16, Mann–Whitney) implies that intrinsic or innate immune responses are not playing a major role in species differences. Rather, adaptive immune responses, in particular cell-mediated immunity, are the likely major determinant of species differences in disease outcome to SIV. Clearly, the relationship between MHC class I alleles and disease outcome is complex and multifactorial, and no single MHC class I allele will define the whole immunologic outcome. Thus, deeper and more comprehensive MHC class I haplotyping of different macaque species will be critical to understand the relationship between host immunogenetics and viral immunity. This is

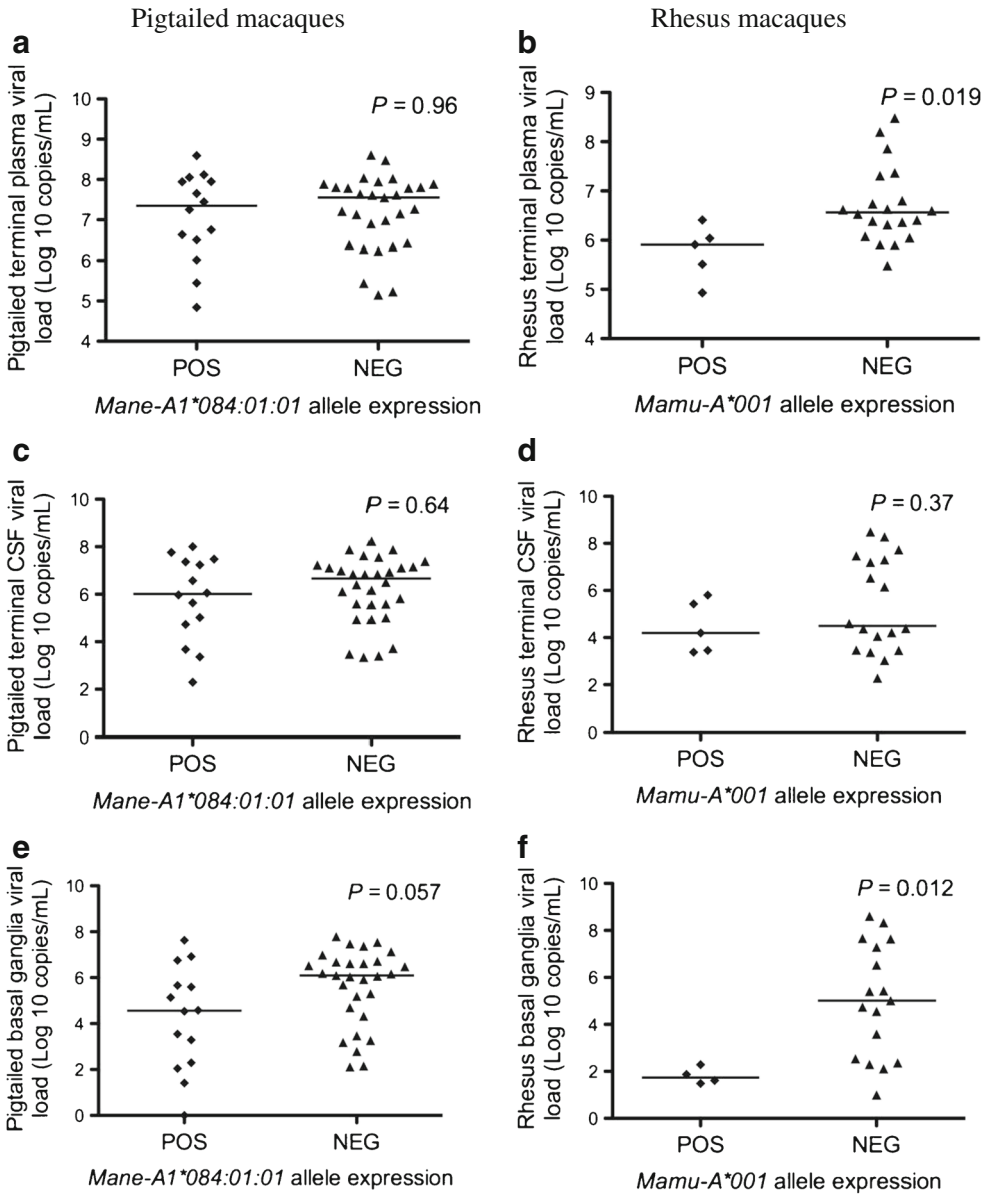

Fig. 5 The effect of host genetics on plasma, CSF, and brain viral loads in pigtailed and rhesus macaques. Terminal viral load levels segregated by MHC class I allele expression were compared in plasma (**a**, **b**), CSF (**c**, **d**), and basal ganglia (**e**, **f**) in pigtailed and rhesus macaques, repectively. Although differences in terminal plasma and CSF viral loads were variable, immunodominant MHC class I alleles in both species were associated with decreased brain viral load (**e** and **f**). *P* values for groupwise comparisons were determined using the Mann–Whitney test. *Horizontal lines* represent medians

an important consideration for preventive and therapeutic vaccine development because vaccination against one single SIV epitope is unlikely to be completely effective. Furthermore, cytotoxic T cell responses do not develop in isolation from other associated inflammatory responses and may be linked to macrophage and microglial immune activation in the CNS; additional study of these potential relationships will clarify casual interactions betweem CTLs and more generalized CNS inflammatory responses. To understand the differences between individual responses to HIV-1 infection, we must try to understand the differences observed in primate models of lentiviral disease, the best animal models available to study HIV pathogenesis.

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Conflict of interest The authors declare they have no conflict of interest.

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