J. Neurovirol. (2018) 24:204–212
DOI 10.1007/s13365-017-0582-4

REVIEW

An SIV/macaque model targeted to study HIV-associated neurocognitive disorders

Sarah E. Beck[1] · Suzanne E. Queen[1] · Kelly A. Metcalf Pate[1] · Lisa M. Mangus[1] · Celina M. Abreu[1] · Lucio Gama[1] · Kenneth W. Witwer[1,2] · Robert J. Adams[1] · M. Christine Zink[1] · Janice E. Clements[1,2,3] · Joseph L. Mankowski[1,2,3]

Received: 13 June 2017 / Revised: 25 August 2017 / Accepted: 14 September 2017 / Published online: 3 October 2017

Abstract Simian immunodeficiency virus (SIV) infection of pigtailed macaques is a highly representative and well-characterized animal model for HIV neuropathogenesis studies that provides an excellent opportunity to study and develop prognostic markers of HIV-associated neurocognitive disorders (HAND) for HIV-infected individuals. SIV studies can be performed in a controlled setting that enhances reproducibility and offers high-translational value. Similar to observations in HIV-infected patients receiving antiretroviral therapy (ART), ongoing neurodegeneration and inflammation are present in SIV-infected pigtailed macaques treated with suppressive ART. By developing quantitative viral outgrowth assays that measure both CD4+ T cells and macrophages harboring replication competent SIV as well as a highly sensitive mouse-based viral outgrowth assay, we have positioned the SIV/pigtailed macaque model to advance our understanding of latent cellular reservoirs, including potential CNS reservoirs, to promote HIV cure. In addition to contributing to our understanding of the pathogenesis of HAND, the SIV/pigtailed macaque model also provides an excellent opportunity to test innovative approaches to eliminate the latent HIV reservoir in the brain.

Keywords SIV · Macaque · Macrophage · HIV · HAND · Anti-retroviral therapy · Latent CNS reservoirs · QVOA

Although antiretroviral treatment (ART) has reduced the incidence of HIV dementia, a high prevalence of HIV-associated neurocognitive disorders (HAND) persists in the ART era ranging from asymptomatic neurocognitive impairment (ANI) to severe HIV-associated dementia (HAD) (Antinori et al. 2007). Prior to ART, HAD developed frequently in HIV-infected individuals with low CD4+ T cell counts and high-HIV plasma viral loads. With effective ART, the incidence of HAD and AIDS decreased (Antinori et al. 2007; Heaton et al. 2010). However, mild to moderate forms of HAND still occur despite long-term suppression with ART (Heaton et al. 2010). Estimates of HAND in HIV+ people on ART range from 15 to 55% in various studies (Saylor et al. 2016). Despite persistent HAND during ART, our understanding of the pathogenesis of ongoing CNS damage underlying HAND remains incomplete. Possible mechanisms include legacy effects (viral or inflammatory-mediated damage present pre-ART), sustained CNS inflammation, various host genetic factors, and neurotoxicity of ART compounds (Levine et al. 2012; Mothobi and Brew 2012).

The SIV/macaque model of HAND

SIV/macaque models have been of great value in elucidating the pathogenesis of HIV-induced nervous system damage. A particularly informative SIV CNS model has been developed and optimized in the Retrovirus Biology Laboratory at Johns Hopkins University over the last 25 years. To develop a reliable SIV model of HIV CNS disease, we mapped the viral determinants of both macrophage-tropism and neurovirulence, thereby generating the neurovirulent molecular clone SIV/17E-Fr. Studies with

✉ Joseph L. Mankowski
jmankows@jhmi.edu

[1] Department of Molecular and Comparative Pathobiology, Johns Hopkins University, Baltimore, MD 21205, USA

[2] Department of Neurology, Johns Hopkins University, Baltimore, MD 21205, USA

[3] Department of Pathology, Johns Hopkins University, Baltimore, MD 21205, USA

recombinant SIV indicated that while changes in *env* sequence were sufficient to confer macrophage tropism, *env*, *nef*, and, 3'LTR sequences all were necessary for virus replication in the CNS and neurovirulence (Mankowski et al. 1997; Ravimohan et al. 2012; Thompson et al. 2003). Subsequent studies discovered that inoculating pigtailed macaques (*Macaca nemestrina*) intravenously with both SIV/17E-Fr and the immunosuppressive CD4+ T cell-depleting SIV swarm SIV/DeltaB670 led to consistent development of AIDS and prototypic SIV encephalitis in the majority of animals within a 3-month period in contrast to inoculating rhesus macaques (*Macaca mulatta*) with the identical combination of SIV (Zink et al. 1999; Mankowski et al. 2002a). We recently detailed the differences between these two macaque species with respect to induction of SIV CNS disease with a focus on the neuroprotective roles of MHC class I alleles in each species (Beck et al. 2015a). This comparison is of note because, in an alternate SIV/rhesus macaque model, SIV encephalitis develops in the majority SIVmac251-infected rhesus macaques only after transient depletion of CD8+ cells (Schmitz et al. 1999; Ratai et al. 2011; Williams and Burdo 2012). Although the SIV CD8+ depletion model illustrates the key role of CD8+ cells in neuropathogenesis and has been informative regarding CNS macrophage biology, study of the specific role of cell-mediated immunity becomes limited when both CD8+ T cells and NK cells are depleted.

For SIV studies, it is crucial to use specific-pathogen-free macaques to avoid confounding co-infections. All pigtailed macaques in our studies are tested negative for SIV, STLV, SRV, and *Cercopithecine herpesvirus* 1 prior to study entry. Macaques are also screened twice yearly for *Mycobacterium tuberculosis*. While SIV macaque studies ideally would include balanced numbers of males and females, most SIV studies use primarily male macaques. Female macaques are highly valuable for breeding colonies and hence are difficult to obtain for most SIV studies.

Nervous system alterations in the SIV/pigtailed macaque model

The SIV/pigtailed macaque model has served as the platform to characterize the successive immunologic and viral parameters developed throughout progressive infection during acute, asymptomatic (an ~ 8-week interval), and terminal stages of disease (Beck et al. 2015b). The SIV/pigtailed macaque model offers many parallels to HIV infection including development of characteristic CNS inflammation that correlates with high-viral load in the brain, cognitive and motor deficits typical of HAND, and classic lesions of HIV/SIV encephalitis (Zink et al. 1999; Brew et al. 1997; Ellis et al. 1997; McArthur et al. 1997; Mankowski et al. 2002b; Weed et al. 2003). SIV-induced neuropathology is characterized by numerous perivascular inflammatory infiltrates in brain and spinal cord predominantly composed of macrophages and multinucleate giant cells that frequently contain replicating SIV, recapitulating classic HIV CNS pathology (Mankowski et al. 2002a; Mangus et al. 2015). Neuroinflammatory changes are accompanied by metabolic alterations including decreased glucose transport across the blood-brain barrier (Mankowski et al. 1999). Neuronal dysfunction manifested by accumulation of amyloid precursor protein (APP) in axons correlated with behavioral outcome measures including decreased performance on bimanual motor tasks (Mankowski et al. 2002b; Weed et al. 2003). Synaptodendritic alterations including increased synaptophysin expression in the brain of untreated infected macaques compared to ART-suppressed macaques also have been characterized (Akay et al. 2014; Helke et al. 2013). In addition, the potential value of PET neuroimaging studies employing the ligand PK1195 to measure peripheral benzodiazepine receptor expression in the brain reflecting microglial activation present in SIV and HIV was first shown in this SIV model (Mankowski et al. 2003).

Peripheral nervous system (PNS) alterations resembling HIV lesions include inflammation in the trigeminal and dorsal root ganglia (DRG; ganglionitis) and loss of epidermal nerve fibers (ENF) (Laast et al. 2007, 2011). DRG inflammation corresponds with loss of epidermal nerve fiber density as well as slowed conduction velocity in small unmyelinated sensory C-fibers in the sural nerve, a feature of various painful neuropathic conditions (Laast et al. 2011). SIV-induced sensory fiber loss extends to the dense sub-basal sensory nerve plexus of the cornea, suggesting that in vivo corneal confocal microscopy, a technique that has shown promise for the assessment of sensory nerve fibers in patients with diabetes mellitus, may be used to track progressive PNS damage caused by HIV as well as neurotoxic antiretroviral treatments (Dorsey et al. 2014). To determine whether suppressive ART altered SIV PNS outcomes, we examined DRG and skin samples from SIV-infected pigtailed macaques receiving ART (ART1, Table 1). Although ART suppressed SIV replication and reduced macrophage activation in DRG, epidermal nerve fiber measurements remained significantly lower as compared to uninfected, untreated pigtailed macaques. These findings demonstrate that significant peripheral nervous system damage persists in SIV-infected macaques despite ART, providing the basis for studying HIV-induced PNS damage that persists in the ART era (Dorsey et al. 2015).

Role of MHC class I alleles in SIV CNS disease

A valuable finding uncovered in the SIV/pigtailed macaque model was that a subset of SIV-infected pigtailed macaques inoculated with both SIV/DeltaB670 and SIV/17E-Fr (approximately one third of animals) did not develop SIV encephalitis despite progressing to AIDS. To compensate for this variability in CNS disease outcomes, especially for experimental design of intervention studies testing novel neuroprotective or latency reactivating

Table 1 ART regimens in the SIV/pigtailed macaque model

Regimen	Compound	Class	Dosage	Route	Frequency	References
ART1	PMPA	NRTI	10 mg/kg*	Subcutaneous	Once/day	Dinoso et al. (2009)
	Saquinavir	Protease inhibitor	205 mg/kg	Oral	Twice/day	
	Atazanavir	Protease inhibitor	270 mg/kg	Oral	Twice/day	
	L-870812	Integrase inhibitor	10 mg/kg	Oral	Twice/day	
ART2	PMPA	NRTI	10 mg/kg	Subcutaneous	Once/day	Gama et al. (2017)
	Darunavir	Protease inhibitor	480 mg/kg	Oral	Twice/day	
	Ritonavir	PI boost	24 mg/kg	Oral	Twice/day	
	L-870812	Integrase inhibitor	10 mg/kg	Oral	Twice/day	
ART3	PMPA	NRTI	20 mg/kg	Subcutaneous	Once/day	Whitney et al. (2014)
	Emtricitabine (FTC)	NRTI	40 mg/kg	Single combined		
	Dolutegravir	Integrase inhibitor	2.5 mg/kg	Injection		

*PMPA dose was initially 30 mg/kg for the first 2 weeks of treatment then reduced to 10 mg/kg

strategies, group sizes would need to be doubled for most outcome measures, greatly increasing costs and effort. By studying animals with and without SIV-induced encephalitis, our lab discovered a neuroprotective MHC class I allele that accounted for the majority of the variation in CNS disease outcomes; animals that expressed the MHC class I allele *Mane-A1*084* were much less likely to develop SIV encephalitis (Mankowski et al. 2008; Queen et al. 2011). This finding substantially refined the SIV model by allowing us to select for or against inclusion of animals expressing this MHC class I allele based on study design. The MHC class I allele *Mane-A1*084:01* (formerly *Mane-A*10*) presents an immunodominant SIV Gag capsid epitope termed KP9 ($KKFGAEVVP_{164-172}$), which is a critical capsid region homologous to HIV Gag KF11, an immunodominant epitope that is recognized by *HLA-B*5701* in humans (Smith et al. 2005a, b). In SIV-infected pigtailed macaques expressing *Mane-A1*084*, immune pressure on the KP9 epitope drives the canonical lysine to arginine escape mutation, K165R (Smith et al. 2005b). Interestingly, in our model, *Mane-A1*084* influence was specific to CNS disease outcomes and did not impact either plasma viral loads or progression to AIDS (Mankowski et al. 2008).

Identification of *Mane-A1*084* as a neuroprotective allele led to additional insights into the role that cytotoxic T cells play in establishing the latent HIV reservoir in the CNS. SIV K165R Gag escape mutations were archived in latent proviral DNA reservoirs including the CNS in animals receiving ART that suppressed viral replication (Queen et al. 2011). Replication-competent SIV Gag K165R escape mutations also were identified in the resting CD4+ T cell reservoir, demonstrating that escape from MHC class I-mediated control occurs during decaying phases of viremia prior to suppression and then persists as replication-competent provirus in tissue sites including the brain (Queen et al. 2011).

We also demonstrated a CNS compartment-specific fitness cost to viral escape from MHC class I-mediated immunologic pressure. After inserting the canonical SIV Gag escape mutation K165R into the neurovirulent molecular clone SIV/17E-Fr, we inoculated *Mane-A1*084*-positive pigtailed macaques with the cloned escape mutant virus and showed decreased viral load in CSF but not plasma. Viral sequencing revealed transient reversion to wild-type Gag KP9 only in the CSF, consistent with decreased CNS fitness of SIV with K165R. In reciprocal experiments, we vaccinated pigtailed macaques with a virus-like particle-based Gag KP9 construct to focus CTL responses on Gag KP9. Subsequent challenge with SIV/17E-Fr demonstrated lower viral load in CSF but not plasma. Combined, these findings demonstrate a CNS-specific loss in viral fitness attributed to a single Gag mutation that permits escape from *Mane-A1*084* control (Laast et al. 2007; Laast et al. 2011). Given that a single-epitope vaccine is not likely to prevent SIV or HIV infection because of potential development of escape mutations, studies in which macaques are vaccinated with multiple SIV Gag epitopes (as well as epitopes beyond Gag) are needed. In addition, our data support development of a therapeutic vaccine approach to control CNS reservoirs given that stimulating *Mane-A1*084*-mediated CTL responses by virus-like particle (VLP) KP9 vaccination prior to SIV infection had a CNS-specific protective effect with lower CSF viral loads (Beck et al. 2016). Ongoing efforts aim to identify whether therapeutic vaccination protocols employing a similar strategy to stimulate CNS-specific MHC class I mediated responses may be pivotal in HIV cure efforts, especially for targeting the CNS compartment.

The SIV/pigtailed macaque model as a biomarker discovery platform

SIV/macaque models are especially valuable for studying the serial events in the neuropathogenesis of HIV as paired samples of plasma and CSF can be obtained from multiple longitudinal

time points throughout the course of infection. To discover biomarkers in blood and CSF that preceded and predicted development of SIV encephalitis, host immune response mediators and viral RNA levels have been characterized. In brief, predictive CSF markers including CCL2, IL-6, neopterin, YKL-40, and SIV RNA load were elevated beginning in the asymptomatic phase of infection and sustained until terminal stages of disease. In blood, the macrophage activation marker sCD163 as well as hemoglobin level and platelet count were all predictive biomarkers for development of CNS disease (Beck et al. 2015b; Mankowski et al. 2004).

One of the most surprising circulating hematologic markers of retroviral-associated CNS disease is decline in platelet count. Platelet decline during asymptomatic infection has been associated with significantly increased risk for the development of HIV-associated dementia (Wachtman et al. 2007; Wachtman et al. 2006). In our cohort of SIV-inoculated pigtailed macaques, we observed pronounced declines in platelet count during the acute (d7-14 p.i.) phase of infection and again in asymptomatic and terminal infection (Metcalf Pate et al. 2013). After day 28 post-inoculation, animals that later developed SIV encephalitis during terminal infection had a greater decrease in platelet count than animals that did not develop SIV encephalitis, though the magnitude of platelet loss during acute infection was similar between SIV-infected animals that progressed to SIV encephalitis and those that did not (Wachtman et al. 2006). These data emphasize the importance of considering absolute change from baseline platelet count as a prognostic indicator of increased risk for the development of HIV-associated CNS disease. This association was further supported by studying HIV-infected patients and additional SIV/macaque model data (Beck et al. 2015b; Wachtman et al. 2007). This predictive platelet loss represents the combined effect of decreased platelet production, increased platelet destruction, and increased association of activated platelets with other cells including CD16+ monocytes. The latter association may reflect a fundamental role for platelet activation in the pathogenesis of HAND (Mudd et al. 2016; Singh et al. 2014).

Anti-retroviral therapy in SIV-infected pigtailed macaques

To study HAND in the context of ART, we have tested a number of ART regimens in the SIV-infected pigtailed macaque model beginning at day 12 post-SIV inoculation for all regimens (Zink et al. 2010). Initially, SIV-infected pigtailed macaques were treated with PMPA, saquinavir, atazanavir, and the Merck integrase inhibitor L-870812, successfully reducing viral load in plasma and in CSF to below the limit of detection in this accelerated model of HIV (ART1, Table 1 and Fig. 1) (Dinoso et al. 2009). As reported for HAND, sustained CNS inflammation persisted despite efficacy of ART, with elevated TNFα and CCL2 in the brain. Furthermore, by comparing blood and CSF samples obtained pre-infection with samples obtained at terminal study time points representing longest duration of SIV replication control, we found strong parallels between our SIV model and reports of HAND biomarkers identified in HIV-infected groups receiving ART (Fig. 2a and b). In CSF, levels of both the inflammation marker neopterin and the neuronal damage marker neurofilament light were elevated despite long-term ART. In the plasma, sCD163, a monocyte/macrophage activation marker, and CCL2 were also higher than pre-infection time points. These findings show that SIV-infected pigtailed macaques receiving suppressive ART nonetheless develop persistent inflammation and neuronal damage that closely corresponds with multiple HIV HAND reports on these biomarkers.

With availability of newer ART options, access to saquinavir for SIV studies has become limited. Given this constraint, we shifted to an ART regimen consisting of PMPA, darunavir with ritonavir boost, and the integrase inhibitor (ART2, Fig. 1 and Table 1). This regimen proved similar to the original ART regimen (ART1) with respect to SIV suppression in plasma and CSF and served as the basis for performing studies aimed at reactivating latent SIV including from potential CNS reservoirs (Gama et al. 2017). A limitation of this regimen was the requirement of twice/day oral dosing of large amounts of three compounds, a treatment scheme that was defined by pharmacokinetic studies prior to use in SIV-infected macaques. To further refine the use of ART in SIV-infected macaques, we next tested the efficacy of a newer regimen consisting of a single once/day subcutaneous injection of PMPA, FTC, and dolutegravir (ART3; Table 1). Previous studies had shown that this combination successfully suppressed SIVmac251 replication in the plasma of rhesus macaques (Whitney et al. 2014). Our studies showed that the ART3 combination also rapidly suppresses SIV/17EFr and SIV/DeltaB670 in both plasma and CSF of pigtailed macaques (Fig. 1). Suppression is sustained in both plasma and CSF over time, providing a long-term suppression model to study both persistent CNS immune activation and neuronal damage that may underlie HAND, as well as the adverse sequelae that follow cessation of ART (Kuller et al. 2008). Furthermore, this ART regimen optimizes the SIV-infected pigtailed macaque model to study HIV latency in the CNS and test novel HIV cure strategies.

Measuring latent reservoirs in the SIV/pigtailed macaque model

There is an extensive literature that details the frequency of HIV infection and latency in resting CD4+ T cells in HIV-infected ART-suppressed humans. To demonstrate that a comparable resting CD4+ T cell reservoir also is established in SIV-infected pigtailed macaques on ART, we developed the rCD4+ QVOA for the SIV model. We used this assay to measure the frequency of rCD4+ cells in blood as well as in both lymph nodes and spleen. This led to the important insight that

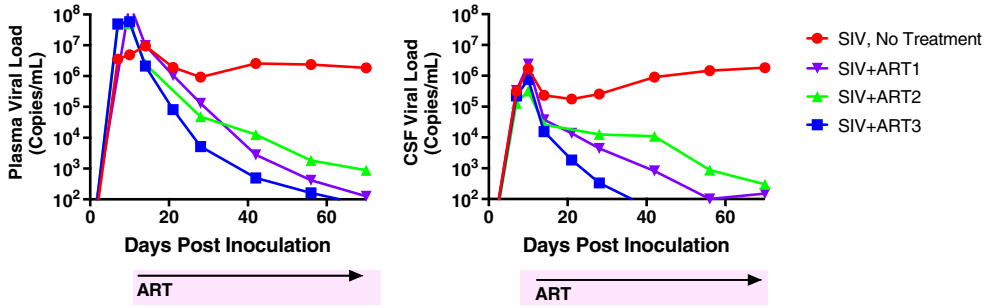

Fig. 1 SIV-infected pigtailed macaques receiving ART beginning day 12 post-inoculation had sustained viral suppression in both *plasma* (left) and *CSF* (right) although time to suppression varied with ART regimen. For comparison, untreated SIV-infected plasma and CSF viral loads are shown in red (circles). ART1 regimen (purple down triangles) consisted of PMPA, saquinavir, atazanavir, and a Merck integrase inhibitor. ART2 (green up triangles) contained darunavir, ritonavir, and the integrase inhibitor. ART3 (blue squares), the most efficacious with shortest interval to suppression, consisted of a single daily subcutaneous injection of PMPA, FTC, and dolutegravir. All anti-retroviral compounds were graciously donated (detailed in acknowledgements)

the frequency of rCD4+ cells that harbor replication competent SIV is very similar in blood, lymph nodes, and spleen. In addition, the frequency of resting CD4+ T cells in blood that contained replication competent virus in our SIV macaque model was equivalent (one cell in a million) to that observed in HIV patients on ART (Dinoso et al. 2009).

To evaluate the SIV model in depth as a platform for studying SIV and HIV latency with a focus on macrophage reservoirs including the CNS compartment, we developed a macrophage (MΦ) quantitative outgrowth assay (Avalos et al. 2016). The assay measures levels of SIV-infected CD11b+ cells isolated from the brain as well as other tissues. Serial dilutions of purified MΦs are plated in poly-L-lysine coated wells and then stimulated with TNF-α, which is known to stimulate HIV-1 gene expression in the U1 MΦ cell line (Folks et al. 1987) and which, in preliminary experiments, enhanced the recovery of infectious SIV. Potential contamination with infected CD4+ T cells is assessed by both flow cytometry and a calibrated RT-qPCR assay for rearranged T cell receptor (TCR) β-chain RNA, which is only found in T cells. Replication-competent SIV released from stimulated MΦs is amplified in culture through the addition of CEMx174 cells (Dinoso et al. 2009; Shen et al. 2003, 2007), and levels of SIV RNA in the supernatant are quantitated by RT-qPCR for 2 weeks. With this approach, infected MΦs have been detected in the brain, lungs, and spleen isolated from viremic SIV-infected macaques (Avalos et al. 2016). The frequency of productively infected MΦs that can release replication-competent SIV in this assay varies from animal to animal in the

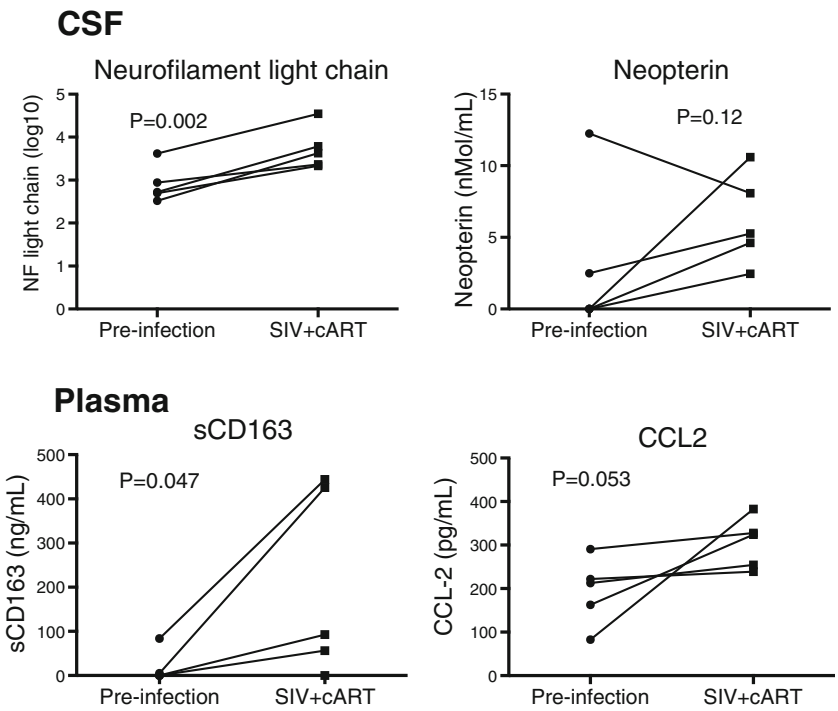

Fig. 2 HAND biomarkers in CSF (top panel) and plasma (bottom panel) samples from SIV-infected pigtailed macaques receiving ART demonstrate parallels between HIV cohort studies and the SIV model

range of 100–10,000/10^6 MΦs and is generally higher in animals with high levels of viremia (Avalos et al. 2016). This striking level of MΦ infection cannot be explained by T cell contamination.

The use of MΦ-QVOAs will be a critical tool for defining myeloid cell reservoirs. Indeed, preliminary studies indicate that the frequency of infected MΦs is dramatically lower in animals on ART. In ART-suppressed macaques, an average of 0.23 circulating monocytes per million harbored replication-competent latent SIV genomes (Fig. 3). Monocytes are released from the bone marrow and have a lifespan of 48–72 h. Some reports demonstrate that monocytes can traffic through tissues (spleen, skin) without differentiating into tissue MΦs (Swirski et al. 2009; van der Laan et al. 2014; McGovern et al. 2014). Although monocytes do not usually fit the definition of "latent viral reservoirs," it is possible that SIV-infected bone marrow promonocytes, which do not express CD34 but express high levels of CD4, proliferate into latently infected blood monocytes that could traffic into tissues and maintain MΦ reservoirs. Levels of infected macrophages in tissues from ART-suppressed macaques varied from 0.23 (brain) to 1 (spleen and lung) per site, suggesting that tissues in suppressed macaques harbor a significant number of latently infected tissue macrophages that have the potential for reactivation after ART interruption (Fig. 3).

A murine viral outgrowth assay for HIV and SIV

A limitation of CD4+ T cell QVOAs is that they underrepresent the actual replication competent reservoir; the macrophage QVOA may have the same limitation (Ho et al. 2013). To overcome this restriction, the Metcalf Pate and Blankson labs at JHU collaboratively developed a novel ultrasensitive murine viral outgrowth assay (MVOA) for HIV and SIV (Metcalf Pate et al. 2015). In the MVOA, the xenoreactivity of human or macaque T cells towards murine antigens induces a high level of T cell activation that reverses latency. Viruses released from infected cells replicate in the xenografted activated $CD4^+$ T cells, producing viremia. Because large numbers of patient or macaque cells can be evaluated (up to 50 million per mouse) and multiple mice can easily be xenografted at once, this assay has a greater potential dynamic range than the QVOA and is anticipated to be especially advantageous in measuring large reservoir reductions induced by curative strategies. An additional important advantage of the MVOA is that the probability that a latently infected CD4+ T cell becomes activated is greatly enhanced by the prolonged and profound immune activation of the xenografted T cells by xenoantigens. Xenografted CD4+ T cells can be further stimulated in vivo in the mouse with anti-CD3 and anti-CD28 antibodies to induce an even higher level of activation. This assay has been validated and refined by two independent groups to date to detect intact, non-induced proviruses (INP) that are missed by the QVOA and thus may provide a more accurate estimate of reservoir size (Charlins et al. 2017; Yuan et al. 2017). We have successfully used this assay to detect SIV in pigtailed macaque peripheral blood mononuclear cells and purified CD4+ T cells (Metcalf Pate et al. 2015) and are in the process of further refining the assay for the detection of SIV in lymphoid and non-lymphoid organs to identify latent viral reservoirs in sanctuary organs.

Conclusion

SIV infection of pigtailed macaques is a highly representative and well-characterized animal model for HIV neuropathogenesis studies including SIV-infected animals on ART. The pigtailed macaque model of accelerated SIV-associated CNS disease provides an excellent opportunity to study and develop prognostic markers of HAND for HIV-infected individuals in a controlled setting that offers high translational value. In addition, similar to observations in ART-treated HIV-infected patients, we have reported evidence of ongoing neurodegenerative and inflammatory

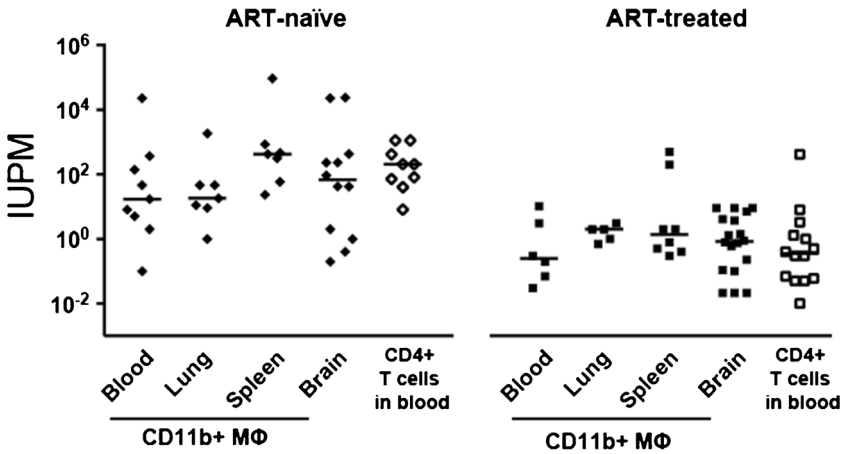

Fig. 3 Number of SIV-infected blood monocytes, tissue macrophages, and blood $CD4^+$ T cells in SIV-infected pigtailed macaques untreated or treated with antiretroviral therapy (ART). Values are shown as infectious units per million (IUPM) cells. Lines represent medians

changes in pigtailed macaques treated with suppressive ART. Biomarkers of HAND provide us with the capability of tracking the CNS status of SIV-infected macaques serially throughout studies, thereby optimizing a biomarker panel that may be valuable for monitoring the development of HAND in clinical settings. In addition to contributing to our understanding of the pathogenesis of HAND, the SIV/pigtailed macaque model also provides an excellent opportunity to test innovative approaches to eliminate the latent HIV CNS reservoir. Finally, our SIV ART studies also have identified potential adjunctive therapies for HAND including maraviroc, minocycline, and a combination of fluconazole and paroxetine (Kelly et al. 2013; Meulendyke et al. 2014; Zink et al. 2005); the SIV ART model we have established will facilitate testing additional therapies for HAND.

Acknowledgements These studies were funded by NIH awards R01NS089482, R01 NS077869, P40OD0131117, R01NS055651, R56AI118753, R01AI127142, P01MH070306, and the Johns Hopkins University Center for AIDS Research P30AI094189.

Anti-retroviral compounds for these studies were kindly donated by Gilead, ViiV Healthcare, Bristol-Meyers Squibb, Merck, Abbvie, Janssen, and Roche. These studies were supported by the excellent technical staff in the Retrovirus Lab at Johns Hopkins.

Compliance with ethical standards

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

References

Akay C, Cooper M, Odeleye A, Jensen BK, White MG, Vassoler F et al (2014) Antiretroviral drugs induce oxidative stress and neuronal damage in the central nervous system. J Neurovirol 20(1): 39–53

Antinori A, Arendt G, Becker JT, Brew BJ, Byrd DA, Cherner M et al (2007) Updated research nosology for HIV-associated neurocognitive disorders. Neurology 69(18):1789–1799

Avalos CR, Price SL, Forsyth ER, Pin JN, Shirk EN, Bullock BT et al (2016) Quantitation of productively infected monocytes and macrophages of simian immunodeficiency virus-infected macaques. J Virol 90(12):5643–5656

Beck SE, Kelly KM, Queen SE, Adams RJ, Zink MC, Tarwater PM et al (2015a) Macaque species susceptibility to simian immunodeficiency virus: increased incidence of SIV central nervous system disease in pigtailed macaques versus rhesus macaques. J Neurovirol 21(2): 148–158

Beck SE, Queen SE, Witwer KW, Metcalf Pate KA, Mangus LM, Gama L et al (2015b) Paving the path to HIV neurotherapy: predicting SIV CNS disease. Eur J Pharmacol 759:303–312

Beck SE, Queen SE, Viscidi R, Johnson D, Kent SJ, Adams RJ et al (2016) Central nervous system-specific consequences of simian immunodeficiency virus gag escape from major histocompatibility complex class I-mediated control. J Neurovirol 22(4):498–507

Brew BJ, Pemberton L, Cunningham P, Law MG (1997) Levels of human immunodeficiency virus type 1 RNA in cerebrospinal fluid correlate with AIDS dementia stage. J Infect Dis 175(4): 963–966

Charlins P, Schmitt K, Remling-Mulder L, Hogan LE, Hanhauser E, Hobbs KS et al (2017) A humanized mouse-based HIV-1 viral outgrowth assay with higher sensitivity than in vitro qVOA in detecting latently infected cells from individuals on ART with undetectable viral loads. Virology 507:135–139

Dinoso JB, Rabi SA, Blankson JN, Gama L, Mankowski JL, Siliciano RF et al (2009) A simian immunodeficiency virus-infected macaque model to study viral reservoirs that persist during highly active antiretroviral therapy. J Virol 83(18):9247–9257

Dorsey JL, Mangus LM, Oakley JD, Beck SE, Kelly KM, Queen SE et al (2014) Loss of corneal sensory nerve fibers in SIV-infected macaques: an alternate approach to investigate HIV-induced PNS damage. Am J Pathol 184(6):1652–1659

Dorsey JL, Mangus LM, Hauer P, Ebenezer GJ, Queen SE, Laast VA, Adams RJ, Mankoski JL (2015) Persistent peripheral nervous system damage in simian immunodeficiency virus-infected macaques receiving antiretroviral therapy. J Neuropathology 74(11)

Ellis RJ, Hsia K, Spector SA, Nelson JA, Heaton RK, Wallace MR et al (1997) Cerebrospinal fluid human immunodeficiency virus type 1 RNA levels are elevated in neurocognitively impaired individuals with acquired immunodeficiency syndrome. HIV neurobehavioral research center group. Ann Neurol 42(5):679–688

Folks TM, Justement J, Kinter A, Dinarello CA, Fauci AS (1987) Cytokine-induced expression of HIV-1 in a chronically infected promonocyte cell line. Science 238(4828):800–802

Gama L, Abreu CM, Shirk EN, Price SL, Li M, Laird GM et al (2017) Reactivation of simian immunodeficiency virus reservoirs in the brain of virally suppressed macaques. AIDS 31(1):5–14

Heaton RK, Clifford DB, Franklin DR Jr, Woods SP, Ake C, Vaida F et al (2010) HIV-associated neurocognitive disorders persist in the era of potent antiretroviral therapy: CHARTER study. Neurology 75(23): 2087–2096

Helke KL, Queen SE, Mankowski JL (2013) 14-3-3 protein in CSF reflects SIV-mediated pre-synaptic damage. Curr HIV Res 11(4): 281–287

Ho YC, Shan L, Hosmane NN, Wang J, Laskey SB, Rosenbloom DI et al (2013) Replication-competent noninduced proviruses in the latent reservoir increase barrier to HIV-1 cure. Cell 155(3):540–551

Kelly KM, Beck SE, Metcalf Pate KA, Queen SE, Dorsey JL, Adams RJ et al (2013) Neuroprotective maraviroc monotherapy in simian immunodeficiency virus-infected macaques: reduced replicating and latent SIV in the brain. AIDS 27(18):F21–F28

Kuller LH, Tracy R, Belloso W, De Wit S, Drummond F, Lane HC et al (2008) Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. PLoS Med 5(10):e203

Laast VA, Pardo CA, Tarwater PM, Queen SE, Reinhart TA, Ghosh M et al (2007) Pathogenesis of simian immunodeficiency virus-induced alterations in macaque trigeminal ganglia. J Neuropathol Exp Neurol 66(1):26–34

Laast VA, Shim B, Johanek LM, Dorsey JL, Hauer PE, Tarwater PM et al (2011) Macrophage-mediated dorsal root ganglion damage precedes altered nerve conduction in SIV-infected macaques. Am J Pathol 179(5):2337–2345

Levine AJ, Service S, Miller EN, Reynolds SM, Singer EJ, Shapshak P et al (2012) Genome-wide association study of neurocognitive impairment and dementia in HIV-infected adults. Am J Med Genet B Neuropsychiatr Genet 159B(6):669–683

Mangus LM, Dorsey JL, Laast VA, Hauer P, Queen SE, Adams RJ et al (2015) Neuroinflammation and virus replication in the spinal cord of simian immunodeficiency virus-infected macaques. J Neuropathol Exp Neurol 74(1):38–47

Mankowski JL, Flaherty MT, Spelman JP, Hauer DA, Didier PJ, Amedee AM et al (1997) Pathogenesis of simian immunodeficiency virus encephalitis: viral determinants of neurovirulence. J Virol 71(8): 6055–6060

Mankowski JL, Queen SE, Kirstein LM, Spelman JP, Laterra J, Simpson IA et al (1999) Alterations in blood-brain barrier glucose transport in SIV-infected macaques. J Neurovirol 5(6):695–702

Mankowski JL, Clements JE, Zink MC (2002a) Searching for clues: tracking the pathogenesis of human immunodeficiency virus central nervous system disease by use of an accelerated, consistent simian immunodeficiency virus macaque model. J Infect Dis 186(Suppl 2): S199–S208

Mankowski JL, Queen SE, Tarwater PM, Fox KJ, Perry VH (2002b) Accumulation of beta-amyloid precursor protein in axons correlates with CNS expression of SIV gp41. J Neuropathol Exp Neurol 61(1): 85–90

Mankowski JL, Queen SE, Tarwater PJ, Adams RJ, Guilarte TR (2003) Elevated peripheral benzodiazepine receptor expression in simian immunodeficiency virus encephalitis. J Neurovirol 9(1):94–100

Mankowski JL, Queen SE, Clements JE, Zink MC (2004) Cerebrospinal fluid markers that predict SIV CNS disease. J Neuroimmunol 157(1–2):66–70

Mankowski JL, Queen SE, Fernandez CS, Tarwater PM, Karper JM, Adams RJ et al (2008) Natural host genetic resistance to lentiviral CNS disease: a neuroprotective MHC class I allele in SIV-infected macaques. PLoS One 3(11):e3603

McArthur JC, McClernon DR, Cronin MF, Nance-Sproson TE, Saah AJ, St Clair M et al (1997) Relationship between human immunodeficiency virus-associated dementia and viral load in cerebrospinal fluid and brain. Ann Neurol 42(5):689–698

McGovern N, Schlitzer A, Gunawan M, Jardine L, Shin A, Poyner E et al (2014) Human dermal CD14(+) cells are a transient population of monocyte-derived macrophages. Immunity 41(3):465–477

Metcalf Pate KA, Lyons CE, Dorsey JL, Shirk EN, Queen SE, Adams RJ et al (2013) Platelet activation and platelet-monocyte aggregate formation contribute to decreased platelet count during acute simian immunodeficiency virus infection in pig-tailed macaques. J Infect Dis 208(6):874–883

Metcalf Pate KA, Pohlmeyer CW, Walker-Sperling VE, Foote JB, Najarro KM, Cryer CG et al (2015) A murine viral outgrowth assay to detect residual HIV type 1 in patients with undetectable viral loads. J Infect Dis 212(9):1387–1396

Meulendyke KA, Queen SE, Engle EL, Shirk EN, Liu J, Steiner JP et al (2014) Combination fluconazole/paroxetine treatment is neuroprotective despite ongoing neuroinflammation and viral replication in an SIV model of HIV neurological disease. J Neurovirol 20(6):591–602

Mothobi NZ, Brew BJ (2012) Neurocognitive dysfunction in the highly active antiretroviral therapy era. Curr Opin Infect Dis 25(1):4–9

Mudd JC, Panigrahi S, Kyi B, Moon SH, Manion MM, Younes SA et al (2016) Inflammatory function of CX3CR1+ CD8+ T cells in treated HIV infection is modulated by platelet interactions. J Infect Dis 214(12):1808–1816

Queen SE, Mears BM, Kelly KM, Dorsey JL, Liao Z, Dinoso JB et al (2011) Replication-competent simian immunodeficiency virus (SIV) gag escape mutations archived in latent reservoirs during antiretroviral treatment of SIV-infected macaques. J Virol 85(17): 9167–9175

Ratai EM, Pilkenton S, He J, Fell R, Bombardier JP, Joo CG et al (2011) CD8+ lymphocyte depletion without SIV infection does not produce metabolic changes or pathological abnormalities in the rhesus macaque brain. J Med Primatol 40(5):300–309

Ravimohan S, Gama L, Engle EL, Zink MC, Clements JE (2012) Early emergence and selection of a SIV-LTR C/EBP site variant in SIV-infected macaques that increases virus infectivity. PLoS One 7(8): e42801

Saylor D, Dickens AM, Sacktor N, Haughey N, Slusher B, Pletnikov M et al (2016) HIV-associated neurocognitive disorder—pathogenesis and prospects for treatment. Nat Rev Neurol 12(4):234–248

Schmitz JE, Kuroda MJ, Santra S, Sasseville VG, Simon MA, Lifton MA et al (1999) Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. Science 283(5403):857–860

Shen A, Zink MC, Mankowski JL, Chadwick K, Margolick JB, Carruth LM et al (2003) Resting CD4+ T lymphocytes but not thymocytes provide a latent viral reservoir in a simian immunodeficiency virus-Macaca nemestrina model of human immunodeficiency virus type 1-infected patients on highly active antiretroviral therapy. J Virol 77(8):4938–4949

Shen A, Yang HC, Zhou Y, Chase AJ, Boyer JD, Zhang H et al (2007) Novel pathway for induction of latent virus from resting CD4(+) T cells in the simian immunodeficiency virus/macaque model of human immunodeficiency virus type 1 latency. J Virol 81(4):1660–1670

Singh MV, Davidson DC, Jackson JW, Singh VB, Silva J, Ramirez SH et al (2014) Characterization of platelet-monocyte complexes in HIV-1-infected individuals: possible role in HIV-associated neuroinflammation. J Immunol 192(10):4674–4684

Smith MZ, Dale CJ, De Rose R, Stratov I, Fernandez CS, Brooks AG et al (2005a) Analysis of pigtail macaque major histocompatibility complex class I molecules presenting immunodominant simian immunodeficiency virus epitopes. J Virol 79(2):684–695

Smith MZ, Fernandez CS, Chung A, Dale CJ, De Rose R, Lin J et al (2005b) The pigtail macaque MHC class I allele mane-a*10 presents an immundominant SIV gag epitope: identification, tetramer development and implications of immune escape and reversion. J Med Primatol 34(5–6):282–293

Swirski FK, Nahrendorf M, Etzrodt M, Wildgruber M, Cortez-Retamozo V, Panizzi P et al (2009) Identification of splenic reservoir monocytes and their deployment to inflammatory sites. Science 325(5940):612–616

Thompson KA, Kent SJ, Gahan ME, Purcell DF, McLean CA, Preiss S et al (2003) Decreased neurotropism of nef long terminal repeat (nef/LTR)-deleted simian immunodeficiency virus. J Neurovirol 9(4): 442–451

van der Laan AM, Ter Horst EN, Delewi R, Begieneman MP, Krijnen PA, Hirsch A et al (2014) Monocyte subset accumulation in the human heart following acute myocardial infarction and the role of the spleen as monocyte reservoir. Eur Heart J 35(6):376–385

Wachtman LM, Tarwater PM, Queen SE, Adams RJ, Mankowski JL (2006) Platelet decline: an early predictive hematologic marker of simian immunodeficiency virus central nervous system disease. J Neurovirol 12(1):25–33

Wachtman LM, Skolasky RL, Tarwater PM, Esposito D, Schifitto G, Marder K et al (2007) Platelet decline: an avenue for investigation into the pathogenesis of human immunodeficiency virus-associated dementia. Arch Neurol 64(9):1264–1272

Weed MR, Hienz RD, Brady JV, Adams RJ, Mankowski JL, Clements JE et al (2003) Central nervous system correlates of behavioral deficits following simian immunodeficiency virus infection. J Neurovirol 9(4):452–464

Whitney JB, Hill AL, Sanisetty S, Penaloza-MacMaster P, Liu J, Shetty M et al (2014) Rapid seeding of the viral reservoir prior to SIV viraemia in rhesus monkeys. Nature 512(7512):74–77

Williams K, Burdo TH (2012) Monocyte mobilization, activation markers, and unique macrophage populations in the brain: observations from SIV infected monkeys are informative with regard to pathogenic mechanisms of HIV infection in humans. J Neuroimmune Pharmacol 7(2):363–371

Yuan Z, Kang G, Lu W, Li Q (2017) Reactivation of HIV-1 proviruses in immune-compromised mice engrafted with human VOA-negative CD4+ T cells. J Virus Erad 3(1):61–65

Zink MC, Suryanarayana K, Mankowski JL, Shen A, Piatak M Jr, Spelman JP et al (1999) High viral load in the cerebrospinal fluid

and brain correlates with severity of simian immunodeficiency virus encephalitis. J Virol 73(12):10480–10488

Zink MC, Uhrlaub J, DeWitt J, Voelker T, Bullock B, Mankowski J et al (2005) Neuroprotective and anti-human immunodeficiency virus activity of minocycline. JAMA 293(16):2003–2011

Zink MC, Brice AK, Kelly KM, Queen SE, Gama L, Li M et al (2010) Simian immunodeficiency virus-infected macaques treated with highly active antiretroviral therapy have reduced central nervous system viral replication and inflammation but persistence of viral DNA. J Infect Dis 202(1):161–170