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In vivo characterization of macrophage-tropic simian immunodeficiency virus molecular clones in rhesus macaques

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Abstract

Macrophages are a major target of HIV/SIV infection and play an important role in pathogenesis by serving as viral reservoirs in the central nervous system. Previously, a unique early SIVmac251 Env variant, deSIV147 was cloned from blood of a rhesus macaque with rapid disease progression and SIV-associated encephalitis. Here, we show that infectious molecular clone deSIV147 caused systemic infection in rhesus macaques following intravenous or intrarectal exposure. Next, we inoculated deSIV147 into macaques depleted of CD4⁺ T cells and found that animals were SIV-positive, with high plasma and CSF viral loads. These macaques also showed SIVp17-positive macrophages in brain, lymph nodes, colon, lung, and liver. Furthermore, accumulation of perivascular macrophages, multinucleated giant cells, and microgliosis was detected. These findings suggest that the neurotropic deSIV147 clone will be useful to study macrophage infection in HIV/SIV-associated neurocognitive disorders, gain insights into myeloid cell reservoirs in brain and other anatomical sites, as well as test strategies for eradication.

Introduction

The hallmark of HIV and SIV pathogenesis is progressive depletion of CD4⁺ T cells and impairment of immune system during the course of infection (Fauci and Desrosiers, 1997; Maartens *et al*, 2014). Macrophages are another major cellular target of HIV and SIV infection, and are relatively resistant to the cytopathic effects of infection (Fauci and Desrosiers, 1997; Igarashi *et al*, 2001; Maartens *et al*, 2014; Perelson *et al*, 1996; Swingle *et al*, 2007). Macrophages are long-lived cells and serve as viral reservoirs in tissues such as

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Conflict of interest

The authors declare that they have no conflict of interest.

the central nervous system (CNS), lymph nodes, and gut (Alexaki *et al*, 2008; Brown and Mattapallil, 2014). In the CNS, macrophages and microglia are the major cells supporting viral replication, which can cause neurological dysfunction and encephalitis (Albright *et al*, 2000; Burdo *et al*, 2013; Dick *et al*, 1997; Wang *et al*, 2002; Wiley *et al*, 1986; Williams *et al*, 2001). Infection of the CNS is characterized by the presence of multi-nucleated giant cells, reactive astrocytes, and white matter abnormalities. CNS infection remains a major obstacle to complete eradication of HIV in infected individuals, since many antiretrovirals cannot easily penetrate the blood-brain barrier, and are therefore less effective in eliminating infected cells (Nwogu *et al*, 2016).

SIV infection in rhesus macaques (RM) follows a similar disease course compared to HIV infection in humans (Fauci and Desrosiers, 1997; Johnson and Hirsch, 1992; Kestler *et al*, 1990). However, most SIV strains do not efficiently infect macrophages and do not cause the same CNS abnormalities seen in HIV-infected humans (Mori *et al*, 1992; Strickland *et al*, 2011). While SIVmac251, a strain used commonly to study HIV pathogenesis in non-human primates, replicates efficiently in both CD4+ T cells and macrophages, there is significant genetic heterogeneity in the uncloned SIVmac251 viral swarm when producing virus stocks in cell culture, resulting in inconsistent disease pathogenesis during infection of non-human primates (Daniel *et al*, 1985; Del Prete *et al*, 2013; Kanki *et al*, 1985; Letvin *et al*, 1985; Miller *et al*, 1998; Strickland *et al*, 2011). The HIV/SIV envelope (Env) is the major viral determinant of macrophage tropism and neurotropism. To gain better understanding of CNS pathology and viral reservoirs, a unique early SIVmac251 Env variant, deSIV147c#4, isolated from blood of a RM with rapid disease progression and SIV-associated encephalitis (SIV-E) was cloned into the SIVmac239 infectious molecular clone (Yen *et al*, 2014). Here, we report the *in vivo* replication kinetics and characteristics of deSIV147c#4 in RMs. The deSIV147c#4 infectious molecular clone replicated in macaques inoculated by intravenous or intrarectal routes, causing macrophage infection in brain, lymph nodes, spleen, colon, lung, and liver, and CD4+ T cell depletion in gut during acute infection. Furthermore, when CD4+ T cells were depleted from blood, lymph nodes, and rectal tissues using an anti-CD4 monoclonal antibody, macrophage infection by deSIV147c#4 was enhanced in multiple organs and tissues including the CNS.

Materials and Methods

Ethical Statement

Juvenile to adult male rhesus macaques (*Macaca mulatta*) 2–4 yrs old Indian origin were used for the studies reported herein (Table 1). All animals were born and maintained at the Yerkes National Primate Research Center of Emory University (Atlanta, GA, USA) in accordance with the regulations of the Committee on the Care and Use of Laboratory Animal Resources, National Research Council and the Department of Health and Human Service guideline titled Guide for the Care and Use of Laboratory Animals, and with the Weatherall report. The animals were fed monkey diet (Purina) supplemented daily with fresh fruit or vegetables and water *ad libitum*. Additional enrichment including the delivery of appropriate safe toys was provided. The facility overseen by the Yerkes enrichment staff, and animal health, was monitored daily by the animal care staff and veterinary personnel

available 24/7. Monkeys were caged in socially compatible same sex pairs to facilitate social enhancement and well-being. Monkeys showing signs of sustained weight loss, disease or distress were subject to clinical diagnosis based on symptoms and then provided either standard dietary supplementation analgesics and/or chemotherapy. Monkeys whose symptoms cannot be alleviated using standard dietary supplementation, analgesics and/or chemotherapy were humanely euthanized using an overdose of barbiturates according to the guidelines of the American Veterinary Medical Association. The studies reported herein were performed under IACUC protocol “Role of Glycosylation in Mucosal Transmission” which was reviewed and approved by the Emory University Institutional Animal Care and Use as well as biosafety review committees. It has been assigned the IACUC protocol number #2001725. The Yerkes National Primate Research Center has been fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International since 1985.

Virus stock generation

The infectious molecular clone (deSIV147c#4) was constructed by cloning full-length deSIV147c#4 Env (a unique early Env variant isolated at two weeks post-infection from blood of an animal infected with SIVmac251) into the infectious molecular clone SIVmac239 (Bixby *et al*, 2010; Yen *et al*, 2014) and transiently transfected into 293T cells. The resulting supernatant containing infectious virus was harvested and used to infect macaque PBMCs to generate large amounts of virus stock. The macaque PBMC-derived virus was titrated in TZM-bl cells and used to infect macaques either intravenously or intrarectally (Table 1).

Virus inoculation

As described in Table 1, one (n=1), the non-CD4+ T cells depleted macaque received 500 TCID₅₀ of the virus via intravenous route. The other two (n=2) received weekly dose of 5,000 TCID₅₀ of the virus via intrarectal route for a total of 4 weeks. All the CD4+ T cells depleted macaques (n=3) received 500 TCID₅₀ of the virus via intravenous route.

Antibodies

The studies conducted utilized both a variety of nonhuman primate reactive commercially available monoclonal antibodies and a series of monoclonal antibodies prepared by our laboratory. The commercially available monoclonal antibodies included PerCP-Cy5.5-CD16, Cat# 338426 (Clone 3G8; BD); FITC-CD14, Cat# 557153, (Clone M5E2; BD); Goat Anti-Mouse Ig, Human ads-PE, Cat# 1010-09, (Isotype IgG, SouthernBiotech); Mouse PE-IgG1 Isotype Control, Cat# IC002P, (Clone 11711, R&D); FITC-CD3, Cat# 556611, (Clone SP34, BD); FITC-CD8, Cat# 347313 (Clone SK1; BD); FITC-CD11b, Cat# 1M0530U, (Clone Bear 1; Beckman Coulter); FITC-CD20, Cat# 556632, (Clone 2H7; BD); APC-CD11c, Cat# 340544, (Clone S-HCL-3; BD); PerCP-Cy5.5-HLA-DR, Cat # 552764 (Clone G46-6; BD); PerCP-Cy5.5-CD123, Cat# 55871, (Clone 7G3; BD); PE-Texas Red-HLADR, Cat# MHLDR17, (Clone Tu36: Life Technologies); PE-anti-human CD200 (clone OX-104, Biolegend), PE-anti-human CD200R (clone OX-108, Biolegend), and PE-anti-human Mincle (clone 15H5, Biolegend). These fluorochrome-conjugated antibodies were utilized at a pre-determined optimum concentration as recommended by the manufacturer.

Polychromatic flow cytometry was performed on stained cell samples using a variety of combinations of these antibodies that identified monocytes and plasmacytoid/myeloid dendritic cells.

Polychromatic Flow Cytometry

Cell populations following staining with the appropriate cocktail of antibody reagents were subjected to analysis using a BD LSRFortessa (BD Immunocytometry Division, Mountain View, CA). Data on a minimum of 10,000 events for lymphoid cells and 100,000 cells for mDC as well as pDCs were acquired. The raw data was then subjected to analysis using the FlowJo Software (ver.9.6.2) (Ashland, OR). The gating strategies utilized for defining the various cell lineages has been published elsewhere (Byrareddy *et al*, 2014; Byrareddy *et al*, 2015; Takahashi *et al*, 2014).

Isolation of cells from blood and tissues

Mononuclear cells were isolated from the PBMCs and colorectal biopsy specimens as described previously (Byrareddy *et al*, 2016; Byrareddy *et al*, 2015). In brief, heparinized blood samples were obtained and mononuclear cells were isolated using ficoll-hypaque gradient centrifugation. A series of 12–16 pinch colorectal biopsies/sample from each of the species of monkeys were subjected to collagenase digestion, homogenization steps and finally isolated by Percoll gradient as described previously (Byrareddy *et al*, 2016; Byrareddy *et al*, 2015). The yields and viability of the pooled cells extracted from the biopsy tissues varied considerably ranging from 62% to 84% as determined by trypan blue dye exclusion method.

Necropsy

Due to study end point and/or weight loss or end of the experiment, the animals were euthanized and a complete necropsy was performed. For histopathologic examination, various tissue samples were fixed in 10% neutral buffered formalin, routinely processed, paraffin-embedded, sectioned at 5 µm, and stained with hematoxylin and eosin (HE).

Immunohistochemistry

Immunohistochemical (IHC) staining of sections of brain, lung, intestine, liver, lymph nodes, spleen from RDn15, RCb15 and RKd15 animals was performed using a biotin-free polymer system. The paraffin-embedded sections were subjected to deparaffinization in xylene, rehydration in graded series of ethanol, and rinsed with double distilled water. Antigen retrieval was performed by immersing sections in DIVA Decloaker (Biocare Medical) at 125°C for 30 seconds in a steam pressure decloaking chamber (Biocare Medical) followed by blocking with Background Sniper Reagent (Biocare Medical) for 10 minutes. The sections were incubated with rabbit anti-human CD68 (clone SP251; Sigma) and mouse anti-SIVmac251 p17 Gag monoclonal (clone KK59; obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH from Dr. Karen Kent and Miss Caroline Powell) (Kent *et al*, 1991) for 1 h, followed by a double detection polymer system (Mach 2 Double Stain 2, Biocare Medical). Labeled antibodies were visualized by development of the chromogen (Warp Red and/or Vina Green Chromogen Kits; Biocare

Medical). Digital images of brain, lung, intestine, liver, lymph nodes, spleen were randomly captured at 200 × and 400 × magnification with an Olympus BX43 microscope equipped with a digital camera (DP26, Olympus) and evaluated using Cellsens® digital imaging software 1.11 (Olympus). Presence of colocalized CD68/SIV labeled cells were semiquantitatively scored as None (- or Grade 0); <10 colocalized cells (+ or Grade 1); 10–25 colocalized cells (++) or Grade 2), >25–50 colocalized cells (+++ or Grade 3) to compare macrophage tropism of SIV strain in CD4 depleted and non CD4 depleted animals.

Results

Infection of deSIV147c#4 in RMs

deSIV147c#4 has been previously shown to infect both RM PBMCs and macrophages *in vitro* (Yen et al, 2014). To determine if deSIV147c#4 is infectious *in vivo* and transmissible via mucosal route, we first infected n=1 RM intravenously and n=2 RMs intrarectally, and then measured plasma viral loads and CD4+ T-cell counts post-infection (Fig. 1A). All three monkeys were successfully infected, causing systemic infection with plasma viral loads ranging from 10⁵ to 10⁷ copies/ml peaking between weeks 2 and 3 post-infection (pi) for animals (Fig. 1B). After peak levels, viral loads decreased and maintained persistent viral set points during the follow-up period. CD4+ T cells levels decreased shortly after infection in all animals, with nadir CD4+ T-cell levels seen at week 2 in RPh15 and week 4 for RFf15 and RFn15, followed by maintenance of CD4+ T-cell levels for the rest of infection (Fig. 2E).

Plasma and CSF viral loads in CD4+ T cells depleted RMs infected with deSIV147c#4

To examine the effect of CD4+ T-cell depletion on deSIV147c#4 infection, we depleted CD4+ T cells in RMs (n=3) by using CD4R1 antibody treatment at week 2 pre-infection and week 3 post-infection (Fig. 1C). Plasma viral loads for RDn15 and RCb15 remained stable during the course of infection with a peak of 1,661,471 copies/ml for RDn15 and 1,160,860 copies/ml for RCb15 at weeks 1 and 11 pi, respectively (Fig. 1D and E). Plasma viral loads for RKd15 increased during the entire course of infection with a peak viral load of 286,139,873 copies/ml at week 9 pi before being euthanized due to weight loss and uncontrolled viral replication (Fig. 1D). CSF viral loads remained low in RDn15 and RCb15, whereas CSF viral loads for RKd15 increased to notably high levels during the course of infection, peaking at week 7 with 18,514,734 copies/ml (Fig. 1E). Overall, we observed low viral loads in CSF in RDn15 and RCb15 as compared to RKd15 which also correlates with brain pathology. The RKd15 animal had higher CSF viral loads, higher inflammation and SIV positive cells in brain sections (see Fig. 3C and Supplementary Fig. S3) as compared to two other animals.

Analysis of CD4+ and CD8+ T cells in blood and lymph node (LN) and rectal biopsies (RB) in CD4+ T-cell-depleted RMs infected with deSIV147c#4

Treatment with CD4R1 effectively depleted circulating CD4+ T cells in all animals until week 9, at which time all animals showed a small increase in the levels of these cells (Fig 2A and B). The % of CD8+ T cells sharply increased after the first dose of CD4R1 and remained high during the entire course of infection (Fig. 2C). Absolute numbers of CD8+ T

cells showed a sharp increase after the first dose of CD4 depleting antibody and varied later on, with RKd15 showing the lowest CD8 cell counts among all animals. Lymph node (LN) biopsies were collected at 3 weeks pre-infection and subsequently at weeks 1, 9, 13 p.i., followed by measurement of the levels of CD4⁺ T-cells in LN by FACS analysis (Fig 2I). All three monkeys had similar levels of CD4⁺ T cells in LN pre-infection, with a mean of 63% (range: 57.6% - 68.5%). At week 1 p.i., CD4⁺ T-cell levels in LN decreased 7.7-fold (mean: 8.1%, range: 4.03% - 15.1%) compared to pre-infection, and continued to decrease through week 9 p.i., with a decrease of 10.9-fold (mean: 5.8%, range: 4.1% - 6.7%) compared to pre-infection levels (Fig. 2I). CD4⁺ T-cell levels in LN increased slightly at week 13 p.i., with a mean of 10.57% (range: 8.2% - 12.2%; Fig. 2I). Administration of CD4R1 antibody effectively depleted CD4⁺ T cells in rectal tissues, and levels remained low during the entire course of infection, even after the last dosage of CD4R1 (Fig. 2J). Mean CD4⁺ T-cell levels in rectal tissues at baseline were 35.5% (range: 26.9% - 33.8%) and decreased by 2.9-fold, 8.6-fold, 16.9-fold, 15.4-fold, and 16.6-fold at weeks 1, 9, 13, 17, and 21 weeks p.i., respectively. We measured changes in the frequencies of classical monocytes (CD14⁺CD16⁻), intermediate monocytes (CD14⁺CD16⁺), and mDCs (Lin⁻CD14⁻CD16⁻HLADR⁺CD11c⁺) in blood of depleted and non-depleted CD4⁺ T cells RMs infected with deSIV147c#4. There was an increase in the frequencies of these subsets after CD4⁺ T cells depletion infusion (Supplementary Fig. S1). The area under curve (AUC) for the classical monocytes data showed that the CD4⁺ T cells depleted group is clearly higher than the non-CD4⁺ T cells depleted group (Supplementary Fig S2). Interestingly, in the CD4⁺ T cells depleted group there was an increase in the intermediate monocytes and mDCs throughout the course of infection (Supplementary Fig. S1 B and C).

Pathological examination of CD4⁺ T-cell-depleted RMs infected with deSIV147c#4

Postmortem examination of gross pathology revealed that the lungs of RKd15 were not completely collapsed and had multifocal dark red-gray areas at the surface as well as within the parenchyma suggestive of interstitial pneumonia. No major gross pathology was observed in the other infected rhesus macaques RDn15 and Rcb15. Histologically, the brain sections (basal ganglia, occipital lobe, frontal lobe and brain stem) from RKd15, the animal with high CSF viral loads post-infection, showed SIV encephalitis characterized by accumulation of perivascular macrophages, multinucleated giant cells, microgliosis, and neuronal injury in gray and white matter in brain. There were multifocal histiocytic nodules composed of macrophages, multinucleated giant cells, and scattered lymphocytes within the brain parenchyma. Other significant histological findings in RKd15 included interstitial pneumonia consistent with SIV giant cell pneumonia and moderate lymphohistiocytic pericholangitis. IHC staining revealed multifocal infiltration of CD68 (macrophage marker)-labeled macrophages (green), SIVmac251 p17 labeled cells (Fuchsin-red), their colocalization in macrophages in the brain parenchyma (Fig. 3A) and perivascular regions (Fig. 3B). In addition, CD68 and SIV mac251 p17 colocalization was observed in spleen (Fig. 3C), lymph nodes (Fig. 3D), lung (Fig. 3E), colon (Fig. 3F) and liver from RKd15, indicative of macrophage infection in multiple organs. The other infected macaques (RDn15, Rcb15) had a lower number of CD68, no brain lesions, and SIVmac251 p17 colocalized staining in macrophages were observed in lymphoid organs, intestine, as well as lung. Furthermore, CD4 depleted macaques showed increased numbers of CD68/SIV colocalized

cells in various organs including brain, lung, intestine, spleen, and lymph nodes (Supplementary Fig. S3) compared to the non-CD4⁺ T cells depleted animals infected with deSIV147c#4 (Table 2). Overall, CD4⁺ T cells depleted animals showed more inflammation and SIV viral antigen in the organs/tissues than non-CD4⁺ T cells depleted animals.

Discussion

The present study demonstrates that 1) the macrophage-tropic strain molecular clone deSIV147c#4 can efficiently infect RMs *in vivo*; 2) increased macrophage tropism in various organs/tissues; 3) increased macrophage tropism leads to development of SIV encephalitis in one of the three CD4⁺ T cells depleted animals. The deSIV147c#4 is a SIVmac251 early variant isolated from the blood of a RM two weeks post-infection (Yen *et al*, 2014). The uncloned SIVmac251 is a viral strain that replicates efficiently in both CD4⁺ T cells and macrophages, and is routinely used for HIV/AIDS studies in non-human primates (Daniel *et al*, 1985; Kanki *et al*, 1985; Letvin *et al*, 1985; Miller *et al*, 1998). However, this strain has a large amount of genetic heterogeneity when isolated from different sources, limiting its usefulness in the study of SIV/HIV pathogenesis (Del Prete *et al*, 2013; Strickland *et al*, 2011). The deSIV147c#4 molecular clone mediates high levels of fusion, replicates efficiently in RM PBMCs as well as macrophages, induces formation of multi-nucleated giant cells during infection of macrophages *in vitro* (Yen *et al*, 2014), and infects macrophages in brain/other tissues of rhesus macaques *in vivo*. Together, these findings demonstrate that deSIV147c#4 is a pathogenic SIVmac251 molecular clone that exhibits unique features compared to other pathogenic SIV clones (e.g., SIVmac239, SIVmac316, and SIVsmE543), including its isolation from an animal during early infection rather than late-stage disease, and its ability to efficiently infect macrophages in multiple organs including brain, lymphoid tissues, gastrointestinal tract, lungs *in vivo*.

To determine the effect of deSIV147c#4 on disease progression *in vivo*, we infected 3 RMs with deSIV147c#4. All 3 monkeys developed infection with deSIV147c#4 based on viral loads in plasma. These animals had an initial increase in viral load, which decreased during the subsequent course of infection. Following infection, CD4⁺ T cells were initially decreased and then stabilized during the rest of infection, a pattern commonly seen in during the course of SIV infection in RMs. To reduce the possible antiviral effects of CD4⁺ T cells and enhance disease progression, we depleted CD4⁺ T-cells in three RMs using the anti-CD4 monoclonal antibody, CD4R1. CD4⁺ T-cells were effectively depleted of CD4⁺ T cells in blood, colon, and LNs following administration of CD4R1. After the last dose, CD4⁺ T cells rose slightly but did not reach pre-infection levels. RMs depleted of CD4⁺ T cells maintained high viral loads during the course of infection compared to animals that were not CD4 depleted. In addition, these RMs' viral loads remained steady during the course of infection compared to the rapid decrease seen in non-CD4 depleted monkeys. CD8⁺ T cells increased rapidly after CD4⁺ T-cell depletion and remained elevated during the entire course of infection, even after the last dose of CD4R1. Thus, deSIV147c#4 infection recapitulates important immunologic aspects of HIV infection *in vivo*.

One CD4⁺ T-cell depleted animal (RKd15) showed rapid progression as evidenced by high viral loads and significant weight loss. In this animal, we found evidence of extensive

macrophage infection in the brain parenchyma, lymph nodes, spleen, colon, lung, and liver, as demonstrated by co-localization of SIVmac251 p17 and the macrophage marker CD68. These results confirm that deSIV147c#4 is a pathogenic clone that infects macrophages *in vivo* in brain as well as other organs and tissues *in vivo*. Overall, these findings suggest that CD4+ T cells depletion is required to induce CNS infection with deSIV147c#4. This was reflected by occurrence of more SIV positive cells in macrophages/other organs in CD4+ T cells depleted animals as compared to non-CD4+ T cells depleted animals. Several studies have used CSF as a surrogate source of virus from the brain, and CSF viral loads often predict the neurological outcome with HIV infection (Ellis *et al*, 1997; McArthur *et al*, 1997; Strain *et al*, 2005; von Giesen *et al*, 2005). This is well supported in our studies, with histopathology and CSF data corroborated in all the animals including RKd15, suggesting that CSF viral loads correlate with virological changes in the brain.

Genetic sequencing of deSIV147c#4 showed that it shares high sequence homology with the gp120 consensus sequence in the brain of animals that developed neurological disease suggesting that this variant is likely to cross the blood-brain barrier and infect cells in the brain (Yen *et al*, 2014). HIV/SIV neurological disease is characterized by infiltration of infected monocytes across the blood-brain barrier, resulting in activation of perivascular macrophages/microglia followed by establishment of CNS infection and chronic inflammation (Bissel *et al*, 2008; Clements *et al*, 2008; Valcour *et al*, 2011; Zink and Clements, 2002). Pathological examination of specimens from SIV-infected non-human primates and HIV-infected individuals show decrease in both white and gray matter, with larger decrease associated with greater decline in neurocognitive abilities (Aylward *et al*, 1995; Babas *et al*, 2003; Cysique *et al*, 2017; Kuper *et al*, 2011; Williams *et al*, 2016; Williams *et al*, 2002; Wohlschlaeger *et al*, 2009). In addition, these animals show multifocal perivascular aggregates composed of macrophages and multinucleated giant cells that are sites of productive infection (Sharer *et al*, 1988; Valcour *et al*, 2011). In this study, one monkey, RKd15, developed SIV encephalitis characterized by accumulation of perivascular macrophages, multinucleated giant cells, microgliosis, and neuronal injury in gray as well as white matter in multiple regions of the brain. These results suggest that deSIV147c#4 is able to enter the CNS and cause neurocognitive dysfunction, particular in the setting of CD4+ T cells depletion.

Herein, we demonstrate that deSIV147c#4 is a unique macrophage-tropic SIV molecular clone that can enter the CNS, infect macrophages in the brain, leading to SIV-associated encephalitis and neurocognitive dysfunction. This model may be useful to study macrophage infection in HIV/SIV-associated neurocognitive disorders, gain insights into myeloid cell reservoirs in brain/other anatomical sites, and test strategies for eradication. However, further studies are warranted to test this model in the context of ART for evaluating its relevance in people living with HIV which in turn will reveal pathophysiology of this model in the context of ART.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

(A & C) Scheme of virus inoculation via intravenous (with and without CD4⁺ T cells depletion) or intrarectal route with SIVmac251 desiv147#C4; red arrows indicate virus is given intravenously; blue arrow indicate virus is inoculated intrarectally by low dose over 4 times; black arrows indicated infusion of CD4R1 antibody to deplete CD4⁺ T cells. (B). Plasma viral loads of animals inoculated intravenously or intrarectally and viral loads were monitored over several weeks. (D & E) Plasma and CSF viral load in the CD4⁺ T cells depleted group.

Fig. 2.

CD4⁺ and CD8⁺ T cell profile. (A, E and B, F), shows the % CD4⁺ T cells and absolute CD4⁺ T cell counts, in the CD4 depleted and non-CD4+ T cells depleted group, respectively. (C, G and D, H), shows the % CD8⁺ T cells and absolute CD8⁺ T cell counts, in the CD4+ depleted group and non-CD4+ T cells depleted group, respectively. I and J, shows the % CD4⁺ T cells in lymph node and rectal biopsies in CD4+ T cells depleted group, respectively.

Fig. 3.

IHC staining from representative tissue sections from a CD4⁺ T cells depleted SIVmac251desiv147#C4 infected rhesus macaque (RKd15). (A) The brain parenchyma contains a histocytic nodule with multiple SIVmac251 p17 (green) and CD68 labeled cells (Fuchsin-red) along with SIV/CD68 colocalization (B) Perivascular cuff in the brain composed of small numbers of SIVmac251 p17- and CD68-labeled cells. Scale bar = 20µm. (C, D) Spleen (C) and lymph node (D) contain many SIVmac251 p17- and CD68- labeled cells. Scale bar = 50µm. (E) The lung shows extensive colocalization of SIVmac251 p17- and CD68-labeled cells. Scale bar = 50µm. (F) Section of colon demonstrates SIVmac251 p17- and CD68-labeled cells in the lamina propria and submucosal lymphoid follicle. Scale bar = 50µm. SIVmac251 p17 and CD68 IHC staining is shown in green and Fuchsin-red color, respectively.

Table 1**Details of the macaques and procedures involved in the study**

Experimental details for the study including route of virus inoculation, sex, age and weight of the animals.

Animal ID	SEX	Age	Weight (Kg)	Mode of virus inoculation	CD4 depletion
RPh15	M	2 Years 3 Months	3.9	Intravenous	No
RFl15	M	2 Years 6 Months	4.5	Intrarectal	No
RFn15	M	2 Years 5 Months	3.5	Intrarectal	No
RDn15	M	3 Years 6 Months	5.1	Intravenous	Yes
RCb15	M	3 Years 8 Months	7.0	Intravenous	Yes
RKd15	M	3 Years 5 Months	4.8	Intravenous	Yes

Comparative numbers of colocalized CD68/SIV labeled cells in CD4 depleted and non-CD4 depleted animals infected with SIV_{mac251} deSIV147c#4.

Table 2

	Non CD4 depleted macaques			CD4 depleted macaques		
	RPh15	RFl15	RFn15	RDn15	RCb15	RKd15
Brain	-	-	-	-	-	+++
Lung	+	+	+	+	++	+++
Intestine	+	+	+	+	++	+++
Spleen	+	-	+	++	++	+++
Lymph nodes	+	+	+	++	++	+++

Scoring criterion: No colocalized cells (- or Grade 0); <10 colocalized cells (+ or Grade 1); 10–25 colocalized cells (++ or Grade 2); >25–50 colocalized cells (+++ or Grade 3)