Characterization of monocyte subsets through the course of AIDS pathogenesis and correlations with the development of SIV-Encephalitis

Author: Hyunjin Shin

Persistent link: http://hdl.handle.net/2345/2221

This work is posted on eScholarship@BC,
Boston College University Libraries.

Boston College

The Graduate School of Arts and Sciences

Department of Biology

Characterization of monocyte subsets through the course of AIDS

pathogenesis and correlations with the development of SIV-Encephalitis

A thesis

by

Hyunjin Shin

Submitted in partial fulfillment of the requirements

For the degree of

Master of Science

December 2010

Characterization of monocyte subsets through the course of AIDS pathogenesis and correlations with the development of SIV-Encephalitis
A thesis by Hyunjin Shin
Advisor: Kenneth Williams, PhD

Abstract

Individuals infected with Human Immunodeficiency Virus (HIV) are susceptible to pathological abnormalities due to the infiltration of virus into different anatomical compartments. Monocytes are a heterogeneous population that undergoes changes in phenotype with HIV infection. It is hypothesized that changes in monocyte subsets observed through the course of infection will correlate with the development of SIV-Encephalitis (SIVE). 14 CD8+ T cell depleted rhesus macaques were infected with SIVmac251 and changes in 3 monocyte subsets, defined by their CD14 and CD16 surface expression as CD14+CD16-, CD14+CD16+, and CD14-CD16+, were tracked through the course of disease. The CD14+CD16- subset increased in the absolute number of cells and decreased in percentage of the total monocyte population. The CD14+CD16+ and CD14-CD16+ subsets increased in both absolute number and percentage. These changes have a biphasic dynamic that occurs during early infection and is pronounced in encephalitic animals. Several markers showed differential expression with infection and between subsets. Mac387, an early monocyte-macrophage marker, demonstrated a considerable decrease in expression. Concomitant with this change, CD68, CD163, CD44v6, CCR2, and CD64 increased expression in the total monocyte population, with the magnitude of these changes occurring in a subset-specific manner. In conclusion, monocyte subsets undergo changes with SIV infection that correspond to the development of encephalitis, highlighting the contribution of monocytes in neuroAIDS.

Table of Contents

Acknowledgements

Abstract

Acknowledgements

First and foremost, I would like to acknowledge and thank my advisor, Dr. Kenneth Williams, for allowing me the opportunity to pursue this avenue of research. His enthusiasm for the process of scientific discovery and desire to push the boundaries of our current understanding of HIV and AIDS pathogenesis truly sets an example and high standards for everyone in our lab. I am grateful to him for his patience with me through the course of this project, and for the guidance he has provided in furthering my understanding of the field. Most importantly, Dr. Williams has shown me the importance of dedication, discipline, and passion for one's pursuits, a lesson that I will value long after my tenure at Boston College.

I thank my committee members, Dr. Junona Moroianu and Dr. Marc-Jan Gubbels, for the time, guidance, and insights they have provided. I have appreciated every interaction we have had and the kindness you have shown through the course of my graduate education.

I thank Dr. Tricia Burdo, who has been a tremendous resource through the course of my project. From the first day I rotated through this lab, she was willing to teach and guide me through learning new techniques and troubleshooting problems. As our "Lab Mom," Dr. Burdo has provided emotional support, well-timed jokes, and conversations that have brought a smile to my face on more than just one occasion. For all of this, thank you.

I thank Dr. Caroline Soulas and Dr. Patrick Autissier, two members of our lab for whom I have incredible respect and gratitude. Dr. Soulas has shown by example and through our conversations a remarkable discipline and mastery of the scientific method that has been nothing short of inspirational. Dr. Autissier processed thousands of my samples over the course of the past two years, and has always shown me great patience and willingness to answer questions and provide technical help for which I thank him.

I thank my past and present fellow Williams lab graduate students, Jennifer Campbell, Arleide Lee, Loseke Pascal Losambe, and Brian Nowlin. I can think of no other group of people with whom I would have wanted to share this experience. I was fortunate to find such a supportive set of colleagues who I now consider my dearest friends. We have shared so many experiences and created memories that I will reflect upon fondly for years to come.

I thank Dr. Barbara Nikolajczyk for her continued friendship, encouragement, and advice throughout my time working in her lab and after I left to pursue graduate school. I feel fortunate to have worked with her for my first true experience as a researcher.

I thank the other students, faculty, and staff of the Biology department for their support, encouragement, and insights. I wish you the best of luck in all of your future endeavors.

Finally, I would like to thank my family for their continued support and encouragement of my personal, academic, and career pursuits.

INTRODUCTION

Monocytes: A heterogeneous population of innate immune cells

Monocytes are a population of bone marrow derived cells that serve as both progenitors to tissue macrophages and immune effector cells that represent one arm of the mononuclear phagocyte system. Monocytes are found in the blood, where they remain in circulation until they extravasate and enter tissues in a chemokine and adhesion receptor mediated fashion. Once in the tissues, monocytes undergo maturation into resident tissue macrophages. Monocytes are essential in the defense against pathogens and foreign entities, playing an important role in antigen-presentation, phagocytosis of foreign matter and debris, and as mediators of immune responses through the production of cytokines. In order to recognize microorganisms, monocytes possess a number of different scavenger receptors and pathogen-associated molecular pattern (PAMP) receptors [1], as well as a number of different chemokine receptors. The heterogeneous nature of this cell population is illustrated in the differential distribution of these receptors amongst the subsets.

Developmentally, monocytes result from successive commitment steps beginning with the differentiation of hematopoietic stem cells (HSC) into common myeloid progenitors (CMP), granulocyte-macrophage precursors (GMP), and then macrophage/DC progenitors (MDP) [2]. While monocytes are thought to develop from the

macrophage/DC progenitors in bone marrow, the heterogeneity of circulating monocytes and the developmental path that produces this diversity is not well understood. Currently, commitment of myeloid progenitors to a monocyte/macrophage/DC lineage is associated with presence of CX3CR1, the fractalkine receptor, as it is only first detected on MDPs [3]. The commitment to monocyte development is restricted by the transcription factors participating in differentiation. PU.1 has been identified as a crucial transcription factor and is required for early commitment of a cell to CMP [4] by acting in an antagonistic manner against other transcription factors that would lead to non-monocyte developmental programming. Additionally, PU.1 activates monocyte/macrophages transcription factors.

Monocytes in human and rhesus blood can be broadly divided into subsets based upon their differential expression of surface markers CD14 and CD16. While nomenclature and delineation of the monocyte subsets varies, for the purposes of this report, the 3 main subsets of interest will be referred to as CD14+CD16-, CD14+CD16+, and CD14-CD16+. In a healthy individual, the "classical" CD14+CD16- subset comprises 80-90% of the total monocyte population. The non-classical, or "inflammatory" monocytes, defined by their relatively increased expression of CD16, CD14+CD16+ cells, comprise 10-20% of the total monocyte population, and have been shown to increase in frequency of the total monocyte population and in absolute cell number. These cells also more resemble mature tissue macrophage, suggesting that they are of a more mature phenotype than the CD14+CD16- subset. The CD14+CD16- and CD14+CD16+ populations can

also be differentiated by their differential expression of other surface makers. CD14+CD16- cells express chemokine receptors CCR2, CX3CR1, and the Fc-gamma receptor 1, CD64, unlike the CD14+CD16+ subset, which expresses lower CCR2 levels, but has higher expression of CX3CR1, CCR5, CD86. Functionally, the CD14+CD16- monocytes exhibit high phagocytic activity, produce IL-10 in response to LPS in-vitro [5], and have lower cytokine production than the CD14+CD16+ subset. In contrast, the CD14+CD16+ monocytes produce TNF-α and IL-1 in response to LPS stimulation [6], but also show high phagocytic capabilities. CD14-CD16+ monocytes remain the least characterized of the three subsets. Previous reports have shown that compared to the CD14+ monocytes, the CD14-CD16+ subset is weakly phagocytic, and does not produce TNF-α and IL-1 in response to LPS [7]. Understanding the diversity within the monocyte population, both in steady-state homeostatic conditions and in times of duress, will provide insight into factors influencing disease progression.

Attempts to characterize differences between the monocyte subsets using gene array data have provided interesting insights into the potential roles of these cells. Genes involved in FcgammaR mediated activity, B cell receptor signaling, and apoptosis signaling were preferentially up-regulated in the CD16+ monocytes, whereas genes involved in antimicrobial processes were up-regulated in CD14+CD16- monocytes [8]. Ancuta et al. have shown with transcriptional profiling a developmental relationship between the CD16- and CD16+ subsets, indicating that the different monocyte subsets originate from a common myeloid progenitor, but have distinct developmental paths. The CD16+ subset

was distinguished by upregulation of transcripts for dendritic cell and macrophage markers. Contrastingly, CD16- subsets showed upregulation of transcripts for myeloid and granulocyte markers. The authors conclude that these subsets are likely to give rise to dendritic cell and macrophage subpopulations with distinct roles in immunity and disease pathogenesis [9]. Kim et al. have reported differences in gene expression between monocyte subsets in uninfected rhesus macaques. Of particular note, the CD16+ subsets exhibited upregulation in genes involved in monocyte-to-macrophage differentiation. Examples cited included monocyte/macrophage phenotype markers, adhesion molecules, macrophage specific enzymes, and transcription factors driving monocyte-to-macrophage differentiation. A comparatively small number of genes involved in lymphotoxic activity distinguished the CD14+CD16+ and CD14-CD16+ subsets [10].

Human Immunodeficiency Virus (HIV) and Simian Immunodeficiency Virus (SIV)
Human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) are closely related retroviruses that are the underlying cause of the Acquired Immune Deficiency Syndrome (AIDS). AIDS is characterized by a severe defect in cell-mediated immunity as a result of the infection and eventual ablation of CD4+ T cells in the host that eventually leads to immune system failure. Upon primary infection, a drop in CD4+ T cells can be observed, along with a concomitant rise in HIV and SIV RNA detected in plasma, indicative of active replication of the virus during this acute stage of infection. Followed by the activation of CD8+ T cells and the ramping up of a humoral response against the virus, CD4+ T cells make a recovery and the host's viral load is decreased,

resulting in a period of clinical latency. Eventually, however, the CD4+ T cell population is compromised to the point where cell-mediated immunity is no longer effective, viral load increases, and opportunistic infections take hold [11].

Most individuals who develop AIDS that do not receive retroviral therapy die from opportunistic infections or malignancies associated with compromised immune systems. While the CD4+ T cell compartment is the most compromised through the course of AIDS pathogenesis, HIV and SIV strains can infect many different cell types. The interaction of CD4 with the viral envelope protein gp120 causes a conformation change that exposes its co-receptor binding site. CCR5 and CXCR4 are the major co receptors identified; virus will infect T cells expressing CXCR4 and macrophage-tropic HIV and SIV strains can gain entrance into macrophages through the engagement of the CD4 receptor and its co-receptor CCR5. The ability of HIV to infect multiple cell types within a host allows for its dissemination through different tissue compartments and in varying states of replication and latency [12]. In addition to the use of different co-receptors, viral replication within macrophages differs from that which occurs in T cells. Upon gaining entry into the host T cell, HIV and SIV will form preintegration complexes that enter the nuclear envelope. Integration and transcription of the viral genome is followed by assembly and budding of virions into the extracellular space. In the case of macrophages, budding and accumulation of viral particles can occur into cytoplasmic vacuoles defined as late endosomes/multivesicular bodies (LE/MVBs). Virus in LE/MVBs may be more stable and are better capable of escaping degradation and evading immune system

recognition, which becomes especially important in the context of central nervous system infiltration by infected monocyte/macrophages acting as viral reservoirs of infectious disease [13].

HIV and SIV infection pertaining to the Central Nervous System

In addition to susceptibility to opportunistic organisms, HIV and SIV infections result in a number of pathological abnormalities due to the infiltration of many anatomical compartments in addition to peripheral blood [14]. HIV and SIV devastate the immune system and results in significant neurological dysfunction in a significant portion of affected individuals. One of the earliest events of HIV infection involves the penetration and infiltration of the CNS, occurring on the scale of hours to days post-infection. Even at this early juncture, virus is detectable in the brain [15]. Neurological complications are common amongst HIV infected individuals, resulting in behavioral, motor, and cognitive perturbances. Approximately 20-40% of infected individuals develop significant cognitive dysfunction [16,17], and postmortem neuropathological abnormalities have been detected in 80% of patient autopsies [18]. Neurological damage can manifest itself at any point during the course of infection, although most develop with more advanced disease.

In the advent of highly active antiretroviral therapy (HAART), the prevalence of HIV-associated dementia (HAD), the clinical correlate to HIVE, has decreased, although milder forms of cognitive impairment are still observed amongst infected individuals.

While there has been a decrease in the incidences of HAD, because of the inability of some retroviral drugs to cross the blood-brain barrier (BBB), and as patients live longer with the disease [19], the overall prevalence of this condition is on the rise. The ability of HAART to attenuate, but not eradicate, HIV-associated disease in the brain indicates events occurring outside of the CNS play a substantial role in neuropathogenesis.

HIV-Encephalitis (HIVE) and SIV-Encephalitis (SIVE) are pathologically defined by lesions consisting of gliosis (the proliferation of astrocytes), microglial nodules, detectable virus in the CNS, perivascular macrophage accumulation, and the presence of activated macrophages, neuronal loss, and multinucleated giant cells (MNGC) [20]. Macrophage-tropic SIV is the predominant virus found in the CNS of macaques with SIVE [21,22]. HIVE is unlike most viral encephalitis in that HIV does not productively infect neurons. Rather, productive replication has only been observed in perivascular macrophages and microglia [23,24,25]. Viral infection also does not appear to be the best predictor of HIVE or SIVE, but rather the number of infected mononuclear phagocytes and their degree of activation are better correlates to disease [26]. It has been shown that both infected and uninfected cells in the CNS produce increased amounts of chemotactic and inflammatory mediators that further recruitment of monocyte macrophage. Concomitantly, immune activation in the periphery leads to the activation of monocytes, both infected and uninfected, causing an increased response to chemokines.

Myeloid lineage cells and HIV/SIV infection

Peripheral blood monocytes and resident tissue macrophages are cellular targets of HIV and may serve as a viral reservoir during the apparent latency period of infection. Both infected and uninfected monocyte/macrophages can extravasate from the blood and cross the blood brain barrier. Initial viral infection of the CNS as well as continuous reseeding with virus from the periphery occurs, and previously uninfected lymphocytes can encounter HIV/SIV at this juncture. Monocytes that remain in the brain and differentiate into resident tissue macrophages, specifically those that become perivascular macrophages, are the major cell type infected in the brain [27,28]. Monocytes have a lower susceptibility to infection compared to more mature macrophage, presumably due to the lower expression levels of coreceptors used for viral entry [29]. This is supported by the upregulation of CCR5, the major coreceptor for the entry of HIV strains into myeloid cells [13], in the more phenotypically mature CD14+CD16+ monocyte subset. When infected, monocytes can support a continuous low-level of virus production throughout their lifetime, and have been found to harbor latent proviral DNA through the course of disease. Additionally, because of their immune privilege status these cells are capable of crossing the blood brain barrier and carrying virus to the brain in a Trojan horse-type manner [27]. These cells serve as immunoregulatory cells through the production of cytokines and chemokines in response to HIV and SIV. After the initial introduction of SIV and HIV in the CNS, viral RNA or proteins are not detected during the asymptomatic period of infection [25,30,31,32], and HIV and SIV DNA is not detected or found in very small amounts during this same period of time[33]. In HIV and

SIV, it has been shown that the virus re-emerges from latency to productive infection with the development of AIDS [34].

Peripheral blood monocyte subsets and SIV-Encephalitis.

The activation and expansion of monocyte/macrophage subsets with HIV and SIV infection is recognized as a critical parameter of AIDS pathogenesis and possibly a marker for the development of neuropathogenesis [35,36]. Bone marrow derived lymphocytes and monocyte subpopulations show dynamic changes and phenotypic shifts with lentiviral infection and the development of AIDS. The CD16+ subset has an increased susceptibility to HIV infection [37], and CNS perivascular macrophages are similar to this subset in both phenotype and HIV and SIV infection. Macrophages derived from the CD14+CD16+ subset are especially efficient at promoting T-cell activation, virus transfer, and HIV-1 replication [38,39]. It has been demonstrated that with HIV and SIV infection, monocytes exhibit an increase in both the percentage and absolute number of cells expressing CD16+ [40]. It is not fully understood whether this increase in circulating CD14+CD16+ cells is due to increased release from the bone marrow or recirculation of cells leaving other tissue compartments. It is unknown whether the recently characterized monocytes originating from the spleen have a contributing role in this phenomenon [41].

This expansion in the CD14+CD16+ monocyte subset has been correlated with the development of NeuroAIDS. It has been shown previously in one longitudinal study

assessing the mechanisms of neuronal injury in the rhesus macaque model that monocyte

activation outside the CNS appears to play a central role [42]. Using the thymidine

analog BrdU, it has been shown that with infection there is an increase in bone marrow

derived monocyte turnover with AIDS progression in macaques [43]. There still remain

questions concerning the definitive roles of the different monocyte subsets and the roles

they play in HIV and SIV driven neuropathogenesis.

See Figure 1.

Peripheral blood monocytes are subject to both peripheral immune activation "push" and

chemokine gradient "pull" forces that direct them towards the CNS [44]. During

infection, the activated monocyte populations will show an increase in CD16 as well as

chemokine and cytokine receptors, allowing for an increased responsiveness to

chemokines such as monocyte chemoattractant protein 1 (MCP-1) and fractalkine

(CX3CL1) [45]. CD16+ cells have an increase in CD163, a hemoglobin/haptogloin

scavenger molecule. It has been suggested that CD163 may play a role in perivascular

macrophage protection from blood brain barrier (BBB) breakdown and leakage [46],

further substantiating a "push" force that draws activated blood monocytes to the brain.

These activated monocytes are recruited to the CNS by increased levels of chemokines

such as MCP-1, Stromal cell-derived factor-1α (SDF-1α, or CXCL12), and fractalkine.

Increases in MCP-1 have also been shown to disrupt the BBB, leading to increased

permeability and reduction in tight junction proteins between endothelial cells [47]. SDF-

1α engagement of its receptor, CXCR4, on monocytes has also been shown to initiate the disengagement of monocytes from inflamed brain microvascular endothelial cells (BMVEC) mediated by β2-integrin/intercellular adhesion molecule-1 (ICAM). In addition, monocytes move toward the SDF-1 gradient and cross the BBB. Within the context of heightened activation and signaling that occurs with HIV/SIV infection, the continuous entry of monocytes into the CNS provides a source of replenishment of resident macrophages that can accumulate in response to increasingly deregulated and damaged neuronal tissue.

Breakdown of the BBB plays an important role in the progression of HIVE and SIVE, as BBB dysfunction is greater in HIV+ patients with dementia than in HIV+ patients without dementia or seronegative controls [48,49]. In addition to exposure to the aforementioned chemokines, viral proteins secreted by infected cells have also been implicated in BBB compromise. Gp120-mediated cytotoxicity has been shown to down-regulate and compromise the tight junction proteins that hold the BMVEC together [50,51], resulting in greater permeability. Without an intact BBB, factors such as endotoxins, free virus and virus particles, activated monocytes, and other lymphoid cells can contribute to CNS damage with greater ease.

Rhesus macaque models of disease

Many aspects of HIV neuropathogenesis cannot be addressed in humans and so are studied in animal models. It is accepted that HIV originated from the transmission of SIV to humans, as is evidenced by the high homology between HIV-1 strains and

chimpanzee-infecting SIV [52]. Unlike HIV, SIV strains can be transmitted between monkeys but not result in the immune system devastation observed in humans[53]. It was recognized that geographically isolated populations of monkeys, however, could transmit virus to one another and develop conditions similar to AIDS [54], resulting in the development of monkey models that could closely recapitulate phenomena observed in humans. Simian models, in particular the rhesus macaque (*Macaca mulatta*), serves as an excellent model for neuropathological studies. SIV strains have high sequence homology, genomic organization and biological properties similar to HIV-1 and HIV-2 [55]. SIV infection in macaques results in 30-40% of animals developing encephalitis, mimicking HIV infection in humans [56]. The CD8 T cell depleted model uses a CD8-depleting monoclonal antibody administered intravenously to the monkeys [57]. This model results in increased levels of plasma virus and recapitulates AIDS onset on a much shorter time frame. Of monkeys that are treated with CD8-depleting antibody, most remain consistently depleted of CD8+ T cells for greater than 28 days, and of these animals 80% develop encephalitis [58].

MATERIALS AND METHODS

Animals, viral infections and CD8+ T lymphocyte depletion

Fourteen rhesus macaques (Macaca mulatta) were utilized in this study. Five were housed at Harvard University's New England Primate Research Center (NEPRC) and nine were housed at the Tulane University's National Primate Research Center (TNPRC) in accordance with standards of the American Association for Accreditation of Laboratory Animal Care. The animals were intravenously inoculated with SIVmac251 (20 ng of SIV p27), kindly provided by Ronald Desrosiers. Blood samples were taken prior to, on the day of infection, and weekly thereafter. CD8+ T lymphocyte depletion was achieved with subcutaneous administration (10 mg/kg) of human anti-CD8 antibody cM-T807 at 6 days post-infection (DPI), proceeded by intravenous administration (5 mg/kg) at 8 and 12 DPI CD8+ T lymphocyte depletion was monitored by flow cytometry prior to antibody treatment and weekly thereafter as previously described [42]. Four of the animals were long-term depleted (>28 days), whereas one animal was short-term depleted (<21 days). Upon development of AIDS, animals were anesthetized with ketamine-HCl and euthanized with intravenous pentobarbital overdose and exsanguinated.

Flow Cytometry

Flow cytometric analyses were conducted on 100 ul aliquots of peripheral whole blood. Erythrocyte lysis was performed (ImmunoPrep Reagent System, Beckman Coulter),

followed by 2 washes with PBS, and incubation with fluorochrome-conjugated antibodies

including anti-CCR2-APC (clone: 48607, R&D Systems), anti-CD14-APC (clone:

M5E2, BD Pharmingen), CD14-FITC (clone: M5E2, BD Pharmingen), anti-CD163

(clone: MAC2-158, Trillium Diagnostics), anti-CD16-PE (clone: 3G8, BD Pharmingen),

anti-CD16-PECy7 (clone: 3G8, BD Pharmingen), anti-CD20-APC (clone: 2H7, BD

Pharmingen), anti-CD3-APC (clone: SP34-2, BD Pharmingen), anti-CD44v6-biotin

(clone: VFF-7, Invitrogen), anti-CD68-FITC (clone: KP1, Dako), anti-CD8-APC (clone:

RPA-T8, BD Pharmingen), anti-CX3CR1 (Torrey Pines), anti-HLA-DR-PerCP-Cy5.5

(clone: L243, BD Pharmingen), and anti-MRP8/14-biotin (clone: 27E10, Bachem).

Intracellular staining was achieved by permeabilization of lymphocytes with

Cytofix/CytopermTM buffer for 15 minutes (BD Biosciences) at room temperature. Cells

were washed with BD Perm WashTM buffer and incubated for 30 minutes with

antibodies including anti-Mac387-FITC (clone:MAC387, Serotec), IgG1 isotype control

(clone: MOPC-21, BD Pharmingen), anti-CD68-FITC (clone: KP1, Dako), and anti-

MRP-8-biotin (clone: 8-5C2, Bachem). Biotinylated antibodies were subsequently treated

with Strepavidin-APC (SAV-APC) (Invitrogen), and anti-CX3CR1 conjugated samples

were incubated with secondary antibody goat anti-rabbit-APC (Invitrogen). All samples

were fixed in 2% paraformaldehyde, and data acquired on a BD FACS Aria (BD

Biosciences). Analysis was performed using FloJo version 8.7 (Tree Star).

Statistical analysis

Statistical and graphical analyses were performed using Prism version 5.0a (GraphPad

Software, Inc., San Diego, CA) software and Microsoft Excel for Mac version 11.3.6.

Viral load determination

Plasma SIV RNA was determined using RT-PCR as previously described [59]. SIV

virions were centrifuged from 0.5 ml EDTA plasma at 20,000g for 1 hour. The

fluorescently labeled, RT-PCR probe employed contained a non-fluorescent quencher,

BHQ-1, at its 3' end. The threshold of sensitivity was 100 copy Eq/ml, with an average

interassay coefficient of variation of less than 25%.

Absolute cell count determination

Absolute counts of monocytes and monocyte subsets were calculated by multiplying the

percentage of each monocyte subpopulation gate within blood by the number of

monocytes per μl of blood as determined by complete blood cell counts (CBC).

RESULTS

Monocyte subsets are defined in SIVmac251 infected animals

Animals enrolled in this study came from three separate cohorts, 5 from the New England

Primate Research Center (NEPRC), 4 from one Tulane University cohort (Tulane I), and

another 5 from Tulane (Tulane II), for a total of 14 animals. All animals except for one

animal (#186-05) maintained long term CD8+ T lymphocyte depletion status, defined as

no repopulation of the CD8+ T lymphocytes before 28 DPI. Of the 14 animals, four

animals developed SIVE, as determined upon necropsy. Two animals, 288-07 and FD05,

were found to be infected with cytomegalovirus (CMV). Survival post-infection ranged

from 56 DPI to 92 DPI for animals that developed SIVE, whereas the non-encephalitic

animals all lived past 56 DPI, with 6 of these animals living past 118 DPI (Table 1).

Blood was drawn prior to infection and on a weekly basis post-infection. Cells from

whole blood were first defined by flow cytometric analysis of forward scatter versus side

scatter properties, gating for myeloid cells at the exclusion of lymphocytes and cells of

high granularity. We further defined the monocyte phenotype by drawing a gate for

HLA-DR positive cells. These cells were delineated into distinct subpopulations as

defined by CD14 and CD16 expression. The three major subpopulations were

characterized by high CD14 and no CD16 expression (CD14+CD16-); high CD14 and

high CD16 (CD14+CD16+); and little to no CD14 and high CD16 (CD14-CD16+)

(Figure 2).

Monocyte subsets show differential marker expression that changes with AIDS

In order to characterize the changes in different monocyte subsets with SIV infection, the

expression levels of monocyte-associated markers were examined. Cells were stained for

flow cytometric analysis of Mac387, CD68, CD163, CD44v6, CCR2, CX3CR1, CD64,

and CCR8 throughout the course of SIV pathogenesis. The average median fluorescence

intensity (MFI) for monocytes was calculated for time points prior to infection and also at

death.

Prior to infection, the relative expression of the different markers between the subsets

varied considerably. The median fluorescence intensities (MFI) of all animals were

averaged to determine the expression of each marker. CD163 showed the highest

expression on the CD14+CD16- and CD14+CD16+ subsets, with a decrease on CD14-

CD16+ monocytes. CD44v6 exhibited a similar pattern of increased expression on the

CD14+ subsets with decreased expression on CD14-CD16+ subsets. CX3CR1 followed a

different distribution of expression between the monocyte subsets, where the highest

relative MFI amongst the three subsets was found on the CD14+CD16+ and CD14-

CD16+ monocytes. CD64 had the greatest expression on the CD14+CD16+ subset, with

relatively lower expression on the CD14-CD16+ monocytes. The expression on this

subset, however, was still considerably higher than that found on the CD14+CD16-

monocytes. CD68 was expressed at increasing levels from CD14+CD16-, CD14+CD16+, to the CD14-CD16+ subsets. Mac387 demonstrated the highest expression on the CD14+CD16+ subset, with decreased expression on the CD14+CD16- monocytes, followed by relatively low expression within the CD14-CD16+ subset (Figure 3A).

At death, virtually all markers exhibited an increase in average MFI relative to the uninfected time point, with the exception of Mac387, which decreased, and CX3CR1, which remained fairly constant (Table 2). CD163 showed an increase in all three subsets from pre-infection time points to death. Expression levels prior to infection and at death were highest in the CD14+CD16- and CD14+CD16+ subsets, and CD14+CD16+ monocytes showed almost a 3-fold increase in CD163 surface expression. Slight increases in CCR2 occurred in the CD14+ subsets, with the strongest fluorescence observed for CD14+CD16-. CD44v6 showed considerable increases for all three monocyte subsets, in particular for the CD14-CD16+ subset. CX3CR1 expression remained relatively stable when comparing MFI from death to pre-infection time points. There even appeared to be a slight decrease in the average MFI of CD14+CD16+ monocytes at death compared to pre-infection, however this falls well within the calculated standard error. CD64 expression on monocytes increased in all three subsets with the greatest relative increase in the CD14-CD16+ subset. Expression in the CD14+CD16- subset was relatively small both pre- and post-infection as compared to the other two subsets. CCR8 showed little change in expression with disease (Table 2). Overall, surface marker expression increases for all three monocyte subsets, but with

differential degrees of increase from pre-infection time points to time of death. The
CD14+CD16+ subset shows considerable increase in CX3CR1 and CD64, whereas the
greatest changes in surface marker expression for the CD14-CD16+ subset occur with
CD163 and CD44v6 (Figure 3b).

Differences in relative expression of markers through the course of pathogenesis

In the NEPRC cohort, there are trends indicative of differential CD163 expression across
the monocyte subsets when comparing SIVE and non-encephalitic animals. CD163
expression is greatest for the CD14+CD16+ monocyte subset, followed by relatively
lower expression in the CD14+CD16- monocytes, and the lowest expression levels found
on the CD14-CD16+ cells (Figure 4A). Similar to the NEPRC cohort, overall expression
of CD163 was higher in the CD14+ monocyte subsets relative to the CD14-CD16+
subset. However, the Tulane I SIVE animals did not exhibit increased CD163 expression
in the CD14+ subsets like that found in NEPRC animals, and in fact demonstrated a
decreased expression in the CD14-CD16+ subset relative to non-encephalitic animals.
There were no significant differences in CD163 MFI between disease states in the CD14-
CD16+ monocytes (Figure 4B). Of the Tulane II animals, none developed encephalitis.
The expression of the different surface markers, however, showed some recapitulation of
trends observed in the previous two animal groups. CD163 was expressed highest in the
CD14-CD16+ group, followed by a modestly lower expression in the CD14+CD16-
subset. Lowest expression was found in the CD14-CD16+ subset (Figure 4C).

CD44v6 exhibits the highest levels of expression on the CD14-CD16+ monocytes, but no differences were observed between SIVE and non-encephalitic animals in the NEPRC animals (Figure 5A). The lack of distinct CD44v6 expression patterns between the SIVE and non-encephalitic animals was recapitulated in the Tulane I cohort. However, unlike the NEPRC animals, monocytes from the Tulane I cohort showed the greatest expression in the CD14-CD16+ subset, with similarly low levels of expression in the two CD14+ subsets (Figure 5B). CD44v6 expression across the subsets in the Tulane II animals was similar to that found in the NEPRC animals, with relatively higher levels of expression on the CD14+CD16+ subset, with similarly low expression found in the CD14+CD16- and CD14-CD16+ subsets (Figure 5C).

Due to CX3CR1 antibody titration issues, data for this marker is not available for NEPRC cohort. However, some patterns in expression could be observed in both the Tulane cohorts. For both the Tulane I and Tulane II animals, there was virtually no expression of CX3CR1 in the CD14+CD16- subset (Figure 6A and 6B). Additionally, CX3CR1 in this subset did not distinguish SIVE animals from non-SIVE animals. CD14+CD16+ monocytes had the highest levels of CX3CR1 expression. In contrast to the CD14+CD16- subset, it is interesting to note that the SIVE animals had higher expression of CX3CR1 in the CD14+CD16+ monocytes (Figure 6A). The CD14-CD16+ monocytes exhibited intermediate expression of CX3CR1 compared to that of the CD14+CD16+ and CD14+CD16- subsets, but unlike the CD14+CD16+ monocytes, SIVE and non-encephalitic animals were indistinguishable based on this parameter.

Overall, relative CCR2 expression levels between the subsets was consistent across the three cohorts, with the highest expression observed with the CD14+CD16- subset, followed by an intermediate phenotype in the CD14+CD16+ subset, and virtually no expression on CD14-CD16+ monocytes. For the NEPRC animals, CCR2 expression appeared to differentiate SIVE animals from those that did not develop neuroAIDS in the CD14+CD16- subset, but no significant differences were found between the different disease stages in the other monocyte subsets (Figure 7A). The Tulane I study cohort demonstrated similar trends but did not fully recapitulate what was found in the NEPRC animals. Differences between disease states were not found in any of the monocyte subsets from the Tulane I animals.

Monocyte subsets undergo dynamic changes in both absolute number and percentage of total monocytes.

With infection, monocyte subsets undergo changes in the relative distribution within the total monocyte population, as well as in the absolute number that are circulating in the periphery. The average frequency and absolute number of monocyte subsets at uninfected, peak viremia, and at terminal AIDS were determined (Figure 8). Prior to infection, the CD14+CD16- subset is dominant in both absolute number and frequency, accounting for about 78% of the total monocyte population. At peak viremia (DPI 12), there is a decrease in both the absolute number and frequency of the CD14+CD16-

monocyte subset, where the frequency of this subset decreases from an average 77.9% prior to infection to 64.7%. The CD14+CD16+ expands to account for an increased percentage of the total monocyte population from an average of 8.2% to 24.1%. The absolute number of CD14+CD16+ cells also increases from 22 cells/ul blood to about 32 cells/ul. The CD14-CD16+ subset remains stable and does not show considerable change from pre-infection time points to peak viremia. At death, all three monocyte subsets maintain similar frequencies as observed at peak viremia, but exhibit an increase in the absolute number of cells present, particularly in the CD14+CD16- and CD14+CD16+ subsets, demonstrating a total increase in monocytes circulating through the periphery.

A longitudinal analysis was performed for both the frequency and absolute numbers of the different monocyte subsets for each animal through the course of infection. Prior to infection, the CD14+CD16- subset is dominant, accounting for 70-90% of total monocytes, as defined by HLA-DR+ cells from the original FSC SSC monocyte gate (Figure 9A-C). CD14+CD16+ cells are relatively fewer in absolute cell number and percentage, accounting for less than 20% of the total monocyte population prior to infection (Figure 9D-F). The CD14-CD16+ subset has a frequency of less than 10% of total monocytes prior to infection for all nine animals (Figure 9G-I). Upon infection, The CD14+CD16- monocytes undergo a decrease in frequency followed by brief periods of recovery. However, the CD14+CD16- subset maintains reduced frequencies in all animals except for animal FB92 (Figure 9E). The relative decrease in frequency is more pronounced in encephalitic animals. Both the CD14+CD16+ and CD14-CD16+ subset

increase in frequency immediately after infection during peak viremia (Figure 9D-I). As early as DPI 5, the degree of change in frequency for all subsets distinguishes animals that develop SIVE from those that do not (Figure 9A, B, D, E, G, H).

The different monocyte subsets exhibit differential expansion in the absolute number of cells. At DPI 12, there is a decrease in the absolute number of CD14+CD16- monocytes for all animals except for Animal #288-07, and 186-05, for which there is a small increase (Figure 10A, D). Despite fluctuations in absolute number through the course of infection, 11 of the 14 animals exhibit an increase in absolute number when compared to the average absolute number of CD14+CD16- cells prior to infection (Figure 10A-C). The CD14+CD16+ subset shows increases in absolute number immediately following infection in all animals (Figure 10D-F). Animal 244-96 in particular exhibits a considerable expansion of CD14+CD16+ cells relative other animals. CD14-CD16+ monocytes exhibit changes in absolute number that are especially pronounced in the severely encephalitic animals 244-96 and DB79 (Figure 10G-I). Interestingly, despite none of the animals from the Tulane II cohort developing SIVE, there is a considerable expansion of the CD14-CD16+ cells.

A biphasic change in monocyte subset frequency and absolute cell number is pronounced in SIVE animals.

Animals infected with SIV exhibit dynamic shifts in monocyte subset frequency and absolute number. These changes follow a biphasic pattern, and are particularly

pronounced in animals that develop encephalitis (Figure 11). The CD14+CD16- subsets

demonstrate an initial decrease, a period of recovery, and then another decrease in

frequency (Figure 11A). This is reflected in the absolute number of cells in this subset,

where there is an initial decrease followed by a surge in cell number (Figure 11B). The

CD14+CD16+ monocytes also exhibit biphasic changes, where initial surges in

frequency are observed immediately following infection, are followed by a decrease and

another increase (Figure 11C). A similar dynamic is observed in the CD14-CD16+

subset, except for in the mildly encephalitic animal CM07, which undergoes a slight

decrease in frequency at DPI 12 (Figure 11E). The CD16-expressing monocyte subsets

undergo a slight expansion immediately following infection for all encephalitic animals.

The absolute number then decreases, and is followed by a relatively greater expansion

than that which occurred during peak viremia (Figure 11D, F).

DISCUSSION

In this report, the longitudinal analysis of peripheral blood monocyte subsets through the course of AIDS pathogenesis in the CD8-T lymphocyte depleted rhesus macaque model demonstrates previously uncharacterized temporal, phenotypic, and distribution changes. When comparing time points prior to infection and at necropsy, the average MFIs of different markers on the three monocyte subsets showed a global increase in expression, with the exception of Mac387, which at death had decreased across subsets. Prior to infection, the CD14+CD16- and CD14+CD16+ populations could be differentiated by their differential expression of these surface makers. CD14+CD16- cells express CCR2, CX3CR1, and CD64, but differs from the CD14+CD16+ subset, which expresses lower CCR2 levels, but more CX3CR1, CCR5, and CD64 is detected.

With infection, the chemokine receptor CCR2 increased but maintained the aforementioned expression patterns. These increases in CCR2 allows for an increased responsiveness to its ligand, MCP-1. With previously reported increases in MCP-1 in the CNS, chemokine gradients established in the periphery lead to increased trafficking of monocytes. The differing affinities and abilities to respond to these gradients, in conjunction with the knowledge that the CD16+ subset has shown preferential susceptibility to HIV infection [37], underscores the importance of such phenotypic changes as they can influence virus-induced CNS pathologies. Interestingly, CX3CR1 did

not exhibit similar increases from pre-infection to terminal disease, although it has been shown that in previous reports its ligand, fractalkine (CX3CL1), has a comparable presence to that of MCP-1. It should be noted that there were certainly fluctuations in the expression of this marker through the course of infection (data not shown). Because the primary cohorts used for analysis of CX3CR1 were those from Tulane, the issue of experimental constraints plays a role; the ability of monocytes to maintain CX3CR1 expression after a questionable period ex-vivo may have some impact on our ability to detect for our target antigen.

CD163 expression is robust on the CD14+CD16- and CD14+CD16+ monocytes prior to infection. With infection, all three monocyte subsets exhibit considerable increases in CD163 expression, but the CD14+CD16+ subset shows the highest relative levels. Accumulation of CD163, a scavenger receptor for the hemoglobulin-haptoglobin complex, on macrophages has been shown to correlate with HIV-1 and SIVE [60,61]. Interestingly, SIVE animals from both the NEPRC and Tulane I cohorts appeared to have higher levels of CD163 in the CD14+CD16- subset when compared to the non-encephalitic animals. This trend was not observed in the CD14+CD16+ subset, although the CD14+CD16+ subset maintained the highest relative levels of expression regardless of disease severity across all three study cohorts. Higher CD163 expression on the CD14+CD16+ subset through the course of infection may contribute to the presence of perivascular macrophages located in brain lesions via reseeding of the resident cells.

The osteopontin receptor, CD44v6, has been reported as a potential marker for SIVE [19]. Marcondes et al. report significant differences in MFI and percent monocytes expressing CD44v6 expression between SIVE and non-encephalitic animals. However, this phenomenon was not recapitulated in our current study. When comparing the average MFI of SIVE and non-encephalitic animals, CD44v6 expression was virtually indistinguishable for all monocyte subsets. Current studies in our group looking at monocytes from HIV-infected individuals have also not supported this published finding (data not shown). This discrepancy between the previously published literature and data generated in our lab may in large part be due to differences in gating strategy and calculation of percent expression that increase the perceived significance of CD44v6 expression. Osteopontin has been implicated in SIVE-associated macrophage accumulation in the brain via the prevention of monocyte/macrophage egress from the brain and prevention of cell death [62]. While the increased presence of its ligand in the brain implicates CD44v6 in the development of NeuroAIDS and the maintenance of viral reservoirs, osteopontin does not behave like other classical chemokines, such as CCL2. Because of its esoteric nature, CD44v6 and its ligand may contribute to the development of neurological disease, but the data presented in this study suggests that this contribution would be secondary to other mediators involved in the recruitment of monocyte/macrophages.

Maturation from monocyte to macrophage is thought of as a phenomenon restricted to the time proceeding extravasation into tissue, and that in homeostatic conditions, monocytes

do not mature when residing in the peripheral blood. This study does not address the phenotypic changes that individual monocytes undergo from bone marrow release to establishment as resident macrophage. However, while observing global and subset-specific changes with disease, it is interesting to note that certain sub-populations developed phenotypes similar to those of maturing or mature macrophages. Mac387, a monocyte marker which is lost during macrophage differentiation, and CD68, which has been used as a marker for differentiated tissue macrophages and blood monocytes [63] were included in our cytometric flow analysis. CD68 showed increases in expression when comparing time points prior to infection and at death, which was concomitant to a decrease in Mac387 (Table 2). These changes in marker expression, when juxtaposed with the increase in CD14+CD16+ cells and simultaneous decrease in CD14+CD16- cells, suggests a possible link between disease severity and monocyte development and differentiation prior to tissue invasion. This possible shift in maturation further underscores the changes that different cell populations undergo, and indicate a change in the role of these subsets through the course of pathogenesis.

While previous studies have characterized the relative contributions of different subsets that comprise the total monocyte population with HIV infection [42,64], the relative frequencies and absolute numbers of the different subsets, and the dynamic changes they undergo through the course of SIV pathogenesis had not been addressed. Almost immediately after infection, there is a decrease in the percentage of CD14+CD16- monocytes that occurs concomitantly with a sharp increase in the CD16+ subsets. This

shift in relative abundance between the two monocyte subsets lends credence to the notion that, with infection, there is a differentiation of the CD14+CD16- subset into the more macrophage-like CD14+CD16+ subset. Using BrDU labeling of proliferating cells, it has been shown that the increase in CD14+CD16+ monocytes is also influenced by the trafficking of CD14+CD16+ cells from the bone marrow [65]. The magnitude of changes in frequency is pronounced in the animals that develop SIVE. This is especially robust in the Tulane I cohort, where clearly the SIVE animals have distinctly different frequencies of CD14-CD16+ monocytes than those found in non-encephalitic animals. This difference is observed very early on during the course of infection, and suggests the importance of early immune activation and deregulation in disease severity.

Our lab has shown previously that perivascular macrophages are the primary reservoirs of active virus in the brain and undergo a high rate of turnover as peripheral monocytes migrate to the brain and differentiate into resident macrophages [25]. Han et al. report the CD14+CD16+ subset of monocytes as the key subset involved in AIDS progression [64]. Because of the increased susceptibility of this subset to infection and its ability to enter tissues, CD14+CD16+ monocytes are of particular interest as potential viral reservoirs that persist within the host despite extensive ART. In animals that develop SIVE, the pronounced increase in both frequency and absolute number of CD14+CD16+ offers one explanation to the observed disease severity. This subset's increased activation and sensitivity to chemokines and other signaling molecules suggests that the expansion of these cells contributes to the reseeding of perivascular macrophages. Also, the

deregulation of inflammatory cytokine production and breakdown of the BBB may become more pronounced, thus accelerating the development of lesions and neuronal damage observed in sicker animals.

All three subsets demonstrate an increase in absolute number, resulting in the total increase of absolute number of monocytes that appear to circulate in the periphery. This increase does not distinguish animals that develop SIVE from those that do not as robustly as the measure of frequency, but it is indicative of a trend in monocyte expansion that may have implications in the development of virus-associated encephalitis. Certainly, the increase in the absolute number of monocytes in the blood could contribute to the inflammatory milieu of and HIV-infected animal through increased cytokine production, as well as turnover and trafficking of newly differentiated macrophages in tissue. Clearance and presentation of foreign matter and cellular debris resultant of leakiness in the gut, from the blood, also becomes an increasingly important role for monocytes [66].

The expansion of the monocyte population exhibited temporal patterns of change that contribute to the overall understanding of AIDS progression and CNS damage. Of the 14 animals enrolled in this study, the four that developed SIVE showed fluctuations within the different subsets that were more pronounced than in the non-encephalitic animals (Figure 10). The biphasic changes observed show an initial shift in the different monocyte populations in response to the initial viral infection. This is followed by an

attenuation of this response where the monocyte population as a whole reverts back to a state similar to that observed before infection. This reversion does not last, however, but is followed by a secondary shift and expansion of the different monocyte subsets. The initial robust response to viral infection is expected and understandable within the context of our understanding of the immune response to HIV and SIV infection. In our CD8+T cell depleted disease model, however, we see that with the introduction of SIV into the animal, there is a sharp increase in virus, and consistently high levels of virus are maintained through the course of infection, suggesting that the monocyte subsets appear to shift and change independent of plasma virus load.

It should be noted that although our CD8 T cell depletion model allows for an excellent recapitulation of neurological pathologies observed in HIV-infected individuals, inherent in this model are limitations in depicting the total immune system response to infection and the environment in which innate immune cells perform their functions. It has been suggested that the monoclonal anti-CD8 antibody used for CD3+CD8+ T cell depletion partially compromises the natural killer cell population in rhesus macaques [67]. The production of cytokines and cross-talk between different immune cell populations has been shown in other inflammatory diseases to provide critical regulatory functions [68] that may have an influence on the phenotype and functionality of monocyte subsets that remain undetermined in the context of this model.

Data from our marker expression analysis shows disparities between the different study cohorts, where observations made in one study cohort were not consistently duplicated in another. Working with monkeys from two different primate center locations situated in different parts of the country quickly became a source of concern, especially with blood samples shipped from Tulane. Differences in shipping conditions and uncontrollable and unknown factors that may influence the blood, as well as the length of time taken to process the samples, all could have contributed do some of the observed disparities between study groups.

For future studies, analysis of the different monocyte subset phenotypes may include a further elucidation of monocyte subset lineages. One study, analyzing the differential expression of genes in human CD16- and CD16+ monocyte subsets, demonstrated distinct differences in transcriptional profiles that suggested different stages of myeloid differentiation as well as unique roles in immune responses and inflammatory disease [9]. The biological functions of analyzed genes showed that in the CD14+CD16+ subset compared to the CD14+CD16- subset, there was an upregulation in adhesion molecule, chemokine, and chemokine receptor genes, indicating recruitment of the different monocyte subsets into tissues via distinct mechanisms. The location of where these changes occur can also become a point of exploration; bone marrow studies may provide some insight into where this differentiation step occurs and whether this changes through the course of infection. Expansion and exploration of other chemokine, cytokine, and scavenger receptors on the monocyte subsets can also be pursued. Clarification of the

development of monocytes, their phenotypes, and role within the context of HIV and SIV

infection will prove critical in understanding the damage observed in the CNS.

REFERENCES

1. Serbina NV, Jia T, Hohl TM, Pamer EG (2008) Monocyte-mediated defense against microbial pathogens. Annu Rev Immunol 26: 421-452.
2. Geissmann F, Manz MG, Jung S, Sieweke MH, Merad M, et al. (2010) Development of monocytes, macrophages, and dendritic cells. Science 327: 656-661.
3. Fogg DK, Sibon C, Miled C, Jung S, Aucouturier P, et al. (2006) A clonogenic bone marrow progenitor specific for macrophages and dendritic cells. Science 311: 83-87.
4. Dakic A, Metcalf D, Di Rago L, Mifsud S, Wu L, et al. (2005) PU.1 regulates the commitment of adult hematopoietic progenitors and restricts granulopoiesis. J Exp Med 201: 1487-1502.
5. Ziegler-Heitbrock HW, Strobel M, Kieper D, Fingerle G, Schlunck T, et al. (1992) Differential expression of cytokines in human blood monocyte subpopulations. Blood 79: 503-511.
6. Grage-Griebenow E, Flad HD, Ernst M (2001) Heterogeneity of human peripheral blood monocyte subsets. J Leukoc Biol 69: 11-20.
7. Skrzeczynska-Moncznik J, Bzowska M, Loseke S, Grage-Griebenow E, Zembala M, et al. (2008) Peripheral blood CD14high CD16+ monocytes are main producers of IL-10. Scand J Immunol 67: 152-159.
8. Zhao C, Zhang H, Wong WC, Sem X, Han H, et al. (2009) Identification of novel functional differences in monocyte subsets using proteomic and transcriptomic methods. J Proteome Res 8: 4028-4038.
9. Ancuta P, Liu KY, Misra V, Wacleche VS, Gosselin A, et al. (2009) Transcriptional profiling reveals developmental relationship and distinct biological functions of CD16+ and CD16- monocyte subsets. BMC Genomics 10: 403.
10. Kim WK, Sun Y, Do H, Autissier P, Halpern EF, et al. (2009) Monocyte heterogeneity underlying phenotypic changes in monocytes according to SIV disease stage. J Leukoc Biol.
11. Piatak M, Jr., Saag MS, Yang LC, Clark SJ, Kappes JC, et al. (1993) High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. Science 259: 1749-1754.
12. Alkhatib G, Combadiere C, Broder CC, Feng Y, Kennedy PE, et al. (1996) CC CKR5: a RANTES, MIP-1alpha, MIP-1beta receptor as a fusion cofactor for macrophage-tropic HIV-1. Science 272: 1955-1958.
13. Carter CA, Ehrlich LS (2008) Cell Biology of HIV-1 Infection of Macrophages. Annual Review of Microbiology 62: 425-443.
14. Stebbing J, Gazzard B, Douek DC (2004) Mechanisms of disease - Where does HIV live? New England Journal of Medicine 350: 1872-1880.
15. Chakrabarti L, Hurtrel M, Maire MA, Vazeux R, Dormont D, et al. (1991) Early viral replication in the brain of SIV-infected rhesus monkeys. Am J Pathol 139: 1273-1280.

16. Gendelman HE, Zheng JL, Coulter CL, Ghorpade A, Che M, et al. (1998) Suppression of inflammatory neurotoxins by highly active antiretroviral therapy in human immunodeficiency virus-associated dementia. Journal of Infectious Diseases 178: 1000-1007.
17. Kolson DL, Gonzalez-Scarano F (2000) HIV and HIV dementia. Journal of Clinical Investigation 106: 11-13.
18. Faulstich ME (1986) Acquired-Immune-Deficiency-Syndrome - an Overview of Central-Nervous-System Complications and Neuropsychological Sequelae. International Journal of Neuroscience 30: 249-254.
19. Marcondes MC, Lanigan CM, Burdo TH, Watry DD, Fox HS (2008) Increased expression of monocyte CD44v6 correlates with the deveopment of encephalitis in rhesus macaques infected with simian immunodeficiency virus. J Infect Dis 197: 1567-1576.
20. Williams KC, Hickey WF (2002) Central nervous system damage, monocytes and macrophages, and neurological disorders in AIDS. Annual Review of Neuroscience 25: 537-562.
21. Stephens EB, Liu ZQ, Zhu GW, Adany I, Joag SV, et al. (1995) Lymphocyte-Tropic Simian Immunodeficiency Virus Causes Persistent Infection in the Brains of Rhesus-Monkeys. Virology 213: 600-614.
22. Mankowski JL, Flaherty MT, Spelman JP, Hauer DA, Didier PJ, et al. (1997) Pathogenesis of simian immunodeficiency virus encephalitis: Viral determinants of neurovirulence. Journal of Virology 71: 6055-6060.
23. Koenig S, Gendelman HE, Orenstein JM, Dalcanto MC, Pezeshkpour GH, et al. (1986) Detection of Aids Virus in Macrophages in Brain-Tissue from Aids Patients with Encephalopathy. Science 233: 1089-1093.
24. Wiley CA, Schrier RD, Nelson JA, Lampert PW, Oldstone MBA (1986) Cellular-Localization of Human Immunodeficiency Virus-Infection within the Brains of Acquired-Immune-Deficiency-Syndrome Patients. Proceedings of the National Academy of Sciences of the United States of America 83: 7089-7093.
25. Williams KC, Corey S, Westmoreland SV, Pauley D, Knight H, et al. (2001) Perivascular macrophages are the primary cell type productively infected by simian immunodeficiency virus in the brains of macaques: implications for the neuropathogenesis of AIDS. J Exp Med 193: 905-915.
26. Glass JD, Fedor H, Wesselingh SL, Mcarthur JC (1995) Immunocytochemical Quantitation of Human-Immunodeficiency-Virus in the Brain - Correlations with Dementia. Annals of Neurology 38: 755-762.
27. Peluso R, Haase A, Stowring L, Edwards M, Ventura P (1985) A Trojan Horse Mechanism for the Spread of Visna Virus in Monocytes. Virology 147: 231-236.
28. Soulas C, Donahue RE, Dunbar CE, Persons DA, Alvarez X, et al. (2009) Genetically Modified CD34(+) Hematopoietic Stem Cells Contribute to Turnover of Brain Perivascular Macrophages in Long-Term Repopulated Primates. American Journal of Pathology 174: 1808-1817.
29. Gendelman HE, Narayan O, Kennedystoskopf S, Kennedy PGE, Ghotbi Z, et al. (1986) Tropism of Sheep Lentiviruses for Monocytes - Susceptibility to Infection

and Virus Gene-Expression Increase during Maturation of Monocytes to Macrophages. Journal of Virology 58: 67-74.

30. Bell JE, Busuttil A, Ironside JW, Rebus S, Donaldson YK, et al. (1993) Human-Immunodeficiency-Virus and the Brain - Investigation of Virus Load and Neuropathologic Changes in Pre-Aids Subjects. Journal of Infectious Diseases 168: 818-824.

31. Donaldson YK, Bell JE, Ironside JW, Brettle RP, Robertson JR, et al. (1994) Redistribution of Hiv Outside the Lymphoid System with Onset of Aids. Lancet 343: 382-385.

32. Reinhart TA, Rogan MJ, Huddleston D, Rausch DM, Eiden LE, et al. (1997) Simian immunodeficiency virus burden in tissues and cellular compartments during clinical latency and AIDS. Journal of Infectious Diseases 176: 1198-1208.

33. Gray F, Scaravilli F, Everall I, Chretien F, An S, et al. (1996) Neuropathology of early HIV-1 infection. Brain Pathology 6: 1-12.

34. Redel L, Le Douce V, Cherrier T, Marban C, Janossy A, et al. (2010) HIV-1 regulation of latency in the monocyte-macrophage lineage and in CD4+ T lymphocytes. J Leukoc Biol 87: 575-588.

35. Fischer-Smith T, Bell C, Croul S, Lewis M, Rappaport J (2008) Monocyte/macrophage trafficking in acquired immunodeficiency syndrome encephalitis: lessons from human and nonhuman primate studies. J Neurovirol 14: 318-326.

36. Kim WK, Corey S, Alvarez X, Williams K (2003) Monocyte/macrophage traffic in HIV and SIV encephalitis. J Leukoc Biol 74: 650-656.

37. Ellery PJ, Tippett E, Chiu YL, Paukovics G, Cameron PU, et al. (2007) The CD16+ monocyte subset is more permissive to infection and preferentially harbors HIV-1 in vivo. J Immunol 178: 6581-6589.

38. Ancuta P, Autissier P, Wurcel A, Zaman T, Stone D, et al. (2006) CD16+ monocyte-derived macrophages activate resting T cells for HIV infection by producing CCR3 and CCR4 ligands. J Immunol 176: 5760-5771.

39. Ancuta P, Kunstman KJ, Autissier P, Zaman T, Stone D, et al. (2006) CD16+ monocytes exposed to HIV promote highly efficient viral replication upon differentiation into macrophages and interaction with T cells. Virology 344: 267-276.

40. Pulliam L, Gascon R, Stubblebine M, McGuire D, McGrath MS (1997) Unique monocyte subset in patients with AIDS dementia. Lancet 349: 692-695.

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

42. Williams K, Westmoreland S, Greco J, Ratai E, Lentz M, et al. (2005) Magnetic resonance spectroscopy reveals that activated monocytes contribute to neuronal injury in SIV neuroAIDS. J Clin Invest 115: 2534-2545.

43. Hasegawa A, Liu H, Ling B, Borda JT, Alvarez X, et al. (2009) The level of monocyte turnover predicts disease progression in the macaque model of AIDS. Blood 114: 2917-2925.

44. Yadav A, Collman RG (2009) CNS inflammation and macrophage/microglial biology associated with HIV-1 infection. J Neuroimmune Pharmacol 4: 430-447.

45. Ancuta P, Moses A, Gabuzda D (2004) Transendothelial migration of CD16+ monocytes in response to fractalkine under constitutive and inflammatory conditions. Immunobiology 209: 11-20.

46. Borda JT, Alvarez X, Mohan M, Hasegawa A, Bernardino A, et al. (2008) CD163, a marker of perivascular macrophages, is up-regulated by microglia in simian immunodeficiency virus encephalitis after haptoglobin-hemoglobin complex stimulation and is suggestive of breakdown of the blood-brain barrier. Am J Pathol 172: 725-737.

47. Eugenin EA, Osiecki K, Lopez L, Goldstein H, Calderon TM, et al. (2006) CCL2/monocyte chemoattractant protein-1 mediates enhanced transmigration of human immunodeficiency virus (HIV)-infected leukocytes across the blood-brain barrier: a potential mechanism of HIV-CNS invasion and NeuroAIDS. J Neurosci 26: 1098-1106.

48. Avison MJ, Nath A, Greene-Avison R, Schmitt FA, Bales RA, et al. (2004) Inflammatory changes and breakdown of microvascular integrity in early human immunodeficiency virus dementia. J Neurovirol 10: 223-232.

49. Toborek M, Lee YW, Pu H, Malecki A, Flora G, et al. (2003) HIV-Tat protein induces oxidative and inflammatory pathways in brain endothelium. J Neurochem 84: 169-179.

50. Kanmogne GD, Kennedy RC, Grammas P (2002) HIV-1 gp120 proteins and gp160 peptides are toxic to brain endothelial cells and neurons: possible pathway for HIV entry into the brain and HIV-associated dementia. J Neuropathol Exp Neurol 61: 992-1000.

51. Kanmogne GD, Primeaux C, Grammas P (2005) HIV-1 gp120 proteins alter tight junction protein expression and brain endothelial cell permeability: implications for the pathogenesis of HIV-associated dementia. J Neuropathol Exp Neurol 64: 498-505.

52. Hahn BH, Shaw GM, De Cock KM, Sharp PM (2000) AIDS as a zoonosis: scientific and public health implications. Science 287: 607-614.

53. Williams KC, Burdo TH (2009) HIV and SIV infection: the role of cellular restriction and immune responses in viral replication and pathogenesis. APMIS 117: 400-412.

54. Mansfield KG, Lerch NW, Gardner MB, Lackner AA (1995) Origins of simian immunodeficiency virus infection in macaques at the New England Regional Primate Research Center. J Med Primatol 24: 116-122.

55. Desrosiers RC (1990) The simian immunodeficiency viruses. Annu Rev Immunol 8: 557-578.

56. Tracey I, Lane J, Chang I, Navia B, Lackner A, et al. (1997) 1H magnetic resonance spectroscopy reveals neuronal injury in a simian immunodeficiency virus macaque model. J Acquir Immune Defic Syndr Hum Retrovirol 15: 21-27.

57. Schmitz JE, Simon MA, Kuroda MJ, Lifton MA, Ollert MW, et al. (1999) A nonhuman primate model for the selective elimination of CD8+ lymphocytes

using a mouse-human chimeric monoclonal antibody. Am J Pathol 154: 1923-1932.

58. Williams K, Alvarez X, Lackner AA (2001) Central nervous system perivascular cells are immunoregulatory cells that connect the CNS with the peripheral immune system. Glia 36: 156-164.

59. Lifson JD, Rossio JL, Piatak M, Jr., Parks T, Li L, et al. (2001) Role of CD8(+) lymphocytes in control of simian immunodeficiency virus infection and resistance to rechallenge after transient early antiretroviral treatment. J Virol 75: 10187-10199.

60. Kim WK, Alvarez X, Fisher J, Bronfin B, Westmoreland S, et al. (2006) CD163 identifies perivascular macrophages in normal and viral encephalitic brains and potential precursors to perivascular macrophages in blood. Am J Pathol 168: 822-834.

61. Roberts ES, Masliah E, Fox HS (2004) CD163 identifies a unique population of ramified microglia in HIV encephalitis (HIVE). J Neuropathol Exp Neurol 63: 1255-1264.

62. Burdo TH, Wood MR, Fox HS (2007) Osteopontin prevents monocyte recirculation and apoptosis. J Leukoc Biol 81: 1504-1511.

63. Micklem K, Rigney E, Cordell J, Simmons D, Stross P, et al. (1989) A human macrophage-associated antigen (CD68) detected by six different monoclonal antibodies. Br J Haematol 73: 6-11.

ANIMAL	STUDY COHORT	CD8+ T LYMPHOCYTE DEPLETION STATUS	PATHOLOGY	SURVIVAL DPI
55-05	NEPRC	Long-Term	**Mild SIVE**	56
168-05	NEPRC	Long-Term	AIDS no E	89
186-05	NEPRC	Short-Term	AIDS no E	296
244-96	NEPRC	Long-Term	**Severe SIVE**	77
288-07	NEPRC	Long-Term	AIDS/CMV	131
CM07	Tulane I	Long-Term	**Mild SIVE**	75
DB79	Tulane I	Long-Term	**Severe SIVE**	92
FB92	Tulane I	Long-Term	AIDS no E	118
FD05	Tulane I	Long-Term	AIDS/CMV	89
FD80	Tulane II	Long-Term	AIDS no E	56
FT73	Tulane II	Long-Term	AIDS no E	56
FC42	Tulane II	Long-Term	AIDS no E	132
FR56	Tulane II	Long-Term	AIDS no E	141
FD37	Tulane II	Long-Term	AIDS no E	142

Table 1: Summary of animals enrolled in study. Fourteen rhesus macaques from three different study cohorts were CD8+T cell depleted and infected with SIVmac251. 2 animals, 288-07 and FD05, had cytomegalovirus (CMV) at death. 5 animals were from the New England Primate Research Center (NEPRC), 4 from one Tulane University cohort (Tulane I), and another 5 from a separate Tulane study (Tulane II).
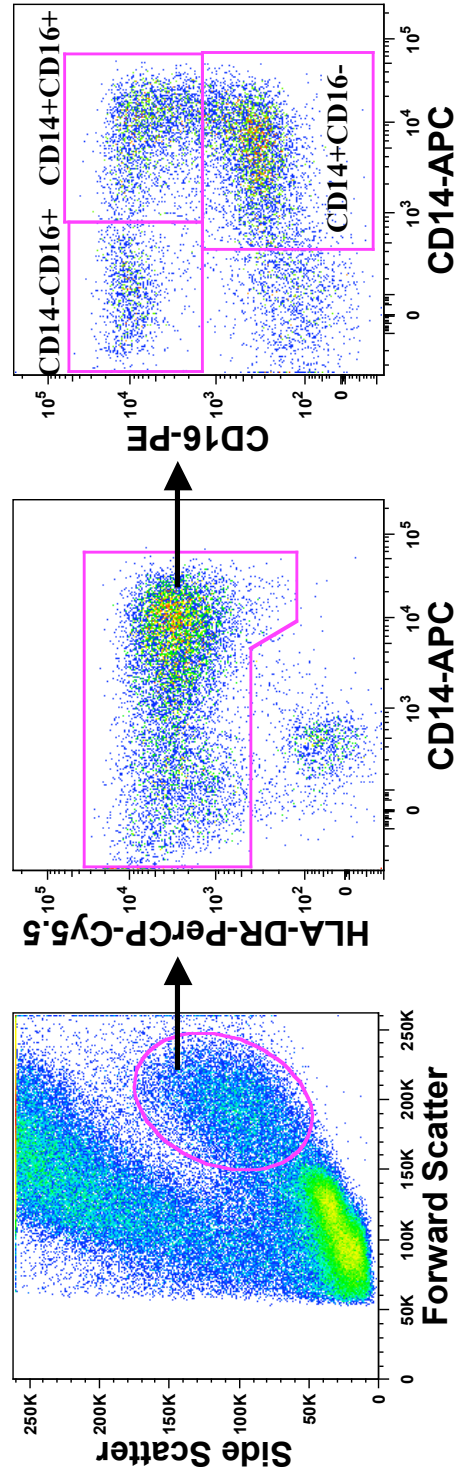
Marker	Time	CD14+CD16-	CD14+CD16+	CD14-CD16+
CD163	**pre-infection**	2440 ± 430	2620 ± 430	520 ± 120
	terminal	5020 ± 1110	7506 ± 1970	3050 ± 1270
CCR2	**pre-infection**	740 ± 120	240 ± 60	10 ±10
	terminal	1270 ± 350	690 ± 250	120 ± 40
CD44v6	**pre-infection**	500 ± 120	900 ± 260	230 ± 40
	terminal	2200 ± 720	5500 ± 2110	2720 ± 1000
CX3CR1	**pre-infection**	530 ± 250	5140 ± 2400	3120 ± 1040
	terminal	520 ± 260	4203 ± 1170	3940 ± 1450
CD64	**pre-infection**	590 ± 30	5550 ± 410	3020 ± 270
	terminal	1950 ± 400	9990 ± 2150	6110 ± 1450
CCR8	**pre-infection**	50 ± 50	0	0
	terminal	220 ± 70	370 ± 200	100 ± 40
CD68	**pre-infection**	2330 ± 150	2960 ± 230	5850 ± 440
	terminal	4720 ± 870	5610 ± 730	8210 ± 1630
Mac387	**pre-infection**	40000 ± 13570	75310 ± 32680	8670 ± 2720
	terminal	21530 ± 8540	35256 ± 19610	4720 ± 2180

Table 2: Differential expression and changes of markers on monocyte subsets.
Median Fluorescence Intensity (MFI) was used to measure the expression of markers on
each monocyte subset. Values derived from the average MFI and standard error for
animals from NEPRC and Tulane II cohorts calculated for time points prior to infection
and at death (n= 10). CD68 values include the Tulane I cohort (n= 14). CX3CR1 values
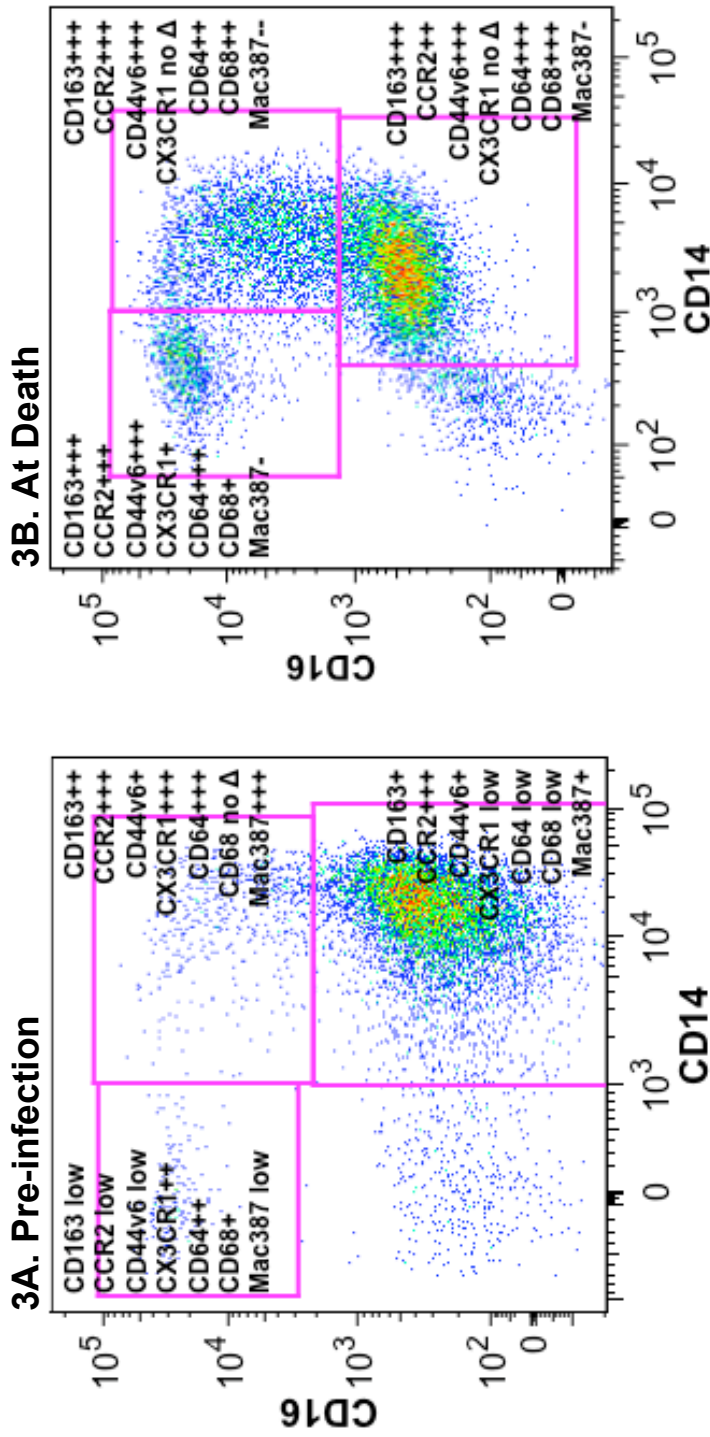derived from the Tulane II cohort only (n= 5).
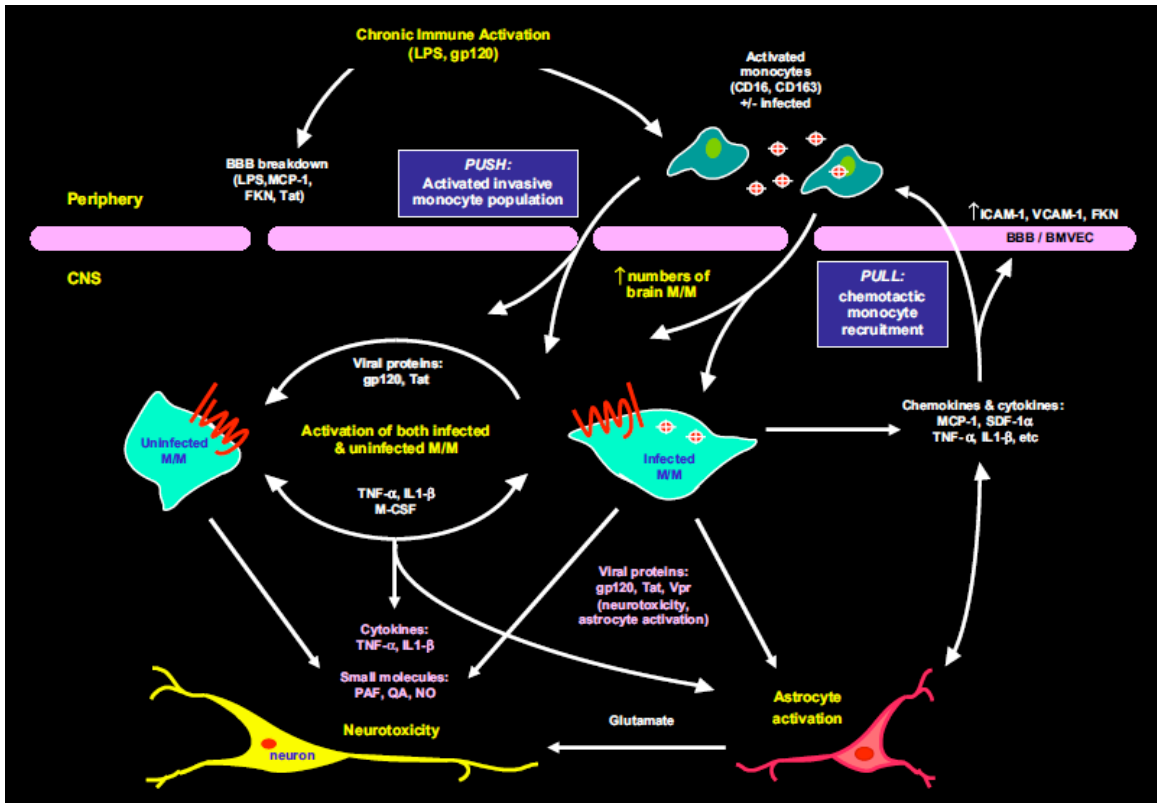
Figure 1: Schematic representation of monocyte/macrophage involvement in HIV-induced neuronal damage. Modified from [44].

Figure 2: Gating strategy for identifying monocyte subsets CD14+CD16–, CD14+CD16+, and CD14-CD16+. Representative dot plots from animal DB79 47 days post-infection.

Figure 3: Subset-specific differences in marker expression and changes that occur with infection. (3A) Relative expression of markers between subsets prior to infection. "Low": lowest marker expression of the 3 monocyte subsets. "No Δ": 0-2 times higher expression than lowest-expressing subset. "+": 2-5 times higher expression than lowest-expressing subset. "++": 5-7.5 times higher expression than lowest-expressing subset. "+++": 7.5-10 times higher expression than lowest-expressing subset. **(3B)** At death, markers have changed expression levels relative to pre-infection time points. The magnitudes of these changes are marker and subset-specific. Magnitude of percent change from pre-infection to death represented as follows. "No Δ": Percent change is less than 25%. "+": Positive percent change is greater than 25% and less than or equal to 50%. "++": Positive percent change is greater than 50% and less than or equal to 100%. "+++": Positive percent change is greater than 100%. "-": Negative percent change at is greater than 25% and less than or equal to 50%. "- -": Negative percent change is greater than 50% and less than or equal to 100%. Relative expression levels based on average MFI values shown in Table 2.
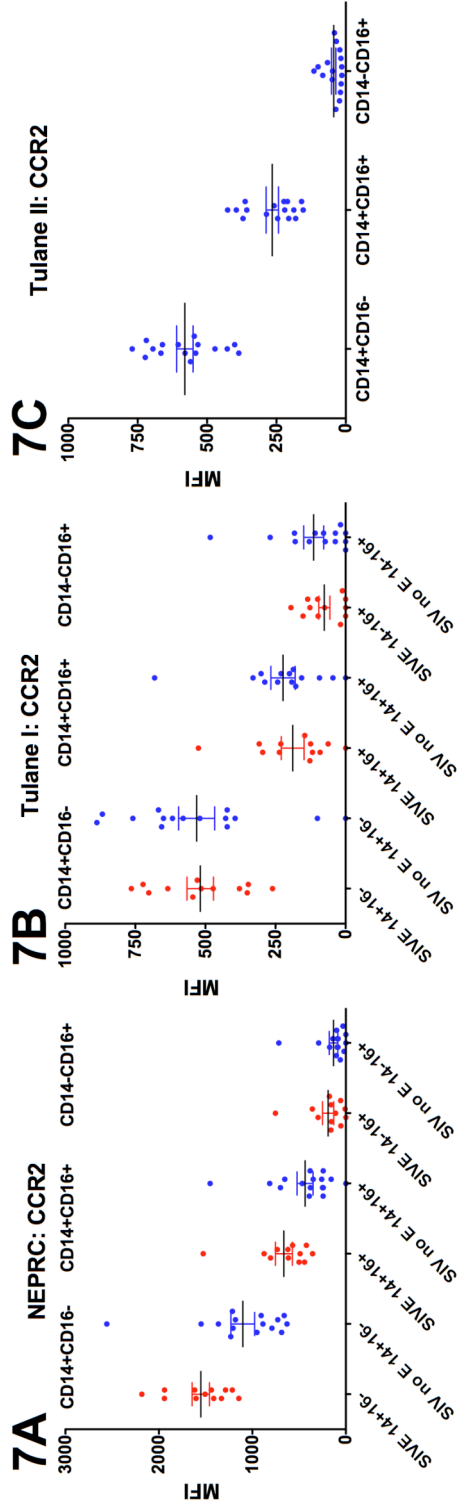
43
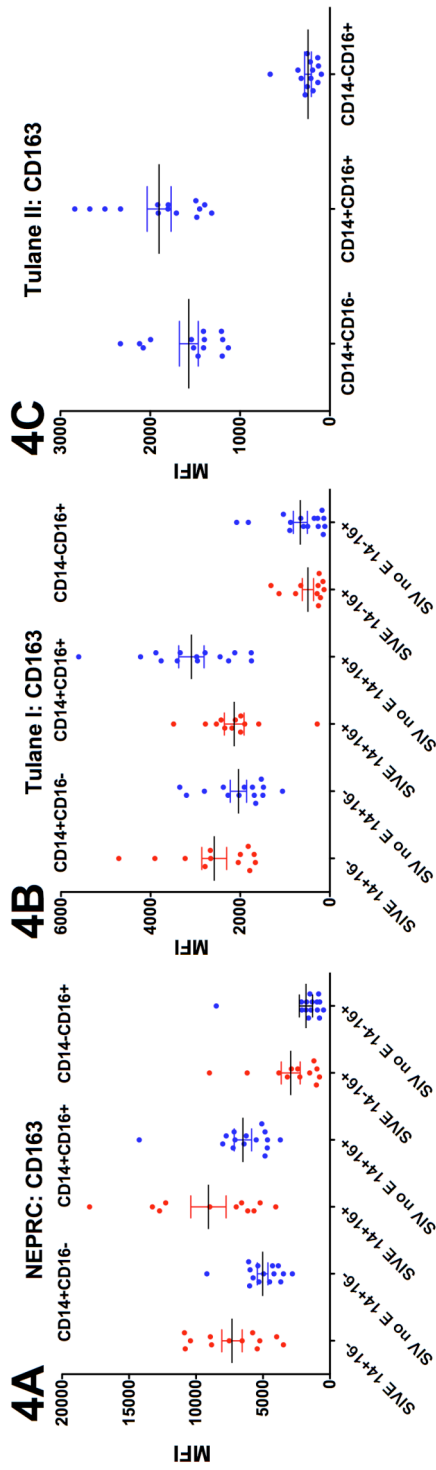
Figure 4: Comparison of CD163 expression on monocyte subsets from SIVE and non-encephalitic animals through the course of infection. Dot plots represent animals grouped according to disease state, and measurements were taken throughout the course of infection. Red dots represent measurements taken from SIVE animals. Blue dots represent measurements taken from non-encephalitic animals. Error bars represent standard error. MFI= Median Fluorescence Intensity; isotype control values were subtracted out to account for background signal. (**4A**) NEPRC cohort (**4B**) Tulane I cohort (**4C**) Tulane II cohort.
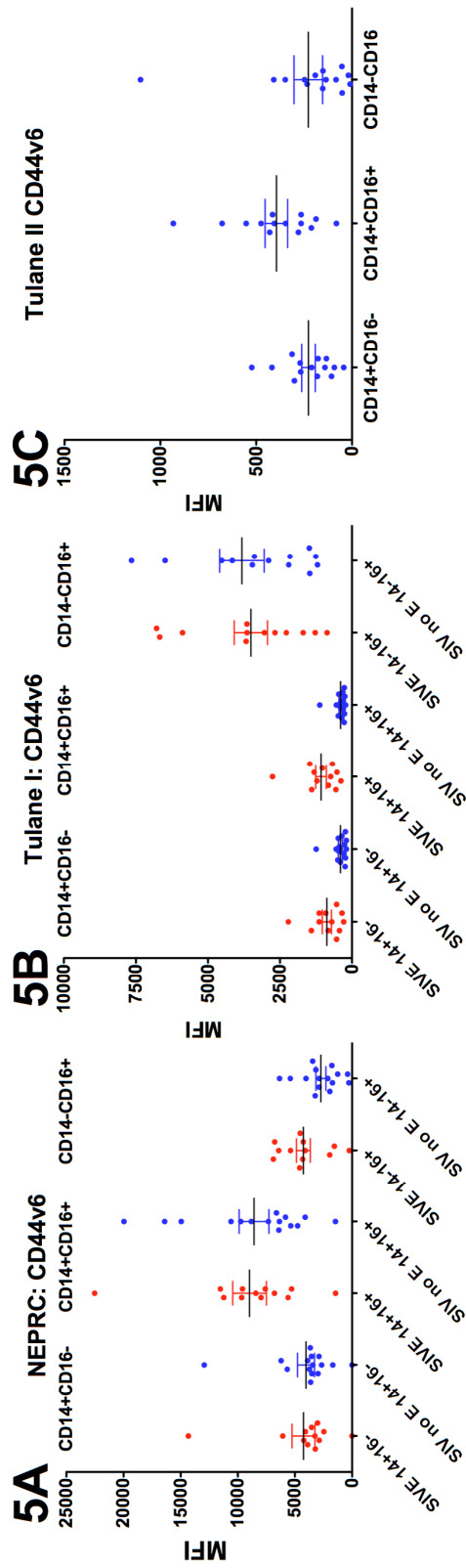
Figure 5: Comparison of CD44v6 expression on monocyte subsets from SIVE and non-encephalitic animals through the course of infection. Dot plots represent animals grouped according to disease state, and measurements were taken throughout the course of infection. Red dots represent measurements taken from SIVE animals. Blue dots represent measurements taken from non-encephalitic animals. Error bars represent standard error. MFI= Median Fluorescence Intensity; isotype control values were subtracted out to account for background signal. (**5A**) NEPRC cohort (**5B**) Tulane I cohort (**5C**) Tulane II cohort.
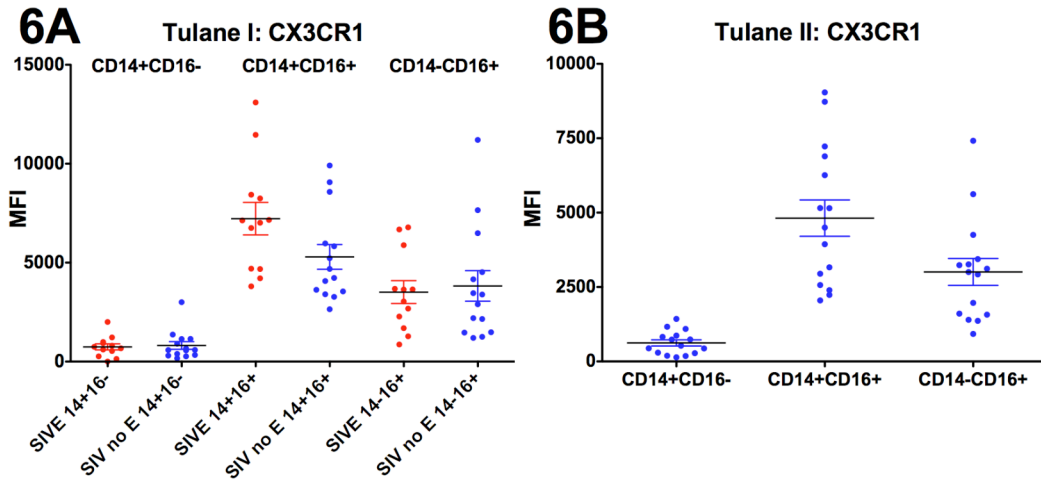
Figure 6: Comparison of CX3CR1 expression on monocyte subsets from SIVE and non-encephalitic animals through the course of infection. Dot plots represent animals grouped according to disease state, and measurements were taken throughout the course of infection. Red dots represent measurements taken from SIVE animals. Blue dots represent measurements taken from non-encephalitic animals. Error bars represent standard error. MFI= Median Fluorescence Intensity; isotype control values were subtracted out to account for background signal. **(6A)** NEPRC cohort **(6B)** Tulane I cohort.

Figure 7: Comparison of CCR2 expression on monocyte subsets from SIVE and non-encephalitic animals through the course of infection. Dot plots represent animals grouped according to disease state, and measurements were taken throughout the course of infection. Red dots represent measurements taken from SIVE animals. Blue dots represent measurements taken from non-encephalitic animals. Error bars represent standard error. MFI= Median Fluorescence Intensity; isotype control values were subtracted out to account for background signal. **(7A)** NEPRC cohort **(7B)** Tulane I cohort **(7C)** Tulane II cohort.
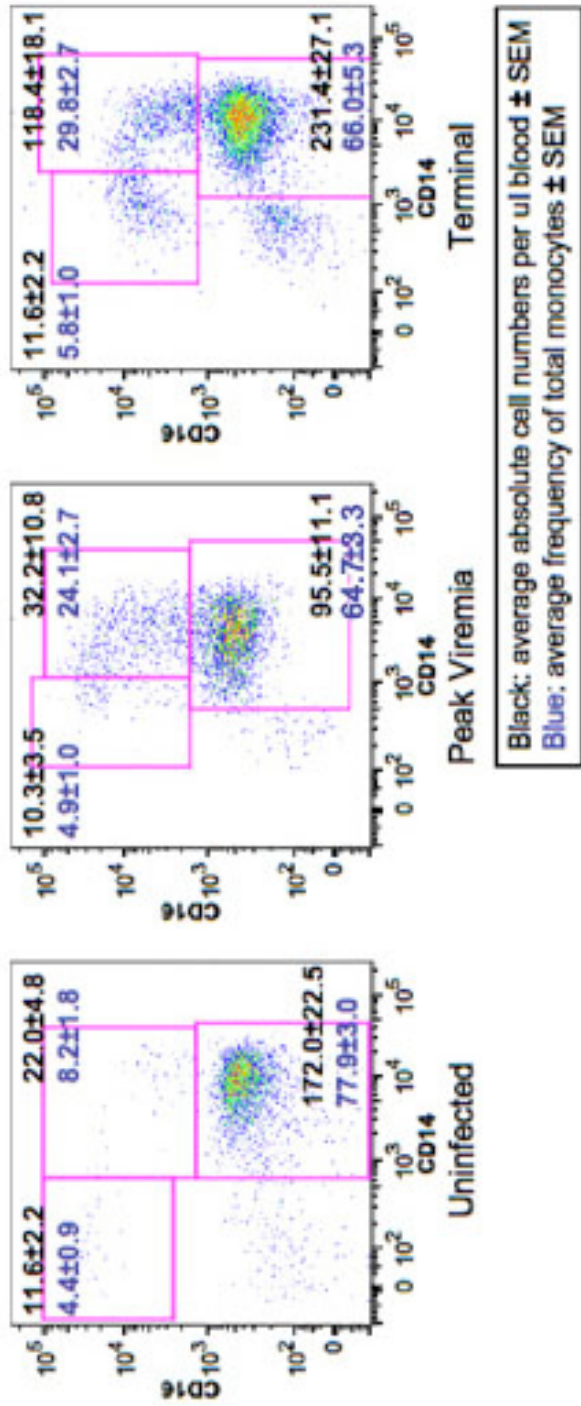
Figure 8: Changes in the relative frequency of HLA-DR+ monocyte subsets and absolute cell counts. CD14+CD16- monocytes are the predominant subset, but with infection, and expansion of the CD14+CD16+ and CD14-CD16+ subsets is observed.

Figure 9: Changes in the frequency of monocyte subsets for individual animals followed over the course of pathogenesis. Top row: NEPRC Middle row: Tulane I Bottom row: Tulane II Left column: CD14+CD16- Middle column: CD14+CD16+ Right column: CD14-CD16+ Red lines represent measurements taken from SIVE animals. Blue lines represent measurements taken from non-encephalitic animals. Dotted line represents the average of pre-infection time point values.

49
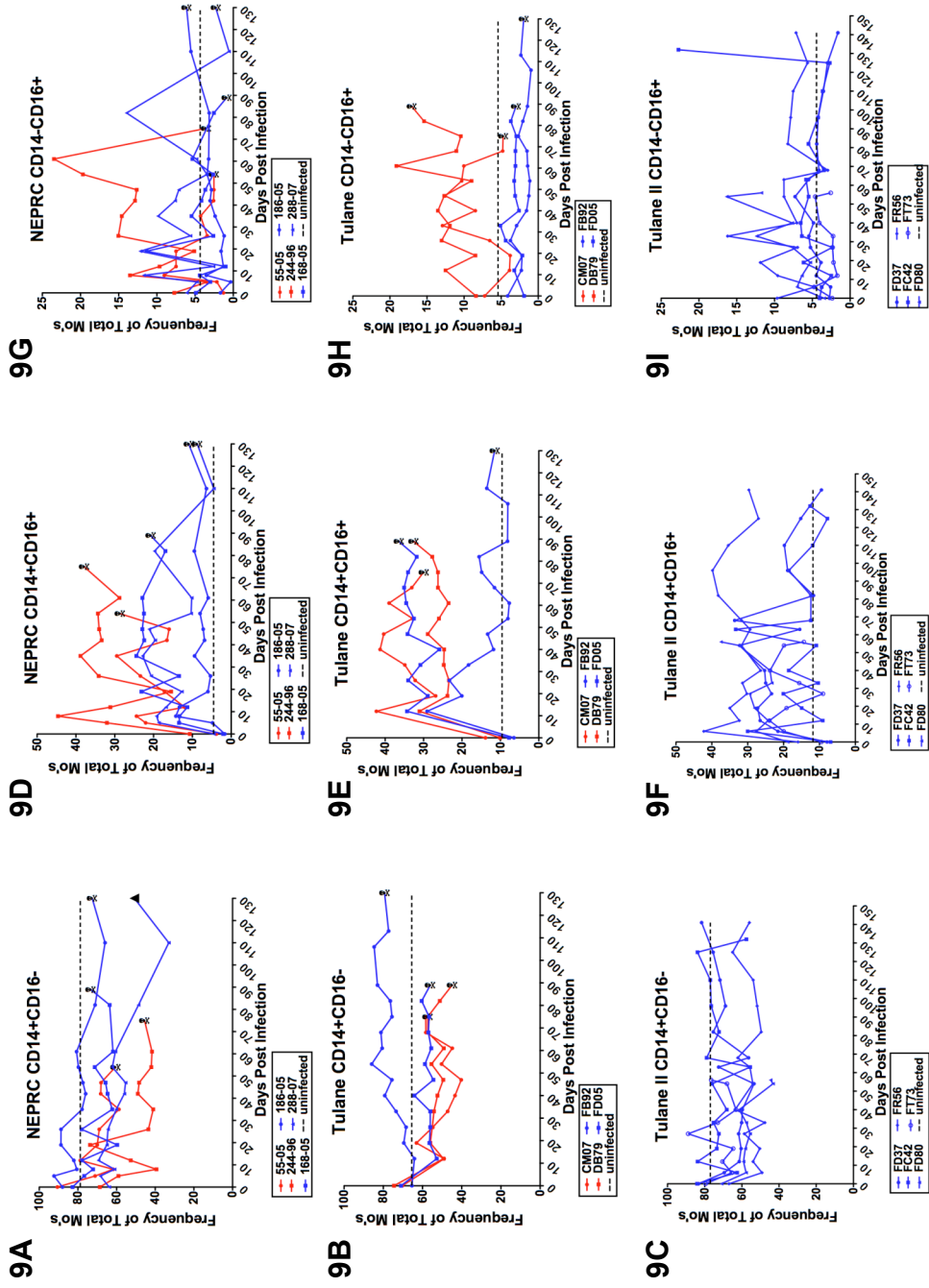
Figure 10: Changes in the absolute number of monocyte within subsets for individual animals followed over the course of pathogenesis.
Top row: NEPRC
Middle row: Tulane I
Bottom row: Tulane II
Left column: CD14+CD16-
Middle column: CD14+CD16+
Right column: CD14-CD16+
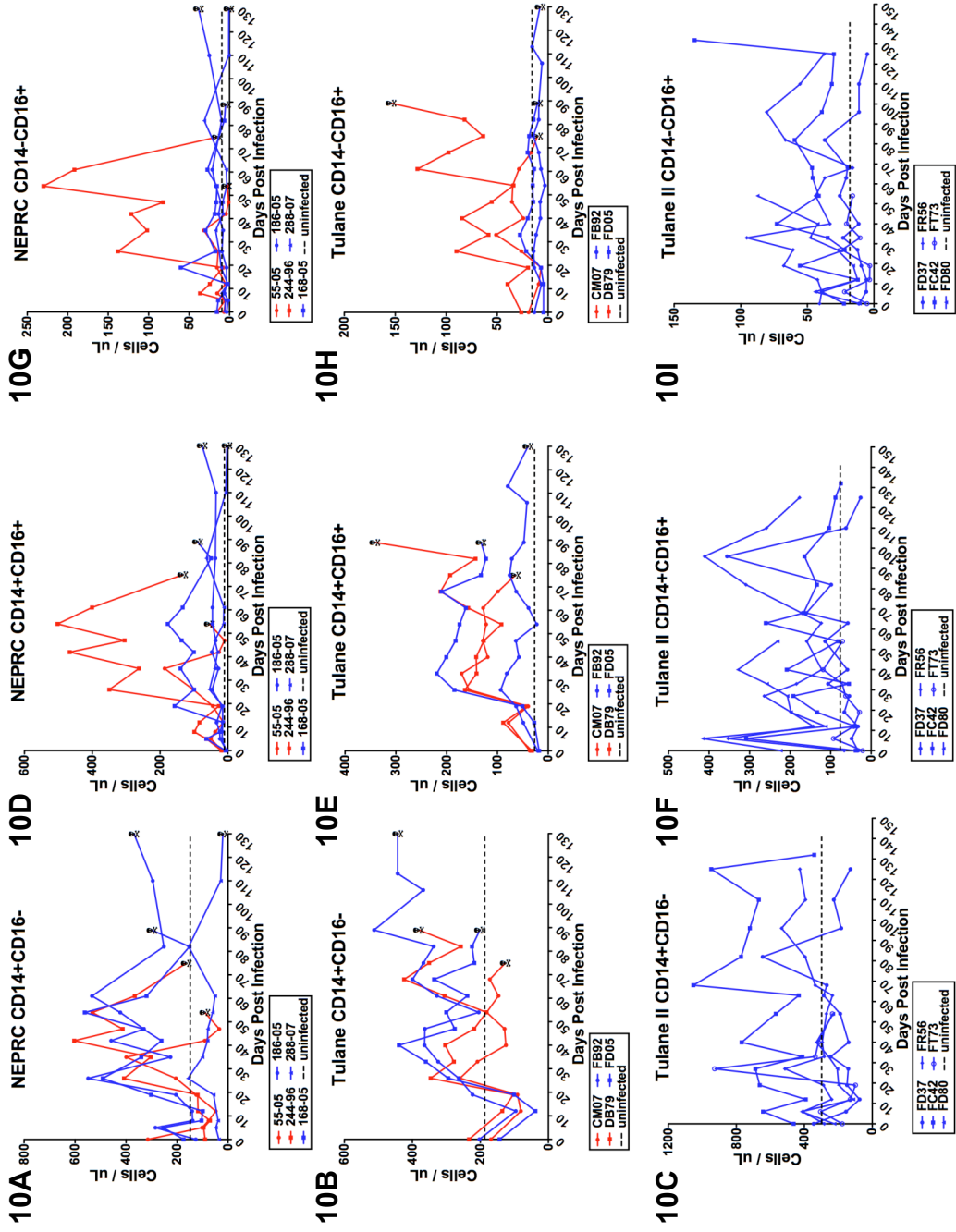Red lines represent measurements taken from SIVE animals. Blue lines represent measurements taken from non-encephalitic animals. Dotted line represents the average of pre-infection time point values.
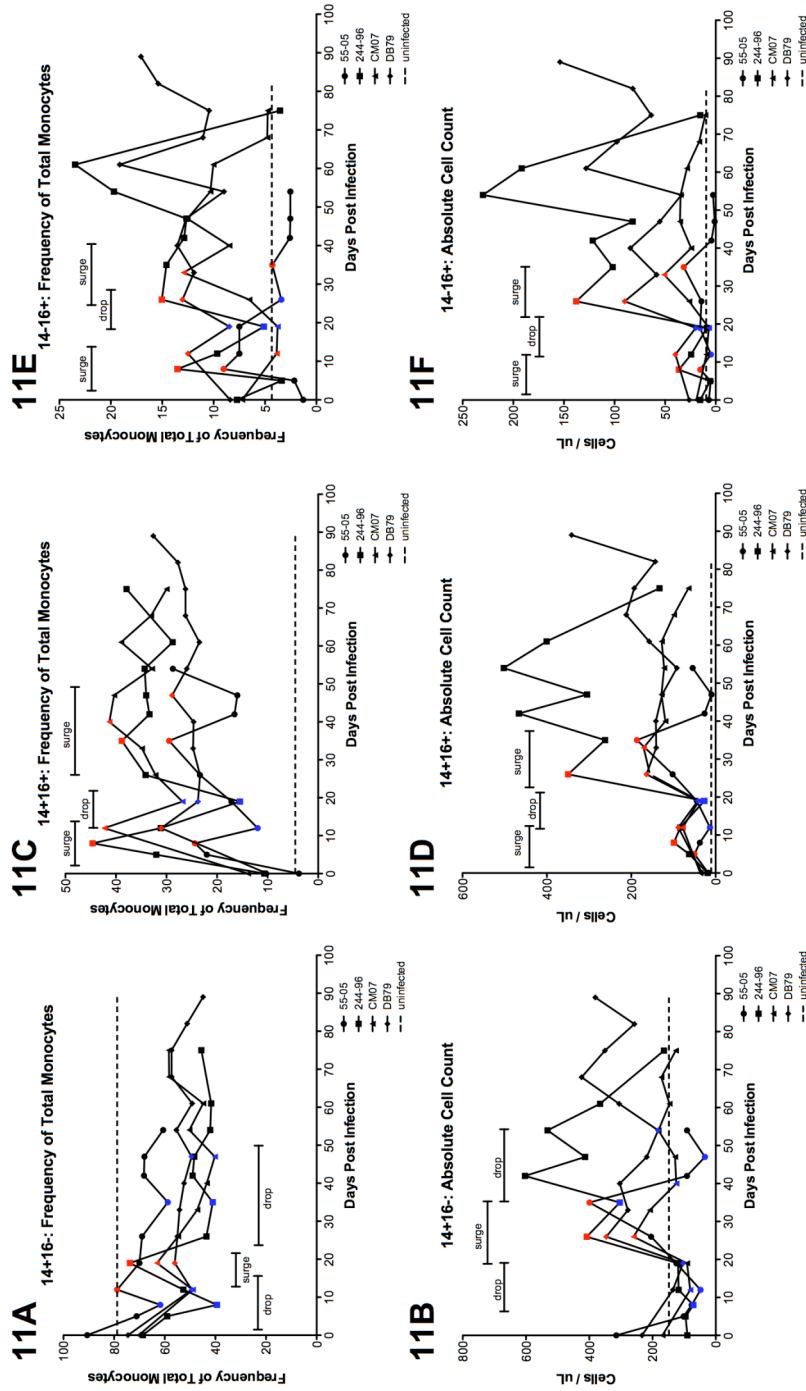
Figure 11. In encephalitic animals, there is a distinct biphasic change in both absolute number and frequency of the different monocyte subsets. The four animals that develop encephalitis are shown. During the early stages of infection, distinct patterns of drops (highlighted in blue) and surges (highlighted in red) can be observed. Dotted line represents the average of pre-infection time point values.