

Monocyte Mobilization, Activation Markers, and Unique Macrophage Populations in the Brain: Observations from SIV Infected Monkeys Are Informative with Regard to Pathogenic Mechanisms of HIV Infection in Humans

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Received: 13 October 2011 / Accepted: 25 November 2011 / Published online: 14 December 2011
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Abstract Non-human primate models of AIDS and neuro-AIDS have been useful to study AIDS in humans in general and neuroAIDS in particular. Important information concerning target cells of infection, mechanisms of immune activation and pathology and cell traffic has been made in non-human primate models. To date observations in SIV infected monkey models have predicted or paralleled monocyte/macrophage biology with HIV infection and neuro-AIDS. In this brief review we discuss a CD8+ T lymphocyte depletion model of rapid AIDS which results in a high incidence of SIV encephalitis. Specifically we review recent observations we have made using this model concerning monocyte turnover, monocyte/macrophage activation, macrophage derived biomarkers of disease and novel therapeutic approaches to AIDS and CNS pathology. Importantly, all observations made in the rapid model of AIDS discussed here are important and relevant to HIV infection of humans, even in the current era of anti-retroviral therapy that maintains HIV in plasma below the limit of detection.

Keywords Monocyte · Macrophage · SIV · HIV · AIDS · neuroAIDS

Introduction

We have used an SIV infection model of SIV-induced AIDS to study the role of monocyte/macrophages in CNS system pathology. For this, we and others have traditionally used an SIV infection model using a viral swarm, SIVmac251 which

until recently was comprised of undefined genotypes (Strickland et al. 2011). Infection with SIVmac251 results in CD4+ T cell lymphocyte depletion, entry of virus into the CNS early after infection, and the eventual development of AIDS and SIV encephalitis (SIVE) in approximately 30% of animals with AIDS. More recently, we have taken advantage of a rapid model of AIDS, using a chimeric, humanized antibody against the CD8 molecule expressed on lymphocytes, that depletes lymphocytes and NK cells resulting in a loss of the cytotoxic arm of the immune system, loss of antiviral immunity, and rapid AIDS (Schmitz et al. 1999a, b). Using this model, animals develop AIDS within 3–4 months in contrast to 1–3 years, and the incidence of SIVE increases from 25% in non depleted animals, to greater than 75% in animals that are persistently CD8+ T lymphocyte depleted (greater than 28 days of depletion). In both models, we have consistently found early expansion in absolute cell number and percentage of activated monocytes that peaks with plasma viremia, subsides, and then peaks again with the development of AIDS, likely preceding SIVE. Interestingly, this bi-phasic wave of activated monocytes occurs regardless of whether SIV infected animals, or HIV infected humans will be rapid disease progressor's, or more chronically long-term progressors. The stimulus for such expansion is not known, but likely critical to our knowledge of factors controlling innate immune responses to HIV and SIV. We have begun to define parameters of monocyte/macrophage expansion and activation including markers which can be used to predict how rapid HIV and SIV induced immune deficiency and AIDS will occur, the relative degree or severity of neuropathogenesis in monkeys, and HIV activity and neurocognitive changes in humans on effective ART. Many times such markers are evident early with infection underscoring the importance of innate immune activation, similar to CD4 nadir that define and

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perhaps set the stage for an individual's response to viral infection and pathogenesis. This review will discuss the importance of animal models of HIV infection in general, and the rhesus macaque model in particular, focusing on our recent findings in a chronic and rapid model of SIV infection with AIDS. We will discuss the biology of monocyte/macrophages with infection; biomarkers on these cells of HIV related disease including neuroAIDS and cardiac disease. We will discuss monocyte/macrophage populations that accumulate in CNS lesions, some which might play a role in expanding lesions by amplifying immune responses and others which may function to down-regulate immune responses. Factors that drive monocyte maturation and traffic from the bone marrow, through blood into parenchymal tissues will be addressed. Lastly, we will present data that when you disrupt monocyte traffic, or effectively target activated and infected monocytes, you can slow or halt AIDS and block or reverse the development of SIVE and possibly neuronal injury.

SIV infection models of AIDS and SIV encephalitis

SIV infection of non-human primates has been an ideal, though expensive model of AIDS and neuroAIDS. First, they are a relevant model of disease. Infected animals actually develop immunodeficiency as a result of viral infection, and key determinants of the virus including *nef* and *env*, similar to HIV in humans have been identified that mediate CD4⁺ T lymphocyte activation and death. Similarly, key epitopes of gp120 are recognized as dominant epitopes that CD8⁺ T lymphocytes recognized, and genetic determinants within Class I molecules are defined that determine susceptibility to disease with SIV infection. Some species of monkeys, Sooty Mangabeys, and African Green monkeys, are endemically infected but non-susceptible hosts that do not develop AIDS despite high viral loads. Using Sooty mangabeys and African Green monkeys, researchers have better defined factors including CD8⁺, NK cell responses, levels of CCR5 and CD4 on target cells of SIV infection, and more recently differences in monocyte responses that may account for lack of AIDS development and importantly AIDS related pathogenesis in tissues. Other advantages of the non-human primate model include the ability to use clinical magnetic resonance (MR) spectroscopy to monitor serially metabolic and structural changes with the CNS of infected animals to unravel clues of neuropathogenesis. Moreover, such approaches allow one to determine beneficial effects of novel therapies to protect the brain, and potentially clear viral reservoirs. In addition, because of the size of monkeys compared to mice it is possible to perform serial sampling studies of lymph nodes, blood, plasma, cerebral spinal fluid (CSF) and perform

bronchoalveolar lavage and gut biopsies to learn about immune changes with infection and therapy. One can inject agents that are restricted in humans including bromodeoxyuridine (BrdU) and monitor T lymphocyte and more recently monocyte/macrophages proliferation, expansion and traffic to learn about signals that result in recruitment of immune cells as a result of viral infection, as well as addressing important questions of cell traffic. Lastly because of the genetic and immune similarities between non-human primates and humans many potential drugs and vaccines candidates can and should be tested non-human primates. The use of the CD8 depletion model in our laboratory (Burdo et al. 2010; Campbell et al. 2011; Hasegawa et al. 2009; Kim et al. 2004, 2006; Ratai et al. 2011a, b, c; Soulas et al. 2011; Williams et al. 2005), and a rapid AIDS model used at Johns Hopkins University (Clements et al. 2005; Mankowski et al. 2002; Zink et al. 1999) result in rapid (3–4 months), consistent and severe AIDS with tissue pathogenesis including neuroAIDS, which make these models ideal for studies of acute and chronic infection that HIV infection in humans. Potential prohibitive factors of these models are high costs associated with non-human primates, the requirement of specialized biosafety (BSL2+) facilities for housing, and in some cases the lack of chronicity of infection which mimic the situation in humans who have been on antiretroviral therapy for years. This is especially critical in the ART era where despite maintaining non-detectable levels of virus, a chronic immune activation occurs perhaps in part from immune reconstitution, which may result in a different array of clinical symptoms. That said, many of the findings we and others have made with rapid models of AIDS and CNS disease in SIV infected monkeys have been informative of HIV infection of humans on effective ART (please see discussion in subsequent sections).

CD8 depletion and SIV infection, a rapid model of AIDS and SIV encephalitis

Our group and others have used rhesus macaque monkeys and infection by a molecular clone, SIVmac239 or viral swarm SIVmac251 for induction of AIDS. This model results in peak plasma virus 7–14 days pi, and virus can be detected in the brain as early as 3 days, consistently by 7–14 days, in perivascular cuffs, the meninges, and the choroid plexus. Similar to HIV infection in humans prior to ART, approximately 25% of SIV-infected rhesus macaques develop SIV encephalitis (SIVE) with multi-nucleated giant cells (MNGC) (Westmoreland et al. 1998). The key to AIDS development is immune deficiency due in part to a loss of CD4⁺ T lymphocytes, which first occurs early, in the gut, where the majority of activated memory CD4 T cells in the

body reside, and then later in the blood. At this time, animals can develop neurological symptoms of disease, including lesions comprised of macrophages, some of which are infected, and in some cases of MNGCs. Importantly, with the development of immune deficiency and AIDS, there is a second wave of activated monocytes in blood, the first having occurred with viremia, and it is quite possible that these activated cells accumulated and actually drive CNS lesion formation and therefore CNS pathogenesis (Kim et al. 2003; Williams and Hickey 2002). Also, a population of these cells in blood are likely infected and probably a source of virus that can continue to enter the CNS, causing a reseeding of virus after the initial wave that occurred early after infection. We and others have hypothesized if you block the traffic of this second wave of monocyte/macrophages into the CNS using ART (Williams et al. 2005) that lowers their activation, or Minocycline that targets their activation and possibly infection and traffic patterns (Campbell et al. 2011), or anti-VLA4 (unpublished) or drugs targeting monocyte to macrophage differentiation and infection (unpublished), you can effectively stop their traffic and accumulation in the CNS, and reverse neuronal injury (Williams and Hickey 2002).

Our laboratory has taken advantage of the fact that CD8⁺ T cells control viral replication and the level of plasma virus in infected monkeys and humans. This was demonstrated by using a humanized antibody administered to SIV infected monkeys at the time of infection on days 0, 3, and 7 (Schmitz et al. 1999a, 2005). This resulted in depletion of CD8⁺ T lymphocytes in blood and LN, and unchecked viral replication. Once CD8⁺ T cells returned in blood, plasma virus is controlled (Schmitz et al. 1999a). Our group took advantage of this model, but administered anti-CD8 mAbs later, at days 6, 8 and 12 post-infection to try to extend the duration of CD8⁺ T lymphocyte depletion. Using this approach, we found that a large percentage of animals were CD8 lymphocyte depleted for greater than 28 days, which we termed persistently CD8 depleted, and within these persistently depleted animals 77% developed SIVE (Table 1). And importantly, these animals developed AIDS and SIVE as early as 50 days (average 91 days pi) and consistently by 4–5 months as apposed to 1–3 years in non-CD8 depleted animals. We found no statistically significant difference in the length of survival between animals that developed SIVE (mean survival dpi=111; n=20) and animals that developed SIV without encephalitis (mean survival dpi=85; n=6) (Mann–Whitney *P*=.18), although the number of animals in the latter group was small. Interestingly, short term CD8 depleted animals, where the CD8 cells return within 21 days, have the same incidence of SIVE as non-depleted animals. The bi-phasic monocyte activation of CD8⁺ T lymphocyte depleted animals was the same as non-depleted animals, but plasma virus never diminished after viremia, similar

Table 1 Greater than three-quarters of persistently CD8⁺ T lymphocyte depleted rhesus develop SIV encephalitis

Animal number	Survival (dpi)	CNS pathology
159-97	117	SIVE
157-97	72	SIVE
272-82	76	SIVE
226-86	78	SIVE
169-94	70	SIVE
217-93	57	SIVE
224-93	63	SIVE
169-90	85	SIVE
N513	50	SIVnoE
E324	89	SIVnoE
BR06	92	SIVE
BP92	146	SIVE
55-05	56	SIVE
244-96	77	SIVE
168-05	89	SIVnoE
288-07	133	SIVE
DB79	91	SIVE
CM07	75	SIVE
FB92	118	SIVnoE
FD05	96	SIVE
CM09	89	SIVE
EJ85	55	SIVE
CB43	146	SIVnoE
CL78	174	SIVnoE
5035	50 ^a	SIVE
5038	50 ^a	SIVE
Average 91 DPI		77% SIVE
Range (50–174)		(20/26)

DPI days post-infection

SIVE SIV encephalitis

SIVnoE = development of simian AIDS without SIVE

^a Timed sacrificed

to what used to be seen in rapidly progressing humans. We have subsequently found, however, that there is a change in the rate and magnitude of monocytes leaving the bone marrow and turnover of perivascular macrophages in the CNS in animals that develop SIVE in this model (please see discussion below).

Expansion of monocytes and monocyte subpopulations in blood with SIV infection

Several groups have demonstrated expansion of the absolute number of monocytes with HIV and SIV infection with a bias toward activated subsets. Monkey studies allow one to know the timing of infection, and how this expansion

correlates with pathology including CNS disease and neuronal injury. We found that absolute numbers of CD14^{high}CD16^{low} and CD14^{low}CD16^{high} monocytes were significantly elevated in acutely and chronically infected animals with detectable plasma virus compared to uninfected controls (Kim et al. 2009), regardless of whether animals were CD8 lymphocyte depleted or not. Expansion of CD16⁺ monocytes was evident both in the acute and chronic phase of infection with high plasma viral load, suggesting such biphasic increases (the first phase early after primary infection and second prior to the development of AIDS) in the absolute number of CD16⁺ monocytes correlates with significant events that set the state for seeding tissues early and loss of immune system control and AIDS late (Williams and Hickey 2002; Williams et al. 2001). We have better defined this expansion using BrdU to label monocyte precursors in bone marrow and studying them in blood (see below). Additionally, we have found if we block the second wave of activated monocyte expansion using ART approaches (Williams et al. 2005), Minocycline (Campbell et al. 2011), MGBG (unpublished) or simply stop traffic of cells using anti-VLA-4 (unpublished), we can block or reverse CNS pathology.

Reconstitution of non human primates with autologous CD34⁺ hematopoietic stem cells demonstrate repopulation of perivascular macrophages

The populations of brain macrophages, either resident parenchymal microglia, perivascular macrophages that are part of the normal CNS but accumulate with disease, and other inflammatory macrophages, are bone marrow derived is not debated. But there have been few actual demonstrations of repopulation of brain macrophages in humans and non-human primates to date, from bone marrow using genetic markers of bone marrow derived cells. We took advantage of a technology developed to introduce retroviral vectors into autologous CD34⁺ hematopoietic stem cells, marked with a retroviral vector that included enhanced green fluorescent protein (EGFP) to study repopulation of CNS macrophages (Soulas et al. 2009). In this work we found a significant percentage of engraftment of stem cells giving rise to monocytes in the vasculature (Mean±SD; 8.1%±3.4), lymphoid tissues and brain 4 years post transplantation! Within the brain of normal, non-infected rhesus monkeys, EGFP⁺ cells were detected in choroid plexus, cerebellum and cerebrum where they were almost exclusively in the vicinity of blood vessels, consistent with perivascular macrophages. Multi-label immunohistochemistry and confocal microscopy studies demonstrated this population of perivascular macrophages were CD163⁺CD68⁺ cells representing cells that could be repopulated by CD34⁺ stem

cells, which had a surprisingly long-term effective turnover, raising the interesting possibility that retroviral vectors could be used in the future to engineer populations of brain perivascular macrophages that are resistant to HIV infected by down regulating HIV receptors for entry including CCR5 and CD4, as well as possibly introducing viral restriction factors such as APOBEC (Peng et al. 2007; An et al. 2004).

MAC387⁺ monocyte/macrophages, a third macrophage population in SIV and HIV encephalitic lesions

It has been long been known the HIV and SIV encephalitic lesions in humans and monkey largely consist of cells of the myeloid lineage. This first major cell type described in such lesions were the parenchymal macrophage of the brain, microglia (Williams et al. 2001; Dickson et al. 1991). Next, perivascular macrophages were defined as important elements of early lesions, perivascular cuffs. Additionally, large lesions comprised of many macrophages, MNGC (likely the fusion of perivascular macrophages (Williams et al. 2001)), and cells that were productively HIV and SIV infected (Williams et al. 2001). We recently reported another monocyte/macrophage lineage cell distinct from the parenchymal microglia and perivascular macrophages, defined by the antibody termed MAC387 (Soulas et al. 2011). These cells are consistently found in HIV and SIV lesions, although their proportion within lesions compared with other macrophages varies, and may be informative with regard to whether lesions studied are early lesion forming lesions, or a more mature, established lesion (Soulas et al. 2011). The MAC387 antibody recognizes a complex of molecules consisting of the macrophage related protein 14 (MRP14), expressed on monocytes as they enter into tissues and thus an early marker on tissues macrophages, and macrophage related protein 8 (MRP8) that is expressed on later stage inflammatory monocyte/macrophages.

We used CNS tissues from animals that were SIV infected and conventional progressors with SIVE (6 months to 2 years pi to AIDS), non-CD8 depleted animals that were rapid disease progressors with SIVE (6 months pi to AIDS), and CD8 lymphocyte depleted animals with SIVE (3 months pi to AIDS) (Soulas et al. 2011). A broad based immunohistochemical analysis demonstrated that CD163 (a type B scavenger receptor) expressed by monocytes and macrophages, CD68 expressed by mature macrophages and resident histiocytes, HAM56 also expressed by mature resident macrophages and histiocytes, We found, as previously reported by us and others (Kim et al. 2006; Fischer-Smith et al. 2008a, b; Roberts et al. 2004; Borda et al. 2008), CD163 is diffusely expressed on macrophages in lesions, and in our hand primarily confined to cells consistent with perivascular macrophages (Kim et al. 2006). CD68 was similarly spread throughout the lesion as

previously described (Hurtrel et al. 1993; Dickson et al. 1991). HAM56 expression was found on a smaller population and number of cells in the lesions (Soulas et al. 2011). In many cases the CD163 and CD68 stain reactivity appeared to overlap, while HAM56 expression seemed much more restricted. Similar to HAM56, MAC387 was expressed on a smaller number and population of cells, and these cells were more rounded and monocyte-like than macrophages (Soulas et al. 2011). Interestingly, MRP8 was not expressed on any of the macrophages within the SIV lesions, though the antigen was detected outside of the CNS on LN tissues from the same animals (Soulas et al. 2011). Thus, we found three macrophage populations in the lesions: 1) resident macrophages (CD68+HAM56+), 2) perivascular macrophages (CD163+CD68+MAC387-) and 3) recently infiltrated MAC387+CD68-CD163-monocyte/macrophages.

With BrdU pulse experiments, administering BrdU 24–48 h prior to sacrifice we found that almost all of the BrdU+ cells in the lesions were MAC387 cells with few scattered CD68 and CD163+ cells, and within lesions approximately 30% of the MAC387+ cells were BrdU+ (Burdo et al. 2010; Soulas et al. 2011). These data support the notion that these cells are recent recruits to the CNS. Interestingly, MAC387+ cells were not always major players in terms of cell numbers within lesions. Importantly, we found MAC387+ cells in SIVE lesions of all animals, chronic progressor, non-depleted rapid progressor, and CD8 lymphocyte depleted animals. But, in contrast the CD163 and CD68 positive cells, we did not find these cells in the CNS of animals without encephalitis, CNS tissues of animals sacrificed peak viremia, and in normal non-infected animals. We found that the relative ratio of CD68+ versus MAC387+ monocyte/macrophages in the CNS defined the relative severity of the CNS lesions. In animals with mild SIV encephalitis there was a higher ratio of MAC387+ cells than CD68+ macrophages, and in more severe and established lesions, there was a higher ratio of CD68+ cells over MAC387+ cells. Lastly, the MAC387+ cells were CCR2-, unlike the perivascular macrophages, and in our hands were not productively infected. The immune phenotype of macrophages in the human CNS was exactly the same as described above, with three populations of cells, but interestingly the MAC387+ cells in the human brain also expressed MRP8+ the later stage inflammatory macrophage marker that we did not find in the CNS of monkeys, likely reflecting the more chronic and long term nature of HIV infection and pathology in the human CNS over that of monkeys.

Our finding of differential numbers of MAC387+ monocyte/macrophages versus perivascular macrophages in lesions may reflect differences in lesion activity. The immune phenotypic markers expressed by perivascular macrophages, including CD163 and CCR2 may suggest they represent M2 macrophages (Soulas et al. 2011; Fischer-

Smith et al. 2008a, b), while the MAC387+ cells, that are only present with inflammation, and early lesion formation, may represent an M1 phenotype. Extending this analogy, one can hypothesize that the M1 MAC387+ monocyte/macrophages may function to expand CNS lesions, while the CD163+ cells may function to try to protect or inhibit immune activation (Soulas et al. 2011). In this scenario, M1 macrophages are early inflammatory monocyte/macrophages that appear only in encephalitic brain. In contrast, the CD163+CD68+ perivascular macrophages are present in the brain normally, but also accumulate in SIVE and HIVE lesions. Whether this scenario is true in vivo requires further experimentation.

Monocyte mobilization from bone marrow correlates with the rate of AIDS development and severity of SIV

Historically, the number of CD4 T cells in blood or plasma virus has been used as an indicator of AIDS development. Indeed, absolute numbers of CD4 T cells less than 200 cells/ μ L of blood are still used as a diagnostic for AIDS, and CD4 cell nadir is predictive of disease outcome (Ellis et al. 2011; Jernigan et al. 2011). We have recently found using non-CD8 lymphocyte depleted SIV Infected monkeys and BrdU injected intravascularly that BrdU is taken up by monocyte precursors (pre-pro monocytes and pro-monocytes) in bone marrow (Hasegawa et al. 2009). Bone marrow biopsies demonstrate the cells are both BrdU+ and Ki-67 positive, consistent with their ongoing proliferation (Ki-67) and having recently taken up BrdU. In blood, 24–144 h post BrdU injection, monocytes are found that are BrdU+, but Ki-67-consistent with cells that have taken up BrdU in bone marrow, but are no longer proliferating outside the marrow (Burdo et al. 2010). Using this approach to study monocyte expansion and mobilization, we found a low basal rate of BrdU+ monocytes in blood (less than 5%) of normal, non-SIV infected animals that were CD8 lymphocyte depleted but not SIV Infected (Burdo et al. 2010). Within CD8 lymphocyte depleted and SIV infected animals we found two sets of animals based on disease outcome: slow progressors, developing AIDS after at least 3 months pi, and rapid progressors that developed AIDS in less than 3 months pi. Within the slow progressing cohort, the magnitude of BrdU label of monocytes ranged from 2% to 5%, similar to non-SIV infected animals that were CD8+ T lymphocyte depleted. In contrast, by 8 days pi the magnitude of BrdU+ monocytes in blood was greater than 5% and continued to increase up to 20–35% in animals that had the most rapid time to development of AIDS (Burdo et al. 2010). In addition the magnitude of BrdU label positively correlates with severity of SIVE (Burdo et al. 2010). Because BrdU injections cannot be used in humans, we looked for surrogate

markers that might parallel BrdU and therefore function as a biomarker of disease. These included LPS, CCL2/MCP-1, and sCD163 (a type B scavenger receptor made and shed exclusively by monocyte/macrophages) (Fabriek et al. 2005; Droste et al. 1999; Moller et al. 2002). Interestingly sCD163 paralleled closely with an increased magnitude of BrdU+ monocytes in blood first, and importantly differentiated between the SIV infected, CD8 lymphocyte depleted animals that developed rapid AIDS with severe CNS disease from those that had slow disease development and less severe or no SIVE. Moreover, sCD163 in plasma could differentiate animals in these two groups as early as 21 days pi (Burdo et al. 2010)! sCD163 has been reported to be elevated in diseases including bacteraemia, Multiple Sclerosis, Tuberculosis, but seems to be a good marker of primarily macrophage mediated diseases including Gaucher's disease (Fabriek et al. 2007; Moller et al. 2004, 2006; Gaini et al. 2008; Knudsen et al. 2005). Our initial interest in sCD163 was from knowledge that LPS, which is reportedly translocated across the gut with severe CD4+ T cell depletion early after HIV and SIV infection is known to be a potent stimulus of CD163 expression on monocyte/macrophages and shedding of CD163 by these cells in vitro and possibly in vivo (Burdo et al. 2010; Weaver et al. 2006, 2007). Our observations in monkeys of correlations with sCD163 with BrdU and also progression to AIDS lead to our interest to ask whether sCD163 was elevated in plasma of HIV infected individuals.

sCD163 is a marker of HIV activity in early and chronically infected patients on effective ART.

To study levels of sCD163 in HIV infected patients we studied two groups, one that was chronically HIV infected, at least 1 year, and a second with early infection, defined as less than 1 year. Patients in the two groups were compared to age-matched HIV-controls. Both groups were also examined after 3 months of effective ART. We found elevated levels of sCD163 in plasma of the chronically infected patients, 2.5–3 fold higher than HIV seronegative patients prior to ART (Burdo et al. 2011a). Following 3 months of ART these levels decreased, but not to the level of that seen in HIV-seronegatives, indicating some level of monocyte/macrophage activation existed that decreased but not to that of HIV-seronegatives (Burdo et al. 2011a). In contrast, in early HIV infected patients, sCD163 was elevated above that of seronegative controls, but half that of chronic infected patients. Similar to the chronic patients with effective ART, sCD163 decreased after ART to a level found in HIV seronegative patients (Burdo et al. 2011a). These results are informative with regard to the level of innate immune activation in HIV infected patients on ART, and

suggest that chronic monocyte/macrophage activation with effective ART likely requires at least a year of HIV infection before it is not able to be ameliorated by effective therapy. Studying monocyte subsets in these patients we found increased percentage of activated CD14+CD16+ monocytes with elevated sCD163 in chronic and early infection, that decreased with ART, and a positive correlation overall with the percentage of CD14+CD16+ monocytes and sCD163 (Burdo et al. 2011a). Similarly, we found increased CD163 on CD14+CD16+ monocytes with a higher level on the early infected patients compared to chronic patients prior to ART, which decreased with effective ART, where the early HIV infected patients still had higher CD163 on CD14+CD16+ monocytes than chronic patients (Burdo et al. 2011a). Overall, we found an inverse relationship between the amount of CD163 on CD14+CD16+ monocytes and the level of sCD163 in plasma of chronic and acute patients when the data were pooled, suggesting that the more CD163 expression there is on CD14+CD16+ monocytes the less there is in plasma, and when there are higher levels of sCD163 in plasma, there is less CD163 on CD14+CD16+ monocytes (Burdo et al. 2011a). Lastly, these data suggest that the CD14+CD16+ monocytes are an indicator of levels of sCD163 in plasma, possibly because they have the highest level of expression, and also perhaps because they are a main monocyte type that is activated and sheds sCD163, a process that is in part mediated by MMP-9 (Hintz et al. 2002). The correlation of sCD163 in plasma with the number of CD14+CD16+ monocytes is consistent with immune activation, in this case monocyte/macrophages. We also found positive correlations with sCD163 levels and lymphocyte activation, specifically CD8+HLA-DR+CD38+ cells also positively correlate, as does plasma virus (Burdo et al. 2011a).

In a group of early HIV infected patients, we studied the effect of ART interruption on sCD163 (Burdo et al. 2011a). We found that with therapy interruption, sCD163 in plasma spikes, in parallel with HIV-RNA in plasma and the absolute number of CD8 T lymphocytes. CD4 T lymphocytes did not appear to increase in number with sCD163. When ART was reinitiated, sCD163 decreased, again in parallel with HIV-RNA in plasma and the absolute number of CD8 T lymphocytes, but not CD4+ T lymphocytes (Burdo et al. 2011a). Overall, these data demonstrate that sCD163 levels in plasma parallel HIV-RNA and correlate with monocyte/macrophage activation and T lymphocyte activation on patients with effective ART. Moreover, we find at successful ART effectively lowers monocyte/macrophage activation if patients are treated within the first year of HIV infection. Patients that are treated after at least 1 year of infection have decreased sCD163, but these levels are higher than those of HIV seronegative controls and HIV infected individuals who had ART within a year of treatment (Burdo et al. 2011a).

sCD163 is a marker of non-calcified plaques in the heart of HIV infected patients

In addition to monocyte activation in blood with HIV infection, chronic immune activation of monocyte/macrophages is found in monkeys in the CNS and heart, as well as LN. We have found this in monkey models without ART with activation macrophages in the CNS with SIVE, myocardium of the heart, and LN. We recently reported that sCD163 is an effective marker of non-calcified (recently formed inflammatory plaque vulnerable to rupture) cardiac plaque in the myocardium of HIV infected patients (Burdo et al. 2011b). In this study we investigated a cohort of 102 HIV infected and 41 seronegative men with equivalent cardiovascular risk factors without a history of coronary artery disease. All had computed tomography coronary angiography. Levels of sCD163 and plaque were significantly higher among antiretroviral-treated subjects with undetectable HIV RNA-levels compared to seronegative controls (Burdo et al. 2011b). Adjusting for age, race, lipids, blood pressure, glucose, smoking, sCD14 and HIV infection, sCD163 was independently associated with non-calcified plaque (Burdo et al. 2011b). This was in contrast to markers of generalized inflammation including C-reactive protein levels and D-dimer that were not associated with sCD163 or plaque in HIV infected patients (Burdo et al. 2011b).

One last note on sCD163 in HIV mediated disease is found with CNS infection in humans. We recently screened an HIV infected cohort of patients examining plasma and CSF for sCD163. We found a significant correlation with elevated sCD163 in plasma in HIV patients with impaired neurocognition but not in patients with normal neurocognitive. Interestingly the level of sCD163 in CSF negligible compared to plasma and did not correlate with CNS disease. Looking within patient groups over time we found that plasma levels of sCD163 decreased on a second visit after 5–42 more months of ART. Thus, this marker appears to serve not only as a marker of monocyte/macrophage activation in blood, HIV activity in plasma, and marker of non-calcified plaques in heart, but also possibly AIDS dementia.

Our observations in the monkey model of neuroAIDS suggest the monocyte/macrophage activation, and traffic of cells to parenchymal tissues is critical for pathogenesis. This is supported by our findings that combination anti retroviral therapy using PMPA and RCV, that did not effectively decrease plasma virus in CD8 lymphocyte depleted monkeys, resulted in a decreased second wave of activated monocytes in blood and reversal of neuronal injury measured by MR spectroscopy (Williams et al. 2005). More recently, we found that that antibiotic minocycline, when administered to CD8 depleted, SIV infected monkeys decreased plasma virus and significantly decreased expansion

of CD14+CD16+ and CD14lowCD16+ monocytes, also reduced the numbers of inflammatory monocyte/macrophages to LN (CD68 and MAC387) and brain. This treatment, similar to the PMPA and RCV therapy also reduced neuronal injury measured by MR spectroscopy, where there was a relationship between decreased CD14+CD16+ and CD14lowCD16+ monocytes and neuronal protection (Campbell et al. 2011). In vitro, Minocycline decreased CD16 expression on monocytes and SIV replication as well as cytokine production following LPS stimulation (Campbell et al. 2011). Thus ART and Minocycline both, by down regulation immune activation of monocytes, viral replication of monocyte/macrophages and cell traffic appear to effectively reverse neuronal injury in the monkey model of neuroAIDS. To date, no studies have equivalently demonstrated that monocyte/macrophage traffic directly results in neuronal injury in this model. We have used an anti-VLA-4 antibody that directly targets monocytes and T cells, stopping their traffic to the CNS, administered to animals 28 days post infection. In this study, we found reversal of neuronal injury measured by MR spectroscopy, and histological evidence of a lack of traffic of myeloid cells as well as virus in the brain, but and LN (Williams et al., unpublished). These data point not only to the importance of continued monocyte traffic to the CNS for ongoing neuronal injury, but suggest such maintenance of traffic in the monkey model also is likely important to maintain productive infection of the CNS.

Overall, these data underscore the utility of sCD163 as a biomarker of HIV activity (HIV-RNA), and more importantly monocyte/macrophage activation that is continued even with effective ART. These data point to the role of monocyte/macrophage in AIDS-related disease again even with effective ART. Such observations point to the importance of monocyte/macrophage responses to HIV infection, which occur early with peak plasma viremia as demonstrated in the animal model and HIV infected humans, regardless of rapid disease progression, chronic infection, or CD8 depletion in monkeys. Lastly, they underscore the importance of ART early after infection, certainly before a year of infection when it appears that sCD163 secretion and monocyte/macrophage activation will not return to baseline levels found in seronegative patients.

Additionally, the studies described above underscore the importance of SIV infected monkey models as they inform about HIV related pathogenesis with AIDS, monocyte/macrophage activation, mechanisms and markers of CNS and cardiac disease with infection. It is important to note that while our studies used a rapid model of CD8 depletion and AIDS with a high incidence of SIVE, they were still informative with regard to HIV infected patients on effective ART. These observations underscore the benefit of the animal model to understand mechanisms of pathogenesis, as

well as biomarkers to monitor disease as new therapies become available.

Funding Sources Work described in this manuscript was support by NIH grants RO1NS40237 (KCW), RO1NS27654 (KCW), U19MH08135 (KCW), RO1NS06897 (KCW).

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

References

- Am P, Bleiber G, Duggal P, Nelson G, May M, Mangeat B, Alobwede I, Trono D, Vlahov D, Donfield S, Goedert JJ, Phair J, Buchbinder S, O'Brien SJ, Telenti A, Winkler CA (2004) APOBEC3G genetic variants and their influence on the progression to AIDS. *J Virol* 78 (20):11070–11076
- Borda JT, Alvarez X, Mohan M, Hasegawa A, Bernardino A, Jean S, Aye P, Lackner AA (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(3):725–737. doi:10.2353/ajpath.2008.070848
- Burdo TH, Soulas C, Orzechowski K, Button J, Krishnan A, Sugimoto C, Alvarez X, Kuroda MJ, Williams KC (2010) Increased monocyte turnover from bone marrow correlates with severity of SIV encephalitis and CD163 levels in plasma. *PLoS Pathog* 6(4): e1000842
- Burdo TH, Lentz MR, Autissier P, Krishnan A, Halpern E, Letendre S, Rosenberg ES, Ellis RJ, Williams KC (2011a) Soluble CD163 made by monocyte/macrophages is a novel marker of HIV activity in early and chronic infection prior to and after antiretroviral therapy. *J Infect Dis* 204(1):154–163. doi:10.1093/infdis/jir214
- Burdo TH, Lo J, Abbara S, Wei J, Delelys ME, Preffer F, Rosenberg ES, Williams KC, Grinspoon S (2011b) Soluble CD163, a novel marker of activated macrophages, is elevated and associated with noncalcified coronary plaque in HIV-infected patients. *J Infect Dis* 204(8):1227–1236. doi:10.1093/infdis/jir520
- Campbell JH, Burdo TH, Autissier P, Bombardier JP, Westmoreland SV, Soulas C, Gonzalez RG, Ratai EM, Williams KC (2011) Minocycline inhibition of monocyte activation correlates with neuronal protection in SIV neuroAIDS. *PLoS One* 6(4):e18688. doi:10.1371/journal.pone.0018688
- Clements JE, Li M, Gama L, Bullock B, Carruth LM, Mankowski JL, Zink MC (2005) The central nervous system is a viral reservoir in simian immunodeficiency virus-infected macaques on combined antiretroviral therapy: a model for human immunodeficiency virus patients on highly active antiretroviral therapy. *J Neurovirol* 11 (2):180–189. doi:10.1080/13550280590922829
- Dickson DW, Mattiace LA, Kure K, Hutchins K, Lyman WD, Brosnan CF (1991) Microglia in human disease, with an emphasis on acquired immune deficiency syndrome. *Lab Invest* 64(2):135–156
- Droste A, Sorg C, Hogger P (1999) Shedding of CD163, a novel regulatory mechanism for a member of the scavenger receptor cysteine-rich family. *Biochem Biophys Res Commun* 256 (1):110–113. doi:10.1006/bbrc.1999.0294
- Ellis RJ, Badiee J, Vaida F, Letendre S, Heaton RK, Clifford D, Collier AC, Gelman B, McArthur J, Morgello S, McCutchan JA, Grant I (2011) CD4 nadir is a predictor of HIV neurocognitive impairment in the era of combination antiretroviral therapy. *AIDS* 25 (14):1747–1751. doi:10.1097/QAD.0b013e32834a40cd
- Fabrick BO, Dijkstra CD, van den Berg TK (2005) The macrophage scavenger receptor CD163. *Immunobiology* 210(2–4):153–160
- Fabrick BO, Moller HJ, Vloet RP, van Winsen LM, Hanemaaijer R, Teunissen CE, Uitdehaag BM, van den Berg TK, Dijkstra CD (2007) Proteolytic shedding of the macrophage scavenger receptor CD163 in multiple sclerosis. *J Neuroimmunol* 187(1–2):179–186. doi:10.1016/j.jneuroim.2007.04.016
- Fischer-Smith T, Bell C, Croul S, Lewis M, Rappaport J (2008a) Monocyte/macrophage trafficking in acquired immunodeficiency syndrome encephalitis: lessons from human and non-human primate studies. *J Neurovirol* 14(4):318–326. doi:10.1080/13550280802132857
- Fischer-Smith T, Tedaldi EM, Rappaport J (2008b) CD163/CD16 coexpression by circulating monocytes/macrophages in HIV: potential biomarkers for HIV infection and AIDS progression. *AIDS Res Hum Retroviruses* 24(3):417–421. doi:10.1089/aid.2007.0193
- Gaini S, Pedersen SS, Koldkaer OG, Pedersen C, Moestrup SK, Moller HJ (2008) New immunological serum markers in bacteraemia: anti-inflammatory soluble CD163, but not proinflammatory high mobility group-box 1 protein, is related to prognosis. *Clin Exp Immunol* 151(3):423–431. doi:10.1111/j.1365-2249.2007.03586.x
- Hasegawa A, Liu H, Ling B, Borda JT, Alvarez X, Sugimoto C, Vinet-Oliphant H, Kim WK, Williams KC, Ribeiro RM, Lackner AA, Veazey RS, Kuroda MJ (2009) The level of monocyte turnover predicts disease progression in the macaque model of AIDS. *Blood* 114(14):2917–2925. doi:10.1182/blood-2009-02-204263
- Hintz KA, Rassias AJ, Wardwell K, Moss ML, Morganello PM, Pioli PA, Givan AL, Wallace PK, Yeager MP, Guyre PM (2002) Endotoxin induces rapid metalloproteinase-mediated shedding followed by up-regulation of the monocyte hemoglobin scavenger receptor CD163. *J Leukoc Biol* 72(4):711–717
- Hurtrel B, Chakrabarti L, Hurtrel M, Montagnier L (1993) Target cells during early SIV encephalopathy. *Res Virol* 144(1):41–46
- Jernigan TL, Archibald SL, Fennema-Notestine C, Taylor MJ, Theilmann RJ, Julaton MD, Notestine RJ, Wolfson T, Letendre SL, Ellis RJ, Heaton RK, Gamst AC, Franklin DR Jr, Clifford DB, Collier AC, Gelman BB, Marra C, McArthur JC, McCutchan JA, Morgello S, Simpson DM, Grant I (2011) Clinical factors related to brain structure in HIV: the CHARTER study. *J Neurovirol* 17(3):248–257. doi:10.1007/s13365-011-0032-7
- Kim WK, Corey S, Alvarez X, Williams K (2003) Monocyte/macrophage traffic in HIV and SIV encephalitis. *J Leukoc Biol* 74 (5):650–656
- Kim WK, Corey S, Chesney G, Knight H, Klumpp S, Wuthrich C, Letvin N, Koralknik I, Lackner A, Veasey R, Williams K (2004) Identification of T lymphocytes in simian immunodeficiency virus encephalitis: distribution of CD8+ T cells in association with central nervous system vessels and virus. *J Neurovirol* 10(5):315–325
- Kim WK, Alvarez X, Fisher J, Bronfin B, Westmoreland S, McLaurin J, Williams K (2006) CD163 identifies perivascular macrophages in normal and viral encephalitic brains and potential precursors to perivascular macrophages in blood. *Am J Pathol* 168(3):822–834
- Kim WK, Sun Y, Do H, Autissier P, Halpern EF, Piatak M Jr, Lifson JD, Burdo TH, McGrath MS, Williams K (2009) Monocyte heterogeneity underlying phenotypic changes in monocytes according to SIV disease stage. *J Leukoc Biol*. doi:10.1189/jlb.0209082
- Knudsen TB, Gustafson P, Kronborg G, Kristiansen TB, Moestrup SK, Nielsen JO, Gomes V, Aaby P, Lisse I, Moller HJ, Eugen-Olsen J (2005) Predictive value of soluble haemoglobin scavenger

- receptor CD163 serum levels for survival in verified tuberculosis patients. *Clin Microbiol Infect* 11(9):730–735. doi:10.1111/j.1469-0691.2005.01229.x
- Mankowski JL, Clements JE, Zink MC (2002) Searching for clues: tracking the pathogenesis of human immunodeficiency virus central nervous system disease by use of an accelerated, consistent simian immunodeficiency virus macaque model. *J Infect Dis* 186 (Suppl 2):S199–S208. doi:10.1086/344938
- Moller HJ, Peterslund NA, Graversen JH, Moestrup SK (2002) Identification of the hemoglobin scavenger receptor/CD163 as a natural soluble protein in plasma. *Blood* 99(1):378–380
- Moller HJ, de Fost M, Aerts H, Hollak C, Moestrup SK (2004) Plasma level of the macrophage-derived soluble CD163 is increased and positively correlates with severity in Gaucher's disease. *Eur J Haematol* 72(2):135–139. doi:10.1046/j.0902-4441.2003.00193.x
- Moller HJ, Moestrup SK, Weis N, Wejse C, Nielsen H, Pedersen SS, Attermann J, Nexø E, Kronborg G (2006) Macrophage serum markers in pneumococcal bacteremia: Prediction of survival by soluble CD163. *Crit Care Med* 34(10):2561–2566. doi:10.1097/01.CCM.0000239120.32490.AB
- Peng G, Greenwell-Wild T, Nares S, Jin W, Lei KJ, Rangel ZG, Munson PJ, Wahl SM (2007) Myeloid differentiation and susceptibility to HIV-1 are linked to APOBEC3 expression. *Blood* 110 (1):393–400
- Ratai EM, Annamalai L, Burdo T, Joo CG, Bombardier JP, Fell R, Hakimelahi R, He J, Lentz MR, Campbell J, Curran E, Halpern EF, Masliah E, Westmoreland SV, Williams KC, Gonzalez RG (2011a) Brain creatine elevation and N-acetylaspartate reduction indicates neuronal dysfunction in the setting of enhanced glial energy metabolism in a macaque model of NeuroAIDS. *Magn Reson Med*. doi:10.1002/mrm.22821
- Ratai EM, Bombardier JP, Joo CG, Annamalai L, Burdo TH, Campbell J, Fell R, Hakimelahi R, He J, Autissier P, Lentz MR, Halpern EF, Masliah E, Williams KC, Westmoreland SV, Gonzalez RG (2011b) Proton magnetic resonance spectroscopy reveals neuroprotection by oral minocycline in a nonhuman primate model of accelerated NeuroAIDS. *PLoS One* 5(5):e10523. doi:10.1371/journal.pone.0010523
- Ratai EM, Pilkenton S, He J, Fell R, Bombardier JP, Joo CG, Lentz MR, Kim WK, Burdo TH, Autissier P, Annamalai L, Curran E, O'Neil SP, Westmoreland SV, Williams KC, Masliah E, Gilberto Gonzalez R (2011c) CD8(+) lymphocyte depletion without SIV infection does not produce metabolic changes or pathological abnormalities in the rhesus macaque brain. *J Med Primatol*. doi:10.1111/j.1600-0684.2011.00475.x
- Roberts ES, Masliah E, Fox HS (2004) CD163 identifies a unique population of ramified microglia in HIV encephalitis (HIVE). *J Neuropathol Exp Neurol* 63(12):1255–1264
- Schmitz JE, Kuroda MJ, Santra S, Sasseville VG, Simon MA, Lifton MA, Racz P, Tenner-Racz K, Dalesandro M, Scallon BJ, Ghayeb J, Forman MA, Montefiori DC, Rieber EP, Letvin NL, Reimann KA (1999a) Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. *Science* 283(5403):857–860
- Schmitz JE, Simon MA, Kuroda MJ, Lifton MA, Ollert MW, Vogel CW, Racz P, Tenner-Racz K, Scallon BJ, Dalesandro M, Ghayeb J, Rieber EP, Sasseville VG, Reimann KA (1999b) A nonhuman primate model for the selective elimination of CD8+ lymphocytes using a mouse-human chimeric monoclonal antibody. *Am J Pathol* 154(6):1923–1932
- Schmitz JE, Johnson RP, McClure HM, Manson KH, Wyand MS, Kuroda MJ, Lifton MA, Khunkhun RS, McEvers KJ, Gillis J, Piatak M, Lifson JD, Grosschupff G, Racz P, Tenner-Racz K, Rieber EP, Kuus-Reichel K, Gelman RS, Letvin NL, Montefiori DC, Ruprecht RM, Desrosiers RC, Reimann KA (2005) Effect of CD8+ lymphocyte depletion on virus containment after simian immunodeficiency virus SIVmac251 challenge of live attenuated SIVmac239delta3-vaccinated rhesus macaques. *J Virol* 79 (13):8131–8141
- Soulas C, Donahue RE, Dunbar CE, Persons DA, Alvarez X, Williams KC (2009) Genetically modified CD34+ hematopoietic stem cells contribute to turnover of brain perivascular macrophages in long-term repopulated primates. *Am J Pathol* 174(5):1808–1817. doi:10.2353/ajpath.2009.081010
- Soulas C, Conerly C, Kim WK, Burdo TH, Alvarez X, Lackner AA, Williams KC (2011) Recently infiltrating MAC387(+) monocytes/macrophages a third macrophage population involved in SIV and HIV encephalitic lesion formation. *Am J Pathol* 178 (5):2121–2135. doi:10.1016/j.ajpath.2011.01.023
- Strickland SL, Gray RR, Lamers SL, Burdo TH, Huenink E, Nolan DJ, Nowlin B, Alvarez X, Midkiff CC, Goodenow MM, Williams K, Salemi M (2011) Significant genetic heterogeneity of the SIVmac251 viral swarm derived from different sources. *AIDS Res Hum Retroviruses*. doi:10.1089/AID.2011.0100
- Weaver LK, Hintz-Goldstein KA, Pioli PA, Wardwell K, Qureshi N, Vogel SN, Guyre PM (2006) Pivotal advance: activation of cell surface Toll-like receptors causes shedding of the hemoglobin scavenger receptor CD163. *J Leukoc Biol* 80(1):26–35. doi:10.1189/jlb.1205756
- Weaver LK, Pioli PA, Wardwell K, Vogel SN, Guyre PM (2007) Up-regulation of human monocyte CD163 upon activation of cell-surface Toll-like receptors. *J Leukoc Biol* 81(3):663–671. doi:10.1189/jlb.0706428
- Westmoreland SV, Halpern E, Lackner AA (1998) Simian immunodeficiency virus encephalitis in rhesus macaques is associated with rapid disease progression. *J Neurovirol* 4(3):260–268
- Williams KC, Hickey WF (2002) Central nervous system damage, monocytes and macrophages, and neurological disorders in AIDS. *Annu Rev Neurosci* 25:537–562
- Williams KC, Corey S, Westmoreland SV, Pauley D, Knight H, deBakker C, Alvarez X, Lackner AA (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(8):905–915
- Williams K, Westmoreland S, Greco J, Ratai E, Lentz M, Kim WK, Fuller RA, Kim JP, Autissier P, Sehgal PK, Schinazi RF, Bischofberger N, Piatak M, Lifson JD, Masliah E, Gonzalez RG (2005) Magnetic resonance spectroscopy reveals that activated monocytes contribute to neuronal injury in SIV neuroAIDS. *J Clin Invest* 115(9):2534–2545
- Zink MC, Suryanarayana K, Mankowski JL, Shen A, Piatak M Jr, Spelman JP, Carter DL, Adams RJ, Lifson JD, Clements JE (1999) High viral load in the cerebrospinal fluid and brain correlates with severity of simian immunodeficiency virus encephalitis. *J Virol* 73(12):10480–10488