SHORT COMMUNICATION

α 4-Integrin Antibody Treatment Blocks Monocyte/Macrophage Traffic to, Vascular Cell Adhesion Molecule-1 Expression in, and Pathology of the Dorsal Root Ganglia in an SIV Macaque Model of HIV-Peripheral Neuropathy

Jessica R. Lakritz,^{*} Derek M. Thibault,^{*} Jake A. Robinson,^{*} Jennifer H. Campbell,^{*} Andrew D. Miller,[†] Kenneth C. Williams,^{*} and Tricia H. Burdo^{*}

From the Department of Biology,^{*} Boston College, Chestnut Hill, Massachusetts; and the Section of Anatomic Pathology,[†] Department of Biomedical Sciences, Cornell University College of Veterinary Medicine, Ithaca, New York

Accepted for publication
March 7, 2016.

Address correspondence to
Tricia H. Burdo, Ph.D.,
Department of Biology, Boston
College, 140 Commonwealth
Ave, Higgins Hall 445, Chest-
nut Hill, MA 02467. E-mail:
burdot@bc.edu.

Traffic of activated monocytes into the dorsal root ganglia (DRG) is critical for pathology in HIV peripheral neuropathy. We have shown that accumulation of recently recruited (bromodeoxyuridine⁺ MAC387⁺) monocytes is associated with severe DRG pathology and loss of intraepidermal nerve fibers in SIV-infected macaques. Herein, we blocked leukocyte traffic by treating animals with natalizumab, which binds to α 4-integrins. SIV-infected CD8-depleted macaques treated with natalizumab either early (the day of infection) or late (28 days after infection) were compared with untreated SIV-infected animals sacrificed at similar times. Histopathology showed diminished DRG pathology with natalizumab treatment, including decreased inflammation, neuronophagia, and Nageotte nodules. Natalizumab treatment resulted in a decrease in the number of bromodeoxyuridine⁺ (early), MAC387⁺ (late), CD68⁺ (early and late), and SIVp28⁺ (late) macrophages in DRG tissues. The number of CD3⁺ T lymphocytes in DRGs was not affected by natalizumab treatment. Vascular cell adhesion molecule 1, an adhesion molecule that mediates leukocyte traffic, was diminished in DRGs of all natalizumab-treated animals. These data show that blocking monocyte, but not T lymphocyte, traffic to the DRG results in decreased inflammation and pathology, supporting a role for monocyte traffic and activation in HIV peripheral neuropathy. (*Am J Pathol* 2016, 186: 1754–1761; <http://dx.doi.org/10.1016/j.ajpath.2016.03.007>)

The primary mechanisms of HIV peripheral neuropathy include immune damage secondary to viral infection and mitochondrial toxicity from the antiretrovirals. Because HIV and SIV do not productively infect neurons or Schwann cells, damage to the dorsal root ganglia (DRG) in peripheral neuropathy is believed to be, in part, because of infected and activated macrophages. *In vitro* and *in vivo* studies suggest that both viral proteins and systemic inflammation secondary to the viral infection may damage neurons and axons.¹ Using a CD8-depleted SIV-infected rhesus macaque model of peripheral neuropathy, we have shown that accumulation of recruited [bromodeoxyuridine

(BrdU)⁺MAC387⁺] monocytes/macrophages was associated with severe DRG pathology.² The number of BrdU⁺ monocytes correlated with DRG histopathology, which included neuronophagia, satellitosis, and Nageotte nodules.² Our data demonstrate that newly recruited MAC387⁺BrdU⁺ macrophages play a significant role in DRG pathogenesis.

Supported by NIH/National Institute of Neurological Diseases and Stroke grants R01 NS082116 (T.H.B.) and R01 NS040237 (K.C.W.). The *in vivo* CD8 T lymphocyte depletion antibodies were provided by the NIH Nonhuman Primate Reagent Resource grants RR016001 and AI040101.

Disclosures: Natalizumab was provided by Biogen Idec, Cambridge, MA.

Natalizumab was approved for the treatment of relapsing-remitting multiple sclerosis and Crohn's disease.³ Natalizumab blocks traffic of leukocytes (monocytes/macrophages, T cells, and B cells) in the central nervous system of patients with relapsing-remitting multiple sclerosis³ and gut of patients with Crohn's disease.⁴ We have shown that natalizumab treatment of SIV-infected rhesus macaques resulted in stabilization of ongoing neuronal injury (N-acetylaspartate to creatine ratio by ¹H magnetic resonance spectroscopy), and decreased numbers of monocytes/macrophages and productive SIV infection in the brain and gut.⁵ We found similar numbers of CD68⁺ and MAC387⁺ monocytes/macrophages in lymph nodes of untreated and treated SIV-infected animals, suggesting that natalizumab did not significantly affect traffic to lymph nodes, as previously described.⁵ Recently, we have also shown that natalizumab treatment of SIV-infected rhesus macaques blocks monocyte traffic to the heart, resulting in decreased cardiac pathology, cardiovascular disease, and fibrosis.⁶ Herein, we extend these studies to determine whether ongoing monocyte/macrophage traffic is required for SIV-associated DRG damage and we found that natalizumab treatment decreased inflammation, monocyte traffic, SIV infection, and pathology of DRGs.

Materials and Methods

Ethical Statement

All animals used in this study were handled in strict accordance with American Association for Accreditation of

Laboratory Animal Care with the approval of the Massachusetts General Hospital Subcommittee on Research and Animal Care, the Institutional Animal Care and Use Committee of Harvard University, and Tulane University.

Animals, Viral Infection, and CD8 Lymphocyte Depletion

Sixteen adult male rhesus macaques (*Macaca mulatta*) were used in this study. Animals were inoculated i.v. with SIVmac251 (5 ng SIV p27; a generous gift from Dr. Ronald Desrosiers, University of Miami, Coral Gables, FL) and were administered 10 mg/kg anti-CD8 antibody s.c. at day 6 after infection, and 5 mg/kg i.v. at days 8 and 12 after infection to achieve rapid AIDS. The human anti-CD8 antibody was provided by the NIH Nonhuman Primate Reagent Resource (RR016001 and AI040101).^{7–12} Eight macaques ($n = 4$ late natalizumab treated, $n = 4$ late untreated) were sacrificed between 49 and 77 days after infection (dpi) and eight animals ($n = 5$ early natalizumab treated, $n = 3$ early untreated) were sacrificed at 21 or 22 dpi.⁵ All animals were anesthetized with ketamine hydrochloride and euthanized by an i.v. pentobarbital overdose and exsanguinated. Both early and late natalizumab-treated groups and the early untreated group were timed sacrificed (days 21, 22, and 49, after infection, respectively) (Table 1). Late untreated animals were sacrificed on the basis of the guidelines for euthanasia of SIV-infected rhesus macaques. All animals were still fully CD8 depleted at the time of necropsy.

Table 1 SIV-Infected CD8-Depleted Rhesus Macaques Used in This Study and Brain and DRG Pathology

Animal groups	ID	DRGs examined*	BrdU administered (dpi)	Survival (days)	Plasma viral load at necropsy (log10)	Brain pathology	Overall DRG pathology	Inflammation/satellite cell proliferation	Neuronophagia	Nageotte nodules
Early untreated ($N = 3$)	A01	M	6, 20	22	6.87	PV cuffs	Moderate	Moderate	Moderate	Rare
	A02	M	6, 20	22	7.15	PV cuffs	Moderate	Moderate	Moderate	Rare
	A03	M	20	22	6.84	Normal	Mild	Mild	Mild	No
Early natalizumab ($N = 5$)	A04	L, S	6, 20	21	7.54	Normal	Mild-mod	Moderate	Mild	No
	A05	L	6, 20	21	7.3	Normal	Mild	Mild	No	No
	A06	M	6, 20	21	7.64	Normal	Mild-mod	Moderate	Mild	No
	A07	C	6, 20	21	7.43	Normal	Mild-mod	Moderate	Mild	No
Late untreated ($N = 4$)	A08	M	6, 20	21	7.54	Normal	Mild	Mild	No	No
	A09	M	Pre, 7, 26, 55	56	7.86	Mild-SIVE	Moderate	Moderate	Moderate	Rare
	A10	T, L, S	Pre, 7, 20, 41, 54	55	7.83	SIVE	Mild-mod	Moderate	Mild	Rare
	A11	T, L, S	41, 62, 76	77	7.23	SIVE	Mod-severe	Severe	Mod-severe	Extensive
Late natalizumab ($N = 4$)	A12	T, L, S	41, 62, 76	77	8.54	SIVE	Moderate	Moderate	Moderate	Rare
	A13	M	Pre, 26, 47	49	7.95	Satellitosis	Mild-mod	Moderate	Mild	No
	A14	M	Pre, 26, 47	49	7.71	Mild-SIVE	Moderate	Moderate	Mild	Rare
	A15	T, S	33, 47	49	7.56	Normal	Moderate	Moderate	Mild	Rare
	A16	S	33, 47	49	7.91	Normal	Moderate	Moderate	Mild	Rare

*DRGs examined were from mixed DRGs and may contain thoracic, lumbar, sacral, and/or cervical DRGs.

BrdU, bromodeoxyuridine; C, cervical; dpi, days after infection; DRG, dorsal root ganglia; L, lumbar DRG; mod, moderate pathology; M, mixed DRGs; Normal, no significant pathology; Pre, before infection; PV, perivascular; S, sacral DRG; Satellitosis, rare neuronal satellitosis in frontal cortex; SIVE, SIV encephalitis; T, thoracic DRG.

Anti- $\alpha 4$ Integrin (Natalizumab) Administration

The recombinant humanized IgG4 monoclonal anti- $\alpha 4$ integrin monoclonal antibody (natalizumab) was kindly provided by Biogen Idec (Cambridge, MA). The antibody was administered once weekly (30 mg/kg) for 3 weeks beginning on the day of infection (early, 0 dpi, $n = 5$) or 28 days after infection (late, 28 dpi, $n = 4$) in a slow bolus. A high dose of natalizumab was given three times with 1-week intervals between each treatment to avoid hypersensitivity responses.⁵ This regimen maintains high serum levels of natalizumab.¹³

BrdU Administration

BrdU was administered as a slow bolus i.v. injection at a dose of 60 mg BrdU/kg body weight, as previously described.¹⁴ To monitor levels of monocyte/macrophage trafficking out of the bone marrow, in blood, and into the DRG, BrdU was administered before infection (−9 dpi), 26 dpi, and 24 hours before necropsy in two macaques given natalizumab beginning on 28 dpi. In the other natalizumab-treated animals, BrdU was administered once natalizumab treatment was initiated, on days 33 and 47 after infection ($n = 2$ late natalizumab) or days 6 and 20 after infection ($n = 5$ early natalizumab). For the early, untreated animals, BrdU was also given at days 6 and 20 after infection ($n = 3$ early untreated). For the late untreated animals, BrdU was administered before infection (−9 dpi), at peak infection (7 and 20 or 26 dpi), day 41 after infection, and 24 hours before necropsy in two macaques and 24 hours and days 41 and 62 before necropsy in the other two macaques (Table 1).

Necropsy and Histopathology

Animals were necropsied immediately after euthanasia, and representative sections of all major organs were collected, fixed in 10% neutral buffered formalin, embedded in paraffin, and divided into sections (5 μ m thick). Sections were stained with hematoxylin and eosin, as previously described.^{2,15}

Histopathological Analysis of DRG Morphology

Hematoxylin and eosin sections of DRG were evaluated blindly for histopathological lesions by a board-certified veterinary anatomical pathologist (A.D.M.) and scored on the basis of the presence and severity of infiltrating mononuclear cells, neuronophagia, and Nageotte nodules, as previously described.^{2,15} Overall pathology was scored via the following criteria: i) mild: scattered infiltrating mononuclear cells with rare evidence of neuronophagia and/or neuronal loss; ii) moderate: increased numbers of infiltrating mononuclear cells with occasional neuronophagia and/or neuronal loss; and iii) severe: abundant infiltrating mononuclear cells, frequent neuronophagia, and neuronal loss were all present.^{2,15,16}

Immunohistochemistry

DRG sections were stained with either anti-SIV protein p28 (Fitzgerald, Acton, MA), the pan-macrophage marker anti-CD68 (clone KP1; Dako, Carpinteria, CA), the scavenger receptor anti-CD163 (clone MCA1853; Serotec, Raleigh, NC), the early inflammatory marker anti-MAC387 (clone M0747; Dako), anti-BrdU (clone M0744; Dako), the T-lymphocyte marker anti-CD3 (clone A0452; Dako), or anti-vascular cell adhesion molecule (VCAM)-1 (4E8 clone; Antibodies-Online, Atlanta, GA), as previously described.^{2,16}

Immunohistochemistry Quantitation in DRGs

For quantitation of monocyte/macrophage and T-cell populations by immunohistochemical analyses, at least eight nonoverlapping fields at $\times 200$ magnification were quantitated per DRG tissue, as previously described.^{2,16} Data were expressed as means $\pm$ SEM. For animals that had more than one region of DRG, the means were averaged.

DRG tissue was stained for VCAM-1, as described above. Eight random, nonoverlapping fields at $\times 200$ magnification were imaged, and the percentage of VCAM-1⁺ area of total DRG tissue area was calculated. The area of VCAM-1⁺ tissue was quantified using ImageJ analysis software version 1.45s (NIH, Bethesda, MD). Images underwent color deconvolution to separate histological dyes into diaminobenzidine (DAB; brown) and hematoxylin (purple) channels. The color threshold on the DAB channel was adjusted uniformly on all images to measure DAB⁺ area. Percentage of VCAM-1⁺ area of total tissue was calculated by dividing the total DAB⁺ area by the total area of tissue and multiplying by 100. For animals that had more than one region DRG, the percentages of each region were averaged.

Statistical Analysis

Prism software version 5.0f (GraphPad Software, Inc., San Diego, CA) was used for statistical analyses. *t*-Tests were used to detect variation in cell numbers between untreated and natalizumab-treated rhesus macaques. $P < 0.05$ was considered significant.

Results

Histopathological Analysis Shows Diminished DRG Pathology with Natalizumab Treatment

Sixteen SIV-infected CD8-depleted rhesus macaques were included in this study (Table 1). Eight animals were sacrificed at 21 or 22 dpi. Five of these animals received natalizumab treatment beginning on the day of infection (0 dpi), and three animals were untreated controls. An additional eight macaques were sacrificed at similar time points (49 to 77 dpi). Four of these animals received natalizumab treatment at day 28, and four animals were untreated controls. In

two of three early sacrificed, untreated animals, there was moderate DRG pathology characterized by increased numbers of satellite cells and lymphocytic and histiocytic infiltration into the ganglia. This was associated with a moderate degree of neuronophagia. Nageotte nodules were either rare or absent in this group of animals (Figure 1A). In the early natalizumab-treated animals, DRG pathology ranged from mild to moderate. In these animals, neuronophagia was mild and no Nageotte nodules were noted (Figure 1B).

Late untreated animals had moderate to severe inflammation, satellite cell proliferation, and neuronophagia. Nageotte nodules were rare in three of the four animals, and one animal had extensive Nageotte nodules (Figure 1C). In the late natalizumab-treated animals, the dorsal root ganglia had a moderate degree of inflammation and satellite cell proliferation, milder degrees of neuronophagia, and no or rare Nageotte nodules (Figure 1D). Thus, overall there was diminished DRG pathology with natalizumab treatment, which included less inflammation, neuronophagia, neuronal loss/degeneration, and Nageotte nodules.

Natalizumab Blocks Traffic of Monocytes into DRGs

To identify monocyte traffic to the DRGs, we counted the number of recently recruited MAC387⁺ monocytes and used BrdU pulse to label monocytes that divide in and egress from bone marrow into the circulation and traffic to tissues. The MAC387 antibody recognizes the myeloid-related protein 14,^{17–19} which is expressed on recently

infiltrating monocytes/macrophages during early acute inflammation.²⁰ In the early, untreated animals, there were 225.6 MAC387⁺ monocytes/mm² in the DRG, which decreased by 2.9-fold in the natalizumab-treated animals (Figure 2A). In the late untreated animals, there were 144.3 MAC387⁺ monocytes/mm² in the DRG. The number of MAC387⁺ monocytes significantly decreased by 9.1-fold to 15.88 MAC387⁺ monocytes/mm² in DRGs of natalizumab-treated animals ($P < 0.0001$) (Figure 2A). In early, untreated animals, there were 94.40 BrdU⁺ cells/mm², which significantly decreased by 3.5-fold to 26.93 BrdU⁺ cells/mm² of DRG tissue in early natalizumab-treated animals ($P < 0.01$) (Figure 2B). In late animals, there was a 1.6-fold decrease, although not statistically significant, in the number of BrdU⁺ cells with natalizumab treatment (Figure 2B). Overall, these data suggest that treatment with natalizumab decreases monocyte traffic into the DRG during SIV infection.

Natalizumab Treatment Significantly Decreases the Number of CD68⁺ Macrophages, but Neither CD163⁺ Macrophages nor CD3⁺ T Lymphocytes in DRGs

In early, untreated animals, there were 1101 CD68⁺ macrophages/mm², which significantly decreased by 17.6-fold to 62.73 CD68⁺ macrophages/mm² of DRG tissue in early natalizumab-treated animals ($P < 0.01$) (Figure 2C). In late untreated animals, there were 1016 CD68⁺ macrophages/mm², which significantly decreased by 9.4-fold to 107.8 CD68⁺ macrophages/mm² of DRG tissue in late

Figure 1 Decreased dorsal root ganglia (DRG) pathology with natalizumab treatment. The terminology used during histological examination is defined as follows: satellitosis, increased numbers of cells around a neuronal cell body; Nageotte nodule, focal proliferations of satellite cells that partially to completely replace foci of neuronal cell loss; and neuronophagia, increased numbers of satellite cells infiltrating a neuronal cell body. Neuronophagia is a precursor lesion to the development of a Nageotte nodule. Cells were identified by morphology and the pattern of tissue reaction detailed above. Neurons were not counted, but loss was examined by the presence of Nageotte nodule and neuronophagia, which are satellite cells that replace foci of neuronal cell loss. **A:** A representative image of a DRG from an early untreated with a mild increase in satellite cells with a Nageotte nodule (arrow). **B:** A representative image of a DRG from an early natalizumab-treated animal with mild increase in satellite cells with no evidence of neuronophagia or Nageotte nodules. **C:** A representative image of a DRG from a late-untreated animal with multifocal foci of inflammation with neuronophagia (arrows). **D:** A representative image of a DRG from a late natalizumab-treated SIV-infected CD8-depleted rhesus macaque with moderate increase in satellite cells with rare neuronophagia (arrow). Hematoxylin and eosin was the stain. Original magnification, $\times 200$ (A–D).

Figure 2 Natalizumab treatment results in a decrease in monocyte traffic, macrophage activation, SIV infection, but not numbers of CD3⁺ T cells in dorsal root ganglia (DRGs). In DRG tissues from early untreated, early naltizumab-treated, late untreated, and late naltizumab-treated SIV-infected CD8-depleted rhesus macaques, the absolute number of MAC387⁺ monocytes/mm² of DRG (A), bromodeoxyuridine (BrdU)⁺ cells/mm² of DRG (B), CD68⁺ macrophages/mm² of DRG (C), CD163⁺ macrophages/mm² of DRG (D), CD3⁺ T cells/mm² of DRG (E), and SIV p28⁺ cells/mm² of DRG (F) was quantitated. At least eight nonoverlapping fields at $\times 200$ magnification were quantitated per DRG tissue. The absolute number of cells was calculated by dividing the number of positively stained cells (diaminobenzidine-positive brown cells) by the area of tissue examined (cells/mm²). *t*-Test was used to detect variation in cell numbers between untreated and naltizumab-treated rhesus macaques. Data were expressed as means $\pm$ SEM (A–F). **P* < 0.05, ***P* < 0.01, and ****P* < 0.001.

naltizumab-treated animals (*P* < 0.01) (Figure 2C). Although not significant, there was a trend toward a decrease in number of CD163⁺ macrophages in both early and late naltizumab-treated animals compared with untreated animals (Figure 2D). These data show that naltizumab significantly decreased number of CD68⁺ macrophages in DRG tissues. Interestingly, there were no significant differences in number of CD3⁺ T lymphocytes between untreated and either early or late naltizumab-treated animals (Figure 2E). Because animals were CD8 depleted, these CD3⁺ T lymphocytes were most likely CD4⁺.

Naltizumab Treatment Significantly Decreases SIV Infection in DRGs

In early, untreated animals, there were 84.22 SIVp28⁺ cells/mm², which decreased 3.2-fold to 25.92 SIVp28⁺ cells/mm² of DRG tissue in early naltizumab-treated animals (Figure 2F). In late animals, there were 39.55 SIVp28⁺ cells/mm², which significantly decreased by 10.9-fold to only 3.63 SIVp28⁺ cells/mm² of DRG tissue in late naltizumab-treated animals (*P* < 0.05) (Figure 2F). These data show that naltizumab significantly decreased SIV-infected cells in DRG tissues.

Naltizumab Treatment Decreases VCAM-1 Expression on the Surface of DRG Blood Vessels

Because naltizumab blocks $\alpha 4$ -integrin binding to VCAM-1, an adhesion molecule involved in mediating leukocyte traffic, DRG tissues were examined for VCAM-1 expression. VCAM-1 expression was detected on blood vessels in DRGs of early and late untreated animals, but was diminished in DRGs from naltizumab-treated animals (Figure 3, A–D). This reduction was quantitated, and VCAM-1 expression was significantly decreased in both early and late naltizumab treatment compared with untreated control animals (*P* < 0.05) (Figure 3E). Thus, reducing expression of VCAM-1 on blood vessels in DRGs by naltizumab may be one mechanism of inhibiting monocyte recruitment to DRGs during SIV infection or less cell traffic because of naltizumab treatment may have caused decreased VCAM-1 expression.

Discussion

Naltizumab is a monoclonal antibody against human $\alpha 4$ -integrin indicated for treatment of multiple sclerosis³ and Crohn's disease⁴ that blocks the extravasation of leukocytes into tissues and, thus, reduces inflammation. Because

Figure 3 Natalizumab treatment reduces vascular cell adhesion molecular (VCAM)-1 expression on surface of blood vessels in dorsal root ganglia (DRG). Representative images of DRG tissues stained with anti-VCAM-1 from an early untreated (A), an early natalizumab-treated (B), late untreated (C), and late natalizumab-treated (D) SIV-infected CD8-depleted rhesus macaque. **Arrows** show VCAM-1⁺ blood vessels. **E:** VCAM-1 expression was quantitated in DRG tissues from early untreated, early natalizumab-treated, late untreated, and late natalizumab-treated SIV-infected CD8-depleted rhesus macaques. The percentages of VCAM-1⁺ area of total area of tissue were calculated. *t*-Test was used to detect variation in cell numbers between untreated and natalizumab-treated rhesus macaques. Data are given as means $\pm$ SEM (E). **P* < 0.05.

$\alpha 4$ -integrins and their receptors are involved in immune cell trafficking, natalizumab interferes with these processes.²¹ We have previously shown that monocyte traffic to the DRG is associated with severe DRG pathology, as well as a loss of intraepidermal nerve fiber density.² Herein, we used natalizumab to determine the effect of blocking leukocyte traffic on DRG pathology. This is the first study that blocked monocyte traffic to the DRG, which resulted in diminished macrophage inflammation and reduced DRG pathology.

In natalizumab-treated animals, there was less severe DRG pathology that included decreased inflammation, mild neuronophagia, and no-to-rare Nageotte nodules. All of these changes are associated with damage to the structure of the ganglion and, in turn, will lead to functional deficits. One manifestation of this is the loss of intraepidermal nerve fiber density, which we have shown is correlated with damage to the DRG,²² whereas others have shown it is correlated with pain.²³

Natalizumab treatment resulted in decreased numbers of recently recruited M1-like MAC387⁺BrdU⁺ monocytes, decreased CD68⁺ macrophages, and fewer productively SIV-infected macrophages in DRGs, suggesting that these all relate to DRG pathology. These results demonstrate that by blocking $\alpha 4$ -integrins and, thus, traffic of monocytes to DRGs, subsequent histopathology is diminished and suggests that monocyte traffic, viral replication, and macrophage activation have causative roles in perpetuating neuronal damage, and are not simply correlative. Thus, drugs that directly target monocyte traffic may slow the progression of or prevent HIV-associated neuropathies.

This study examined if stopping leukocyte traffic prevents DRG pathology during either early or late infection. In the late-treated animals, natalizumab was not administered until 28 dpi, and thus damage may have already been initiated during early infection. However, by stopping late leukocyte traffic, animals did not develop severe DRG pathology, as seen in untreated animals sacrificed at similar time points.² Unlike in the central nervous system, peripheral nervous system neurons can regenerate over time, and thus even late treatment may allow for the peripheral nervous system to recover fully over a longer period of time.

The induction of integrin receptors is the limiting event in initiating monocyte recruitment and extravasation during inflammation. VCAM-1 is expressed on the vasculature and is known to mediate adhesion of mononuclear cells to endothelial cells via binding of very late antigen-4, which is essential for transmigration across the endothelium. A recent study, using an experimental model of early-stage lumbar disk herniation, has demonstrated that macrophage infiltration into the DRG is related to up-regulation of VCAM-1.²⁴ It was also shown that systemic administration of a tumor necrosis factor (TNF)- α inhibitor prevented macrophage infiltration and up-regulation of VCAM-1.²⁴ Another study has demonstrated that the administration of a TNF- α inhibitor, given after the onset of inflammation, prevents both macrophage invasion and VCAM-1 up-regulation in the DRG in a rat model of arthritis.²⁵ TNF- α and IL-1 β are secreted by activated resident macrophages during inflammatory events and induce expression of VCAM-1 and other adhesion molecules on the local vasculature. In this study, by blocking very late antigen-4 (the ligand for VCAM-1), we prevented additional monocytes from entering the tissue, as indicated by the reduced number of MAC387⁺ and BrdU⁺ cells. We showed a significant reduction in VCAM-1 expression in DRG tissues with natalizumab treatment.

We examined if decreased expression of VCAM-1 was because of enhanced shedding of VCAM-1 and, thus, elevated plasma soluble (s)VCAM-1 or decreased TNF- α , because TNF- α induces VCAM-1 expression. We found low to undetectable levels of TNF- α in all untreated and treated animals (data not shown). sVCAM-1 was not significantly increased in any of the treatment groups from preinfection to necropsy (data not shown). sVCAM-1 in

serum has been used as a surrogate marker of VCAM-1 endothelial expression, and studies in multiple sclerosis patients show that sVCAM-1 is decreased with natalizumab.²⁶ Herein, sVCAM-1 in plasma collected at necropsy positively correlated to VCAM-1 expression in DRG tissue, suggesting that reduced tissue expression is not because of increased shedding (data not shown). Thus, we hypothesize that the reduced VCAM-1 expression is because of a feedback mechanism where VCAM-1 is down-regulated in natalizumab-treated animals because of the absence of its ligand.

In this study, we also found a difference in the effect of natalizumab treatment on CD68⁺ and CD163⁺ macrophage populations in the DRG. There is overlap between CD68 and CD163 cell populations, although CD68⁺CD163⁻ and CD68⁻CD163⁺ cells have been detected both in the brain²⁷ and in the DRG (J.R.L. and T.H.B., unpublished data). Herein, we found that natalizumab treatment significantly reduced the number of CD68⁺ macrophages in both early and late groups and a trend in a reduction of CD163⁺ macrophage numbers, although not significant. There is a reduction of CD68⁺ antigen expression with decreased activation. The kinetics and turnover of CD68⁺ and CD163⁺ macrophage with natalizumab are unknown, but our data suggest differences in the populations.

We have recently shown that natalizumab treatment in SIV⁺ macaques decreased traffic of CD3⁺ T cells in the brain and the gut,⁵ but not cardiac tissues.⁶ In this study, we showed that CD3⁺ T lymphocyte numbers in DRGs were not decreased with natalizumab treatment. This suggests that CD3⁺ T lymphocytes may use different integrins to traffic to the DRG and cardiac tissue than to the brain or gut. Alternatively, we have previously shown no difference in CD3⁺ T cells in the DRG in infected and uninfected animals,^{2,15} suggesting that T cells do not home to the DRG during SIV-induced inflammation. Thus, blocking all leukocyte traffic will not modulate these numbers because few CD3⁺ T cells traffic to the DRG in untreated, SIV-infected animals.

Other drug studies regarding the role of monocytes in HIV infection have focused on either reducing the infectivity of monocytes/macrophages or suppressing inflammation.^{28,29} For example, maraviroc, a CCR5 inhibitor, reduced the size of the viral reservoir in monocytes and macrophages and may also reduce inflammation.³⁰ Statins have also been under investigation for their anti-inflammatory properties. Rosuvastatin reduced sCD14 and tissue factor expression on monocytes in HIV⁺ patients.³¹ However, natalizumab is the only drug known to block leukocyte traffic and reduce inflammation.

We have recently shown that natalizumab treatment in SIV⁺ macaques reduces cell traffic to the brain and heart, thus reducing the incidence of encephalitis and fibrosis.^{5,6} Herein, we have demonstrated that blocking leukocyte traffic reduced the severity of DRG pathology. Long-term natalizumab treatment (>1 year) in HIV⁺ patients is not

recommended because of the risk of developing progressive multifocal leukoencephalopathy.³² However, our data suggest that it may be beneficial for patients to receive natalizumab for short periods of time to slow progression of DRG tissue damage because of continuous leukocyte traffic. Regardless of natalizumab's benefit or risk in a clinical setting, this study has demonstrated, in a proof-of-concept manner, that stopping leukocyte traffic by blocking very late antigen-4 and down-regulation of VCAM-1 results in less severe DRG pathology during SIV infection.

Acknowledgments

We thank the veterinary staff at the New England Primate Research Center and Tulane National Primate Research Center for animal care, pathology residents and staff for assisting with necropsies and tissue collection, the NIH Nonhuman Primate Reagent Resource for providing the *in vivo* CD8 T-lymphocyte depletion antibodies, and Dr. Ronald Desrosiers (University of Miami) for providing SIVmac251.

T.H.B. is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Cashman CR, Hoke A: Mechanisms of distal axonal degeneration in peripheral neuropathies. *Neurosci Lett* 2015, 596:33–50
2. Lakritz JR, Bodair A, Shah N, O'Donnell R, Polydefkis MJ, Miller AD, Burdo TH: Monocyte traffic, dorsal root ganglion histopathology, and loss of intraepidermal nerve fiber density in SIV peripheral neuropathy. *Am J Pathol* 2015, 185:1912–1923
3. Polman CH, O'Connor PW, Havrdova E, Hutchinson M, Kappos L, Miller DH, Phillips JT, Lublin FD, Giovannoni G, Wajgt A, Toal M, Lynn F, Panzara MA, Sandrock AW, Investigators A: A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. *N Engl J Med* 2006, 354:899–910
4. Sandborn WJ, Yednock TA: Novel approaches to treating inflammatory bowel disease: targeting alpha-4 integrin. *Am J Gastroenterol* 2003, 98:2372–2382
5. Campbell JH, Ratai EM, Autissier P, Nolan DJ, Tse S, Miller AD, Gonzalez RG, Salemi M, Burdo TH, Williams KC: Anti-alpha4 antibody treatment blocks virus traffic to the brain and gut early, and stabilizes CNS injury late in infection. *PLoS Pathog* 2014, 10:e1004533
6. Walker JA, Beck GA, Campbell JH, Miller AD, Burdo TH, Williams KC: Anti-alpha4 integrin antibody blocks monocyte/macrophage traffic to the heart and decreases cardiac pathology in a SIV infection model of AIDS. *J Am Heart Assoc* 2015, 4:pii:e001932
7. Lackner AA: Pathology of simian immunodeficiency virus induced disease. *Curr Top Microbiol Immunol* 1994, 188:35–64
8. Sasseville VG, Lackner AA: Neuropathogenesis of simian immunodeficiency virus infection in macaque monkeys. *J Neurovirol* 1997, 3:1–9
9. Schmitz JE, Kuroda MJ, Santra S, Sasseville VG, Simon MA, Lifton MA, Racz P, Tenner-Racz K, Dalesandro M, Scallan BJ, Ghayeb J, Forman MA, Montefiori DC, Rieber EP, Letvin NL, Reimann KA: Control of viremia in simian immunodeficiency virus infection by CD8⁺ lymphocytes. *Science* 1999, 283:857–860

10. Westmoreland SV, Halpern E, Lackner AA: Simian immunodeficiency virus encephalitis in rhesus macaques is associated with rapid disease progression. *J Neurovirol* 1998, 4:260–268
11. 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: Magnetic resonance spectroscopy reveals that activated monocytes contribute to neuronal injury in SIV neuroAIDS. *J Clin Invest* 2005, 115:2534–2545
12. Williams KC, Hickey WF: Central nervous system damage, monocytes and macrophages, and neurological disorders in AIDS. *Annu Rev Neurosci* 2002, 25:537–562
13. Wehner NG, Gasper C, Shopp G, Nelson J, Draper K, Parker S, Clarke J: Immunotoxicity profile of natalizumab. *J Immunotoxicol* 2009, 6:115–129
14. Burdo TH, Soulas C, Orzechowski K, Button J, Krishnan A, Sugimoto C, Alvarez X, Kuroda MJ, Williams KC: Increased monocyte turnover from bone marrow correlates with severity of SIV encephalitis and CD163 levels in plasma. *PLoS Pathog* 2010, 6:e1000842
15. Burdo TH, Orzechowski K, Knight HL, Miller AD, Williams K: Dorsal root ganglia damage in SIV-infected rhesus macaques: an animal model of HIV-induced sensory neuropathy. *Am J Pathol* 2012, 180:1362–1369
16. Laast VA, Pardo CA, Tarwater PM, Queen SE, Reinhart TA, Ghosh M, Adams RJ, Zink MC, Mankowski JL: Pathogenesis of simian immunodeficiency virus-induced alterations in macaque trigeminal ganglia. *J Neuropathol Exp Neurol* 2007, 66:26–34
17. Hessian PA, Fisher L: The heterodimeric complex of MRP-8 (S100A8) and MRP-14 (S100A9): antibody recognition, epitope definition and the implications for structure. *Eur J Biochem* 2001, 268:353–363
18. Goebeler M, Roth J, Burwinkel F, Vollmer E, Bocker W, Sorg C: Expression and complex formation of S100-like proteins MRP8 and MRP14 by macrophages during renal allograft rejection. *Transplantation* 1994, 58:355–361
19. Goebeler M, Roth J, Teigelkamp S, Sorg C: The monoclonal antibody MAC387 detects an epitope on the calcium-binding protein MRP14. *J Leukoc Biol* 1994, 55:259–261
20. Odink K, Cerletti N, Bruggen J, Clerc RG, Tarcsay L, Zwadlo G, Gerhards G, Schlegel R, Sorg C: Two calcium-binding proteins in infiltrate macrophages of rheumatoid arthritis. *Nature* 1987, 330:80–82
21. Mitroulis I, Alexaki VI, Kourtzelis I, Ziogas A, Hajishengallis G, Chavakis T: Leukocyte integrins: role in leukocyte recruitment and as therapeutic targets in inflammatory disease. *Pharmacol Ther* 2015, 147:123–135
22. Lakritz JR, Robinson JA, Polydefkis MJ, Miller AD, Burdo TH: Loss of intraepidermal nerve fiber density during SIV peripheral neuropathy is mediated by monocyte activation and elevated monocyte chemoattractant proteins. *J Neuroinflammation* 2015, 12:237
23. Polydefkis M, Yiannoutsos CT, Cohen BA, Hollander H, Schifitto G, Clifford DB, Simpson DM, Katzenstein D, Shriver S, Hauer P, Brown A, Haidich AB, Moo L, McArthur JC: Reduced intraepidermal nerve fiber density in HIV-associated sensory neuropathy. *Neurology* 2002, 58:115–119
24. You C, Zhu K, Liu X, Xi C, Zhang Z, Xu G, Yan J: Tumor necrosis factor- α -dependent infiltration of macrophages into the dorsal root ganglion in a rat disc herniation model. *Spine* 2013, 38:2003–2007
25. Segond von Banchet G, Boettger MK, Fischer N, Gajda M, Brauer R, Schaible HG: Experimental arthritis causes tumor necrosis factor- α -dependent infiltration of macrophages into rat dorsal root ganglia which correlates with pain-related behavior. *Pain* 2009, 145:151–159
26. Millonig A, Hegen H, Di Pauli F, Ehling R, Gneiss C, Hoelzl M, Künz B, Lutterotti A, Rudzki D, Berger T, Reindl M, Deisenhammer F: Natalizumab treatment reduces endothelial activity in MS patients. *J Neuroimmunol* 2010, 227:190–194
27. Soulas C, Conerly C, Kim WK, Burdo TH, Alvarez X, Lackner AA, Williams KC: Recently infiltrating MAC387(+) monocytes/macrophages a third macrophage population involved in SIV and HIV encephalitic lesion formation. *Am J Pathol* 2011, 178:2121–2135
28. Campbell JH, Hearn AC, Martin GE, Williams KC, Crowe SM: The importance of monocytes and macrophages in HIV pathogenesis, treatment, and cure. *AIDS* 2014, 28:2175–2187
29. Mehraj V, Jenabian MA, Vyboh K, Routy JP: Immune suppression by myeloid cells in HIV infection: new targets for immunotherapy. *Open AIDS J* 2014, 8:66–78
30. Wilkin TJ, Gulick RM: CCR5 antagonism in HIV infection: current concepts and future opportunities. *Annu Rev Med* 2012, 63:81–93
31. Funderburg NT, Jiang Y, Debanne SM, Storer N, Labbato D, Clagett B, Robinson J, Lederman MM, McComsey GA: Rosuvastatin treatment reduces markers of monocyte activation in HIV-infected subjects on antiretroviral therapy. *Clin Infect Dis* 2014, 58:588–595
32. Walker A, Watson C, Alexopoulos ST, Deniz B, Arnold R, Bates D: A benefit-risk analysis of natalizumab in the treatment of patients with multiple sclerosis when considering the risk of progressive multifocal leukoencephalopathy. *Curr Med Res Opin* 2014, 30:629–635