Pathogenesis of Simian Immunodeficiency Virus-Induced Alterations in Macaque Trigeminal Ganglia

Victoria A. Laast, DVM, PhD, Carlos A. Pardo, MD, Patrick M. Tarwater, PhD, Suzanne E. Queen, MS, Todd A. Reinhart, ScD, Mimi Ghosh, PhD, Robert J. Adams, DVM, M. Christine Zink, DVM, PhD, and Joseph L. Mankowski, DVM, PhD

INTRODUCTION

Peripheral neuropathy is the most common neurologic complication associated with human immunodeficiency virus (HIV) infection, yet its pathogenesis remains poorly understood. To study the mechanisms causing HIV-induced peripheral nervous system disease, we examined trigeminal ganglia obtained from simian immunodeficiency virus (SIV)-inoculated macaques. SIV-infected macaques developed multifocal trigeminal ganglionitis of varying severity characterized by multifocal mononuclear infiltrates, neurophagia, and neuronal loss resembling reports of HIV-associated changes present in dorsal root ganglia. Neuronal density, measured by calculating the fractional area of trigeminal ganglia occupied by neurons, was significantly lower in SIV-infected macaques versus uninfected macaques (p = 0.001). To characterize the inflammatory cell population and measure productive viral infection in ganglia, trigeminal ganglia from SIV-infected macaques were immunostained for macrophage or cytotoxic lymphocyte markers and for SIV gp41. The extent of macrophage infiltration in trigeminal ganglia was inversely correlated with neuronal loss (p = 0.001), whereas cytotoxic lymphocyte infiltration was not associated with neuronal loss. These studies demonstrate that alterations in the somatosensory ganglia of SIV-infected macaques closely parallel those observed in HIV-infected individuals and show that study of SIV-infected macaques may help elucidate the pathophysiology of HIV-induced peripheral neuropathy.

Key Words: Macaque, Macrophages, Peripheral nervous system (PNS), Simian immunodeficiency virus, Trigeminal ganglia.

From the Department of Molecular and Comparative Pathobiology (VAL, SEQ, RJA, MCZ, JLM), Department of Pathology (VAL, CAP, MCZ, JLM), and Department of Neurology (CAP), Johns Hopkins University School of Medicine, Baltimore, Maryland; the Department of Biostatistics and Epidemiology (PMT), Graduate School of Public Health, University of Texas Health Science Center at Houston, School of Public Health, El Paso Regional Campus, El Paso, Texas; and the Department of Infectious Diseases and Microbiology (TAR, MG), University of Pittsburgh, Pittsburgh, Pennsylvania.

Send correspondence and reprint requests to: Joseph L. Mankowski, DVM, PhD, Johns Hopkins University School of Medicine, Department of Molecular and Comparative Pathobiology, 733 N. Broadway, 835 Broadway Research Bldg., Baltimore, MD; E-mail: jmankows@jhmi.edu

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has not been examined in detail for the presence of lesions in somatosensory PNS ganglia that parallel pathologic features of HIV-induced PNS disease. Our laboratory has developed an accelerated, consistent SIV/macaque model of HIV CNS disease in which over 90% of macaques develop encephalitis by 84 days postinoculation. The timing of progression from acute to asymptomatic and then late-stage infection is highly reproducible in this model. Given that our SIV model has close similarities to HIV-induced CNS disease (13), we hypothesized that PNS lesions also would develop in SIV-infected macaques, thus providing a model for the study of the mechanisms of HIV PNS disease.

MATERIALS AND METHODS

Animals and Histopathologic Evaluation

All SIV-infected pigtailed macaques in this study were simultaneously inoculated intravenously with both SIV/17E-Fr, a neurovirulent clone, and SIV/DeltaB670, an immunosuppressive strain, as previously described, and killed at varying times postinoculation (13). To determine whether lesions developed in somatosensory ganglia of SIV-infected macaques, 12 SIV-infected animals were killed approximately 12 weeks postinoculation at terminal stages of infection when encephalitis typically develops (average time postinoculation = 84 days, range = 67–94 days postinoculation). Exact times of killing postinoculation for these 12 animals are listed in the Table. To determine when SIV replication first occurred in sensory ganglia postinoculation, trigeminal ganglia also were examined from SIV-infected animals killed at 7, 10, 21, and 56 days postinoculation (6 animals at each time point). Six uninfected pigtailed macaques served as control animals. Trigeminal ganglia from all animals were fixed in Streck Tissue Fixative (Streck Laboratories, Omaha, NE) before embedding in paraffin.

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Hematoxylin and eosin (H&E)-stained sections of trigeminal ganglia also were examined from SIV-infected animals killed at 7, 10, 21, and 56 days postinoculation (6 animals at each time point). Six uninfected pigtailed macaques served as control animals. Trigeminal ganglia from all animals were fixed in Streck Tissue Fixative (Streck Laboratories, Omaha, NE) before embedding in paraffin.

TABLE. Relationship Between Peripheral Nervous System and Central Nervous System Disease in Simian Immunodeficiency Virus (SIV)-Infected Macaques at Terminal Stages of Disease

<table>
<thead>
<tr>
<th>Macaque ID</th>
<th>Days Postinoculation</th>
<th>Terminal CD4 Counts (cells/mm³)</th>
<th>Terminal Plasma SIV Load (copy eq/mL)</th>
<th>Ganglionitis Severity</th>
<th>SIV Burden in Ganglia (log pixels)</th>
<th>Encephalitis Severity</th>
<th>SIV Burden in Brain (log pixels)</th>
</tr>
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<tbody>
<tr>
<td>CC33</td>
<td>73</td>
<td>918</td>
<td>1.7 × 10⁷</td>
<td>None</td>
<td>1.92</td>
<td>Severe</td>
<td>3.95</td>
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<tr>
<td>708</td>
<td>88</td>
<td>260</td>
<td>9.8 × 10⁶</td>
<td>Mild</td>
<td>1.79</td>
<td>Moderate</td>
<td>1.91</td>
</tr>
<tr>
<td>713</td>
<td>93</td>
<td>340</td>
<td>2.7 × 10⁶</td>
<td>Mild</td>
<td>1.93</td>
<td>Mild</td>
<td>1.48</td>
</tr>
<tr>
<td>BP33</td>
<td>84</td>
<td>84</td>
<td>6.9 × 10⁵</td>
<td>Mild</td>
<td>2.13</td>
<td>None</td>
<td>1.88</td>
</tr>
<tr>
<td>389</td>
<td>87</td>
<td>330</td>
<td>1.1 × 10⁵</td>
<td>Mild</td>
<td>2.93</td>
<td>Severe</td>
<td>3.91</td>
</tr>
<tr>
<td>387</td>
<td>92</td>
<td>360</td>
<td>2.2 × 10⁵</td>
<td>Mild</td>
<td>3.05</td>
<td>Moderate</td>
<td>2.41</td>
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<tr>
<td>715</td>
<td>94</td>
<td>390</td>
<td>6.7 × 10⁵</td>
<td>Moderate</td>
<td>1.69</td>
<td>Mild</td>
<td>2.32</td>
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<tr>
<td>BK09</td>
<td>82</td>
<td>84</td>
<td>2.8 × 10⁵</td>
<td>Moderate</td>
<td>2.34</td>
<td>Mild</td>
<td>2.37</td>
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<tr>
<td>BM03</td>
<td>67</td>
<td>663</td>
<td>4.2 × 10⁷</td>
<td>Moderate</td>
<td>3.94</td>
<td>Severe</td>
<td>3.87</td>
</tr>
<tr>
<td>B155</td>
<td>82</td>
<td>79</td>
<td>1.9 × 10⁵</td>
<td>Moderate</td>
<td>4.14</td>
<td>Severe</td>
<td>4.21</td>
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<tr>
<td>BP41</td>
<td>84</td>
<td>266</td>
<td>2.4 × 10⁵</td>
<td>Severe</td>
<td>2.23</td>
<td>Moderate</td>
<td>2.11</td>
</tr>
<tr>
<td>394</td>
<td>86</td>
<td>20</td>
<td>4.1 × 10⁷</td>
<td>Severe</td>
<td>3.14</td>
<td>Moderate</td>
<td>2.50</td>
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harboring SIV, 7-μm-thick sections of paraffin-embedded trigeminal ganglia were deparaffinized and rehydrated. After antigen retrieval in sodium citrate buffer for 8 minutes, sections were washed and then blocked in 3% normal goat serum containing 1% triton for 1 hour at room temperature followed by incubation with the anti-SIV gp41 antibody KK41 (1:4000; NIH AIDS Research and Reference Reagent Program) and antibody directed against the macrophage marker Iba-1 (1:250; WAKO Lab Chemicals, Richmond, VA) for 48 hours at 4°C. A polyclonal antibody was used to detect the macrophage marker Iba-1 rather than monoclonal anti-CD68 antibody because the anti-SIV antibody KK41 is a monoclonal antibody and a combination of monoclonal and polyclonal antibodies was necessary to perform double-labeling studies. Sections were then incubated in fluorescent-tagged secondary antibodies (Cy3-conjugated goat anti-rabbit IgG (1:2000; Jackson ImmunoResearch Lab Inc., West Grove, PA), AlexaFlour-488-conjugated goat anti-mouse IgG (1:1000; Molecular Probes, Eugene, OR) for 2 hours at room temperature in the dark. Sections were subsequently washed, coverslipped, and visualized in a single X:Y plane using a Nikon C1 confocal laser microscopy system mounted on a Nikon Eclipse TE2000-E microscope.

To detect SIV RNA in trigeminal ganglia, tissue microarray slides containing 2.0-mm-diameter punch samples of STF-fixed trigeminal ganglia (approximately 40% of the area of a macaque trigeminal ganglion/microarray tissue punch) were used for SIV in situ hybridization, providing a total sample area of 6.3 mm². Each tissue microarray slide included trigeminal ganglia punches from all SIV-infected macaques for uniformity. To identify cells in the trigeminal ganglia that were productively infected with SIV, in situ hybridization was performed using a pool of 4 SIV-specific riboprobes as previously described on 2 tissue microarray slides (14). Two additional tissue microarray slides were probed with sense riboprobes as controls. Autoradiographic exposure times were 3 days. After development and fixation of the autoradiographic signal, tissues were counterstained with cresyl violet, cleared in xylene, and mounted.

To evaluate infiltrating lymphocyte subpopulations in SIV-infected animals and control animals, trigeminal ganglia were double immunostained with both TIA-1, an antibody directed against the cytoplasmic granule-associated protein, a component of the cytotoxic granules of both cytotoxic T lymphocytes and natural killer cells (1:100; Coulter, Hialeah, FL), and an anti-CD3 antibody (1:400; Dako) as described previously for immunohistochemical staining. We have previously used this labeling technique to measure numbers of TIA-1+, CD3+ cytotoxic T cells and TIA-1+, CD3- natural killer cells in macaques (15–17). To count these cell types, the total number of each cell type in 7 nonoverlapping grid areas (grid size 0.25 mm × 0.25 mm) was counted in double-stained trigeminal ganglion.

**Measurement of Neuronal Density in Trigeminal Ganglia**

To measure neuronal density in SIV-infected animals at terminal stages of disease, neurons in H&E-stained sections of trigeminal ganglia were counted in SIV-infected and control animals using the unbiased stereologic method of fractional area quantification using Stereo Investigator Software (MicroBright Field Inc., Colchester, VT) as previously described (18–21). Thirty-five to 40 counting frames were randomly placed within the delineated area of each trigeminal ganglia section. Each counting grid had a group of 20 counting points, and for each sample, the sum of the points covering neurons was divided by the total number of points sampled to estimate the fraction of the area occupied by neurons (fractional area) (18).

**Plasma Simian Immunodeficiency Virus RNA Measurement and CD4+ T Cell Counts**

To measure SIV RNA in plasma from the 12 SIV-infected animals killed at terminal stages of disease, virus was pelleted from 1 mL of plasma and viral RNA was extracted and quantitated by real-time reverse transcriptase–polymerase chain reaction using primers to quantitate gag as previously described (13). Complete blood counts with differentials were performed on blood samples and the absolute number of lymphocytes determined using a CellDyn 3200 hematology analyzer (Abbott, Abbott Park, IL). Mononuclear cells were separated on Percoll discontinuous gradients and labeled with a fluochrome-conjugated monoclonal antibody (CD4-Leu3a; Becton Dickinson, San Jose, CA) to identify CD4+ lymphocytes. Absolute CD4+ cell counts were determined by multiplying the percentage of CD4+ cells by the absolute lymphocyte count.

**RESULTS**

**Lesions in the Trigeminal Ganglia of Simian Immunodeficiency Virus-Infected Macaques**

Microscopic lesions closely resembling those described in the dorsal root ganglia of HIV-infected patients with peripheral neuropathy were observed in the trigeminal ganglia of 11 of 12 SIV-inoculated macaques examined at terminal stages of disease, approximately 12 weeks post-infection (1, 22). Lesions in the trigeminal ganglia consisted of diffuse aggregates of infiltrating mononuclear cells, including macrophages accompanied by scattered lymphocytes (Fig. 1A, B). The presence of infiltrating macrophages abutting neurons within the neuronal compartment, consistent with neuronophagia, was frequent in animals with moderate to severe ganglionitis (Fig. 1C). In addition, Nageotte nodules, characterized by overt neuronal loss with replacement by both satellite cells and infiltrating mononuclear cells, were present in animals with severe ganglionitis (Fig. 1D). Taken together, the morphologic changes, including neuronophagia and the formation of Nageotte nodules, show that neuronal loss occurs in the trigeminal ganglia of SIV-infected macaques. Interestingly, multinucleate giant cells, a common occurrence in SIV-induced CNS disease, were rare and only observed in the trigeminal ganglia of one SIV-infected animal (B155), although multinucleate giant cells were commonly detected in the CNS of several of these SIV-infected macaques examined at terminal stages of disease. Trigeminal ganglionitis was classified as moderate to severe in 6 of 12 SIV-infected animals and...
mild in 5 animals. One of the 12 SIV-infected macaques had no lesions in trigeminal ganglia (CC33, Table). Uninfected control macaques (6 pigtailed macaques) did not have any lesions resembling those seen in SIV-infected macaques. These lesions in trigeminal ganglia of SIV-infected macaques bear a strong morphologic resemblance to the lesions reported in dorsal root ganglia of HIV-infected individual with distal sensory polyneuropathy (6, 22).

**Inflammatory Infiltrates in Trigeminal Ganglia**

To characterize and measure the infiltrating mononuclear cells identified in SIV-infected macaques with trigeminal ganglionitis, sections of trigeminal ganglia were immunostained for the macrophage marker CD68 and the amount of immunostaining was then measured by quantitative image analysis as described previously (12, 13). Total immunostaining for infiltrating CD68-positive cells in the trigeminal ganglia was significantly higher in SIV-infected macaques examined at terminal stages of infection than in uninfected control animals, indicating that increased numbers of macrophages are present in trigeminal ganglia during SIV infection (p = 0.003, Figs. 1E, F, 2). Interestingly, CD68 immunostaining also demonstrated that uninfected control macaques had a substantial resident population of CD68-positive macrophages located diffusely in the perineuronal compartment of the trigeminal ganglia (Fig. 1E). Although macrophage activation reflected by the increased intensity of CD68 immunostaining of endogenous macrophages distributed diffusely throughout the perineuronal compartment appeared to be higher in SIV-infected macaques with

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**FIGURE 1.** Trigeminal ganglionitis in simian immunodeficiency virus (SIV) -infected macaques. Representative hematoxylin and eosin-stained sections of trigeminal ganglia from control macaques (A) and SIV-infected macaques demonstrated multifocal to coalescing mononuclear infiltrates in the perineuronal compartment of the SIV-infected macaques (B). Trigeminal ganglia from SIV-infected macaques also had infiltrating mononuclear cells within the neuronal compartment indicative of neuronophagia (C, arrows) and contained Nageotte nodules, focal areas of neuronal loss with replacement by mononuclear cell infiltrates, and activated Schwann cells (D, arrows). Sections of trigeminal ganglia from control macaques contained scattered CD68-immunopositive resident macrophages (E), whereas SIV-infected macaques had increased immunostaining for CD68 (F) primarily reflecting infiltrating macrophages. Scale bars = (A, B, E, F) 100 μM; (C, D) 50 μM.
ganglionitis than in control animals, the lack of markers to differentiate between endogenous versus infiltrating macrophages precludes measurements of endogenous macrophage activation separate from infiltrating macrophages.

Immunostaining was also performed to characterize and count infiltrating lymphocytes including cytotoxic T cells (TIA-1+, CD3+) and natural killer cells (TIA-1+, CD3-). Median NK cell numbers/mm² were higher in SIV-infected macaques at terminal stages of infection (13.8 NK cells/mm²) than in control animals (4.6 cells/mm²; p = 0.07), whereas numbers of cytotoxic T cells declined in the trigeminal ganglia of SIV-infected animals (118.4 cytotoxic T cells/mm²) versus uninfected control macaques (155.2 cytotoxic T cells/mm²; p = 0.28). In SIV-infected macaques examined at terminal stages of disease, both of these lymphocyte subsets were present in variable numbers in trigeminal ganglia and, furthermore, the total numbers of cytotoxic lymphocytes did not correlate with decreases in neuronal density (cytotoxic T cells, p = 0.54, r = 0.18 and NK cells p = 0.076, r = −0.51).

FIGURE 2. Scatterplot depicting the amount of CD68 immunostaining for activated macrophages in the trigeminal ganglia of control macaques (denoted by triangles) and simian immunodeficiency virus (SIV)-infected macaques (denoted by squares) demonstrates a statistically significant increase in macrophage infiltration in the trigeminal ganglia of SIV-infected macaques compared with controls (p = 0.003, Kruskal-Wallis). Bars represent median values.

FIGURE 3. Simian immunodeficiency virus (SIV) in trigeminal ganglia of SIV-infected macaques. Immunostaining for SIV gp41 protein (gp41) shows numerous SIV-positive cells in the perineuronal compartment of trigeminal ganglia (A). In situ hybridization was performed on trigeminal ganglia sections using 35S-labeled SIV antisense riboprobes to identify viral replication in the trigeminal ganglia. Silver grains marking cells containing SIV RNA indicative of viral replication were present in the trigeminal ganglia of SIV-infected macaques (B), whereas sense ribobase controls performed in parallel showed no signal (C). To identify which cell types in the trigeminal ganglia were infected with SIV, confocal laser scanning microscopy was performed on double-immunostained sections of trigeminal ganglia from SIV-infected macaques for the macrophage marker Iba-1 (D, red) and SIV transmembrane protein gp41 (E, green). A composite image showed colocalization of Iba-1 and SIV gp41 (F, yellow) indicating that macrophages were the predominant SIV-infected cell type in trigeminal ganglia.
To determine whether alterations in lymphocyte infiltration in the trigeminal ganglia developed during earlier stages of infection, immunostaining was performed to count cytotoxic lymphocytes present in the trigeminal ganglia of animals killed at 21 days and 56 days post-SIV inoculation. The median number of NK cells present in trigeminal ganglia at day 21 (2.3 NK cells/mm²) and day 56 days postinoculation (8.0 NK cells/mm²) did not significantly differ from control animals. A similar pattern was found with cytotoxic T cells with no significant change detected between numbers of cytotoxic T cells present in control animals versus either 21 days (193.2 cells/mm²) or 56 days (154.1 cells/mm²) postinoculation. Together, these data demonstrate that NK cells are likely recruited to the trigeminal ganglia predominantly during terminal stages of infection rather than at earlier time points but do not appear to be associated with extent of neuronal loss.

Simian Immunodeficiency Virus in Trigeminal Ganglia

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Virus Detection During Acute and Asymptomatic Infection

It is not known when in the course of HIV infection the cells in somatosensory ganglia are infected. To determine whether SIV infection could be detected in trigeminal ganglia during acute and asymptomatic stages of SIV infection, sections of trigeminal ganglia obtained from groups containing 6 SIV-infected macaques killed at each of 7, 10, 21, and 56 days postinoculation were evaluated by in situ hybridization to detect SIV RNA. During acute infection with SIV, viral RNA was present in the trigeminal ganglia in 2 of 6 animals examined at both 7 and 10 days postinoculation by in situ hybridization. In contrast, no animals (0 of 6) had replicating SIV in trigeminal ganglia 21 days postinoculation. At 56 days postinoculation, virus replication was detected in one of 6 SIV-infected macaques. This trend, in which low-level SIV replication occurs during acute infection in somatosensory ganglia, is suppressed by 21 days postinoculation and then begins to recrudesce at 56 days postinoculation paralleling the trend in SIV replication reported in the CNS of SIV-infected animals (23).

FIGURE 4. Neuronal density in trigeminal ganglia. A scatter-plot of the neuronal area fraction in the trigeminal ganglia of control macaques (denoted by triangles) and simian immunodeficiency virus (SIV)-infected macaques (denoted by squares) demonstrates a statistically significant decrease in neuronal density in the trigeminal ganglia of SIV-infected macaques compared with controls (p = 0.001, Kruskal-Wallis). Bars represent median values.

Simian Immunodeficiency Virus in Trigeminal Ganglia

To establish whether SIV was present in sensory ganglia of SIV-infected macaques at terminal stages of infection, trigeminal ganglia sections were immunostained for the SIV transmembrane protein gp41. Numerous SIV-infected cells were detected in regions containing infiltrating mononuclear cells. In addition, SIV-positive cells were located diffusely in the perineuronal compartment, consistent with the distribution pattern of endogenous macrophages in ganglia (Fig. 3A). SIV protein was detected by immunohistochemistry in the trigeminal ganglia of 8 of 12 SIV-infected animals examined at terminal stages of disease. Immunostaining for SIV was then measured by quantitative image analysis to compare with the amount of macrophage infiltration. The extent of productive SIV infection was strongly correlated with amount of CD68 immunostaining (p = 0.003, r = 0.8), indicating that SIV expression was colinear with macrophage infiltration in the trigeminal ganglia in SIV-infected macaques. In situ hybridization using riboprobes to detect SIV RNA confirmed viral replication in the trigeminal ganglia in 9 animals (Fig. 3B, C). In total, 10 of 12 SIV-infected animals examined approximately 12 weeks postinoculation contained SIV in the trigeminal ganglia detected either by immunohistochemistry or in situ hybridization.

To identify the specific cell type(s) in trigeminal ganglia infected with SIV, sections were double immunostained for SIV gp41 and the macrophage marker Iba-1 and then examined by laser confocal microscopy. Colocalization of SIV gp41 within macrophages demonstrated that macrophages were clearly the major cell type harboring SIV in the trigeminal ganglia (Fig. 3D–F).

FIGURE 5. To examine the relationship between neuronal loss and macrophage infiltration, neuronal area fraction was compared with the amount of CD68 immunostaining in the trigeminal ganglia. A significant inverse correlation was found between neuronal loss and macrophage infiltration (p = 0.001, Spearman’s rank).
Neuronal Loss in Trigeminal Ganglia of Simian Immunodeficiency Virus-Infected Macaques

To determine whether neuronal loss developed in trigeminal ganglia of SIV-infected macaques, the neuronal density in trigeminal ganglia was measured using the area fraction fractionator technique, an unbiased stereologic method (18–21). These measurements revealed a significant decrease in the fractional area occupied by neurons in the trigeminal ganglia of SIV-infected macaques as compared with control macaques, indicating a loss of neurons in the trigeminal ganglia (p = 0.001, Fig. 4). To determine whether macrophage infiltration was associated with neuronal loss, CD68 immunostaining was compared with the decrease in neuronal density. The decline in neuronal density in trigeminal ganglia was inversely correlated with the extent of macrophage infiltration (p = 0.001, r = −0.6, Fig. 5).

Relationship Between Simian Immunodeficiency Virus-Induced Peripheral Nervous System and Central Nervous System Disease

To examine the relationship between development of PNS lesions in trigeminal ganglia and CNS lesions induced by SIV, lesion severity, macrophage infiltration, and levels of SIV productive infection in trigeminal ganglia and the brain were compared in 12 SIV-infected macaques examined approximately 12 weeks postinoculation (Table). CNS findings have been reported previously for these animals (11, 24). A nonsignificant trend was found between severity of trigeminal ganglionitis and CNS lesion severity (p = 0.09, r = 0.4). The severity of trigeminal ganglionitis was also compared with the severity of SIV-induced lesions specifically in the brainstem (pons and midbrain); trigeminal ganglia lesion severity also was not highly correlated with brainstem lesion severity (p = 0.44, r = 0.25). Similarly, CD68 immunostaining for activated macrophages in trigeminal ganglia was not strongly correlated with CD68 immunostaining in the CNS (p = 0.21, Fig. 6). In contrast, levels of SIV in the trigeminal ganglia and CNS of SIV-infected animals were strongly correlated (p = 0.036, r = 0.80, Fig. 6). However, there was no clear relationship between SIV burden in ganglia and plasma viral load (Table).

DISCUSSION

Although HIV-induced peripheral neuropathy is now the most common neurologic complication of HIV infection in the developed world, the SIV/macaque model has not been evaluated in detail as a model for HIV peripheral neuropathy (1, 5, 7). This study demonstrates that statistically significant decreases in neuronal density develop in the trigeminal ganglia of pigtailed macaques inoculated with both SIV/17E-Fr and SIVDeltaB670, illustrating the value of this particular SIV/macaque model for elucidating mechanisms underlying neuronal damage and loss in somatosensory ganglia induced by HIV. The decline in neuronal density measured in SIV-infected macaques was inversely correlated with the amount of macrophage infiltration in trigeminal ganglia measured by CD68 immunostaining, suggesting that macrophage infiltration and/or activation of endogenous macrophages plays a central role in causing damage to the PNS in HIV infection. Although the decrease in neuronal density measured in the trigeminal ganglia of SIV-infected macaques may in part be attributable to macrophage infiltration, the morphologic findings of infiltrating macrophages replacing lost neurons, overt neurophagia, and the presence of Nageotte nodules clearly show that neuronal loss occurs in the trigeminal ganglia in SIV-infected macaques.

As the extent of macrophage infiltration in ganglia was colinear with levels of productive SIV infection, neurotoxic products of activated macrophages (including viral proteins...
from SIV-infected, activated macrophages) likely mediate neuronal damage and loss in sensory ganglia. In SIV-infected macaques, neuronal damage is thus an indirect event rather than the consequence of SIV infection of neurons in trigeminal ganglia. Double-labeling studies confirmed that SIV protein was predominantly contained within macrophages in the trigeminal ganglia consistent with a role for macrophage-tropic SIV in neuronal loss in somatosensory ganglia. Although CD68 immunostaining revealed a population of endogenous macrophages in the trigeminal ganglia that may be susceptible to SIV infection, at present, a lack of specific cell markers makes it impossible to separate the role of endogenous macrophages versus infiltrating macrophages recruited to the ganglia with respect to either supporting productive SIV replication or production of proinflammatory mediators. It is possible that both endogenous and recruited macrophages develop an activated phenotype and support SIV replication in somatosensory ganglia. Endogenous macrophages in particular are attractive candidates for harboring latent SIV in the CNS. In addition, immune activation of endogenous macrophages independent of SIV infection also may contribute to production of proinflammatory neurotoxic mediators. The increases in CD68 immunostaining observed in the trigeminal ganglia of SIV-infected macaques are consistent with immune activation of both endogenous macrophages as well as infiltrating cells.

To determine when in the course of infection macrophages in somatosensory ganglia were infected by SIV, we examined trigeminal ganglia from SIV-infected macaques killed during acute (7 and 10 days postinoculation) and asymptomatic infection (days 21 and 36 postinoculation). Of 2 of 6 animals evaluated at each of 7 and 10 days postinoculation, replicating SIV was detected by in situ hybridization, whereas SIV RNA was not detected in animals at 21 days postinoculation. This trend, with low levels of SIV replication during acute infection in ganglia that is suppressed by 21 days postinoculation, parallels the kinetics of virus replication in the CNS of SIV-infected animals (23). During later stages of disease, as SIV-infected macrophages transition into terminal disease when both AIDS and CNS disease develop, SIV is again actively replicating in ganglia. Although these data demonstrate that sensory ganglia can be infected during primary infection, it remains to be determined whether gangliotropic viral strains remain latent in sensory ganglia archived as proviral DNA during asymptomatic infection and then reemerge at later stages of disease. Although we identified SIV RNA by in situ in one-third of SIV-infected macrophages during acute infection, it is probable that the use of more sensitive techniques, including reverse transcriptase–polymerase chain reaction, would uncover active infection in additional animals during acute infection. The SIV/macaque model offers the opportunity to address these critical questions.

These studies focused on trigeminal ganglia from SIV-infected macaques because we had access to archived trigeminal ganglia from SIV-infected macaques that had been killed at 84 days postinoculation, when all macaques developed AIDS and over 90% had SIV encephalitis, whereas fewer archived dorsal root ganglia samples (from animals other than those used for trigeminal ganglia studies) were available to us. Somatosensory neurons in the trigeminal ganglia are structurally and functionally homologous to those of the dorsal root ganglia. Furthermore, previous studies have demonstrated that HIV-induced alterations in somatosensory ganglia appear to be similar regardless of specific anatomic location of sensory ganglia (25).

Interestingly, SIV CNS disease is characterized by replication of SIV in macrophages, similar to the observations of SIV replication in trigeminal ganglia (12). To determine whether PNS disease paralleled SIV CNS disease, we investigated whether a correlation existed among host immune responses, neuronal damage, and productive viral infection in SIV PNS disease versus SIV CNS disease. The presence of either ganglionitis or sensory neuronal loss in trigeminal ganglia was not associated with concurrent presence of encephalitis in the CNS. In contrast, extent of SIV productive infection in the trigeminal ganglia and CNS was correlated. This lack of concordance between SIV PNS and CNS disease suggests that although similarities exist, including replication of SIV in macrophages associated with neuronal loss, there also are likely fundamental differences between the pathogenesis of lentiviral-induced CNS and PNS disease. One interesting morphologic difference between both HIV and SIV-induced lesions in the CNS versus the brain is the relative lack of multinucleate giant cell formation in affected sensory ganglia that contrasts sharply with the large numbers of multinucleate giant cells present in the brain. This disparity may be the result of differences in macrophage biology and/or the fusogenic properties of the particular strains of HIV/SIV present in the CNS versus the PNS. For example, although resident populations of macrophages have been described in both the CNS and the brain, it is likely that differences in barrier properties between the blood and these separate macrophage populations (i.e. the lack of a blood–ganglia barrier versus the blood–brain barrier) greatly impact regulation of macrophage activation as well as susceptibility to HIV infection (26, 27).

The presence of replicating SIV within macrophages in somatosensory ganglia coupled with neuronal loss demonstrates that this SIV/macaque model may be used to study the pathogenesis of HIV neuropathy. Additional studies in the SIV/macaque model focused on measuring contemporaneous physiological and pathologic alterations in distal sensory pathways, including lumbar dorsal root ganglia, peripheral nerve, and intraepidermal nerve fibers, should help define the mechanisms underlying HIV-induced peripheral nervous system disease.

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