Journal of NeuroVirology (1999) 5, 685–694

http://www.jneurovirol.com

Role of HIV-1 Tat and CC Chemokine MIP-1α in the pathogenesis of HIV associated central nervous system disorders

Robert Bonwetsch[2], Sidney Croul[1,4], Max W Richardson[1], Carlos Lorenzana[1], Luis Del Valle[1], Andrij E Sverstiuk[1], Shohreh Amini[1], Susan Morgello[3], Kamel Khalili[1] and Jay Rappaport*[,1]

[1]Center for Neurovirology and Cancer Biology, Temple University, 1900 North 12th Street, Philadelphia, PA 19122, USA; [2]Department of Neurology, MCP Hahnemann University, 245 North 15th Street, Philadelphia, PA 19102, USA; [3]Mount Sinai Medical Center, 1 Gustave L. Place, New York, NY 10029, USA; [4]Department of Pathology and Laboratory Medicine, MCP Hahnemann University, 245 North 15th Street, Philadelphia, PA 19102, USA

Two syndromes affecting cognitive and motor function in the setting of AIDS have been described as HIV encephalopathy (HIVE) and progressive multifocal leukoencephalopathy (PML). HIVE is characterized by the presence of microglial nodules with accompanying astrocytosis. PML is a fatal demyelinating disease of the white matter induced by the human papovavirus JCV which causes cytolytic destruction of glial cells. In addition to the effect of HIV-1 induced immune suppression, HIV may act directly as a co-factor for stimulation of JCV replication in AIDS patients, in part due to Tat-induced activation of JCV gene transcription. Since Tat has been implicated in CNS pathogenesis, we examined its localization in CNS specimens from HIV infected patients with HIVE and PML as well as controls. Based on the observation of CC chemokine induction in monocytes by Tat, we also examined the cellular localization of the CC chemokine Macrophage Inflammatory Protein-1α (MIP-1α) and its cognate receptor CCR-5 in these samples. In HIVE, Tat was primarily localized in astrocytes and microglia, within the nodular lesions. In PML, a marked increase in the number of Tat positive astrocytes was observed. In both HIVE and PML, prominent expression of MIP-1α and CCR-5 was found within areas containing histopathological lesions. CCR-5 positivity of microglia was localized primarily to nodular lesions in HIVE. In PML, increased numbers of cells with monocyte/microglial morphology were observed relative to HIVE. The increased MIP-1 alpha positivity, and potentially other chemokines, may contribute to the pathogenesis of PML in the setting of HIV infection. Tat may play an important role in the pathogenesis of both HIV associated CNS disease states, acting indirectly through cytokine and chemokine dysregulation.

Keywords: HIV-1; Tat; chemokine; central nervous system

Introduction

HIV infection results in CNS abnormalities in approximately two-thirds of infected individuals, manifested as behavioral and motor disorders including dementia (Gabuzda *et al*, 1987). Histologically, brain tissue from individuals with HIV associated neurological diseases exhibits several characteristic features now recognized as hallmarks of HIV infection in the CNS. In HIV encephalopathy, multinucleated giant cells with other chronic inflammatory cells constitute 'microglial nodules', usually with accompanying white matter degeneration as well as other abnormalities (Budka *et al*, 1987; Nielson *et al*, 1984; Sharer and Kapila, 1985). Increased numbers of perivascular macrophages are also observed in the CNS in HIV infection and in the non-human primate SIV model, which may represent infected and/or activated cells traversing the blood-brain barrier (Lane *et al*, 1996; Power *et al*, 1993; Price *et al*, 1988; Rostad *et al*, 1987). It has been proposed that HIV infection of the CNS occurs

*Correspondence: J Rappaport
Received 14 July 1999; revised 18 August 1999; accepted 13 September 1999

through migration of infected monocyte/macrophages across the blood-brain barrier leading to infection of resident microglia and, to a lesser degree, astrocytes (Georgsson, 1994; Lane *et al*, 1996; Nottet *et al*, 1996; Persidsky *et al*, 1997). Although the brain macrophages/microglia are believed to be the major reservoir of HIV in the CNS, the role of astrocytes in CNS HIV infection may be substantially underestimated since a defect exists in the production of structural proteins in this cell type (Gorry *et al*, 1999; Neumann *et al*, 1995; Tornatore *et al*, 1994). HIV infection of microglia and astrocytes in CNS is presumably mediated by interaction of HIV with the chemokine receptors (Ghorpade *et al*, 1998; He *et al*, 1997; Sanders *et al*, 1998).

HIV infection may also activate chemokine secretion, in addition to interaction with the receptors for these chemoattractant cytokines. The β-chemokines MIP-1α and MIP-1β are induced upon infection of human monocyte cultures with HIV and increased levels of MIP-1α and MIP-1β are found in the brains from patients with HIV dementia when compared to patients without dementia (Schmidtmayerova *et al*, 1996). Similarly, in the macaque model of SIV encephalitis, MIP-1α and MIP-1β are elevated in brain tissue along with increased RANTES and the interferon inducible protein IP-10 (Sasseville *et al*, 1996; Westmoreland *et al*, 1998). Studies investigating the levels of chemokines in cerebral spinal fluid (CSF) from patients with HIV dementia have demonstrated elevated levels of Macrophage Chemotactic Protein-1 (MCP-1) (Conant *et al*, 1998; Kelder *et al*, 1998) as well as RANTES (Kelder *et al*, 1998) relative to CSF from patients without dementia. IP-10 has also been a major chemoattractant found in CSF derived from patients with HIV associated neurologic disorders (Kolb *et al*, 1999). Abnormal levels of chemokines in CSF may contribute to the influx of activated T cells into that compartment and provides a contributory mechanism for enhanced viral replication and access of virus to the brain parenchyma. The multiple chemokines induced by HIV infection may contribute to the development of HIV associated central nervous system disorders. The role of chemokines/receptors in the neuropathogenesis of AIDS may not be limited to chemotaxis of inflammatory cells since chemokine receptors CCR5, CCR3 and CXCR4 are present on neurons; the interaction of these G protein-coupled receptors with HIV envelope protein can induce signaling pathways leading to neuronal cell death (Zheng *et al*, 1999; Klein *et al*, 1999; Meucci *et al*, 1998). Chemokines can also block the neurotoxic effects of envelope protein demonstrating the specificity of the interaction with chemokine receptors and distinguishing the effects of envelope from the natural chemokine ligands (Kaul and Lipton, 1999).

Although there has been considerable attention to HIV envelope protein interactions with chemokine receptors both at the level of viral entry and neuronal cell death, the level of neurological impairment in AIDS appears to be disproportionate to the subtle histologic lesions that are observed and the levels of HIV envelope detected in CNS tissue. Indirect pathways involving virus induced cytokine/chemokine regulation have been proposed to account for the behavioral, cognitive and motor deficits occurring in the setting of HIV infection. Such pathways presumably amplify the effects of virally infected cells through cytokine networks and/or the release of biologically active viral proteins (i.e. envelope protein and Tat), leading to the invasion of inflammatory cells, namely macrophages/microglia into affected areas of the CNS. In support of this model, inflammatory cytokine and chemokine (MIP-1α and MIP-1β) secretion are increased in areas of the CNS with virus infected cells, yet surprisingly, cytokine dysregulation is most prominent in virus negative cells (Nuovo and Alfieri, 1996).

Inflammatory cytokine and chemokine induction may provide a mechanism for a continuous supply of fresh target cells for viral infection. The invasion of monocyte/macrophages represents an important step in the pathogenesis of HIV induced neurological disorders. In fact, the number of activated macrophages in CNS appears to be a better correlate of neurologic dysfunction than the number of productively infected cells (Glass *et al*, 1995). In addition, changes in immune surveillance, alterations in macrophage subsets, or alternatively, changes in cytokine environment may account for the susceptibility of the CNS to AIDS pathogenesis. These changes may foster increases in HIV viral gene expression in CNS leading to further CNS damage.

The HIV-1 Tat protein has been implicated in the induction of β-chemokine secretion including Macrophage Inflammatory Protein-1 alpha (MIP-1α) and beta (MIP-1β) *in vitro* in macrophages and dendritic cells (Zagury *et al*, 1998a). These findings might suggest a role for Tat in chemokine induction in CNS, potentially driving a cascade of events resulting in trans-blood-brain barrier migration of perivascular macrophages as well as an increased number of susceptible target cells for HIV infection.

Tat has also been proposed to play a role in the activation of the human papovavirus JCV, leading to Progressive Multifocal Leukoencephalopathy (PML) in HIV infected individuals. Tat activates the JCV late promoter and increases JCV replication (Chowdhury *et al*, 1990; 1992; Tada *et al*, 1990). In view of the relatively high incidence of latent JCV infection in the normal population (approximately 80%), and the low incidence among HIV infected individuals (5–10%), one might predict that if PML is 'Tat driven', higher levels of Tat would be required to induce PML than normally seen in HIV

encephalopathy. If so, one might also expect to observe increased Tat in PML lesions, along with increased chemokine secretion and increased numbers of macrophages/microglia in PML brains. To

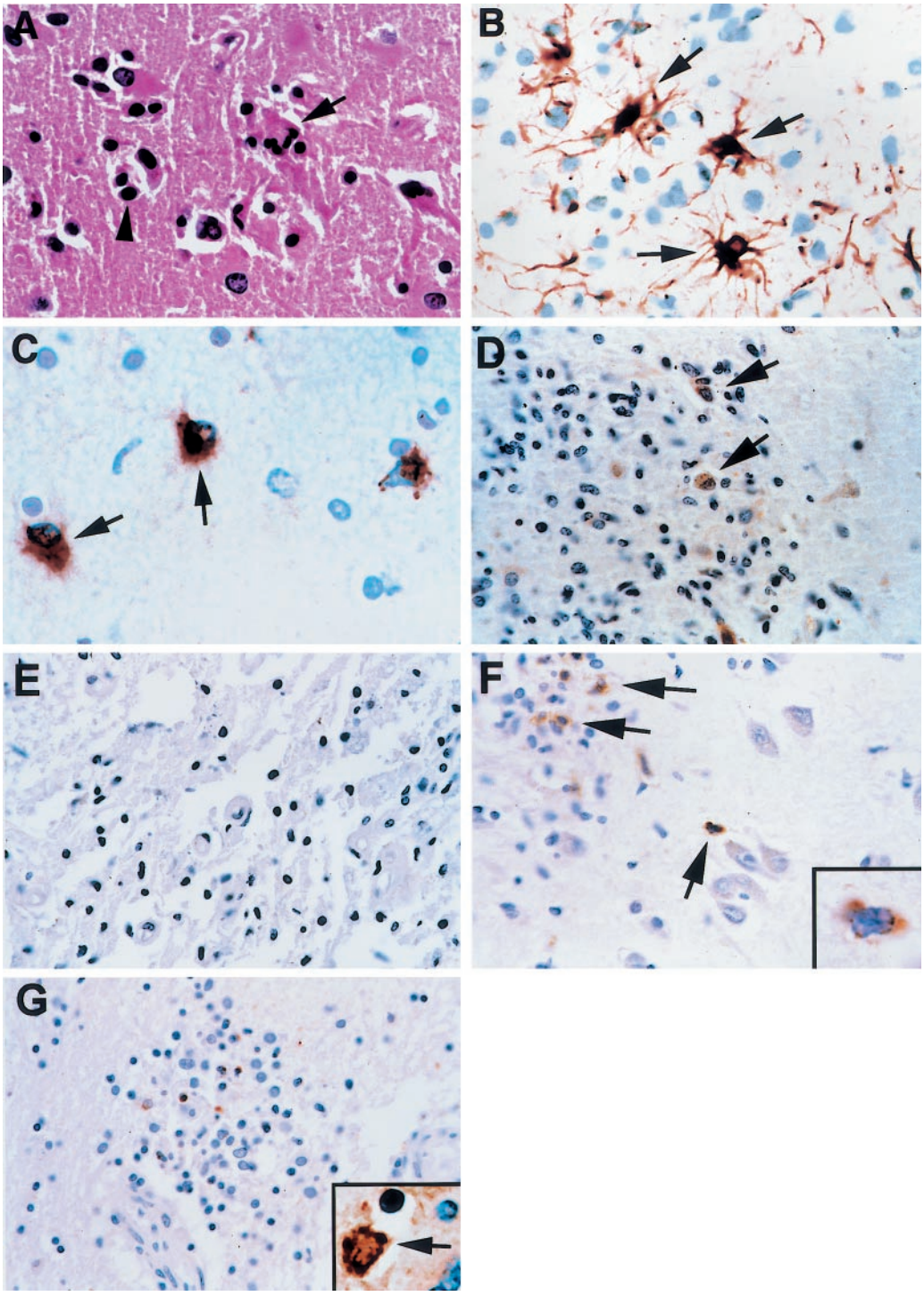

Figure 1 HIV encephalopathy. (**A**) H&E stains of 6–8 micron thick paraffin embedded sections demonstrate multinucleated cells (arrow) and microglia (arrowhead). (**B**) GFAP immunocytochemistry highlights reactive astrocytes within the inflammatory nodules (arrows). (**C**) HIV-1 Tat immunocytochemistry also highlights reactive astrocytes within and adjacent to the lesions (arrows). (**D**) Tat immunocytochemistry also shows positivity in microglia (arrows). (**E**) Tat immunocytochemistry shows no reaction product in a section of spinal cord with both gliosis and monocytes/macrophages from an HIV negative patient. (**F**) MIP-1α immunocytochemistry shows staining (arrows) in the cytoplasm of microglia which at higher power (inset) are cytoplasmic and granular. (**G**) CCR-5 immunocytochemistry shows positive microglial cells in the inflammatory nodules (arrows). At higher magnification (inset, arrow) the stain product is distributed on the cell surface. (Original magnification **A–G**, 40×; insets **F** and **G**, 100×).

test this hypothesis, we have examined the expression of Tat in CNS tissue samples from patients with both HIVE and PML. We have also probed these tissues for chemokine dysregulation by investigating the cellular distribution of MIP-1α (as an example of a β chemokine) and its cognate receptor CCR-5.

Results

Tat has been implicated in CNS disorders associated with HIV infection. Tat may act directly as well as indirectly through the induction of cytokines, including chemokines, resulting in the recruitment of additional inflammatory cells, notably activated macrophages into the CNS compartment (for review see Rappaport *et al*, 1999). In order to explore the role of Tat in HIV associated CNS disorders, we examined the level of the viral Tat protein, Macrophage Inflammatory Protein-1 alpha (MIP-1α) as a candidate chemokine, and the chemokine receptor CCR-5 in brain samples from HIV-infected patients with HIV encephalopathy or Progressive Multifocal Leukoencephalopathy (PML).

Immunohistochemistry of HIV and PML lesions
H&E stained tissue sections from patients with HIV encephalopathy and control cases were screened for pathologic changes. All of the control and HIVE specimens contained large areas of normal grey and white matter. HIVE specimens averaged 1.5 inflammatory nodules per section ranging from 200 to 1000 microns in diameter. They demonstrated multinucleated cells (Figure 1A, arrow) and activated microglia (Figure 1A, arrowhead) as has previously been described in AIDS encephalopathy (Nielson *et al*, 1984; Sharer and Kapila, 1985). No other pathologic changes were found. Within the HIVE nodules, GFAP immunohistochemistry demonstrated the expected reactive astrocytosis (Figure 1B, arrows). These astrocytes were also found in the adjacent brain within several hundred microns of the nodules but did not extend further into the histologically normal tissue. Immunohistochemistry with antibody to HIV-1 Tat protein demonstrated reaction product in the cytoplasm of the reactive astrocytes within and adjacent to the

Table 2 Intensity of immunostaining

	Accession number	*Tat*	*MIP-1α*	*CCR-5*
HIVE	MHBB37	+	+	+
	MHBB56	+	+	++
	MHBB76	+	++	++
	MHBB79	+	++	+
	A97-86	+	+	++
	A98-20	+	+	++
	A98-74	+	+	++
	A98-97	+	++	++
PML	MHBB26	++	++	+++
	A97-183	++++	+++	++++
	06809	++++	++++	++++
Controls	A98-117	–	–	–
	A96-105	–	+	+
	A99-2	–	–	–
	A99-30	–	–	–
	A98-25	–	–	–

Table 1 Human cerebral cortex samples

Accession number	*Pathology*	*Age/sex/risk factors*	*Associated diseases*
MHBB 26	PML	44/M/IVDU	Pulmonary TB/adenocarcinoma lung
MHBB 37	HIVE	50/M/Heterosexual	Pneumonia/microsspordia/CMV
MHBB 76	HIVE	38/F/IVDU/Prostitute	Pneumonia/CMV/ITP/MAI/PCP
MHBB 79	HIVE	45/M/IVDU	Endocarditis/Pneumonia/Amyloid
A97-86	HIVE	34/M/Unknown	PCP/CMV/CNS Lymphoma
A98-20	HIVE	32/M/Unknown	CNS lymphoma (brain only autopsy)
A98-74	HIVE	66/F/Unknown	CMV/Chronic Pancreatitis/Pneumonia
A98-97	HIVE	41/M/IVDU/Heterosexual	Acute Pancreatitis/Hepatitis/Pneumonia
A97-183	PML	48/F/Haitian/Heterosexual	Pneumonia
MHBB 56	HIVE	6/M/Parent	Interstitial pneumonia/bronchopneumonia
06809	PML-HIV+	25/F/Unknown	Unknown
A98-117	HIV+ No pathology	44/F/Heterosexual	B cell lymphoma/bronchopneumonia/ Cirrhosis
A96-105	Multiple Cerebral infarcts	41/M/None known	None known (brain only autopsy)
A99-2	HIV– Creutzfeld Jacob disease with severe CNS astrocytosis	41/F/None known	None
A99-30	HIV– No pathology	56/M/None known	Cardiac transplant/chronic renal failure
A98-25	HIV– No pathology	47/M/None known	Hodgkin's disease/endocarditis

nodules (Figure 1C, arrows) and in the cytoplasm of microglia within the nodules (Figure 1D, arrows). HIV negative controls did not show immunopositivity with the antibody to HIV-1 Tat (Figure 1E). Staining for MIP-1α revealed granular staining within the cytoplasm of non-ramified microglia (Figure 1F, arrows and 1F inset). Control brains, both HIV+ and HIV− without pathology, showed negligible MIP-1α staining (Tables 1 and 2). Antibodies to the CC chemokine receptor CCR-5 revealed positive microglia within the inflammatory nodules as well (Figure 1G) which were not found in non-pathologic control white matter. At higher power, much of this positivity could be localized to the cell surface (Figure 1G inset, arrow). Regions of the brain more than 200 to 300 microns distant from the HIVE nodules showed no difference in immunopositivity compared to control brain section stained with Tat, MIP-1α, and CCR-5.

The PML samples and controls were screened with H&E stains in the same fashion as the HIVE brains. In contrast to the HIVE samples, the majority of each of the PML positive tissue blocks exhibited pathologic alterations. They demonstrated the classic findings of demyelination, bizarre reactive astrocytes (Figure 2A, arrowhead) and viral inclu-

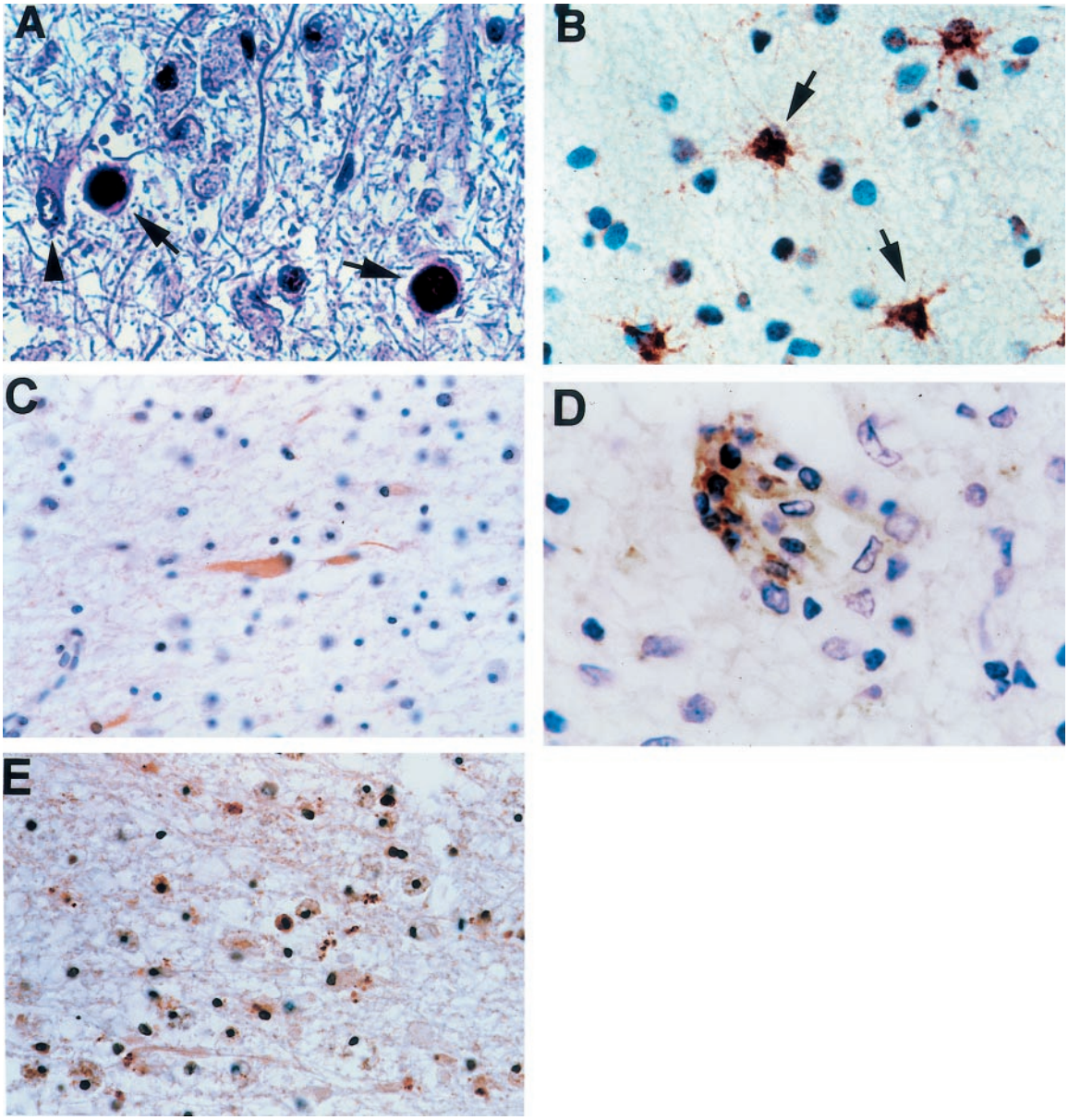

Figure 2 Progressive Multifocal Leukoencephalopathy. (**A**) H&E stain exhibiting demyelination, bizarre astrocytes (arrowhead), and viral inclusions in oligodendrocytes (arrows). (**B**) Tat immunocytochemistry again highlights reactive astrocytes (arrows). MIP-1α immunocytochemistry shows positivity in astrocytes (**C**) and microglia (**D**). (**E**) CCR-5 immunocytochemistry showing positivity in monocytes/macrophages. (Original magnification **A–E**, 40×).

sions in oligodendrocytes (Figure 2A, arrows showing infected oligodendrocyte nuclei). In HIV-associated PML, prominent Tat immunopositivity was found within reactive astrocytes (Figure 2B, arrows). MIP-1α immuno-reactivity was found within both astrocytes (Figure 2C) and microglia (Figure 2D). CCR-5 positive monocytes/macrophages were also found (Figure 2E). In control HIV− tissues with infarcts (Table 1, A96-105), MIP-1α positivity was not found in astrocytes and the intensity of both MIP-1α and CCR-5 positivity in monocytes/microglia was much less than that found in the PML brains.

There was a striking difference in the extent of pathology between the HIVE and PML brains as demonstrated by H&E stains. This difference extended to the immunocytochemical results as well. While the same cell types were immunopositive for Tat, MIP-1α, and CCR-5 in both HIVE and PML, the intensity of staining was consistently greater for all these antibodies in the PML brains. Comparison of the panels of MIP-1α and CCR-5 immunoreactivity for HIVE and PML (Figure 1F and G *versus* Figure 2C, D and E) reveals the difference in staining qualitatively. Quantitation of the immunoreactivity demonstrates the difference most clearly (Table 2). Quantitation of stain intensities for 15 of 24 scores in HIVE were 1+ and the remaining nine were 2+ out of a possible 4+. In PML, five of nine were 4+; two of nine were 3+; and two were 2+.

Discussion

The role of HIV in the pathogenesis of central nervous system disorders presumably involves the direct effect of viral proteins on neurons as well as indirect pathways involving induction of host cytokines by viral factors. Although mounting evidence suggests a role for HIV envelope protein in HIV pathogenesis in CNS, considerable attention has been focused recently on the viral regulatory protein Tat. Tat protein is required for viral replication and acts at the level of RNA transcription via interaction with an RNA sequence element, TAR, at the 5′ end of viral mRNAs. The action of Tat involves interactions with various cellular factors including cyclin T/CDK9 (Wei *et al*, 1998) as well as Purα (Gallia *et al*, in press; Krachmarov *et al*, 1996). In addition to this intracellular activity, Tat is secreted from infected cells and exerts biologic activities on non-infected cells (Ensoli *et al*, 1993). Exposure of non-infected T cells to Tat protein *in vitro* increases the susceptibility of these cells to HIV infection (Li *et al*, 1997), and this process presumably occurs through Tat activation of the seven transmembrane chemokine receptors (Huang *et al*, 1998). Tat also inhibits T cell proliferation in response to activation signals, possibly by dysregulation of cell cycle pathways and/or apoptosis induction (Viscidi *et al*, 1989; Chirmule *et al*, 1995; Li *et al*, 1995; Zagury *et al*, 1996). Based on these activities, it is not surprising that antibodies to Tat have been associated with long-term non-progression in HIV infected individuals (Zagury *et al*, 1998b).

Tat may play a particularly important role in the pathogenesis induced by HIV in the CNS. HIV Tat is neurotoxic *in vitro* via its ability to interact with NMDA as well as non NMDA receptors (New *et al*, 1998; Strijbos *et al*, 1995). Indirect mechanisms may be particularly important as well since Tat activates the secretion of several cytokines, notably TNFα, TGFβ and IL-1 (Biswas *et al*, 1995; Cupp *et al*, 1993; Nath *et al*, 1999; Rappaport *et al*, 1999; Rasty *et al*, 1996; Sawaya *et al*, 1998; Zauli *et al*, 1992). These Tat induced host factors may have potentially significant effects on astrocytes as well as on monocyte-microvascular endothelial cell interactions via adhesion molecules and their receptors. Tat may also play a critical role in CNS pathogenesis through the interaction of HIV with the human papova virus, JCV. Oligodendrocytes infected with JCV may be stimulated by Tat released from HIV infected CNS cell types including macrophages/microglia and astrocytes. *In vitro* studies have demonstrated the ability of HIV-1 Tat protein to activate JCV late promoter transcription by a TAR independent mechanism as well as via a TAR homologous element present in the promoter region of JCV (Chowdhury *et al*, 1990; 1992; Tada *et al*, 1990).

To further define the role of HIV Tat protein in CNS, we have examined distribution and localization of HIV-1 Tat protein in brain tissue from patients with HIV encephalopathy and HIV associated PML. In HIV encephalopathy, Tat positive staining was localized to astrocytes and microglia within nodular lesions. In HIV associated PML, we observed a marked increase in the number of Tat positive astrocytes. We have also observed Tat positivity in JCV transformed oligodendrocytes (Del Valle *et al*, in preparation). The increase in Tat positivity in PML relative to HIVE specimens and the localization of Tat within JCV transformed oligodendrocytes supports the hypothesis that Tat may directly induce JCV replication and gene expression and contribute to the pathogenesis observed in PML.

One of the major pathways leading to CNS involvement in HIV infection presumably involves the upregulation of chemokines in activated macrophages/microglia. MIP-1α and MIP-1β have been demonstrated to be induced in human monocytes by HIV infection and it has been proposed that such chemokine activation may play a role in abnormal trafficking of monocytes and lymphocyte subsets into lymph nodes as well as the CNS (Schmidtmayerova *et al*, 1996). Although chemotactic factors such as MIP-1α, MIP-1β and RANTES can block HIV infection through competition with CCR-5 receptors on target cells (Cocchi *et al*, 1995), chemokine

secretion may be contributory to the HIV life cycle by recruitment of susceptible target cells to foci of HIV infection. In CNS, this mechanism may recruit additional activated macrophages into the brain parenchyma. In this study, we examined the localization of MIP-1α in HIVE and PML brain samples. In HIVE, MIP-1α was localized primarily to microglia in the areas of nodular lesions which were in proximity to the Tat positive astrocytes and microglia of these lesions. In PML, a dramatic increase in the number of Tat positive astrocytes was observed with a concomitant increase in the level of MIP-1α expression, as observed by prominent staining of PML lesions. These results are consistent with a role for extracellular Tat in CC chemokine induction, providing a mechanism for monocyte/macrophage invasion of the CNS in HIV infection. Indeed, an increase in perivascular inflammatory cells observed in AIDS associated PML, relative to non-AIDS PML could be consequent to this pathway (Aksamit *et al*, 1990).

These results also suggest that Tat may play a role in the pathogenesis of HIVE and PML by activation of monocyte/macrophage trafficking into the CNS through MIP-1α upregulation. We examined the distribution of the receptor for MIP-1α, CCR-5, in these tissues. Immunohistochemical staining for CCR-5 revealed prominent microglial expression throughout the PML lesion. Since the lesion actually dominated the sections we examined, it was not possible to determine the distribution of CCR-5 in the normal areas of brain tissue. Analysis of brain from HIVE demonstrated CCR-5 positivity in macrophages/microglia within the nodular lesions. The expression of CCR-5 in areas of histopathological alterations in HIV infected brain is consistent with previous studies of HIV as well as SIV encephalitis (Sanders *et al*, 1998; Westmoreland *et al*, 1998). The detection of CCR-5 positive macrophages/microglia with the HIV associated lesions is consistent with the role of CCR-5 as a co-receptor for HIV in microglia (He *et al*, 1997) and with the role of Tat in upregulating chemokine receptors (Huang *et al*, 1998).

Materials and methods

Human tissue samples

Paraffin embedded Central Nervous System (CNS) tissue samples were obtained from 15 autopsy brains and one surgical biopsy (see Table 1). Of these, 15 were from adults and one was a pediatric specimen. Pathologically, eight of the autopsy specimens showed HIV encephalopathy (HIVE). The one pediatric autopsy specimen had Progressive Multifocal Leukoencephalopathy (PML) in the setting of HIV infection, as did one adult autopsy specimen and the one adult surgical biopsy. Controls consisted of two HIV negative brains with no pathology, one HIV positive brain from an individual without histologic evidence of CNS pathology, one brain from an HIV-negative patient with resolving infarcts and one brain from an HIV negative patient with Creutzfeld Jakob disease and severe CNS astrogliosis. Lymph nodes from patients who were HIV negative and positive served as further non CNS controls. These are not shown in Table 1.

Immunohistochemistry

Six to eight micron thick sections, taken from each sample at 500 micron intervals through the block, were examined by staining with hematoxylin and eosin (H&E) to confirm that the lesions of interest were present. Unstained sections were then processed for immunohistochemistry. Briefly, tissues were rehydrated with dH_2O, incubated in 3% H_2O_2 for 15 min at room temperature to quench endogenous peroxidase, incubated for 30 min in 0.01 M citrate buffer, pH 6.0 at 95°C for antigen retrieval and washed with PBS. The purified IgG fraction of a rabbit polyclonal antibody against an HIV-1 Tat peptide was used at a dilution of 1 : 250. Mouse monoclonal antibodies to MIP-1α (Santa Cruz Biotechnology M-20, 1 : 1000 dilution) and CCR-5 (NIH AIDS Research and Reagent Reference program 5C7, 1 : 250 dilution), GFAP, and a monoclonal antibody to GFAP (Dako) were also used. Primary antibodies were revealed with an Avidin-Biotin complex technique using diaminobenzidine (DAB) as the chromagen (Vecta-stain Elite Kit, Novocastra). All tissues were lightly counterstained with hematoxylin prior to coverslipping. The intensity of immunohistochemical signal was graded by examination of the sections, blinded to diagnosis and grading stain product on a scale of + to ++++ based on the degree of stain product density on a cell-by-cell basis. For negative controls, primary monoclonal antibodies were replaced by buffer, and polyclonal antibodies were replaced by normal serum. All antibodies on each tissue block were run in duplicate in separate immunohistochemical runs.

Acknowledgments

We would like to thank the NIH AIDS Research and Reference Reagent Program (AIDS Reagent Program) for providing the CCR-5 antibody and the Manhattan HIV Brain Bank (1R24MH59724) for providing the HIV brain specimens. We would like to thank past and present members of the Center for NeuroVirology and NeuroOncology for their support and sharing of ideas and reagents, in particular we extend our thanks to Dr Mahendra Bhopale for providing microglial cell cultures. We thank Cynthia Schriver for editorial assistance and preparation of this manuscript. This work was made possible by grants awarded by NIH to K Khalili, S Amini, S Morgello and J Rappaport.

References

Aksamit AJ, Gendelman HE, Orenstein JM, Pezeshkpour GH (1990). AIDS-associated progressive multifocal leukoencephalopathy (PML): comparison to non-AIDS PML with in situ hybridization and immunohistochemistry. *Neurology* **40:** 1073–1078.

Biswas DK, Salas TR, Wang F, Ahlers CM, Dezube BJ, Pardee AB (1995). A tat induced auto-up-regulatory loop for superactivation of the human immunodeficiency virus type 1 promoter. *J Virol* **69:** 7347–7354.

Budka H, Costanzi G, Cristina S, Lechi A, Parravicini C, Trabattoni R, Vago L (1987). Brain pathology induced by infection with the human immunodeficiency virus (HIV). A histological, immunocytochemical, and electron microscopical study of 100 autopsy cases. *Acta Neuropathol (Berl)* **75:** 185–198.

Chirmule N, Than S, Khan SA, Pahwa S (1995). Human immunodeficiency virus Tat induces functional unresponsiveness in T cells. *J Virol* **69:** 492–498.

Chowdhury M, Taylor JP, Tada H, Rappaport J, Wong-Staal F, Amini S, Khalili K (1990). Regulation of the Human neurotropic virus promoter by JCV-T antigen and HIV-1 tat protein. *Oncogene* **5:** 1737–1742.

Chowdhury M, Taylor JP, Chang C-F, Rappaport J, Khalili K (1992). Evidence that a sequence similar to TAR is important for induction of the JC virus late promoter by human immunodeficiency virus type 1 by Tat. *J Virol* **66:** 7355–7361.

Cocchi F, DeVico AL, Garzino-Demo A, Arya SK, Gallo RC, Lusso P (1995). Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by CD8+ T cells. *Science* **270:** 1811–1815.

Conant K, Garzino-Demo A, Nath A, McArthur JC, Halliday W, Power C, Gallo RC, Major EO (1998). Induction of monocyte chemoattractant protein-1 in HIV-1 Tat-stimulated astrocytes and elevation in AIDS dementia. *Proc Natl Acad Sci USA* **95:** 3117–3121.

Cupp C, Taylor JP, Khalili K, Amini S (1993). Evidence for stimulation of the transforming growth factor β-1 promoter by HIV-1 Tat in cells derived from CNS. *Oncogene* **8:** 2231–2236.

Ensoli B, Buonaguro L, Barillari G, Fiorelli V, Gendelman R, Morgan RA, Wingfield P, Gallo RC (1993). Release, uptake, and effects of extracellular human immunodeficiency virus type 1 Tat protein on cell growth and viral transactivation. *J Virol* **67:** 277–287.

Gabuzda DH, Hirsch MS (1987). Neurologic manifestations of infection with human immunodeficiency virus. Clinical features and pathogenesis. *Ann Intern Med* **107:** 383–391.

Gallia GL, Darbinian N, Tretiakova A, Ansari SA, Rappaport J, Brady J, Wortman MJ, Johnson EM, Khalili K (1999). Association of HIV-1 Tat with the cellular protein, Puralpha, is mediated by RNA. *Proc Natl Acad Sci USA* **96:** 11572–11577.

Georgsson G (1994). Neuropathologic aspects of lentiviral infections. *Ann NY Acad Sci* **724:** 50–67.

Ghorpade A, Xia MQ, Hyman BT, Persidsky Y, Nukuna A, Bock P, Che M, Limoges J, Gendelman HE, Mackay CR (1998). Role of the beta-chemokine receptors CCR3 and CCR5 in human immunodeficiency virus type 1 infection of monocytes and microglia. *J Virol* **72:** 3351–3361.

Glass JD, Fedor H, Wesselingh SL, McArthur JC (1995). Immunocytochemical quantitation of human immunodeficiency virus in the brain: correlations with dementia. *Ann Neurol* **38:** 755–762.

Gorry PR, Howard JL, Churchill MJ, Anderson JL, Cunningham A, Adrian D, McPhee DA, Purcell DF (1999). Diminished production of human immunodeficiency virus type 1 in astrocytes results from inefficient translation of gag, env, and nef mRNAs despite efficient expression of Tat and Rev. *J Virol* **73:** 352–361.

He J, Chen Y, Farzan M, Choe H, Ohagen A, Gartner S, Busciglio J, Yang X, Hofmann W, Newman W, Mackay CR, Sodroski J, Gabuzda D (1997). CCR3 and CCR5 are co-receptors for HIV-1 infection of microglia. *Nature* **385:** 645–649

Huang L, Bosch I, Hofmann W, Sodroski J, Pardee AB (1998). Tat protein induces human immunodeficiency virus type 1 (HIV-1) coreceptors and promotes infection with both macrophage-tropic and T-lymphotropic HIV-1 strains. *J Virol* **72:** 8952–8960.

Kaul M, Lipton SA (1999). Chemokines and activated macrophages in HIV gp120-induced neuronal apoptosis. *Proc Natl Acad Sci USA* **96:** 8212–8216.

Kelder W, McArthur JC, Nance-Sproson T, McClernon D, Griffin DE (1998). Beta-chemokines MCP-1 and RANTES are selectively increased in cerebrospinal fluid of patients with human immunodeficiency virus-associated dementia. *Ann Neurol* **44:** 831–835.

Klein RS, Williams KC, Alvarez-Hernandez X, Westmoreland S, Force T, Lackner AA, Luster AD (1999). Chemokine receptor expression and signaling in macaque and human fetal neurons and astrocytes: implications for the neuropathogenesis of AIDS. *J Immunol* **163:** 1636–1646.

Kolb SA, Sporer B, Lahrtz F, Koedel U, Pfister HW, Fontana A (1999). Identification of a T cell chemotactic factor in the cerebrospinal fluid of HIV-1-infected individuals as interferon-gamma inducible protein 10. *J Neuroimmunol* **93:** 172–181.

Krachmarov CP, Chepenik LG, Barr-Vagel S, Khalili K, Johnson EM (1996). Activation of the JC virus Tat-responsive transcriptional control element by association of the Tat protein of human immunodeficiency virus 1 with cellular protein Purα. *Proc Natl Acad Sci USA* **93:** 14112–14117.

Lane JH, Sasseville VG, Smith MO, Vogel P, Pauley DR, Heyes MP, Lackner AA (1996). Neuroinvasion by simian immunodeficiency virus coincides with increased numbers of perivascular macrophages/microglia and intrathecal immune activation. *J Neurovirol* **2:** 423–432.

Li CJ, Friedman DJ, Wang C, Metelev V, Pardee AB (1995). Induction of apoptosis in uninfected lymphocytes by HIV-1 Tat protein. *Science* **268:** 429–431.

Li CJ, Ueda Y, Shi B, Borodyansky L, Huang L, Li YZ, Pardee AB (1997). Tat protein induces self-perpetuating permissivity for productive HIV-1 infection. *Proc Natl Acad Sci USA* **94:** 8116–8120.

Meucci O, Fatatis A, Simen AA, Bushell TJ, Gray PW, Miller RJ (1998). Chemokines regulate hippocampal neuronal signaling and gp120 neurotoxicity. *Proc Natl Acad Sci USA* **95:** 14500–14505.

Nath A, Conant K, Chen P, Scott C, Major EO (1999). Transient exposure to HIV-1 tat protein results in cytokine production in macrophages and astrocytes. A hit and run phenomenon. *J Biol Chem* **274:** 17098–17102.

Neumann M, Felber BK, Kleinschmidt A, Froese B, Erfle V, Pavlakis GN, Brack-Werner R (1995). Restriction of human immunodeficiency virus type 1 production in a human astrocytoma cell line is associated with a cellular block in Rev function. *J Virol* **69:** 2159–2167.

New DR, Maggirwar SB, Epstein LG, Dewhurst S, Gelbard HA (1998). HIV-1 Tat induces neuronal death via tumor necrosis factor-alpha and activation of non-N-methyl-D-aspartate receptors by a NFkappaB-independent mechanism. *J Biol Chem* **273:** 17852–17858.

Nielsen SL, Petito CK, Urmacher CD, Pasner JB (1984). Subacute encephalitis in acquired immunodeficiency syndrome: A post-mortem study. *Am J Clin Pathol* **82:** 678–682.

Nottet HS, Persidsky Y, Sasseville VG, Nukuna AN, Bock P, Zhai QH, Sharer LR, McComb RD, Swindells S, Soderland C, Gendelman HE (1996). Mechanisms for the transendothelial migration of HIV-1-infected monocytes into brain. *J Immunol* **156:** 1284–1295.

Nuovo GJ, Alfieri ML (1996). AIDS dementia is associated with massive, activated HIV-1 infection and concomitant expression of several cytokines. *Mol Med* **2:** 358–366.

Persidsky Y, Stins M, Way D, Witte MH, Weinand M, Kim KS, Bock P, Gendelman HE, Fiala M (1997). A model for monocyte migration through the blood-brain barrier during HIV-1 encephalitis. *J Immunol* **158:** 3499–3510.

Power C, Kong PA, Crawford TO, Wesselingh S, Glass JD, McArthur JC, Trapp BD (1993). Cerebral white matter changes in acquired immunodeficiency syndrome dementia: alterations of the blood-brain barrier. *Ann Neurol* **34:** 339–350.

Price RW, Brew B, Sidtis J, Rosenblum M, Scheck AC, Cleary P (1988). The brain in AIDS: central nervous system HIV-1 infection and AIDS dementia complex. *Science* **239:** 586–592.

Rappaport J, Joseph J, Croul S, Alexander G, Amini S, Khalili K (1999). Molecular Pathway Involved in HIV-1 Induced CNS Pathology: Role of Viral Regulatory Protein, Tat. *J Leuk Biol* **65:** 458–465.

Rasty S, Thatikunta P, Gordon J, Khalili K, Amini S, Glorioso J (1996). Human immunodeficiency virus tat gene transfer to the murine central nervous system using a replication-defective herpes simplex virus vector stimulates transforming growth factor beta 1 gene expression. *Proc Natl Acad Sci USA* **93:** 6073–6078.

Rostad SW, Sumi SM, Shaw CM, Olson K, McDougall JK (1987). Human immunodeficiency virus (HIV) infection in brains with AIDS-related leukoencephalopathy. *AIDS Res Hum Retroviruses* **3:** 363–373.

Sanders VJ, Pittman CA, White MG, Wang G, Wiley CA, Achim CL (1998). Chemokines and receptors in HIV encephalitis. *AIDS* **12:** 1021–1026.

Sasseville VG, Smith MM, Mackay CR, Pauley DR, Mansfield KG, Ringler DJ, Lackner AA (1996). Chemokine expression in simian immunodeficiency virus-induced AIDS encephalitis. *Am J Pathol* **149:** 1459–1467.

Sawaya BE, Thatikunta P, Denisova L, Brady J, Khalili K, Amini S (1998). Regulation of TNFα and TGFβ-1 gene transcription by HIV-1 Tat in CNS cells. *J Neuroimmunol* **87:** 33–42.

Schmidtmayerova H, Nottet HS, Nuovo G, Raabe T, Flanagan CR, Dubrovsky L, Gendelman HE, Cerami A, Bukrinsky M, Sherry B (1996). Human immunodeficiency virus type 1 infection alters chemokine beta peptide expression in human monocytes: implications for recruitment of leukocytes into brain and lymph nodes. *Proc Natl Acad Sci USA* **93:** 700–704.

Sharer LR, Kapila R (1985). Neuropathologic observations in the acquired immunodeficiency syndrome. *Acta Neuropathol (Berl)* **66:** 188–198.

Strijbos PJ, Zamani MR, Rothwell NJ, Arbuthnot G, Harkiss G (1995). Neurotoxic mechanisms of transactivating protein Tat of Maedi-Visna virus. *Neurosci Lett* **197:** 215–218.

Tada H, Rappaport J, Lashgari M, Amini S, Wong-Staal F, Khalili K (1990). Transactivation of the JC virus late promoter by the Tat protein of type-1 human immunodeficiency virus in glial cells. *Proc Natl Acad Sci USA* **87:** 3479–3483.

Tornatore C, Meyers K, Atwood W, Conant K, Major EO (1994). Temporal patterns of human immunodeficiency virus type 1 transcripts in human fetal astrocytes. *J Virol* **68:** 93–102.

Viscidi RP, Mayur K, Lederman HM, Frankel AD (1989). Inhibition of antigen-induced lymphocyte proliferation by Tat protein from HIV-1. *Science* **246:** 1606–1608.

Wei P, Garber ME, Fang SM, Fischer WH, Jones KA (1998). A novel CDK9-associated C-type cyclin interacts directly with HIV-1 Tat and mediates its high-affinity, loop-specific binding to TAR RNA. *Cell* **92:** 451–462.

Westmoreland SV, Rottman JB, Williams KC, Lackner AA, Sasseville VG (1998). Chemokine receptor expression on resident and inflammatory cells in the brain of macaques with simian immunodeficiency virus encephalitis. *Am J Pathol* **152:** 659–665.

Zagury J-F, Lachgar A, Bizzini B, Astgen A, Lecoq H, Fouchard M, Chams V, Feldman M, Richardson M, Rappaport J, Burny A, Zagury D, Gallo RC (1996). A critical role of Tat and IFNα in the HIV-1 induced immunosuppression leading to AIDS. *AIDS Sciences* **3:** 104–108.

Zagury D, Lachgar A, Chams V, Fall LS, Bernard J, Zagury J-F, Bizzini B, Gringeri A, Santagostino E, Rappaport J, Feldman M, Burny A, Gallo RC (1998a). Interferon alpha and Tat involvement in the immunosuppression of uninfected T cells and C-C chemokine decline in AIDS. *Proc Natl Acad Sci USA* **95:** 3851–3856.

Zagury J-F, Abderrahim-Lachgar A, Le Buanec H, Rappaport J, Hendel H, Bizzini B, Gringeri A, Carcagno M, Criscuolo M, Burny A, Blattner W, Gallo RC, Zagury D (1998b). Antibodies to the HIV-1 Tat protein correlate with non-progression to AIDS: a rationale for the use of a Tat toxoid as an HIV-1 vaccine. *J Hum Virol* **1:** 282–292.

Zauli D, Davis BR, Re MC, Visani G, Furlini G, La Placa M (1992). Tat protein stimulates production of transforming growth factor-β1 by marrow macrophages: A potential mechanism for human immunodeficiency virus-1 induced hematopoietic suppression. *Blood* **80:** 3036–3043.

Zheng J, Thylin MR, Ghorpade A, Xiong H, Persidsky Y, Cotter R, Niemann D, Che M, Zeng YC, Gelbard HA, Shepard RB, Swartz JM, Gendelman HE (1999). Intracellular CXCR4 signaling, neuronal apoptosis and neuropathogenic mechanisms of HIV-1-associated dementia. *J Neuroimmunol* **98:** 185–200.