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Epidemiology Report

Epidemiological evidence and molecular basis of interactions between HIV and JC virus

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The epidemiology of PML in the era of AIDS

Impairment of cell-mediated immunity is the chief predisposing condition for the development of PML. Lymphoproliferative disorders, either chronic lymphocytic leukemia or lymphoma, were the underlying immunosuppressive conditions in Astrom, Mancall, and Richardson's initial characterization of PML as a distinct clinical entity in 1958. Through the beginning of the AIDS epidemic in 1981, lymphoproliferative disorders remained the most common predisposing illness for PML. In a review of 230 cases of PML published in 1984 by Brooks and Walker, PML was seen in association with lymphoproliferative diseases in 62.2% of the cases (Brooks and Walker, 1984). Other predisposing illnesses included myeloproliferative diseases in 6.5%, carcinoma in 2.2%, granulomatous and inflammatory diseases, such as, tuberculosis and sarcoidosis, in 7.4%, and other immune deficiency states in 16.1%. Although AIDS was included in the latter category, there were only 2 reported cases of PML complicating AIDS at that time (Brooks and Walker, 1984), the first of which was reported in 1982, only 1 year after the seminal description of AIDS.

Until the AIDS pandemic, PML remained a rare disease. Indeed, for most practicing neurologists, it remained a medical curiosity that was seldom observed in practice. Following the AIDS pandemic, the incidence of PML changed very dramatically. In major metropolitan areas, the disease can hardly be regarded as rare. By the late 1980s, AIDS was reported to be the most common underlying disorder predisposing to the development of PML at institutions in New York (Krupp *et al*, 1985) and Miami (Berger *et al*, 1987). Gillespie and colleagues (Gillespie *et al*, 1991) studying the prevalence of AIDS-related illnesses in the San Francisco Bay area estimated a prevalence for PML of 0.3%. The investigators acknowledged that this might have been a significant underestimate (Gillespie *et al*, 1991). Based on death-certificate reporting of AIDS to the Centers for Disease Control (CDC) between 1981 and June 1990, 971 (0.72%) of 135,644 individuals with AIDS were reported to have PML (Holman *et al*, 1991). Due to the notorious inaccuracies in death-certificate reporting (Messite and Stillman, 1996) and the requirement of pathologic confirmation for inclusion in this study, this is also likely a significant underestimate of the true prevalence. Other studies have suggested that the prevalence of PML in AIDS cases is substantially higher than that reported by the CDC. Most estimates range between 1% to 5% in clinical studies and as high as 10% in pathological series (Krupp *et al*, 1985; Stoner *et al*, 1986; Berger *et al*, 1987; Lang *et al*, 1989; Kure *et al*, 1991; Kuchelmeister *et al*, 1993; Whiteman *et al*, 1993). In 1987, a large, retrospective, hospital-based, clinical study (Berger *et al*, 1987) found PML in approximately 4% of patients hospitalized with AIDS.

Four percent of all patients dying with AIDS had PML in a combined series of 7 separate neuropathological studies comprising a total of 926 patients with AIDS (Kure *et al*, 1991). Two other large neuropathologic series found PML in 7% (Lang *et al*, 1989) and 9.8% (Kuchelmeister *et al*, 1993) of autopsied AIDS patients. The authors of the latter study acknowledged that an unusually high estimate might have resulted from numerous referral cases from outside the study center and the inherent bias imposed (Kuchelmeister *et al*, 1993). However, a study of 548 consecutive, unselected autopsies between 1983 and 1991 performed on HIV-seropositive individuals by the Broward County (Florida) Medical Examiner revealed that 29 (5.3%) had PML confirmed at autopsy (Whiteman *et al*, 1993). Similarly, the Multicenter AIDS Cohort Study also identified a dramatic rise in the incidence of PML over a similar time period. Specifically, the Multicenter AIDS Cohort Study identified 22 cases of PML among the cohort of AIDS cases studied from 1985 to 1992: the average annual

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incidence of PML was 0.15 per 100 person-years with a yearly rate of increase of 24% between 1985 and 1992 (Bacellar *et al*, 1994).

An excellent illustration of the change of PML frequency since the advent of AIDS is indicated by the south Florida experience. During the quarter century between the description of the disorder by Astrom *et al* (Astrom *et al*, 1958), to the extensive review of PML by Brooks and Walker in 1984, just over 200 cases had been reported in the world's literature. In south Florida, over a 14-year interval from 1980–1994, 156 cases of PML were identified from the records of the University of Miami Medical Center and the Broward County Medical Examiner's Officer (Berger *et al*, 1998b). All but two of these cases (Hodgkin's disease and Wiskott–Aldrich syndrome) were AIDS-related. When comparing the 4-year intervals (1980–1984 and 1990–1994) in this series, there was a 20-fold increase in the number of cases of PML (Berger *et al*, 1998b). In comparison, no cases of PML were observed among the substantial number of transplant patients who had received long-term pharmacologically induced immunosuppression. On average, there were 100 patients undergoing renal transplant and 50 undergoing other major organ transplantation annually at the University of Miami/Jackson Memorial Hospital during this period. Additionally, with the exception of the one patient with Hodgkin's disease, no instances of PML were identified among the rather substantial number of patients with cancer of hematological malignancy. Similarly, no cases of PML were reported in two large series of the complications of renal transplantation (Yoshimura and Oka, 1990; Harmon, 1991). Although the HIV-infected population in south Florida is large, these data strongly suggest that there is a particular predilection for the development of PML in the setting of AIDS when compared to other immunosuppressive conditions that may be of equal or greater prevalence in some populations.

Overall, AIDS has been estimated to be the underlying cause of immunosuppression in 55% to more than 85% of all current cases of PML (Major *et al*, 1992). In some geographic regions, this incidence of AIDS as a predisposing factor for PML, for example, south Florida, may be an underestimate. However, in other geographic regions, for example, Africa and southern India, PML complicating AIDS appears to be significantly more rare. A neuropathological study from southern India found an incidence of 1% (Satishchandra *et al*, 2000). The overall frequency of PML in these areas and the underlying predisposing illnesses remain unknown.

In 1996, there was an expansion of available anti-retroviral therapies with the development of highly active anti-retroviral therapies (HAART). Opportunistic infections, for example, cytomegalovirus (Baril *et al*, 2000), toxoplasmosis (Maschke *et al*, 2000), and primary central nervous system lymphomas (Sparano *et al*, 1999) appear to have declined significantly following their introduction. The effect of this therapy on the incidence of PML remains uncertain. D'Arminio Monforte and colleagues (d'Arminio Monforte *et al*, 2000) detected a 95% risk reduction in all CNS AIDS-related conditions following the adoption of HAART in their cohort. Twenty cases of PML were identified in this study, but a specific analysis for PML was not undertaken. Others (Maschke *et al*, 2000) have similarly noted a decline in HIV-related CNS disorders but have had too few cases to comment specifically about PML.

Despite the introduction of HAART and effects of selection and other biases on the studies of PML frequency in AIDS, there is an indisputable marked increased frequency of PML since the inception of the AIDS pandemic. Furthermore, it appears that the incidence of PML complicating HIV/AIDS is higher than that of any other immunosuppressive disorder relative to their frequencies.

Why is PML so prevalent in association with HIV infection?

What accounts for the higher frequency of PML in HIV infection? A number of possibilities exist and need not be mutually exclusive. Among the possibilities are the following:

1. Difference in the degree and duration of the cellular immunosuppression in HIV infection as compared to other immunosuppressive conditions.
2. Facilitation of the entry into the brain of JC virus–infected B-lymphocytes (Houff *et al*, 1988) by alterations in the blood–brain barrier due to HIV (Power *et al*, 1993).
3. Facilitation of entry into the brain of JC virus–infected B-lymphocytes as a consequence of upregulation of adhesion molecules on the brain vascular endothelium due to HIV infection (Hofman *et al*, 1994).
4. Transactivation of JCV by the HIV *tat* protein (Tada *et al*, 1990).
5. Transactivation of JCV by cytokines and chemokines induced by HIV infection.

AIDS immunosuppression versus that seen with other disorders associated with PML

Perhaps the most simple explanation is that the nature, degree, and duration of immunosuppression occurring in the face of HIV infection is greater than that occurring in other conditions. However, given the rather substantial number of individuals with hematological malignancy, solid tumors undergoing chemotherapy, underlying tuberculosis, or status post organ transplantation and their potentially long survival, this explanation is unsatisfactory.

As PML is typically a late complication of HIV infection, the late immune abnormalities that accompany HIV infection will be reviewed briefly. The striking abnormality is a defect in both the numbers and function of CD4+ T cells. The loss of CD4+ T cells is by both direct and indirect mechanisms

(D'Souza and Fauci, 1999). Accompanying the loss of CD4+ T cells are other immunological defects. These include deficiencies in chemotaxis, monocyte-dependent T-cell proliferation, Fc-receptor function, C3 receptor–mediated clearance, and oxidative burst responses (D'Souza and Fauci, 1999). Infection of monocyte/macrophages with HIV has been demonstrated to result in impairment of antibody-dependent, cell-mediated cytotoxicity, intracellular antimicrobiocidal activity, and the induction of interferon alpha secretion. Abnormalities of B-cell activation are characteristic of HIV infection. There is a decrease in the number of circulating B cells early that progresses as the diseases advances. Additionally, in association with HIV infection, B cells secrete markedly high levels of cytokines. The specific immunological abnormality or abnormalities that lead to a predisposition to the development of PML remains unknown. A common thread to the underlying illnesses is a loss of effective cell-mediated immunity. One may not be able to rely on absolute numbers of CD4+ T cells as a marker of susceptibility, as PML has been observed in rare occasions in the face of normal CD4+ T cells in PML (Berger *et al*, 1998a) as well as in the absence of any underlying identifiable immunosuppressive disorder. In the Brooks and Walker review of PML, 5.6% of all cases were unassociated with known underlying disease (Brooks and Walker, 1984).

Following organ transplantation, pharmacological suppression of the immune system continues indefinitely. Therefore, the duration is similar to that of AIDS. However, as with other forms of immunosuppression, the specific nature of the impairment is likely different than that observed with HIV infection. For example, granulocytopenia is more common in the transplant population. It remains unknown if cytotoxic immune responses to viruses such as JCV remain unaltered in these patients.

Alterations in the blood–brain barrier and upregulation of adhesion molecules on cerebral endothelium in the face of HIV infection

Seroepidemiologic studies for JC virus reveal that the majority of the world's population develops antibody to this virus at an early age (Walker and Padgett, 1983). By middle adulthood, 80–90% of the population have IgG antibodies against JC virus and seroconversion rates have exceeded 90% in some urban areas (Walker and Padgett, 1983). It is likely that the initial infection is an inapparent upper respiratory tract or oropharyngeal infection (Monaco *et al*, 1998).

The reticuloendothelial system seems to be the sight of JC virus latency. By polymerase chain reaction and immunocytochemistry, the virus can be demonstrated in tonsils, spleen, lymph nodes, lymphocytes, lungs, and kidney (Berger and Major, 1999). *In situ* DNA hybridization has revealed JCV-infected cells near brain–blood vessels in the brain, in B lymphocytes in bone marrow (Houff *et al*, 1988), and in the brain (Major *et al*, 1990). PCR technology reveals the presence of JCV DNA in the peripheral blood lymphocytes of approximately 95% of patients with biopsy-proven PML (Tornatore *et al*, 1992). Other studies of the presence of JC viral DNA in the peripheral B cells in as many as 50% of immunosuppressed persons in comparison to approximately 5% of normal, healthy volunteers (Berger and Major, 1999). Concordant with the hypothesis that lymphocytes, particularly B cells, harbor JCV in a latent state and, upon activation, carry virus to the brain, has been the demonstration of nuclear DNA binding proteins in B cells that recognize important sites on the JCV regulatory region (Major *et al*, 1990). Using both gel retention assays and DNase footprinting experiments, several human B-cell lines have been described as permissive for JCV multiplication and possessing DNA binding proteins that recognize the same sequences on the JCV genome as the highly permissive human glial cells (Atwood *et al*, 1992). These proteins may not be identical, but may represent similar members of an entire family of transcription factors, and perhaps others not yet identified.

Unlike monocytes and T cells (Williams and Hickey, 1995), B cells do not cross the blood–brain barrier effectively (Williams and Hickey, 1995). Therefore, JCV-infected B cells would not be anticipated to cross the BBB and establish infection in the absence of other factors that assist this passage. The disruption of the BBB in the face of HIV infection and the expression of adhesion molecules on cerebral endothelial as a consequence of HIV infection are likely contributory. With respect to the former, abnormalities of the blood–brain barrier in the face of HIV infection have been observed in both laboratory models (Toneatto *et al*, 1999; Persidsky *et al*, 2000), pathologically (Petito and Cash, 1992; Dallasta *et al*, 1999), and by radiographic contrast enhancement (Berger *et al*, 2000). The expression of cytokines (Couraud, 1998; Woodman *et al*, 1999) or HIV Tat protein in perivascular space in association with HIV infection may contribute to an increased expression of adhesion molecules and the migration of B cells into the brain. Intracisternal injection of TNF-α and IL-1β into rats has been shown to increase BBB permeability (Quagliarello *et al*, 1991). Both of these cytokines are shown to be induced by HIV infection and Tat (Nath *et al*, 1991). These very same cytokines, which are induced in HIV-infected monocytes and astrocytes, induce the expression of endothelial cell expression of adhesion molecules. This is borne out by the observation that activated HIV-infected monocytes induce higher levels of E-selectin and VCAM-1 on brain microvascular endothelial cells as compared to activated uninfected monocytes (Nottet *et al*, 1996). Last, direct infection of brain endothelial cells by HIV may contribute to a disruption of the blood–brain barrier (Moses and Nelson, 1994).

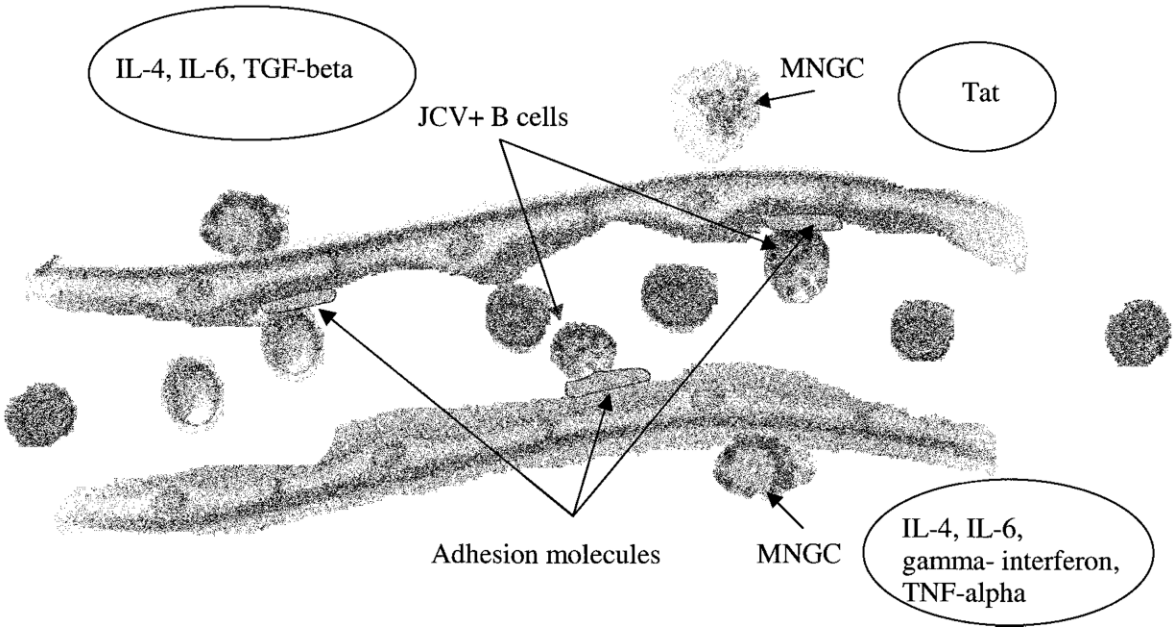

Figure 1 Interactions of cytokines, adhesion molecules, and HIV-Tat protein in the transmigration of JCV-infected cells across the blood–brain barrier. Products in upper ovals increase expression of adhesion molecules. Products in lower oval activate JC virus.

There appears to be a number of mechanisms by which HIV infection can potentially increase the migration of JC virus-infected B cells into the brain. These same mechanisms are not found in the presence of other immunosuppressive conditions that predispose to PML. Perhaps this facilitation of passage of B cells into the brain of HIV-infected persons may explain the increased frequency of PML with AIDS relative to other immunosuppressive conditions (see Figure 1).

HIV strains, PML, and genetic susceptibility

Our current knowledge is rudimentary with regard to interactions of various strains of HIV with JCV and with regard to genetic susceptibility to developing PML. However, some epidemiological studies raise important questions. Curiously, there seems to be a higher degree of prevalence of PML in white males compared to African American males (Holman *et al*, 1991). Additionally, there may be some geographical differences in the prevalence of PML. For example, PML is considered rare in Africa and a neuropathological study from Southern India suggests an incidence of 1% (Satishchandra *et al*, 2000). The population differences observed may be the consequence of the nature of medical care rendered. PML is typically observed in advanced HIV infection, and therefore, it is not unlikely that patients succumbing to other AIDS-related disorders early in the course of their infection may not live long enough to develop PML. In Africa and southern India, there is also a decreased prevalence on Kaposi's sarcoma and HIV encephalitis. Because the HIV Tat protein has been shown to play an important role in the pathogenesis of all of these complications of AIDS, one possibility might be that the HIV clade C virus Tat is not as potent as HIV clade B virus in its interactions with HHV-8, the etiological agent of Kaposi's sarcoma, or on causing neurotoxicity and glial cells activation that are important mediators of HIV encephalitis, or in its interactions with JCV. Certainly, secondary structural modeling shows that there may be important structural differences between the Tat proteins of HIV clade B and C viruses. These differences are present in the transactivating regions and the nuclear localizations sequence of Tat. However, functional studies are necessary to confirm the significance of these observations (see Figure 2).

JCV tissue tropism and virus replication

JCV has a marked specificity for glial cells and reactivation of latent virus in individuals suffering from immunosuppression can lead to selective infection and cytolytic destruction of myelin-producing oligodendrocytes and results in severe demyelination. The tissue tropism of JCV is at the level of transcription. One of the transcriptional regulators present in myelinating glial cells is Tst-1/SCIP/Oct-6, a member of POU domain family. This factor is required for normal development of myelinating cells (Arroyo *et al*, 1998). There is a striking correlation between expression of Tst-1 and replication of JCV likely by stimulation of the viral early and late gene promoters (Renner *et al*, 1994). The Tst-1/SCIP/Oct-6 functions synergistically with large T antigen of JCV. The J domain of T antigen is responsible for

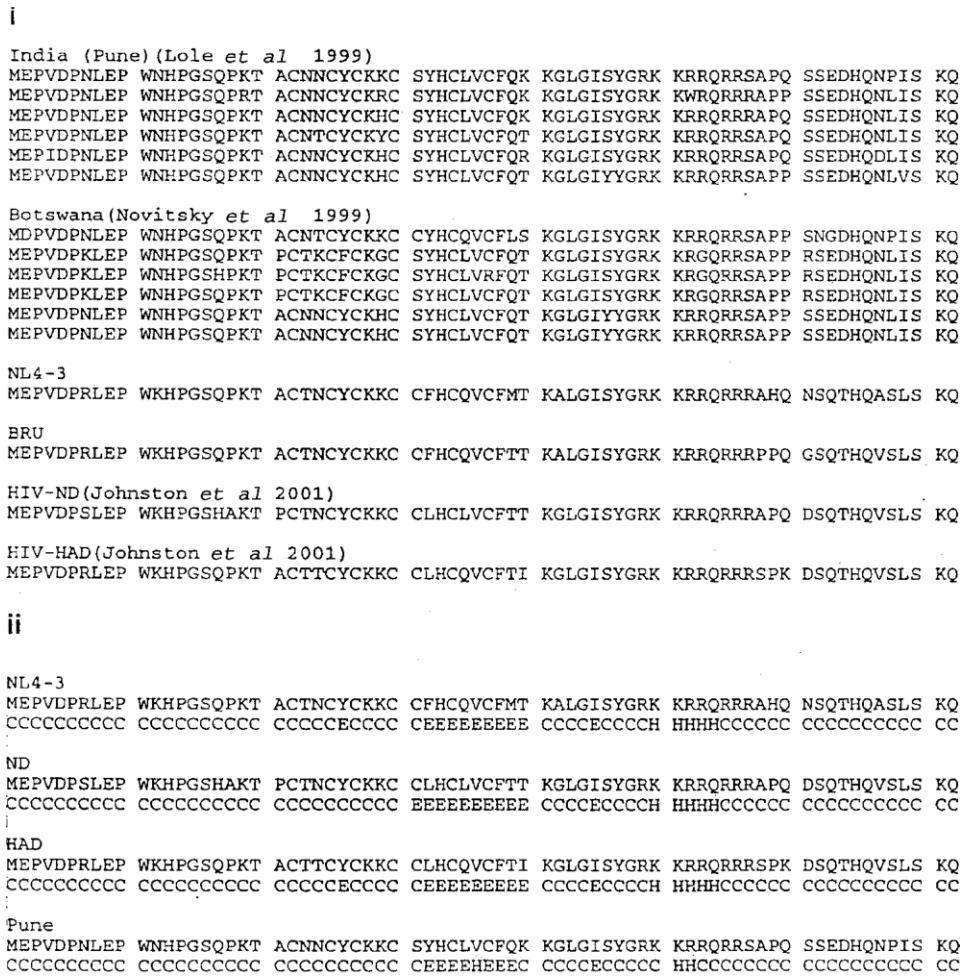

Figure 2 Comparison of Tat protein from HIV-1 clade B and C sequences. (i) Significant sequence differences are noted between clade C (India and Botswana) when compared to clade B Tat sequences are noted in positions 35 (Q-L; polar to nonpolar), 39 (T-Q; nonpolar to polar), and 66 (R-S; positive to negative charge). Clade B strains shown are from HIV strains NL4-3, BRU, and brain-derived sequences from a patient without dementia (HIV-ND) and with dementia (HIV-HAD). (ii) Secondary structure modeling is shown below the amino acid sequence, which demonstrates an interruption of the beta sheet (E) at position 36 with a helix (H) and the substitution of the helix with a loop (C) at positions 50–54 is noted in clade C Tat-Pune when compared to clade B Tat. The secondary structures of these regions in the clade B derived Tat proteins are well preserved. Secondary structure modeling was done using Predict Protein software.

the protein–protein interaction with Tst-1 (Sock *et al*, 1999). Keeping in mind the activation potential of HIV Tat protein on production of T antigen of JCV, it is necessary to determine the effect of Tat on Tst-1 and other glial specific factors that may regulate JCV expression.

Effect of cytokines on JC virus replication

Cytokines regulate gene expression by activating transcription factors via well-characterized signal transduction pathways. GBP-i is a novel inducible protein that binds to a double-stranded GGA/C-rich region of the transcriptional control region of JCV, specifically within the origin of viral DNA replication. GBP-i is distinct from previously characterized GC box–binding proteins with respect to both its sequence specificity and its electrophoretic mobility on native and denaturing gels. GBP-i responds within 90 min to phorbol myristate acetate stimulation; however, unlike typical phorbol myristate acetate-inducible factors, this rapid induction is regulated primarily at the transcriptional level. Further, the induction of GBP-i appears to be widespread and mediated by many inflammatory cytokines, including interleukin-1 beta, tumor necrosis factor alpha, gamma interferon, and transforming growth factor beta. Interestingly, the induced protein acts as a transcriptional repressor in its native context in the JCVL promoter (Raj and Khalili, 1994). However, it remains unknown if GBP-i is present in glial cells. Moreover, TNF-alpha may not be a major player in JCV replication (Atwood *et al*, 1995). Clearly, more experimental studies are needed to determine the role of cytokines in JCV replication.

Effect of HIV cytokine and chemokine induction

HIV infection induces a wide variety of cytokines, chemokines, and adhesion molecules in the brain (Nottet and Gendelman, 1995; Persidsky *et al*, 1997). There is also an upregulation of IL-8, RANTES, and several chemokine receptors in astrocytes following infection by HIV. Increased expression of these chemokines, along with their receptors, would not only have the effect of attracting monocytes and lymphocytes into the brain, but also establish an autocrine/paracrine loop for further activation of astrocytes that may contribute to the inflammatory state in the brain (Cota *et al*, 2000). The mechanisms by which HIV causes an inflammatory response are not fully understood. However, some of the HIV proteins have been implicated in mediating such responses. HIV Tat–derived peptide, when injected into rat brain, increased levels of proinflammatory cytokines (Philippon *et al*, 1994). Our *in vitro* studies show that Tat induces TNF-α and IL-1 in monocytes/macrophages and IL-6 in astrocytes. In fact, Tat was more potent than even LPS in inducing TNF-α production (Chen *et al*, 1997). Cytokine induction in both cell types is NF-κB dependent (Chen *et al*, 1997; Nath *et al*, 1999). The role of these cytokines in Tat-mediated neurotoxicity remains to be determined. Tat also induces MCP-1 expression in astrocytes, and the levels of this chemokine are elevated in the CSF and brains of patients with HIV dementia (Conant *et al*, 1998). We have also shown that Tat induces the transmigration of monocytes across an *in vitro* blood–brain barrier model. This Tat-induced transmigration can be blocked with antisera to MCP-1 (Weiss *et al*, 1999). Gp120, the envelope protein of HIV, can also cause cytokine induction in monocytes (Zembala *et al*, 1995) and glial cells (Ilyin and Plata-Salaman, 1997).

Detection of HIV Tat in PML and HIVE

Tat protein can be detected in brains of patients with HIV encephalitis (Hofman *et al*, 1994; Kruman *et al*, 1999) and in rhesus macaques with encephalitis due to a chimeric strain of HIV and the simian immunodeficiency virus (Nath *et al*, 1998). Additionally, mRNA levels for *tat* and the envelope gene, *env*, are also elevated in brain tissue of patients with HIV dementia (Wesselingh *et al*, 1993; Wiley *et al*, 1996). A unique feature of this protein is that it does not become incorporated into the structure of the virus but is actively released in two forms (Tat1-72 formed by the first exon only and Tat1-86-101 formed by the first and second exons) by persistently and productively infected cells without rupture of the cell membrane (Chang *et al*, 1997). Consistent with this observation, Tat can also be detected in the serum of patients with HIV infection in concentrations of about 1 ng/ml (Westendorp *et al*, 1995) and in the extracellular matrix in the perivascular compartments in the brain. Recent data from our laboratory also confirm the presence of Tat in supernatants of HIV-infected monocytes (Turchan *et al*, 2001).

Effect of Tat on JC virus replication

The HIV-1-encoded trans-regulatory protein *Tat* increases the basal activity of the JCV late promoter, JCVL, in glial cells (Tada *et al*, 1990). The ability of HIV-1 Tat protein and JCV T antigen in inducing transcription from the JCV late promoter, JCVL, were compared. A JCVL promoter-chloramphenicol acetyltransferase plasmid (pJCL-CAT) was transfected into human glial cells alone or together with plasmids producing T antigen and Tat protein. CAT enzyme activity obtained from the transfected cells indicated that both JCV T antigen and HIV-1 Tat proteins stimulated JCV late gene expression. However, the level of induction mediated by Tat protein was significantly higher than that obtained with T antigen (Chowdhury *et al*, 1990).

A specific RNA sequence located in the leader of all human immunodeficiency virus type 1 (HIV-1) mRNAs, termed the transactivation response element, or TAR, is a primary target for induction of HIV-1 long terminal repeat activity by the HIV-1-derived trans-regulatory protein, Tat. JCV contains sequences in the 5′-end of the late RNA species with an extensive homology to HIV-1 TAR. Site-directed mutagenesis studies show that critical G residues required for the function of HIV-1 TAR that are conserved in the JCV-TAR homolog play an important role in Tat activation of the JCV promoter. In addition, *in vivo* competition studies suggest that shared regulatory components mediate Tat activation of the JCV late and HIV-1 long terminal–repeat promoters. These results suggest that the TAR homolog of the JCV late promoter is responsive to HIV-1 Tat induction and thus may participate in the overall activation of the JCV late promoter mediated by this transactivation (Chowdhury *et al*, 1990; Chowdhury *et al*, 1992). Further, JCV activation at transcriptional level is mediated by interaction of several inducible regulatory proteins such as NF-κB, C Jun/Ap-1, and NF-1 (Amemiya *et al*, 1992; Wortman *et al*, 2000). These regulatory proteins can be induced by HIV Tat protein in glial cells or by cytokines that are induced by HIV proteins in glial cells (Atwood *et al*, 1995; Chen *et al*, 1997). The elusive behavior of Tat protein to enter into other cells, as well as to induce production of cytokines, may thus make the JCV dormant in oligodendrocytes or astrocytes target for activation and may be involved in the neuropathogenesis of PML in patients with HIV infections.

Co-infection of HIV and JC virus

Valle *et al* (2000) demonstrated high-level expression of the JCV capsid protein, VP1, in oligodendrocytes and to some degree in astrocytes of AIDS with PML. In HIV-1+ samples, expression of HIV-1 core protein, p24, was detected in perivascular monocytic

cells and to a lesser extent in astrocytes and endothelial cells. A lack of p24 expression in oligodendrocytes suggested no infection of these cells with HIV-1. Interestingly, HIV-1 Tat was detected in various infected cells as well as in uninfected oligodendrocytes from HIV-1+ tissue, supporting the earlier *in vitro* findings that secreted Tat from the infected cells can be localized in the neighboring uninfected cells. The presence of Tat in oligodendrocytes was particularly interesting as this protein can upmodulate JCV gene transcription and several key cell cycle regulatory proteins including cyclin E, Cdk2, and pRb. These data provide *in vivo* evidence for a role of HIV-1 Tat in the pathogenesis of AIDS/PML by acting as a positive regulatory protein that affects the expression of JCV. Interestingly, Tat also interacts with MEF-1/Purα, a DNA binding transcription factor (Gallia *et al*, 1999; Wortman *et al*, 2000), which activates the myelin basic protein gene promoter (Tretiakova *et al*, 1999b). One of the factors contributing to demyelination in PML probably is the downregulation of myelin basic protein transcription by the JCV T antigen (Tretiakova *et al*, 1999a). Therefore, Tat apart from JCV activation may quench the MEF-1/Purα factor and inhibit its activation potential for the myelin basic protein gene promoter, or Tat may stimulate the JCV early promoter utilizing Purα, resulting in JCV T antigen production. Above all, JCV T antigen has been demonstrated in oligodendrocytes where Tat has also been seen (Valle *et al*, 2000).

Release of Tat from HIV-infected cells and uptake by uninfected cells

Tat protein is produced during HIV infection (Mace and Gazzolo, 1993) and can be released by unruptured HIV-infected lymphocytes (Ensoli *et al*, 1990; Ensoli *et al*, 1993) and monocytic cells (Tardieu *et al*, 1992). The relative amounts of Tat produced by lymphocytes and monocytes is similar (Robert-Guroff *et al*, 1990). Furthermore, extracellular Tat is functionally active (Ensoli *et al*, 1993). Tat exits from intact cells through a leaderless secretion pathway that shares several features with that of fibroblast growth factor. The released Tat binds to heparan sulfate proteoglycans through its basic region and this determines its storage into the extracellular matrix (Chang *et al*, 1997). It may also cause activate metalloproteases in the extracellular space, leading to degradation of the blood–brain barrier (Johnston *et al*, 2001) and thus potentially lead to an influx of JCV-infected cells. Concentrations of Tat are approximately 1 ng/ml in the serum of HIV-infected patients and 4 ng/ml in the conditioned media of HIV-infected cells (Westendorp *et al*, 1995; Albini *et al*, 1998).

Tat can be readily taken by several types of cells. We measured the cellular uptake of length Tat (amino acids 1 to 86) Tat(1–86) and Tat(1–72) (first exon) in human fetal astrocytes, neuroblastoma cells, and human fetal neurons and demonstrated that the uptake of Tat(1–72) without the second exon was much lower than that of Tat(1–86). This suggests an important role for the C-terminal region of Tat for its cellular uptake (Ma and Nath, 1997). Following uptake, Tat is readily detected in the nucleus of uninfected cells (Ensoli *et al*, 1993; Ma and Nath, 1997). Tat binds to low-density lipoprotein receptor-related protein (LRP), which promotes efficient uptake of Tat. LRP-mediated uptake of Tat is followed by translocation to the neuronal nucleus (Liu *et al*, 2000). In one study, a Tat peptide was injected intraperitoneally in rats and detected in the brain 8 hours later, demonstrating its ability to cross the intact blood–brain barrier (Schwarze *et al*, 1999).

Summary and therapeutic approaches

In summary, the epidemiology of PML suggests that dysfunction of cellular immune response is the single most important determining factor that predisposes to the development of PML, although gender, genetic factors, and viral strains may also play a role. PML seems to occur disproportionately in the HIV-infected population in contrast to other populations with underlying immunosuppressive conditions. Despite this strong association of HIV and PML, very few experimental studies have addressed this interaction. Nonetheless, these experimental studies suggest that the pathophysiology of PML in HIV-infected patients may be intimately related to products released from HIV-infected cells. Thus, in the absence of specific anti-JCV therapy, any approach aimed at controlling HIV replication would potentially be useful in these patients. Future experimental approaches may include therapeutic vaccines using HIV gene products. In this regard, the HIV Tat protein may be an excellent candidate because it is an early gene product, and immune responses targeted against this protein could potentially not only prevent its interaction with JCV but may also cause cytolysis of HIV-infected cells before viral replication occurs in these cells. Certainly, Tat vaccine seems to be well tolerated in preliminary human studies (Gringeri *et al*, 1998; Gringeri *et al*, 1999).

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