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Human polyomavirus JCV and expression of myelin genes

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Myelin basic protein (MBP) is a major component of the myelin sheath of both the central and peripheral nervous systems. A number of neurological diseases in humans are associated with demyelination of the central and/or peripheral nervous systems, including multiple sclerosis and its variants such as acute disseminated encephalomyelitis (AD), acute hemorrhagic leukoencephalopathy, and idiopathic polyneuritis (Guilliame-Barre syndrome), as well as tropical spastic paraparesis (TSP), and progressive multifocal leukoencephalopathy (PML). Multiple sclerosis (MS) is perhaps the most common demyelinating disease and is one of great importance to the clinical neurologist. The underlying cause of the demyelination seen in multiple sclerosis patients is unknown. However, patients frequently have unusually high antibody titers to a number of common viruses, leading to speculation that viral infections may participate in the pathogenesis of MS. On the other hand, studies on maternal and paternal twins have suggested the involvement of genetic factors in the predisposition of an individual toward developing MS. PML, once a rare demyelinating disease of elderly patients with lymphoproliferative disorders, is now a much more common disease affecting patients of all ages due to the increasingly widespread use of immunosuppressive chemotherapy and the prevalence of AIDS. PML is the result of productive infection of oligodendrocytes, the myelin producing cells of the CNS, with the human polyomavirus, JCV. In this article, we have focused our attention on PML, and the role of JCV in disrupting myelin sheaths by affecting myelin basic gene expression, ultimately leading to demyelination. *Journal of NeuroVirology* (2000) **6,** S92–S97.

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Progressive multifocal leukoencephalopathy (PML) is a demyelinating disease of the central nervous system (CNS), which is most often seen in individuals with immunosuppressive diseases that result in impaired T cell-mediated immunity (Berger and Concha 1995). At present, the most frequent underlying disease for PML is acquired immunodeficiency syndrome (AIDS) (Berger *et al*, 1987). Other conditions associated with PML include Hodgkin's lymphoma and other lymphoproliferative diseases, myeloproliferative diseases, renal and bone marrow transplantation, exogenous immunosuppression, autoimmune diseases, granulomatosis, inflammatory diseases such as sarcoidosis and tuberculosis, and antineoplastic therapy. In a small percentage of patients, PML occurs without any underlying disease (Berger and Concha 1995; Brooks and Walker 1984).

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Macroscopically, the common feature of PML is demyelination of the CNS which results from the cytolytic destruction of oligodendrocytes, the myelin producing cells of the CNS. PML usually begins subacutely with either unifocal or multifocal neurologic disorders. Regardless of the initial presentation, patients develop multiple neurologic signs reflecting the multifocal areas of demyelination usually detectable by magnetic resonance imaging (MRI). The classic triad most often seen in PML patients include hemiplegia, visual disturbances such as cortical blindness and homonymous hemianopsia, and subcortical dementia (Berger and Concha 1995; Brooks and Walker 1984). Patients may also develop neurologic symptoms including dipoplia, monoplegia, bradykinesia/akinesia, rigidity, and sensory disturbances. Cerebellar dysfunction as a result of involvement of the cerebellar white matter has been frequently seen in AIDS patients and can result in ataxia and dysarthria. The clinical symptoms and signs result from multifocal

areas of demyelination in the white matter of the CNS. Although the cerebral hemispheres are most commonly involved, PML may develop in the white matter in any part of the CNS axis. The prominent histopathological finding of PML is multiple foci of myelin loss, hyperchromatic, enlarged oligodendroglial nuclei, and enlarged bizarre astrocytes with lobulated hyperchromatic nuclei (Figure 1). The enlarged astrocytes occasionally contain mitotic figures quite similar to malignant astrocytes of pleomorphic glioblastomas (Richardson and Webster, 1983).

The human polyomavirus, JCV, is the established etiological agent of PML (Berger and Concha, 1995; Frisque and White 1992; Major *et al*, 1992; Padgett *et al*, 1971), possessing a circular genome of double-stranded DNA with an icosachedral capsid. As shown in Figure 2, the prototype strain of JCV, Mad-1, contains 5130 nucleotides (Frisque *et al*, 1984), which can be functionally divided into three regions; an early coding region, a late coding region, and a regulatory (non-coding) region (for review see Raj and Khalili 1995). The regulatory region, which contains the promoter/enhancer for early and late gene transcription as well as the origin of DNA replication is located between the early and late coding regions. The viral early genes encode the viral regulatory protein, T-antigen, whereas the late genes encode the structural capsid proteins VP1, VP2, and VP3 as well as Agnoprotein (Raj and Khalili, 1995).

The viral lytic cycle begins by expression of the viral early protein, T-antigen, which occurs before replication of viral DNA. T-antigen is a multifunctional protein which interacts with several host regulatory proteins and orchestrates subsequent steps of the viral life cycle including viral DNA replication and activation of late gene transcription by manipulating host gene expression and/or function. The products of the late genes, the capsid proteins, accumulate in the nucleus and associate with the replicated viral DNA forming virions which, in turn, lyse the host cells. Thus, T-antigen acts as the central regulator of the viral lytic cycle. To exert its regulatory action on both DNA replication and gene transcription, JCV T-antigen requires the participation of host factors. As JCV displays a narrow species and host cell tropism which restricts its productive replication to human brain cells, many laboratories, including our own,

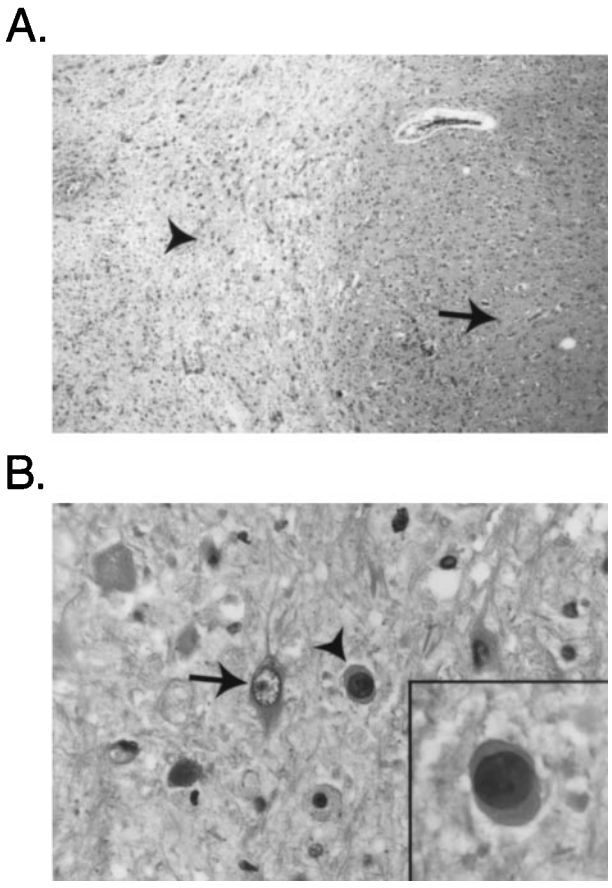

Figure 1 Pathological features of Progressive Multifocal Leukoencephalopathy. (**A**) Hematoxylin and eosin staining of a PML lesion at low magnification demonstrates plaques of demyelination (arrowhead) adjacent to areas of normal myelination (arrow). (**B**) The appearance of bizarre astrocytes (arrow) and oligodendrocyte inclusion bodies (arrowhead and inset) are readily noted within active lesions at higher magnification. (Original magnification: **A**, ×100; **B**, ×400; inset×1000).

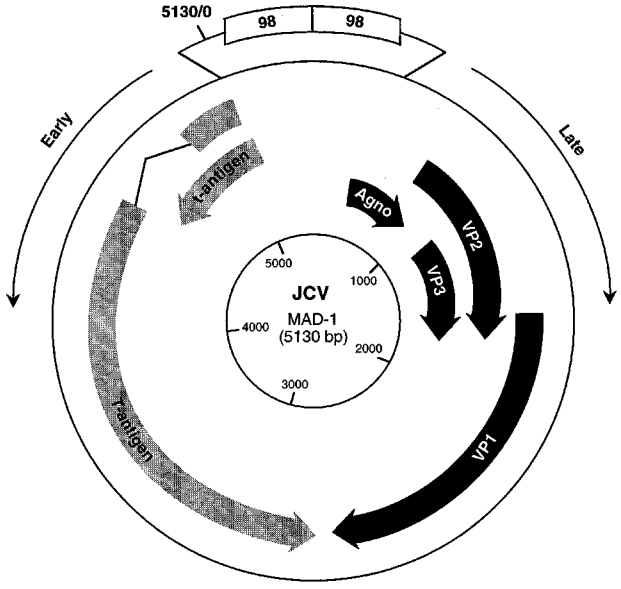

Figure 2 Genomic organization of the human polyomavirus JCV. Schematic depiction of the JCV genome illustrating coding regions for the early structural proteins, large and small T-antigen (light grey), and the late capsid proteins, VP-1, VP2, and VP3, as well as the Agnoprotein (dark grey). The inside circle indicates the map positions according to Frisque *et al* (1984) with 0 designated at the *Eco*RI site. The outer circle represents the regulatory non-coding region of the viral genome which contains two 98 base pair repeat sequences and the origin of viral DNA replication.

have focused their attention on deciphering the molecular basis for host cell restriction of JCV and have identified participant factors (for review see Frisque and White 1992; Raj and Khalili 1995). Comparison of JCV expression in CNS (glial-origin cells) and non-glial cell types has demonstrated that JCV early promoter activity is significantly higher in glial cells (Feigenbaum *et al*, 1987; Kenney *et al*, 1984; Lashgari *et al*, 1989; Tada *et al*, 1989) suggesting that the promoter/enhancer region of JCV acts in a cell type-specific manner. Because activation of the early genes is essential for productive infection, non-glial tissues, which cannot sufficiently support expression of the JCV early genome (i.e. large T-antigen) fail to stimulate viral DNA replication or late gene transactivation. In support of this hypothesis, earlier studies have indicated that JCV DNA replication and late gene transcription occur in non-glial cells which contain an exogenous source of the JCV early gene product (Feigenbaum *et al*, 1987; Lashgari *et al*, 1989). These experiments suggest that the host cell restriction of JCV in humans is predicated on the ability of glial cells, but not non-glial cells, to produce transcription factors which transactivate early gene expression resulting in production of T-antigen.

The observation that the non-coding regulatory region of JCV is responsible for the host range and glial cell tropism prompted many laboratories to perform genetic and molecular biology studies to identify important *cis*-acting regulatory elements within the viral promoter and define the *trans*-acting cellular factors from CNS cells that recognize specific sequences within the regulatory region of JCV to induce viral entry and late gene expression. The regulatory region of JCV contains the origin of viral DNA replication and several transcriptional control elements including the binding site for nuclear factor-kappa B (NF-κB) and two 98 bp tandem repeats positioned on the early and late sides of the viral origin, respectively (Figure 3). Evidently, NF-κB enhances JCV early and late gene transcription at the basal level and is responsive to phorbol-12-myristate-13-acetate (PMA) and TNF-α induction (Mayreddy *et al*, 1996; Ranganathan and Khalili 1993). The 98 bp tandem repeats contain several regulatory regions which are recognized as distinct DNA binding motifs (for review see Raj and Khalili 1995).

A region designated the lytic control element (LCE) has received special attention as it differentially affects viral gene expression by positively and negatively regulating JCV early and late promoter transcription (Tada and Khalili 1992; Tada *et al*, 1991), and plays an important role in viral DNA replication (Chang *et al*, 1994; Lynch and Frisque, 1990). This region contains a pentanucleotide repeat sequence (AGGGAAGGGA) in juxtaposition to a poly(dA/dT) tract which displays a single-stranded configuration (Amirhaeri *et al*, 1988). Earlier studies from our laboratory have led to the identification of two single-stranded DNA-binding proteins, YB-1 and Purα, with the ability to interact with nucleotides on the early (C/T-rich) and late (G/A-rich) strand of the LCE. Of interest is the notion that the unique interplay between these proteins and JCV T-antigen determine their binding activities with the LCE. As T-antigen, through its interaction with these and other cellular factors, navigates the process which ensures cytolytic destruction of oligodendrocytes infected with JCV, it may directly or indirectly influence host function and contribute to the pathological abnormalities which are seen in the PML brain.

As a model for understanding the pathogenesis of JCV-induced demyelination, transgenic mice were

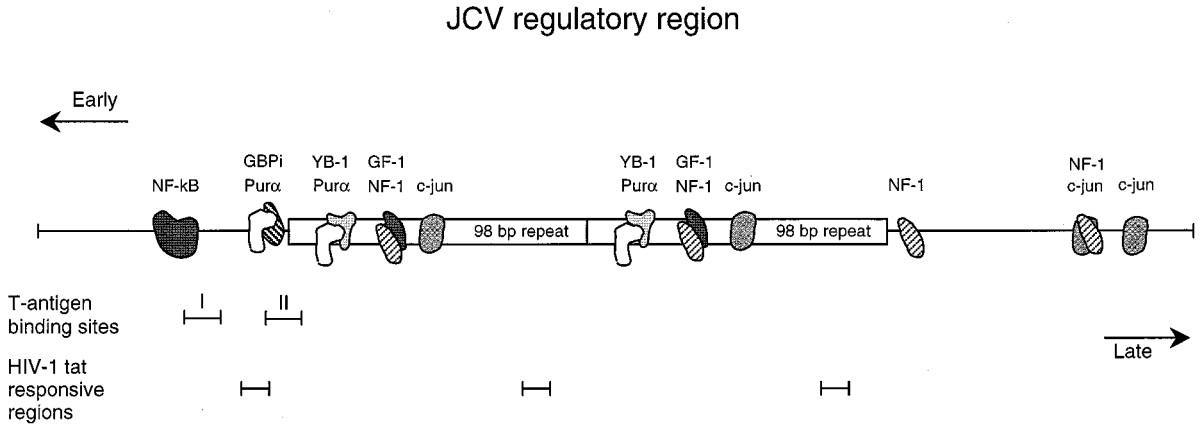

Figure 3 Structural organization of the JCV regulatory non-coding region. The schematic in the center of the figure illustrates the structural organization of the JCV promoter region, with boxed 98 base pair motifs representing binding sites for both potential and known transcription factors. Established binding sites for several well-characterized cellular proteins are indicated. The direction of early and late gene synthesis is shown at either side of the schematic. Also depicted are large T-antigen binding sites and HIV-1- tat-responsive regions.

constructed by insertion of the JCV early region, which includes the viral regulatory proteins large T-antigen and small t-antigen, expressed under control of their natural JCV regulatory sequences (Small *et al*, 1986a,b). The transgene encompassed the JCV origin of replication, the transcriptional control elements, and the large and small T-antigen coding sequences (Small *et al*, 1986b). Of note, the genes encoding the structural proteins VP1, VP2, and VP3 were removed, thus preventing any possibility of productive viral infection resulting in an ideal animal model with which to determine the role of the viral T-antigens in demyelination.

Two lines of transgenic mice, JC48 and JC91, were produced which exhibited neurological disorders due to dysmyelination of the CNS (Small *et al*, 1986a). Electron microscopy revealed a reduction in the thickness of the myelin sheath as well as abnormal compaction of existing myelin lamellae. In these mice, large T-antigen was expressed at high levels in the brain, as determined by Northern blot analysis. These studies indicated that the severity of the neurological disorder, as determined both by physical phenotype (ataxia) and extent of dysmyelination in the CNS, was directly correlated with the level of T-antigen RNA expression in the brain (Small *et al*, 1986a). Furthermore, T-antigen RNA was detected by *in situ* hybridization of brain tissue and was found to be expressed predominantly in oligodendrocytes in white matter tracts (Trapp *et al*, 1988).

The molecular mechanisms of JCV T-antigen induced dysmyelination and its effect on myelin gene expression is not clear. In general, papovaviral early proteins exhibit regulatory activity on transcription of several eukaryotic promoters including their own early and late genes. Accordingly, our previous studies have demonstrated that JCV T-antigen stimulates transcription directed by JCV late regulatory sequences (Lashgari *et al*, 1989). Polyomavirus T-antigens have also been shown to be involved in the differential regulation of transcription of viral early genes and in determining transcription initiation sites. Evidently, T-antigen acts during the lytic cycle alternatively to suppress expression from one initiation site and stimulate expression from others, resulting in a net shift of the RNA start site (Khalili *et al*, 1987).

Neuropathological analysis of the JCV T-antigen mice revealed dysmyelination in the CNS and high levels of JCV T-antigen in oligodendrocytes. In addition, immature oligodendrocytes and hyperproliferating astrocytes with abnormal morphologies were also observed in these mice (Trapp *et al*, 1988). These observations demonstrate that dysmyelination in the CNS of transgenic animals, and perhaps demyelination in PML patients, is related at least partially to T-antigen expression in CNS cells, and that T-antigen may affect the normal structure and function of glial cells. In support of this notion, results from transgenic mice expressing polymomavirus large T-antigen have indicated that the expression of T-antigen in astrocytes may alter glial-glial interaction causing impaired oligodendroglial development and eventually dysmyelination of the brain (Baron-van Evercooren *et al*, 1992). More recently, we have developed a cell culture system derived from a bipotential glial cell line, CG-4, that express JCV T-antigen (Tretiakova *et al*, 1999). The salient feature of CG-4 cells rests in their ability to differentiate *in vitro* into either mature oligodendrocytes or type 2 astrocytes under appropriate inducing conditions (Lillien *et al*, 1990; Noble *et al* 1990). The expression of T-antigen in CG-4 cells dramatically effects the morphology of the differentiated astrocytes which normally contain a round cell body and thin radial processes. The T-antigen producing astrocytes exhibit a fibroblastoid shape with significantly less processes, whereas differentiated T-antigen-negative oligodendrocytes are characterized by a phase-bright cytoplasm and numerous long processes branching extensively into a complex network. The T-antigen-producing oligodendrocytes showed much less complicated and reduced number of processes with abnormal nuclei. Further molecular biological studies have revealed the deregulation of several proteins including cyclins and their associated kinases in cells expressing T-antigen. As oligodendrocytes are responsible for synthesis of myelin and expression of myelin-associated proteins, JCV T-antigen may affect the level of myelin gene production in these cells. Interestingly, it was noted that myelin-specific genes including myelin basic protein (MBP) in the brains of JCV T-antigen transgenic mice were decreased at the mRNA level suggesting that T-antigen may impair transcriptional regulation of the MBP promoter (Haas *et al*, 1994). Similar to other cellular genes, transcription of the MBP gene is regulated by upstream promoter sequences which have the ability to interact with specific DNA-binding transcription factors (Lashgari *et al*, 1990; Devine-Beach *et al*, 1990). Previous studies from our laboratory have identified a cellular protein, named MEF-1, from mouse brain nuclear extract that binds to a specific GC-rich region of the MBP promoter and stimulates

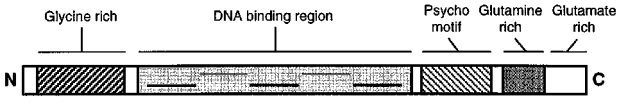

Figure 4 Schematic diagram of the single-stranded DNA-binding protein, Purα. Several of the modular domains of Purα are demonstrated, including the central aromatic and basic repeats interspersed with acidic leucine-rich repeats within the DNA binding region (indicated as thick and thin horizontal lines, respectively). The amino terminal glycine-rich region, the amphipathic α-helix region, and the glutamate-glutamine-rich region near the carboxy terminus are also shown.

its expression both *in vivo* and *in vitro* (Haas *et al*, 1993; 1995). Results from amino acid analysis of MEF-1 along with several of its characteristics indicate that this protein is the single-stranded DNA-binding protein, Purα (Haas *et al*, 1995). Purα interacts preferentially with single-stranded DNA containing the GGN motif (Figure 4). This protein has also been shown to interact with several viral and cellular proteins including the HIV-1 Tat protein (Krachmarov *et al*, 1996), the JCV early protein T-antigen (Chen *et al*, 1995), and the product of the retinoblastoma gene Rb (Johnson *et al*, 1995). Since protein-protein interaction may play a major role in the activity of this regulatory protein, the level of expression and the association of Purα with JCV T-antigen in the brains of dysmyelinated mice transgenically expressing T-antigen was examined. Results from these studies have indicated that Purα in extracts from transgenic mice brains is associated with JCV T-antigen (Tretiakova *et al*, 1999). Interestingly, infection of oligodendrocytes with JCV in cell culture results in the inhibition of Purα gene expression, suggesting that at least two distinct mechanisms which involve interaction of the viral early protein with Purα and suppression of Purα expression may participate in JCV-induced hypomyelination of the CNS. Prior to active viral infection, expression of JCV T-antigen and its association with Purα may decrease the level of MBP gene expression and myelination. We have previously demonstrated that association of Purα with T-antigen also decreases the ability of T-antigen to transactivate the JCV late promoter (Gallia *et al*, 1998), an event which is important for a productive JCV lytic cycle. Thus, one can envision a model in which at the earlier stage of the disease when the level of T-antigen is low, interaction of T-antigen with Purα may prevent Purα from exerting its regulatory activity in oligodendrocytes. This interaction, which also blocks T-antigen from stimulating the late stage of the viral infection cycle, prolongs the early stage and permits continuous production of T-antigen in the affected cells. As the level of T-antigen is increased, the unoccupied protein eventually navigates the virus through the lytic cycle, causing cytolytic destruction of oligodendrocytes. Consistent with a number of animal viruses, the lytic infection of JCV seems to be associated with inhibited expression of some cellular genes including MEF-1/Purα.

Thus, based on the *in vivo* animal and *in vitro* cell culture models, it is conceivable to hypothesize that the expression of JCV T-antigen, in both oligodendrocytes and astrocytes, may have several effects which accounts for the pathological damage in the brains of PML patients. On one hand, in concert with cellular factors, T-antigen orchestrates the JCV lytic cycle and results in the destruction of myelin producing cells, and on the other hand, it may impair the normal development and function of oligodendrocytes and astrocytes by associating with cell regulatory proteins and interfering with their regulatory actions. The use of such models of JCV T-antigen induced demyelination will enable a better understanding of the events at the cellular as well as the molecular level which occur during the process of demyelination.

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