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Human Polyomavirus Receptor Distribution in Brain Parenchyma Contrasts with Receptor Distribution in Kidney and Choroid Plexus

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The human polyomavirus, JCPyV, is the causative agent of progressive multifocal leukoencephalopathy, a rare demyelinating disease that occurs in the setting of prolonged immunosuppression. After initial asymptomatic infection, the virus establishes lifelong persistence in the kidney and possibly other extraneural sites. In rare instances, the virus traffics to the central nervous system, where oligodendrocytes, astrocytes, and glial precursors are susceptible to lytic infection, resulting in progressive multifocal leukoencephalopathy. The mechanisms by which the virus traffics to the central nervous system from peripheral sites remain unknown. Lactoseries tetrasaccharide c (LSTc), a pentasaccharide containing a terminal α2,6—linked sialic acid, is the major attachment receptor for polyomavirus. In addition to LSTc, type 2 serotonin receptors are required for facilitating virus entry into susceptible cells. We studied the distribution of virus receptors in kidney and brain using lectins, antibodies, and labeled virus. The distribution of LSTc, serotonin receptors, and virus binding sites overlapped in kidney and in the choroid plexus. In brain parenchyma, serotonin receptors were expressed on oligodendrocytes and astrocytes, but these cells were negative for LSTc and did not bind virus. LSTc was instead found on microglia and vascular endothelium, to which virus bound abundantly. Receptor distribution was not changed in the brains of patients with progressive multifocal leukoencephalopathy. Virus infection of oligodendrocytes and astrocytes during disease progression is LSTc independent. *(Am J Pathol 2015, 185: 2246—2258; http://dx.doi.org/10.1016/j.ajpath.2015.04.003)*

The human polyomavirus (JCPyV) is the causative agent of progressive multifocal leukoencephalopathy (PML), a rapidly progressing, often fatal neurodegenerative disease. Although PML is rare, JCPyV infection is widespread, infecting approximately 50% to 80% of the healthy adult population.[1,2] As the initial infection is asymptomatic, the mode of JCPyV transmission is unknown. The virus establishes a persistent infection in the kidney and urinary tract of immunocompetent hosts,[3] and about 20% of these infected individuals shed virus in their urine.[4] JCPyV DNA has also been detected in other tissues, including B lymphocytes in the bone marrow, tonsillar stromal cells, lungs, spleen, and brain,[5—13] suggesting additional sites of viral persistence. The route of viral transmission from the initial site(s) of infection and latency to the central nervous system (CNS), the main site of pathogenesis, is not clearly understood.

Under conditions of immunosuppression, JCPyV infects and destroys the myelin-producing oligodendrocytes, resulting in demyelination, which is the hallmark of this fatal disease; to a

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lesser degree, astrocytes and neurons are infected as well.[14,15] When PML was first described, it was a rare disease that primarily affected patients with B-cell lymphoproliferative disorders.[16,17] During the AIDS pandemic, the prevalence of PML in patients rose significantly, with 3% to 5% of HIV/AIDS patients developing PML.[18,19] With the advent of combined antiretroviral therapy, the number of HIV/AIDS patients with PML has declined, although it has decreased less significantly than that of other opportunistic infections.[20] While the occurrence of PML historically has been linked to HIV/AIDS, recently the rate of PML has risen again with the introduction of immunomodulatory therapy for autoimmune diseases, such as multiple sclerosis, rheumatoid arthritis, psoriasis, and Crohn disease.[21–25] PML has been reported to occur in patients receiving treatment with drugs including the monoclonal antibodies natalizumab, efalizumab, and rituximab.[22,26] One action of these therapies is to inhibit leukocyte migration into the CNS, suggesting that a key to JCPyV pathogenesis in the brain is the suppression of cells that normally perform immune surveillance. In addition to PML, JCPyV causes other diseases of the CNS, including JCPyV granule cell neuronopathy[27] and JCPyV encephalopathy,[28] and has been associated with isolated cases of JCPyV-associated nephropathy in kidney transplant recipients.[29–32]

JCPyV has a circular, double-stranded DNA genome that is enclosed by a nonenveloped, icosahedral capsid, which is composed of three proteins, viral proteins (VP)-1, -2, and -3.[33] VP1 is the main component of the capsid and is the primary means by which the virus engages receptors to initiate infection of host cells.

JCPyV requires at least two known functional receptors for attachment and subsequent entry. Previous *in vitro* experiments have demonstrated that the virus initially binds to an α2,6 sialic acid on the cell surface.[34–36] Crystallographic and functional studies with VP1 demonstrated that JCPyV VP1 binds to the host cell via the α2,6-linked glycan lactoseries tetrasaccharide c (LSTc).[37] Although LSTc recognition is required for JCPyV attachment, it is not sufficient for viral infection. In addition to engaging LSTc on the cell surface, JCPyV entry requires the presence of a serotonin (5-HT)-2 receptor family member. Virus internalization in a nonpermissive cell line was markedly enhanced when the cell line was transfected with any of the three 5-HT_2 receptor family subtypes, 5-HT_{2A}, 5-HT_{2B}, or 5-HT_{2C}, and shown to act early in the virus life cycle by facilitating virus entry.[38,39] JCPyV infection is blocked by antibodies to 5-HT_2s and other specific inhibitors of these receptors.

Despite JCPyV being a significant human pathogen, many questions about its etiology remain unresolved. The species specificity of JCPyV is highly restricted to humans, an obstacle that has stymied efforts to develop an animal model to follow the path of the virus from initial infection to CNS penetration.

The current study focused on identifying determinants of tissue tropism of JCPyV in two known sites of JCPyV infection, the brain and the kidney, using labeled virus, and identified receptors as markers to trace JCPyV interaction with specific cell types in the human host. This work has relevance in understanding the basic mechanism by which JCPyV engages its target cell(s) in the host and its route to the CNS, as well as clinical interest in that it suggests possibilities for specific interference of infection.

Materials and Methods

Virus Purification and Labeling

Generation of the JCPyV virus strain Mad-1/SVEΔ has been described previously.[40,41] Purified JCPyV was labeled with Alexa Fluor 488 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Labeled virus was added to kidney sections at concentrations of 2 and 20 μg/mL on brain sections.

Tissue Preparation and IHC Staining

Human brain (from frontal and parietal lobe), choroid plexus, meningeal, and kidney tissues (Table 1) were obtained from the National NeuroAIDS Tissue Consortium[42] and from Rhode Island Hospital (Providence, RI) in accordance with protocols approved by the Institutional Review Boards at Brown University (Providence, RI) and Rhode Island Hospital. Neural tissues selected as normal controls were from patients who were HIV-seronegative and died of causes not related to HIV/AIDS. Formalin-fixed, paraffin-embedded slides were deparaffinized in xylene followed by a series of graded ethanol washes and subjected to sodium citrate antigen retrieval if necessary. Fresh-frozen brain tissues were embedded in OCT, sectioned, and fixed for 10 minutes in 70% acetone and 30% ethanol at 4°C before staining. Sections were stained with antibodies against mucin (MUC)-1 (sc-53377; Santa Cruz Biotechnology, Dallas, TX), 5-HT_{2A} [S1001-16 (US Biological, Salem, MA) and LS-A1106 (LSBio, Seattle, WA)], 5-HT_{2B} [S-1001-17A (US Biological) and HPA012867 (Sigma, St. Louis, MO)], 5-HT_{2C} [AB9507 (Millipore, Billerica, MA) and LS-A1119 (LSBio)], T antigen (Ab-2; Millipore), JCPyV VP1 (pAb 597[43]; gifted by Dr. Edward Harlow, Harvard Medical School, Boston, MA), carbonic anhydrase II[44] (PC076; The Binding Site, Birmingham, UK), glial fibrillary acidic protein (G-A-5; Sigma), OLIG2 (MABN50; Millipore), and CD68 (Cell Marque, Rocklin, CA). For immunofluorescence detection, slides were then incubated in secondary antibody conjugated to Alexa Fluor 488 or 594 at 10 μg/mL (Invitrogen) and mounted in media containing DAPI (Vector Laboratories, Burlingame, CA). For lectin staining, slides were blocked with carbohydrate-free blocking solution and streptavidin/biotin block (Vector Laboratories) and incubated with biotinylated *Polyporus squamosus* lectin (PSL) at 20 μg/mL (E-Y Laboratories, San Mateo, CA), followed by streptavidin conjugated to Alexa Fluor 488 or 594 at 3.3 μg/mL (Vector Laboratories). For immunohistochemistry, primary antibody incubation slides were stained with ImmPRESS Universal secondary antibody (Vector Laboratories) and developed using the DAB system