Immunohistochemical Detection of JC Virus in Nontumorous Renal Tissue of a Patient with Renal Cancer but without Progressive Multifocal Leukoencephalopathy

NAOTO AOKI,1* TADAICHI KITAMURA,2 TAKASHI TOMINAGA,3 NOBUTAKA FUKUMORI,1 YOSIMITSU SAKAMOTO,1 KENZO KATO,4 AND MAYUMI MORI5

Division of Pathology, Department of Toxicology, Tokyo Metropolitan Research Laboratory of Public Health, Hyakunincho, Shinjuku, Tokyo 169,1 Department of Urology, Branch Hospital, Faculty of Medicine, The University of Tokyo, Mejirodai, Bunkyo-ku, Tokyo 112,2 Department of Urology, Mitui Memorial Hospital, Izumicho, Kanda, Tokyo 101,3 Department of Virology II, National Institute of Health, Toyama, Shinjuku, Tokyo 162,4 and Department of Hematology, Tokyo Metropolitan Institute of Gerontology, Sakaecho, Itabashiku, Tokyo 173,5 Japan

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We performed immunohistochemical staining on the nontumorous renal tissue of 45 patients with renal cancer but without progressive multifocal encephalopathy using JCV-specific antibody. For one patient we found positive staining of the nuclei of the renal collecting ducts. Immunoelectron microscopic examination of the positive cell nuclei revealed electron-dense polyomavirus-like particles.

JC virus (JCV) is a member of the polyomavirus group of viruses and is the causative agent of progressive multifocal leukoencephalopathy (PML) (18). JCV infects humans mostly at a young age without obvious clinical manifestations and persists in the kidney tissue (6, 15, 19, 21). Although the renal infection with JCV is not associated with clinically apparent tissue damage, PML is a devastating central nervous system disease that mostly occurs in immunocompromised patients (5, 18, 22). JCV has been detected in urine and renal tissue not only of PML and immunocompromised patients but also those of the immunocompetent general population (6, 8, 10, 12, 13, 14). Although there have been many reports of studies of JCV in urine and kidney tissue by DNA hybridization and/or PCR (6, 4, 7), histological examinations of the JCV replication site in the kidney are scarce. Dörries and ter Meulen (9) reported on the detection of the JCV genome in the cells of renal collecting ducts in a PML patient by in situ hybridization; however, renal JCV localization by immunohistochemistry (IHC) and immunoelectron microscopy (IEM) has not been reported. Recently, we developed a JCV-specific antibody (JCAb1) that is effective with formalin-fixed paraffin-embedded tissue (3). Formalin-fixed paraffin sections of brain tissues from patients with PML and negative staining of SV40-infected IMR32 cells, negative staining of BKV-infected 293 cells, and negative staining of SV40-infected IMR32 cells. The specificity has also been confirmed by positive staining of the typical JCV-infected oligodendrocytes and astrocytes of formalin-fixed paraffin sections of brain tissues from patients with PML (3; unpublished data). Immunohistochemical staining has been performed as reported previously with the peroxidase LSAB kit (DAKO), with visualization with diaminobenzidine (DAB) (3). Formalin-fixed paraffin sections of brain tissues from patients with PML were used as positive controls throughout this experiment. For the negative controls, JCAb1 was replaced by normal rabbit serum.

For examination by IEM, paraffin sections were processed in the same way as they were for light microscopic IHC through the DAB coloring step, but the microwave treatment was skipped to avoid tissue damage. After visualization with DAB, the sections were processed as described previously (2).

IHC-positive staining was obtained for tissue from one patient (patient 881073), whose kidney tissue was positive for JCV DNA but whose urine was not available for examination. The patient had a nephrectomy on 25 April 1988, when he was 51 years old. His postoperative course has been uneventful,
with no signs or symptoms of either PML or a recurrence of renal cell carcinoma until the time of submission of the manuscript of this report. In the kidney tissue of patient 881073, strong staining was observed in the lining epithelial cells of the collecting ducts in a single area of the renal papilla of the section (Fig. 1A and B), which shows the focal nature of the intrarenal distribution of JCV infection. Granular positive staining was recognized in the nuclei of these cells. Some cells with strongly positive nuclei showed faint cytoplasmic staining. A single to several positive cells were found in each positive duct, but these were not associated with degenerative or necrotic changes. Some of the positive cells appeared to be protruding into the tubular lumen, with the positive nuclei of the cells protruding into the luminal side. This might reflect a process of exfoliation into the tubular lumen (Fig. 1B). No specific staining was observed in the other segments of the

FIG. 1. IHC of the renal medulla. The tissue was counterstained with hematoxylin. (A) The arrowheads and the arrow indicate positive collecting ducts. The ducts indicated by arrowheads each contain one positive cell. Magnification, $\times 120$. (B) High-power view of the positive duct indicated by an arrow in panel A. Note the two strongly positive (black in this photo) nuclei and the three weakly positive nuclei that can be seen to be protruding into the lumen. Magnification, $\times 480$.

FIG. 2. IEM of the positive duct. The tissue was counterstained with uranyl acetate. (A) Low-power view of the positive collecting duct. Arrow, a positive cell; L, the lumen; *, a negative cell. Note that the positive cell protrudes into the lumen and overlies the negative cells. Bar, 4 $\mu$m. Magnification, $\times 2,600$. (B) High-power view of the positive nuclei in panel A. Arrowheads indicate representative areas containing electron-dense polyomavirus-like particles. Bar, 200 nm. Magnification, $\times 50,000$. 
renal tubules, in the glomerulus, in the interstitial kidney tissues, or in the tumorous tissues adjacent to the normal kidney tissue. The discrepancy between the number of PCR-positive patients ($n = 20$) and the number of IHC-positive patients ($n = 1$) may be due to (i) the relative low sensitivity of the IHC method compared with that of PCR, (ii) viral latency (the presence of viral DNA without VP1 antigen expression), and (iii) a focal distribution of cells with viral replication.

IEM revealed that the collecting ducts had positive epithelial cells, which concurred with our light microscopic findings. The electron-dense staining was localized in the nuclei of ductal cells. Some of these positive cells were located in the luminal side and were lying on the negative cells (Fig. 2A). JCV replication may affect the cellular adherence to the basement membrane and/or to the adjacent cells, or JCV replication may be facilitated in the exfoliating cells. The cells loaded with high viral contents may exfoliate into the lumen and may serve as a source of the urinary JCV (7, 12, 24). Although most of the IEM-positive electron-dense material was of a particular or a granular nature, the virus-like nature of the particles was clear only in some limited areas. These virus-like particles measured 35 to 45 nm in diameter (16, 17, 20) and aligned in beads or clusters (Fig. 2B), which were compatible with polyomavirus particles. Filamentous forms were not observed. Cytoplasmic virus-like particles were not readily discernible, although the cytoplasmic structures were poorly preserved.

No inflammatory cell infiltration was observed in the area containing JCV-positive collecting ducts either by IHC or by IEM. Although Dörries and ter Meulen (9) also found no inflammatory cell infiltration in the area with ISH-positive cells in a patient with PML, the implication suggested by our findings is different. In typical patients with PML, the lack of inflammatory reactions in central nervous system lesions has been ascribed to the presence of generalized immunodeficiency, including specific cellular immunity against JCV (1, 11, 23). By the same token, the renal reaction may lack a cellular immune response in patients with PML. On the other hand, our findings suggest a lack of an apparent local cellular immune response against the JCV-infected tubular epithelial cells even in the immunocompetent patient. This kind of virus-host interaction may closely be related to mechanisms of persistent infection of JCV in renal tissue.

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