

## BK and JC Polyomaviruses Are Not Associated with Idiopathic Pulmonary Fibrosis

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**We sought to determine if the BK and JC polyomaviruses were associated with idiopathic pulmonary fibrosis (IPF). We did not detect the BK or JC polyomaviruses in lung tissue extracts from 33 patients with IPF by using real-time PCR, which suggests that an etiologic association is unlikely.**

Idiopathic pulmonary fibrosis (IPF) is a devastating disease that can be fatal without lung transplantation (1, 6). The exact cause of the extensive pulmonary fibrosis in these patients is not fully understood, but dysregulation of repair processes that are associated with the inflammatory pathways is under investigation (7, 16). Fibrosis is a nonspecific histopathologic reaction to injury. The injuries that invoke fibrosis are protean and include ischemia, damage by toxins, and a variety of infections.

Several of the members of the family *Herpesviridae* have been associated with pulmonary fibrosis, but the etiologic significance of this association remains to be determined (14, 15). An association between IPF and other viruses, such as hepatitis C virus and adenovirus, has also been suggested (10). More recently, the human herpes virus type 8 has also been associated with another proliferative pulmonary disease, primary pulmonary hypertension (4, 5). The pathological findings in cases of IPF exhibit major similarities to those for chronic interstitial nephritis, which also is characterized by interstitial fibrosis and mononuclear cell infiltrate. Previous studies have demonstrated that human polyomavirus infection is associated with chronic interstitial nephritis in immunocompromised hosts (12, 13). Therefore, we sought to determine if the BK and JC polyomaviruses, which, like the herpesviruses, may remain latent in the body and later become reactivated, were present in the tissues of patients with IPF by using real-time PCR.

We studied a collection of DNA extracts from patients with IPF and from controls for the presence of the JC and BK polyomaviruses. Thirty-three patients with IPF and 23 patients with ( $n = 20$ ) and without ( $n = 3$ ) other lung diseases were studied. The histopathologic diagnoses of the patients with IPF consisted of usual interstitial pneumonitis (27), nonspecific interstitial pulmonary pneumonitis (2), and end-stage fibrosis (4). The negative control specimens were from patients who had cancer (9), primary or secondary pulmonary hypertension (4), sarcoidosis (4), bronchiolitis obliterans with organizing pneumonia (2), or *Streptococcus pneumoniae* pneumonia (1) or were from donor transplant lungs (3). The histopathologic diagnoses were rendered by an experienced pulmonary pathologist.

In the majority of instances, fresh lung tissue was obtained, rapidly frozen in liquid nitrogen, and stored at  $-80^{\circ}\text{C}$  for later PCR analysis. For six of the controls and two of the IPF patients, formalin-fixed, paraffin-embedded lung tissue was used for PCR analysis. Nucleic acids were extracted from lung tissue with a QIAamp DNA Mini Kit according to the protocol of the manufacturer (QIAGEN Inc., Valencia, Calif.). For the formalin-fixed, paraffin-embedded lung specimens, approximately 25 mg of each specimen was treated with xylene in order to remove the paraffin before tissue lysis and DNA extraction were performed. A human  $\beta$ -actin gene was amplified on every extracted DNA to ensure the quality of the extracted DNA samples (9). A LightCycler real-time PCR was performed on these extracts, as previously described for the BK and JC polyomaviruses (2). The assay detects both of these polyomaviruses, but differentiates them by postamplification melt curve analysis and is a modification of the assay reported by Whiley et al. (17). We have successfully used this assay for the detection of the JC virus from formalin-fixed, paraffin-embedded brain biopsies from patients with progressive multifocal leukoencephalopathy, for the detection of BK virus from formalin-fixed, paraffin-embedded kidney tissue from patients with BK nephropathy, and for the detection and differentiation of these viruses from fresh blood and urine specimens (2). To avoid amplification product contamination, reagent preparation and PCR setup were performed in a dead-air box in a room separate from the amplification area.

The 33 specimens from patients with IPF and the 23 specimens from control lungs were uniformly negative for the BK and JC polyomaviruses. All of the extracts demonstrated PCR amplification of the human  $\beta$ -actin gene, which excludes inhibition of PCR amplification.

Furthermore, since 32 of 33 specimens from the experimental group and 9 of 23 specimens from the control group were found to have a PCR product for a member of the family *Herpesviridae* in a previous study, it is unlikely that the negative PCR results reported here are secondary to PCR inhibition (15).

The BK and JC polyomaviruses, like the *Herpesviridae*, are DNA viruses that cause human disease and may reside in the human body in a latent state after an acute infection (8, 11). These viruses are often considered to be low-virulence viruses, because they cause disease only in immunocompromised pa-

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tients. The JC virus causes progressive multifocal leukoencephalopathy, a progressive demyelinating disease of the brain, in patients with AIDS (3). The BK virus causes hemorrhagic cystitis, often in bone marrow transplant recipients, and renal allograft nephropathy in kidney transplant recipients (8, 11). The natural history and the extent and types of disease that may be produced by these polyomaviruses are still being studied (12, 13). Therefore, we sought to determine if these viruses were present in the lung tissue of patients with IPF, since the cause of this disease has not yet been determined.

We used a sensitive and specific real-time PCR assay that has previously been shown to be able to detect polyomavirus DNA, even when the extracts were from formalin-fixed, paraffin-embedded tissues (2). None of the 33 specimens from patients with documented IPF and none of the specimens from the controls demonstrated the presence of either the BK or the JC polyomavirus by PCR, although the housekeeping gene was appropriately amplified in all instances. Although this study requires corroboration, it suggests that the BK and JC polyomaviruses are not associated with IPF and that the chronic interstitial fibrosis in the kidney and lung may develop through different mechanisms. In addition, the failure to detect these viruses in the extracts from the specimens from patients with a variety of conditions provides information about the natural history of this infection, and suggests that these viruses do not reside in latency in the lung, since some of these patients would, by chance, be expected to have been infected by a BK or JC polyomavirus.

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