A Rearranged Form of Epstein–Barr Virus DNA Is Associated with Idiopathic Pulmonary Fibrosis

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An association between idiopathic pulmonary fibrosis (IPF) and productive Epstein–Barr virus (EBV) infection has been found previously. Productive EBV replication can be associated with a rearrangement in EBV genomes termed WZhet. We hypothesized that WZhet genomes might be present in patients with IPF. Thirty-nine patients with IPF, 26 lung transplant recipients, and 24 normal subjects were studied. When EBV DNA–positive lung tissue biopsy samples from IPF patients were analyzed, 11 of 18 (61%) were positive for WZhet. Buffy coat DNA analysis showed that 75–85% were EBV DNA–positive in both IPF and control groups. Buffy coat analysis for WZhet was positive in 16 of 27 (59%) IPF patients, compared with none of 32 lung transplant recipients and 1 of 24 (4%) normal blood donors (p = 0.001). There was thus a good correlation between the presence of WZhet in lung tissue and peripheral blood. However, there was no significant association between the presence of WZhet and immunosuppressive therapy. These data further confirm the association between active EBV infection and IPF and provide a potential marker in the peripheral blood for the tracking of EBV in this disease.

Keywords: Epstein–Barr virus infections; polymerase chain reaction; pulmonary fibrosis

Idiopathic pulmonary fibrosis (IPF) is a progressive lung disease characterized by thicken, fibrotic alveolar walls and focal cuboidalization of the alveolar epithelium. Clinically this results in shortness of breath and inspiratory crackles. Bilateral radiologic shadowing, mainly of the lower lung zone, impaired gas transfer, and a restricted ventilatory pattern are characteristic. Prognosis is poor, and lung transplantation in selected patients is the only current therapy with any degree of success. Both environmental exposure and viral infections have been associated with IPF, suggesting that different injuries may trigger the clinical disease in different individuals (1–3).

The Epstein–Barr virus (EBV) is a herpesvirus prevalent in all human populations and associated with a number of diseases. These include infectious mononucleosis, B cell lymphoproliferative disease, Burkitt’s lymphoma, Hodgkin’s disease, and nasopharyngeal carcinoma (4). Although normally associated with infection of the upper respiratory mucosa and B lymphocytes, EBV can infect and replicate in the lower respiratory tract (5, 6). Previous serologic analysis found an association between active EBV infection and IPF (7). Here, Vergnon and coworkers showed raised anti-EBV viral capsid antigen immunoglobulin A levels in a study of patients with IPF (7). We have reported immunohistochemical evidence of EBV-productive cycle antigens in Type II alveolar epithelial cells in IPF (8). This was confirmed by detecting EBV DNA by polymerase chain reaction (PCR) in the lung tissue of patients with IPF (9). Recent observations by Tsukamoto and colleagues (10) have confirmed and extended these observations showing that expression of the EBV latent membrane protein 1 in infected epithelial cells was associated with poor prognosis in patients with IPF.

Some conditions are associated with spontaneous, productive EBV replication. For instance, oral hairy leukoplaikia, thymic carcinoma, and Hodgkin’s disease have been shown to contain rearranged EBV genomes (11–14). These genomes were first identified in vitro in the Burkitt’s lymphoma cell line P3J-HR-1 by the presence of aberrant DNA restriction fragments, termed heterogeneous (het) fragments (15–17). The deleted and rearranged genome (het DNA) in defective virus forms self-contained replicons that multiply independent of standard EBV and are capable of cell-to-cell spread (18). The most salient rearrangement in these het genomes is the juxtapositioning of sequences from the BamHI W and Z restriction fragments that are separated in the standard genome by more than 55 kb (19) (see Figure 1). This so-called WZhet recombinant fragment contains the entire BZLF1 open reading frame encoding a product (ZEBRA) that trans activates not only the lytic (productive) origin of EBV DNA replication but also EBV lytic cycle promoter elements (20, 21). The ability of WZhet DNA to activate EBV lytic replication stems from dysregulation of expression of BZLF1 by the ectopic positioning during rearrangement of positive regulatory elements on either side of the gene (22). It has been postulated that this rearrangement may represent a novel mechanism by which a persistent virus can move, via recombinational events, from a latent to a productive phase of its life cycle (11).

As defective EBV is associated with productive EBV infection in other clinical lesions, we hypothesized that patients with IPF might also harbor defective EBV. Most human subjects are EBV-positive and harbor EBV DNA in their peripheral blood, but almost none harbor EBV DNA in lung tissue (4, 9). The aim of this study was to assess whether the presence of rearranged EBV (WZhet) DNA in either the lung tissue or the peripheral blood was associated with IPF.

METHODS

Patient Selection

Two premises were used in patient selection. First, 90–95% of all human subjects are EBV-positive and harbor EBV DNA in their peripheral blood lymphocytes (4). Second, EBV DNA is almost never detected in the lung tissue of normal subjects (9).

In an initial pilot screen of lung tissue for WZhet-rearranged EBV DNA, 18 archived, surgical lung biopsies from patients with IPF (Patients 1 to 18; Table E1 in the online data supplement) were selected for study on the basis of lung tissue containing EBV DNA. This had been determined in a previous study (9). All patients were independently reassessed and analyzed blind.
RESULTS

WZhet DNA Analysis of Lung Tissue from EBV-Positive Patients with IPF

Archival lung tissue sections from patients with IPF that had been used in our previous analysis (9) and that were EBV DNA–positive (n = 18; Patients 1–18, Table E1 in the online data supplement) were selected. They were reanalyzed for the presence of EBV DNA and WZhet DNA by PCR. As before, all were positive for EBV DNA, and 11 of 18 patients (61%) were positive for WZhet DNA.

Analysis of Buffy Coat

DNA was extracted from buffy coat cells from patients with IPF, lung transplant recipients, and normal blood donors and analyzed by PCR for both EBV DNA and WZhet DNA. The results are shown in Tables E1 and E2 in the online data supplement and summarized in Table 1. Twenty-three of 27 patients (85%) with IPF were positive for EBV compared with 21 of 26 (81%) transplant recipients (p = 0.94) and 20 of 24 (83%) normal blood donors (p = 0.83). In contrast, 16 of 27 patients (59%) with IPF were positive for WZhet DNA compared with none of 26 transplant recipients (p = 0.00001) and 1 of 24 (4%) blood donors (p = 0.000078). All of the patients with IPF who were WZhet-positive were also EBV-positive. Likewise, all of the patients who were EBV DNA–negative were also WZhet DNA–negative. A representative example of a PCR assay for WZhet DNA from patients with IPF is shown in Figure 2.

Subgroup Analysis of Data

In buffy coat samples, 16 of 23 (69%) patients with IPF who were positive for EBV DNA were positive for WZhet-rearranged DNA. This compares well with 11 of 18 (61%) patients in the case of lung tissue (p = 0.57). In six cases (Patients 13 to 18; Table E1 in the online data supplement), we were able to directly correlate the presence of EBV DNA with WZhet DNA in lung tissue and in the buffy coat. In these cases, there was a 100% correlation between the presence of EBV DNA and WZhet DNA in both the lungs and buffy coat.

Impact of Immunosuppression on the Presence of WZhet

Twenty-five (64%) patients with IPF had received immunosuppression. Sixteen of 21 (76%) patients with IPF who were WZhet-positive had received immunosuppression, compared...
The clinical reactivation of other herpes viruses is associated with immunosuppressive therapy. Our previous data detected no significant association between the presence of EBV DNA or EBV antigens in lung tissue and immunosuppression in patients with IPF (9). Likewise, there was no significant association between immunosuppressive therapy and the presence of WZhet in either patients with IPF or lung transplant recipients. Thus, the detection of WZhet in IPF does not appear to be associated with the reactivation of EBV replication because of iatrogenic immunosuppression.

WZhet-rearranged DNA has been seen previously in tissue derived from diseases such as oral hairy leukoplakia (11), thymic carcinoma (13), and Hodgkin’s disease (14). However, we also detect WZhet DNA in the peripheral blood. This suggests that WZhet-containing EBV may be generated in lung tissue and spread to the peripheral circulation. Conversely, it is also possible that WZhet-containing EBV is being produced or is multiplying in systemic lymphoid tissue and spreading to the lungs via the peripheral circulation. This is entirely feasible because it has recently been shown that distinct EBV strains can traffic in both directions between the epithelium and the peripheral blood in oral hairy leukoplakia (23). The resolution of this question in IPF would require a longitudinal study of WZhet in blood and pulmonary tissue.

WZhet-rearranged EBV is associated with productive EBV replication. Indeed, the introduction of DNA containing this rearrangement reactivates EBV from latency (19). Thus, WZhet EBV may contribute to the pathogenesis of disease where EBV production is seen. Infection with WZhet-containing EBV or random generation of the mutation within a patient may be responsible for triggering reactivation of EBV. However, the presence of WZhet may simply be a by-product of productive EBV replication.

This study confirms the association between EBV and IPF. More importantly, it describes a highly amenable method of detecting a form of EBV that is more than likely associated with productive viral replication in patients with IPF. The establishment of a causal association between EBV and IPF requires a clinical response to an antiviral agent combined with evidence of a reduction in viral replication. The assay described here provides the means with which to perform such a study.

**References**

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