EDITORIAL

Viruses and idiopathic pulmonary fibrosis

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Idiopathic pulmonary fibrosis (IPF) is an increasingly recognized problem with a generally poor prognosis [1]. Fifty per cent of patients diagnosed as having IPF may be expected to die within 4 yrs of the diagnosis [2]. Although termed idiopathic or cryptogenic it is increasingly acknowledged that an occult inhaled environmental injury precipitates a common histological response, which manifests as the heterogeneous clinical syndrome, IPF [3, 4]. Since many patients date the onset of their symptoms to a viral infection or a "cold", there has been a suggestion that IPF may be an expression of injury precipitated or potentiated by a diagnostically occult viral infection. Previously, hepatitis C virus (HCV) [5] and Epstein-Barr virus (EBV) [6] have been implicated in the aetiology of IPF, and in this issue Kuwano et al. [7] present data evaluating adeno-virus as a factor in the aetiology of IPF. Such viruses have the ability to actively replicate and to assume an endogenous latent state (adenovirus, EBV) [7] or a state of chronic infection (HCV) [8]. The study by Kuwano et al. [7] highlights the difficulties in investigating and interpreting findings related to viruses and IPF. With the advent of highly sensitive molecular based diagnostic techniques, there is the potential for erroneous associations between virus and conditions of unclear aetiology, such as IPF. This review will attempt to place in context the identification and potential role of viruses in patients with IPF.

Hepatitis C virus

HCV, a ribonucleic acid (RNA) virus, is now recognized as the major cause of non-A, non-B transfusion-associated hepatitis [9]. With improved diagnostic techniques, HCV infection is increasingly identifiable and is recognized as being associated with conditions other than hepatitis. The background rate of HCV infection is important in interpreting the relationship between HCV and extra-hepatic disease. Of people exposed to HCV, 50–70% may be expected to develop chronic infection [9]. This contrasts with viruses such as adeno-virus and EBV where almost 100% of the population would be expected to have experienced an acute infection.

There is controversy as to whether IPF is associated with HCV. Ueda et al. [5] first suggested a relationship between IPF and HCV infection by studying 66 Japanese patients with a clinical diagnosis of IPF, using the first generation enzyme-linked immunsorbent assay (ELISA) tests for evidence of HCV infection. Twenty eight per cent of the IPF patients studied were found to be HCV antibody positive. Confirmatory testing using recombinant immunoblotting assay (RIBA) was used, and 12 of the 19 index cases were found to be RIBA positive. Unfortunately, in this study it was not specified which RIBA assay was used. The study was then refuted by Irving et al. [10] who studied stored sera from 62 patients in the UK with a clinical diagnosis of IPF. This study utilized the next, or "second generation" ELISA tests and RIBA for four HCV antigens, C100, 5-1-1, C33 and C22. Irving et al [10] found equivocal evidence of HCV antibody in only one patient, and confirmatory reverse transcriptase polymerase chain reaction (RT/PCR) was negative. The apparent disparity between these two studies can be explained by the high false-positive rates for the first generation ELISA tests used by Ueda et al. [6]. False-positive results can occur with raised immunoglobulin G (IgG) levels, which are recognized in approximately 50% of IPF patients [5, 10].

The difficulties in interpretation of HCV and IPF are further emphasized by Meliconi et al. [11], who studied 60 Italian patients. Control groups included 130 patients with obstructive and suppurative lung disease and 4,614 blood donors. The blood donors, as expected, had a lower rate (0.3%) of HCV positivity than the general population. Of the IPF patients, eight of the 60 (13%) had evidence of HCV infection, using second generation ELISA tests. All eight IPF patients were RIBA positive and had evidence of HCV RNA by RT/PCR which did not differ significantly from the 6% of patients in the control group of mixed lung diseases who were also HCV positive. Multiple hospital admissions, a risk factor for HCV exposure, was common to both the IPF group and the mixed lung disease group [11]. The study of Meliconi et al. [11] clarified the issue of diagnostic techniques, but emphasized that the baseline prevalence of HCV infection in the population studied determines the results. In the USA and western Europe, the prevalence of HCV positivity is approximately 1%, in northern Italy it is 3.4% and in some parts of Japan it is as high as 30% [11, 12]. Therefore, it is most likely that the relationship between IPF and HCV is spurious and reflects the background rate of HCV infection.

Adenovirus

Adenovirus is a ubiquitous virus recognized as causing a spectrum of clinical disease, including both upper and lower respiratory tract infections from which most...
people recover. Matsuse et al. [13] have demonstrated using the polymerase chain reaction (PCR) that adenoviral deoxyribonucleic acid (DNA) exists in the lower respiratory tract of human lungs. Kuwano et al. [7], with nested PCR, have studied the prevalence of adenovirus DNA in the lung tissue of patients with IPF, using primers directed against the early gene E1A, a gene that facilitates cell transformation [8]. In this study, 16% of IPF cases had evidence of adenovirus DNA. Using nested PCR with an in vitro sensitivity of one single copy of adenovirus DNA, the prevalence is unexpectedly low, because the lower respiratory tract is a potential reservoir for adenovirus [13].

The low rate of adenovirus DNA positivity may be explained firstly by enhanced clearance of infected cells by the background inflammatory and repair process. This process has been proposed to explain the low prevalence of adenovirus DNA in follicular bronchiectasis [14]. Secondly, the low prevalence of adenovirus DNA in IPF may be due to sampling error by the use of transbronchial biopsy for the acquisition of lung tissue. Three transbronchial biopsies in the setting of lung fibrosis are probably not comparable to studying surgically resected tissue specimens. In the study by Matsuse et al. [13] using primers for E1A adenoviral DNA, serial tissue sections alternated between PCR positivity and PCR negativity. This suggested that E1A DNA was randomly present in the bronchial epithelial cells. A similar distribution in the lung of IPF patients would, therefore, explain the low prevalence in tissue obtained by transbronchial biopsy specimens despite the sensitivity of nested PCR.

The finding by Kuwano et al. [7] that adenovirus DNA is more common in those patients receiving corticosteroid therapy (67%) compared to those not receiving therapy (10%) is interesting. Considering that transbronchial biopsies may have underestimated the incidence of adenovirus DNA positivity, this may indicate that the viral load in such immunocompromised patients is higher than normal. Potentially, this is an important finding, which suggests that adenovirus replication is promoted by immunosuppression. Whether viral replication in the presence of immunosuppression then contributes to disease progression, could only be proven by demonstrating a response to antiviral therapy.

Conflicting data have been presented on the application of antiviral therapy against adenovirus in patients with IPF. Prieto et al. [15] described a case in which the patient responded to the administration of nebulized ribivarin. The case appeared to be consistent with a pattern of cellular, usual interstitial pneumonia (UIP). If it is assumed that cellular UIP represents an "early" phase of IPF, then such a response to ribivarin would support the hypothesis that adenovirus infection was involved in the initiation of the process that resulted in IPF. In contrast, Agusti et al. [16], studied 10 immunocompromised patients with advanced IPF and observed no benefit from aerosolized ribivarin. Although one patient exhibited an objective improvement, it is often difficult to demonstrate any therapeutic benefit in patients with advanced IPF because of the degree of pulmonary remodelling that has occurred [17]. Therefore, to date there is no clear evidence of a response to therapy for adenovirus in IPF patients.

Epstein-Barr virus

EBV is a gamma herpes virus that is present in >90% of the general population [8]. Acute infection may manifest as the syndrome of infectious mononucleosis. Following acute infection, EBV assumes a latent state. Whether epithelial cells or B-lymphocytes are the site of EBV persistence is an area of controversy [18]. Host T-cell mediated immune surveillance maintains EBV latency. Despite apparent host immune competence, EBV has oncogenic potential and is associated with Burkitt's lymphoma, Hodgkin's lymphoma and nasopharyngeal carcinoma [19]. Overt modification of the host immune state (human immunodeficiency virus (HIV), following transplantation) can allow latent EBV-driven B-cell proliferation, which manifests as lymphoproliferative disease. Immunosuppression can also give rise to a productive EBV replication in epithelial cells, as seen in oral hairy leukopapulosis (OHL) [19].

EBV was first implicated in the aetiology of IPF in 1984 by a French study based on EBV blood serology [6]. Elevated levels of IgG and immunoglobulin A (IgA) against viral capsid antigen (VCA) were observed in 11 patients with IPF. Although, in general, serological testing for EBV infection has a low sensitivity and specificity, the specificity of IgA against VCA is high and suggests the presence of active EBV replication at an epithelial site [20]. Subsequently, a tissue-based study was undertaken using immunohistochemistry [21]. Of 20 open lung biopsies taken from patients with IPF, 14 had positive staining for both VCA and the membrane antigen gp340/220. EBV antigens expressed during viral replication. These findings were compared to resected tissue taken from a control group that was predominantly composed of patients undergoing surgical treatment of lung cancer. In the control group, lung tissue remote from the neoplasia was studied for EBV. Of 21 controls studied, two were EBV VCA and gp340/220 positive [21].

EBV antigen-positive staining needs to be interpreted in the context of a number of factors, including whether the viral antigens studied are expressed during viral latency or viral replication [8]. The most commonly used reagents for the diagnosis of EBV are those antibodies specific for proteins associated with EBV transformation, which are expressed during virus latency. These include monoclonal antibodies specific for EBV nuclear antigen (EBNA) 2 and latent membrane protein (LMP), which are able to detect EBV-transformed proliferating cells [22]. Examples of this include the detection of EBV in association with the post-transplant lymphoma and lymphocytic interstitial pneumonia [23].

In contrast, antibodies specific for cells containing EBV that is undergoing productive replication, i.e. producing viral particles, are also available in a diagnostic context. Examples of these include antibodies specific for immediate early protein BZLF1, components of the VCA or the membrane antigen gp340/220 [24]. These reagents are used in the detection of productively replicating EBV in epithelial lesions such as oral hairy leukopapulosis, and have been studied in IPF [21, 25].

The interpretation of EBV positivity is further influenced by the target cell type of interest [8]. The infected host cell characteristics influence the phase of the EBV
cell cycle. EBV infection of undifferentiated basal epithelial cells or B-lymphocytes results typically in EBV establishing a latent phase characterized by expression of EBERs (EBV-related small messenger RNA detected by in situ hybridization) and the EBNA/LMP antigens [8, 26]. Conversely, viral replication characterized by VCA and membrane antigen expression typically occurs in terminally differentiated epithelial cells, including the parotid gland, OHL and, it would appear, in IPF [21, 25].

A further difficulty in interpreting immunohistochemistry is that there is the potential for antibodies directed against viral antigens to cross react with host antigens ("molecular mimicry") [27]. Up to 5% of all monoclonal antibodies developed for the identification of viruses react against autoantigens. In order to minimize this possibility, two antibodies directed against two different EBV antigens (VCA, gp340/220) were used in the tissue-based study of EBV in lung tissue [21].

Another technique for EBV detection and cellular localization is the use of in situ hybridization (ISH) for EBERs [26]. Whilst ISH for EBER and immunohistochemistry have the advantage of allowing localization of EBV, they do not necessarily complement or mirror each other. EBERs are not expressed in lesions of active viral replication such as OHL, where the use of antibodies are more informative [25]. Such a biological model as OHL, in which EBV viral replication occurs in differentiated epithelial cells in the absence of EBER expression may be comparable to EBV replication in IPF, in which EBER expression is absent in cells expressing VCA and membrane antigen (unpublished observations). The identification of EBV-related antigens, therefore, demands the application of corroborative diagnostic techniques.

The PCR is an extremely sensitive technique that has been applied in the diagnostic context for a number of viruses. Preliminary data has been presented on PCR for EBV DNA in IPF. In a small study of lung tissue (n=4), PCR for the EBV gene BAM W was negative [28]. In contrast, in a study of 20 cases of IPF using nested PCR with a sensitivity of 1–10 copies of EBV DNA (RAJI I), 11 cases had evidence of EBV DNA (versus 1 of 8 normals and 1 of 8 patients with sarcoidosis) [29]. Another group has also observed that six of 12 cases with IPF were EBV DNA PCR positive [30]. These study differences may reflect EBV activity in discrete areas of alveolar tissue; patient selection; or the sensitivity of the PCR assay. A major problem with PCR for the diagnosis of EBV is the ubiquitous nature of the virus. EBV DNA can be detected in the blood of most individuals and the lung is recognized as a reservoir for EBV [31].

Quantitation of the EBV DNA load by PCR may allow a study of the relationship between the viral load and IPF, and has proven useful in assessing individuals in the context of post-transplant lymphoproliferative disease [32]. A study of quantitative PCR in IPF would be a laborious and difficult study in a heterogeneous group of individuals. Therefore, the study of EBV in animal models may give insight into the behaviour of EBV in lung tissue. EBV, like all gamma herpes viruses, has an extremely narrow host range. The best-known model for EBV infection, the cotton top tamarin, does not breed well in captivity and is an endangered species. The mouse model of murine gamma herpes virus 68 (MHV68) infection, which, like EBV, is transmitted via the nasal route, results in an interstitial pneumonia and transient splenomegaly reminiscent of infectious mononucleosis. It then becomes latent in B-cells, persisting for the lifetime of the mouse, and is associated with the development of lymphoma [33, 34].

The presence of EBV, whether detected by immunohistochemistry or PCR, has to be judged in the context of the presence or absence of immunosuppression. EBV-associated pulmonary fibrosis has also been described in a heart-lung transplant recipient [35]. Such an association in an immunocompromised transplant recipient emphasizes the issue of whether a ubiquitous herpes virus like EBV represents "a passenger or a pathogen" when localized in lung tissue. Further examples of this controversy are with cytomegalovirus (CMV) in acquired immune deficiency syndrome (AIDS) patients and human herpes virus 6 in bone marrow transplant recipients [36, 37]. In the study by Kuwano et al. [7], an increased incidence of adenovirus seen in patients receiving corticosteroid therapy is a further example of this controversy.

If latent viruses such as adenovirus and EBV exist in groups of IPF patients, how might such viruses contribute to disease progression? Firstly, viral proteins that are already present in cells can promote chronic inflammation and repair. For instance, EBV LMP increases class II antigen expression in previously EBV negative B-lymphocytes [38]. Furthermore, viral proteins may promote the persistence of inflammation that was originally initiated by a different (environmental) injury. The repeat GPPAA sequence is observed both in the HLA-DQβ chain and the EBV EBNA 3 antigen [27]. This could promote antibody cross-reactivity between viral and host antigens as is seen with CMV infection following solid organ transplantation [39]. Secondly, viral infection may activate type 1 collagen genes in alveolar epithelium. It has been demonstrated that rat neonatal alveolar cells immortalized by the adenoviral 12SE1A gene produce large amounts of type 1 collagen [40].

Finally, viral genes may act as transactivating factors. Transactivating factors are nuclear proteins which, because of specific census sequences, bind to or interact with DNA, thereby regulating RNA protein transcription and modifying cell behaviour [41]. Proto-oncogenes are an example of such target genes for viral proteins. Proto-oncogenes are normal, "wild" growth regulating genes while "mutant" proto-oncogenes are expressed in a deregulated manner and play a role in tumorigenesis. Such oncogenes are expressed in lung tissue. A recent report (Kuwano et al. [42]) suggests that overexpression of wild p53 gene occurs in the lung tissue of IPF patients. This may reflect regulation of cells with damaged DNA. Fibroblasts obtained from patients with Li-Fraumeni syndrome which inherently have p53 mutations, demonstrate in vitro prolongation of their fibroblast life span and, sometimes immortalization of the fibroblast [43].

EBV has the potential to interact with oncogenes. The EBV nuclear antigen (EBNA 5) can form a molecular complex with both the retinoblastoma (RB) gene and p53 gene [44]. The EBV immediate early protein BZLF1 interacts with p53 both in vivo and in vitro [45]. BZLF1 inhibits p53-dependent transactivation. Adenovirus also has the ability to interact with p53 tumour suppressor...
gene [46]. The adenoviral protein E1A has the potential to bind to the RB oncogene [47]. The RB gene controls the genesis of the cell replication phase. E1A promotes cell replication and growth by binding to RB.

Therefore, the interaction of viral proteins (transactivating factors) with genes involved in the regulation of cell growth may provide a common mechanism by which different viruses play a role in a single fibroproliferative disease process such as IPF. However, further study is required in this area.

How can one reconcile the concept of virus-mediated IPF and IPF precipitated by an inhaled environmental injury [3, 4, 6, 21]? Within the heterogeneous group of patients labelled as IPF, it is possible that different aetiological groups exist, some with an environmental trigger, and others with a viral trigger. In some IPF patients, a primary environmental injury may be potentiated at a later phase in the disease process by viral replication within the injured tissue. Ultimately each individual or combination of aetiological factors may contribute to a common clinical and histological response to injury.

The application of sensitive diagnostic techniques has allowed the detection of ubiquitous viruses in the lung tissue of patients with IPF. This does not prove cause and effect. The identification of viruses is influenced by many factors, particularly the sensitivity and specificity of the diagnostic techniques, the distribution of the virus within the lung and the presence or absence of immunosuppression.

Mechanisms by which viruses may promote an inflammatory response and influence cell behaviour have been identified. IPF may not necessarily be related to a single virus; rather, different ubiquitous viruses may potentiating factors) with genes involved in the regulation of cell replication and growth by binding to RB. Suppression. The identification of viruses is influenced by many factors, particularly the sensitivity and specificity of the diagnostic techniques, the distribution of the virus within the lung and the presence or absence of immunosuppression. Potentiating factors) with genes involved in the regulation of cell replication and growth by binding to RB. Suppression.

Mechanisms by which viruses may promote an inflammatory response and influence cell behaviour have been identified. IPF may not necessarily be related to one single virus; rather, different ubiquitous viruses may potentiate the disease process via a common pathway. As many patients deteriorate while receiving immunosuppressant therapy, at issue is whether an increasing viral load may be a cofactor contributing to disease progression. In idiopathic pulmonary fibrosis, a pathogenic role can only be ascribed to viruses if a clinical response is demonstrated by the administration of antiviral therapy.

References


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