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SHORT COMMUNICATION

Idiopathic pulmonary arterial hypertension in Dutch Caucasian patients is not associated with human herpes virus-8 infection

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Summary
Samples of lung tissue, taken at time of lung transplant, from 13 Dutch Caucasian patients with idiopathic pulmonary arterial hypertension (iPAH) and 14 patients with non-idiopathic PAH were studied for the presence of human herpes virus-8 (HHV-8). By immunohistochemical staining, in none of patients expression of HHV-8 latency-associated nuclear antigen 1 (LANA-1) was demonstrated. Using two nested polymerase chain reactions (PCR) to amplify part of the open reading frame (ORF) 65 and ORF 73, we failed to detect HHV-8 DNA in all samples studied. These results argue strongly against a role for HHV-8 in the pathogenesis of iPAH.

Introduction

Human herpes virus-8 (HHV-8), also known as kaposi's sarcoma (KS)-associated herpesvirus, is a vasculotropic virus that plays an etiological role in Kaposi sarcoma, primary effusion lymphoma and multicentric Castleman disease.[1] In the past, HHV-8 has also been tentatively linked to various other malignant and non-malignant diseases. The etiological relationship between HHV-8 and these disorders, however, has been clearly disproved by additional analysis after the initial positive reports. Recently, HHV-8 infection was reported to be associated with idiopathic pulmonary arterial

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hypertension (iPAH).[2] Idiopathic PAH is a rare, but fatal disease, clinically characterized by progressive pulmonary hypertension and right heart failure. Histopathologically, the disease is characterized by an arteriopathy of the small precapillary arterioles with proliferation of endothelial cells and smooth muscle cells. Over time, these conditions induce luminal obstruction, resulting in a gradual increase in pulmonary vascular resistance. Moreover, generally, in more advanced stages characteristic complex vascular (plexiform) lesions can be observed. The etiology of these lesions as well as the observed arteriopathy is far from understood yet. Cool and coworkers[2] reported the presence of HHV-8 DNA and the expression of the HHV-8 latency-associated nuclear antigen 1 (LANA-1) in lung tissue from 10 out of 16 iPAH patients. Surprisingly, LANA-1 expression was described not to be confined to cells within plexiform lesions, but was also described to be present in bronchoepithelial cells, inflammatory cells, and endothelial cells lining patent vessels. Since, however, in studies in German,[3] French[4] and Northern-American[5] populations, serologically, the incidence of HHV-8 infection did not differ between iPAH patients and controls, these findings were questioned. It was argued, however, that serologic tests might fail to detect organ-specific HHV-8 infection.[6] Subsequently, however, two Japanese groups were unable to detect HHV-8 DNA in lung samples of Japanese iPAH patients.[7,8] Since these studies were performed in Asian populations, it was argued that the pathogenesis of iPAH may differ between patients with different ethnic background.

In the present study, we investigated the expression of LANA-1 and the presence of HHV-8 DNA in lung biopsies of Dutch Caucasian patients with iPAH ($n = 13$) and non-idiopathic PAH ($n = 14$) who underwent a lung transplant at the University Medical Center Groningen.

Subjects, materials and methods

Lung biopsy specimens were obtained at lung transplant in all patients between 1991 and 2003. All patients had a Caucasian ethnic background. Diagnoses were revised according to international guidelines prior to the study. Thirteen patients with iPAH (40 ± 4 yr) and 14 patients with non-idiopathic PAH (38 ± 3 yr) were included in the study.

Immunohistochemical staining was performed to identify LANA-1 of HHV-8 encoded by the open reading frame 73 (ORF 73). Briefly, formalin-fixed, paraffin embedded tissue was used for extraction of DNA, and performed as described by Cool et al.[2] To detect HHV-8 DNA two nested PCR reactions were performed to amplify part of ORF 65 and ORF 73, as described by Goudsmit et al.[9] Positive controls (paraffin embedded Kaposi Sarcoma skin) and negative controls were included in each reaction set. By a homemade chemokine receptor CCR5 PCR on each extracted DNA the presence of PCR inhibitors was ruled out. The assay detection level for HHV-8 was five copies. The study was approved by the medical ethics committee of the UMCG, and informed consent was obtained from all patients.

Results

Hematoxylin-eosin staining revealed that characteristic pathological arteriolar abnormalities were present in all biopsy specimen. Moreover, plexiform lesions were identified in 11 out of 13 iPAH, and in six out 14 non-iPAH biopsy specimen, respectively. In none of the iPAH patients and none of the non-idiopathic PAH, LANA-1 expression was demonstrated, whereas LANA-1 was demonstrated in all KS samples. In addition, PCR amplification failed to detect HHV-8 DNA in all samples studied, whereas it was positive in all KS control samples. The control gene CCR5 was detected in all samples.

Discussion

In the present study, using identical methods as described by Cool et al.[2] that is immunohistochemical staining for LANA-1 and HHV-8 DNA amplification by PCR, we failed to detect HHV-8 in lung tissue from 13 Dutch caukasian patients with iPAH or 14 patients with non-idiopathic PAH.

Previously, two studies performed in Japanese patients[7,8] also failed to confirm the observations by Cool and coworkers. This difference, however, could be explained by possible differences in the etiology of iPAH in patients with a different ethnic background. The patients studied here, however, all had a Caucasian background, ruling out such racial differences. Recently, also a study in German iPAH patients was reported.[10] In this study, by immunohistochemistry, using a commercial monoclonal antibody directed against LANA-1, a positive signal reminiscent of the ''speckled'' nuclear pattern typical for HHV-8 infected cells was demonstrated in 16 out of 26 iPAH lung biopsies. The expression was, however, confined to bronchial and alveolar epithelial cells. Only in a single case LANA-1 positive mesenchymal cells were demonstrated adjacent to plexiform lesions. By different PCR assays (based on ORF 26, ORF K6 and ORF 72, respectively), HHV-8 infection could not be confirmed. Taken together, the present data appear to put an end to the ongoing debate on the possible etiological role of HHV-8 in iPAH.

The recent data are also in line with studies on the seroprevalence of HHV-8 in patients with iPAH as compared to non-iPAH patients and healthy individuals. In a German study,[3] no difference was found between 49 iPAH patients and 17 patients with other forms of pulmonary hypertension. In this respect, using highly specific and sensitive serological assays, based on both lytic and latent HHV-8 antigens, Laney and coworkers[5] found no evidence of HHV-8 infection in any of the 19 iPAH patients studied. In a recent French study,[4] the prevalence of antibodies against HHV-8 was found similar in iPAH patients and healthy blood donors, as well as in patients with HIV infection with or without associated PAH.

How to explain the differences between the initial observation by Cool and coworkers and all the subsequent studies? As shown,[10] immunochemistry may be prone to false-positive signals. The specificity of the PCR amplification products generated by the primers used by Cool and coworkers was questioned by Laney and coworkers.[7] Indeed, one round (35 cycles) of PCR may be prone to false positive

reactions. This can be overcome by using different PCR assays,[10] or a nested PCR, like we did. A nested PCR increases the specificity by a second round of amplification with two HHV-8 specific primers. Moreover, we also used two different PCR assays to detect HHV-8 DNA based on sequences of ORF 65 and ORF 73.

In conclusion, the results of the recent studies, in which no evidence for HHV-8 infection was found in Caucasian iPAH patients, argue strongly against an etiological role for HHV-8 in the pathogenesis of iPAH. In our view, iPAH can be added to the list of disorders that in the end were not proven to be causally linked with HHV-8 infection.

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