

## Expression of Human Herpesvirus 8 in Primary Pulmonary Hypertension

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### ABSTRACT

#### BACKGROUND

Severe pulmonary hypertension constitutes a group of diseases characterized by complex, lumen-occluding vascular lesions that develop in genetically susceptible persons. The only viral infection associated with severe pulmonary hypertension has been that due to human immunodeficiency virus type 1, but neither the viral genome nor viral antigens have been demonstrated in pathologic lesions.

#### METHODS

We examined lung-tissue samples from 16 patients with sporadic primary pulmonary hypertension and 14 patients with secondary pulmonary hypertension for evidence of infection with human herpesvirus 8 (HHV-8). HHV-8 infection was ascertained immunohistochemically with use of an antibody directed against latency-associated nuclear antigen 1 (LANA-1), and a polymerase-chain-reaction (PCR) assay was performed on lung DNA to detect the viral cyclin gene of HHV-8. Sequence analysis was also performed.

#### RESULTS

In lung tissue from 10 of 16 patients with primary pulmonary hypertension (62 percent), cells within the plexiform lesions as well as cells outside the lesions were positive for LANA-1 on immunohistochemical analysis. Tissue from the same 10 patients contained viral cyclin on PCR analysis. No LANA-1 was detected in lung tissue from patients with secondary pulmonary hypertension, although one such patient had PCR evidence of viral cyclin. Plexiform lesions from patients with primary pulmonary hypertension had a histologic and immunohistochemical resemblance to cutaneous Kaposi's sarcoma lesions.

#### CONCLUSIONS

The spectrum of trigger factors and molecular mechanisms leading to severe pulmonary hypertension and the formation of plexiform lesions is apparently wide, including both genetic and epigenetic factors. Our data suggest that infection with the vasculotropic virus HHV-8 may have a pathogenetic role in primary pulmonary hypertension.

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SEVERE PULMONARY HYPERTENSION constitutes a group of pulmonary vascular abnormalities characterized clinically by marked elevation of the pulmonary-artery pressure and the development of right ventricular failure. Primary (idiopathic) pulmonary hypertension occurs sporadically and in a familial form, in which germ-line mutations of bone morphogenetic protein receptor 2 (BMPR2) have been identified.<sup>1</sup> Although the clinical spectrum of severe pulmonary hypertension is large and includes primary forms and secondary forms (e.g., in association with congenital cardiac abnormalities, pulmonary embolism, portal hypertension, various collagen vascular disorders, sarcoidosis, and human immunodeficiency virus type 1 [HIV-1] infection), the histologic features, characterized by complex, lumen-occluding vascular lesions (plexiform lesions), are shared and are therefore not specific for any of the clinical associations.<sup>2,3</sup>

Analysis of lung tissue from patients with primary pulmonary hypertension has established that the endothelial cells of the plexiform lesions proliferate in a monoclonal fashion<sup>4</sup> and that they can have somatic mutations of the BMPR2 and BAX genes.<sup>5</sup> Although the histopathological findings are not specific, the molecular pathological characteristics of the various forms of severe pulmonary hypertension are distinct, as suggested by the fact that the gene-expression profile of whole-lung tissue can distinguish between patients with sporadic and those with familial primary pulmonary hypertension.<sup>6</sup> However, not all plexiform lesions from patients with primary pulmonary hypertension have somatic mutations, and only 50 percent of patients with familial primary pulmonary hypertension have BMPR2 mutations.<sup>1,5</sup> Therefore, additional molecular factors must be involved in the acquisition of the selective growth advantage of endothelial cells in patients with severe pulmonary hypertension.

Our understanding of the role of human herpesvirus 8 (HHV-8), a newly discovered gamma herpesvirus, in human disease is evolving. HHV-8 is thought to be the cause of all clinical types of Kaposi's sarcoma<sup>7,8</sup> and rare lymphoproliferative disorders associated with HIV-1 infection, such as primary effusion lymphoma and multicentric Castleman's disease.<sup>9</sup> Seroepidemiologic studies have established that the prevalence of HHV-8 infection varies geographically and is influenced by behavioral risk factors. In the United States, antibodies

against HHV-8 latency-associated nuclear antigen 1 (LANA-1) are present in approximately 33 percent of homosexual men without Kaposi's sarcoma, 8 percent of patients without HIV-1 infection who are attending sexually transmitted disease clinics, and up to 3 percent of blood donors without HIV-1 infection.<sup>10,11</sup>

Several observations led us to explore the potential relation between HHV-8 infection and primary pulmonary hypertension. First, there is a well-described association between HIV-1 infection and severe pulmonary hypertension,<sup>12-14</sup> and although the effect of the coexistence of HHV-8 infection in these patients has not been studied, the prevalence of HHV-8 infection is increased among patients who are infected with HIV-1.<sup>11,15</sup> Second, primary pulmonary hypertension has been described in two patients with HHV-8-associated Castleman's disease, and lung tissue from one of these patients was positive for LANA-1.<sup>16</sup> Third, elevated levels of inflammatory cytokines are present in the serum of patients with primary pulmonary hypertension,<sup>17,18</sup> and T and B lymphocytes are present in plexiform lesions from such patients.<sup>19,20</sup> These observations raise the possibility that an immune response to an undefined antigenic stimulus may be present in these patients or may even be involved in the pathogenesis of this disorder. Finally, in the course of evaluating a large number of patients with primary pulmonary hypertension, we noted a histologic resemblance between the plexiform lesions of the disorder and the endothelial abnormalities of cutaneous Kaposi's sarcoma. For these reasons, we examined lung tissue from patients with primary and various secondary forms of severe pulmonary hypertension for evidence of infection with HHV-8.

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## METHODS

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### SCREENING STUDY

In a screening study, we extracted DNA from 15 microdissected plexiform lesions from six patients with primary pulmonary hypertension and 5 skin lesions from five patients with Kaposi's sarcoma, with the use of laser capture microdissection (PixCell II LCM unit, Arcturus Engineering). Only the core of the lesions containing endothelial cells was dissected from serial sections that were 10  $\mu$ m thick (approximately 100 cells per section). DNA was extracted and amplified with use of a polymerase-chain-reaction (PCR) assay (Expand Long Template

PCR System, Roche Diagnostics) in combination with nested PCR in order to amplify DNA from a single tumor cell. PCR analysis of the DNA representing the HHV-8 open reading frame 26 (ORF26)<sup>21,22</sup> was performed.

To corroborate the findings, we also analyzed formalin-fixed, paraffin-embedded samples of lung tissue from 30 patients with severe pulmonary hypertension, 4 patients with other lung diseases, and 1 control patient with normal lung tissue. A lymph node from one of the patients with Castleman's disease and primary pulmonary hypertension was analyzed because lung tissue was unavailable. Cutaneous-biopsy specimens from five patients with Kaposi's sarcoma served as positive controls. We examined the tissue sections for HHV-8 LANA-1 using immunohistochemical techniques and for viral cyclin using DNA extraction followed by PCR analysis.

#### PATIENTS

The institutional review board of the University of Colorado Health Sciences Center approved the study. Patients with severe pulmonary hypertension who were on the waiting list for lung transplantation granted written permission for their explanted lung tissue to be used for investigations, and their next of kin gave written permission to use the tissue samples for a postmortem examination as well as for research. Lung tissue banked at the Pulmonary Hypertension Center of the University of Colorado Health Sciences Center from 30 patients with severe pulmonary hypertension was identified, and the patients' medical history, clinical presentation, and data obtained by right heart catheterization were reviewed (see Supplementary Appendix 1, available with the full text of this article at <http://www.nejm.org>). Most of the patients had been followed for many years at the center. Some of the archived tissue (e.g., from Patients 7 and 15) had been obtained at autopsy 10 to 20 years earlier. The histologic findings in the lungs and pulmonary vessels were independently reviewed by two pathologists, using tissue sections stained with hematoxylin and eosin.

Patients 1, 2, 3, and 4 had recently received a lung transplant; Patients 2, 3, and 4 had done so after the failure of long-term prostacyclin infusion therapy. Patient 13 had died 2.5 years after the development of fenfluramine-induced pulmonary hypertension; Patient 7 had a history of injection-drug abuse. Birefringent crystals, as well as plexiform lesions, were noted in the lung tissue from Patient 7. Patient 6 had

initially been treated for sleep-apnea-associated pulmonary hypertension before receiving a diagnosis of primary pulmonary hypertension, and Patient 22 had been given a diagnosis of primary pulmonary hypertension before a skin biopsy documented sarcoidosis. Patient 8 had primary pulmonary hypertension associated with Castleman's disease<sup>16</sup>; Patients 19, 20, and 21 had pulmonary hypertension associated with HIV-1 infection; and Patients 27, 28, 29, 30, and 31 had been given a diagnosis of severe pulmonary hypertension due to atrial septal or ventricular septal defects. Patient 18 had pulmonary capillary hemangiomatosis. Lung tissue from two patients with cryptogenic organizing pneumonia, one patient with nonspecific interstitial pneumonitis, one patient with smoking-induced emphysema, and one patient with normal lung tissue served as controls (Table 1).

#### IMMUNOHISTOCHEMICAL ANALYSIS

Immunohistochemical staining was performed as previously described to identify the LANA-1 of HHV-8 encoded by ORF73 (dilution, 1:1500; Advanced Biotechnologies).<sup>23</sup> For each antigen, skin tissue from the five patients with Kaposi's sarcoma and lung tissues were stained simultaneously. Briefly, after paraffin-embedded blocks had been cut into 5- $\mu$ m sections and mounted onto slides, the specimens were deparaffinized and rehydrated. High-temperature antigen retrieval involved boiling the slides in citrate buffer (10 mM per liter, pH 6.0) for 20 minutes, followed by incubation with the avidin-biotin-peroxidase complex (Vectastain Elite ABC kit, Vector Laboratories) according to the manufacturer's instructions. For the detection of LANA-1, the samples were incubated with primary antibody (dilution, 1:1500) at room temperature for one hour. The samples were then stained with 3,3'-diaminobenzidine as the chromogen and counterstained with Mayer's hematoxylin. Normal blocking serum without primary antibody was used for the negative control.

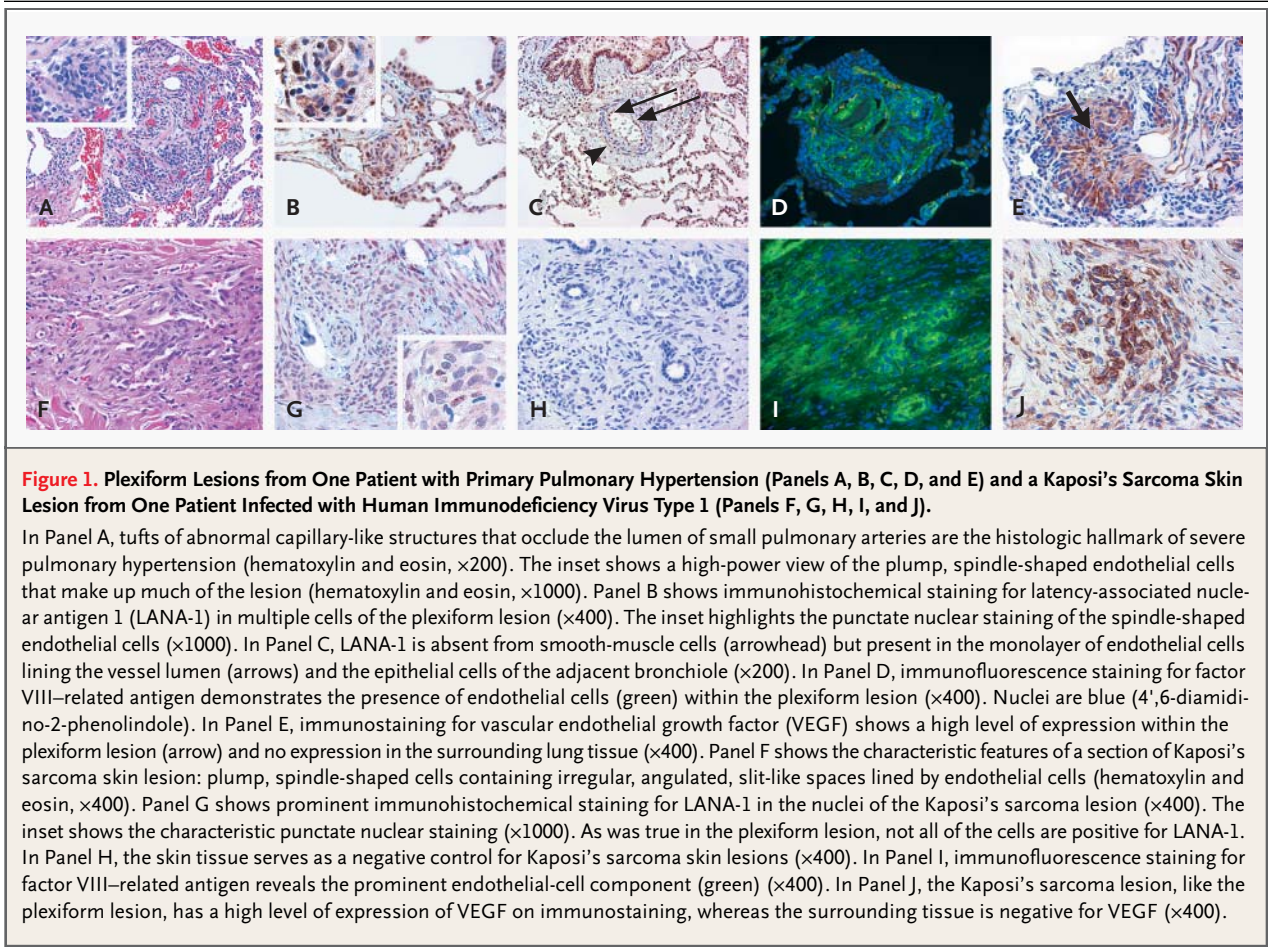
For the comparison of Kaposi's sarcoma and plexiform lesions, selected slides were immunostained with an antibody against vascular endothelial growth factor (VEGF) (dilution, 1:300; Dako). An immunofluorescence assay for factor VIII-related antigen was also performed (dilution, 1:1000; Dako) in which the samples were incubated with goat-antirabbit secondary antibody (dilution, 1:200) conjugated to fluorescein (Molecular Probes).

**Table 1. Results of Immunohistochemical (IHC) and Polymerase-Chain-Reaction (PCR) Analysis of Lung Tissue.\***

Subject	Sex	Condition	Associated Condition	LANA-1 on IHC Analysis	Viral Cyclin Gene on PCR	BMP2 Mutation
<b>Patients</b>						
Patient 1	M	PPH		Yes	Yes	No
Patient 2	F	PPH		Yes	Yes	No
Patient 3	F	PPH		Yes	Yes	No
Patient 4	M	PPH		Yes	Yes	No
Patient 5	NA	PPH		No	No	No
Patient 6	F	PPH		Yes	Yes	No
Patient 7	M	PPH		No	No	Yes
Patient 8	F	PPH	Castleman's disease	Yes	Yes	Yes
Patient 9	F	PPH		No	No	No
Patient 10	M	PPH		No	No	Yes
Patient 11	F	PPH		Yes	Yes	No
Patient 12	NA	PPH		No	No	No
Patient 13	F	PH	Fenfluramine use	No	No	
Patient 14	F	PPH		Yes	Yes	No
Patient 15	F	PPH		Yes	Yes	No
Patient 16	M	PPH		Yes	Yes	Yes
Patient 17†	M	PPH	Castleman's disease	No	No	No
Patient 18	M	PCH		No	No	
Patient 19	M	PH	HIV-1 infection	No	No	
Patient 20	NA	PH	HIV-1 infection	No	Yes	
Patient 21	F	PH	HIV-1 infection	No	No	
Patient 22	F	PH	Sarcoidosis	No	No	
Patient 23	F	PH	CREST	No	No	
Patient 24	F	PH	CREST	No	No	
Patient 25	F	PH	CREST	No	No	
Patient 26	M	PH	CREST	No	No	
Patient 27	F	PH	Eisenmenger's syndrome	No	No	
Patient 28	F	PH	Eisenmenger's syndrome	No	No	
Patient 29	F	PH	Eisenmenger's syndrome	No	No	
Patient 30	F	PH	Eisenmenger's syndrome	No	No	
Patient 31	M	PH	Eisenmenger's syndrome	No	No	
<b>Controls</b>						
Patient 32	F	COP		No	No	
Patient 33	F	COP		No	No	
Patient 34	M	NSIP		No	No	
Patient 35	M	Normal		No	No	
Patient 36	M	Emphysema		No	No	

\* LANA-1 denotes latency-associated nuclear antigen 1; *BMP2* bone morphogenetic protein receptor 2; PPH primary pulmonary hypertension; NA not available; PH pulmonary hypertension; PCH pulmonary capillary hemangiomas; HIV-1 human immunodeficiency virus type 1; CREST calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasis; COP cryptogenic organizing pneumonia; and NSIP nonspecific interstitial pneumonitis.

† A lymph-node–biopsy specimen was analyzed rather than lung tissue.



**Figure 1.** Plexiform Lesions from One Patient with Primary Pulmonary Hypertension (Panels A, B, C, D, and E) and a Kaposi's Sarcoma Skin Lesion from One Patient Infected with Human Immunodeficiency Virus Type 1 (Panels F, G, H, I, and J).

In Panel A, tufts of abnormal capillary-like structures that occlude the lumen of small pulmonary arteries are the histologic hallmark of severe pulmonary hypertension (hematoxylin and eosin,  $\times 200$ ). The inset shows a high-power view of the plump, spindle-shaped endothelial cells that make up much of the lesion (hematoxylin and eosin,  $\times 1000$ ). Panel B shows immunohistochemical staining for latency-associated nuclear antigen 1 (LANA-1) in multiple cells of the plexiform lesion ( $\times 400$ ). The inset highlights the punctate nuclear staining of the spindle-shaped endothelial cells ( $\times 1000$ ). In Panel C, LANA-1 is absent from smooth-muscle cells (arrowhead) but present in the monolayer of endothelial cells lining the vessel lumen (arrows) and the epithelial cells of the adjacent bronchiole ( $\times 200$ ). In Panel D, immunofluorescence staining for factor VIII-related antigen demonstrates the presence of endothelial cells (green) within the plexiform lesion ( $\times 400$ ). Nuclei are blue (4',6-diamidino-2-phenylindole). In Panel E, immunostaining for vascular endothelial growth factor (VEGF) shows a high level of expression within the plexiform lesion (arrow) and no expression in the surrounding lung tissue ( $\times 400$ ). Panel F shows the characteristic features of a section of Kaposi's sarcoma skin lesion: plump, spindle-shaped cells containing irregular, angulated, slit-like spaces lined by endothelial cells (hematoxylin and eosin,  $\times 400$ ). Panel G shows prominent immunohistochemical staining for LANA-1 in the nuclei of the Kaposi's sarcoma lesion ( $\times 400$ ). The inset shows the characteristic punctate nuclear staining ( $\times 1000$ ). As was true in the plexiform lesion, not all of the cells are positive for LANA-1. In Panel H, the skin tissue serves as a negative control for Kaposi's sarcoma skin lesions ( $\times 400$ ). In Panel I, immunofluorescence staining for factor VIII-related antigen reveals the prominent endothelial-cell component (green) ( $\times 400$ ). In Panel J, the Kaposi's sarcoma lesion, like the plexiform lesion, has a high level of expression of VEGF on immunostaining, whereas the surrounding tissue is negative for VEGF ( $\times 400$ ).

**HHV-8 VIRAL CYCLIN ANALYSES**

Formalin-fixed, paraffin-embedded tissue was used for DNA extraction. Five-micrometer sections were cut from each paraffin block and placed in 0.5-ml MicroAmp tubes (Perkin-Elmer Applied Biosystems). The tissue sections were deparaffinized twice in xylene for five minutes each, followed by a two-step rehydration in 100 percent ethanol. The air-dried pellet was then resuspended in buffer with proteinase K, incubated for 18 hours at 37°C and 3 hours at 55°C, and then heat inactivated for 10 minutes at 98°C.

The PCR primers were synthesized to amplify the viral cyclin of HHV-8 encoded by ORF72. Primer set KS1 and KS2 (KS1, 5'CGCCTGTAGAACGGAAACAT; KS2, 5'TTGCCCGCCTCTATTATCAG) amplifies a 138-bp fragment of HHV-8. The PCR conditions have been described previously.<sup>24</sup>

DNA from each PCR product was quantified with the use of ultraviolet photospectrometry, then di-

luted to a concentration of 20 ng per microliter of water. ORF72-specific primers were diluted to a concentration of 10  $\mu$ M in water. The samples were sequenced at the Diabetes and Endocrinology Research Center's Molecular Biology Core Facility (Barbara Davis Center, University of Colorado Health Sciences Center). We used the Basic Local Alignment Search Tool 2 (BLAST2) Sequences program of the National Center for Biotechnology Information, which aligns two sequences, to determine the degree of homology between the two sequences.<sup>25</sup>

**ANALYSIS OF BMPR2 FOR MUTATIONS**

DNA from the 16 patients with primary pulmonary hypertension was screened for at least seven of the most commonly reported mutations in the BMPR2 gene. Mutations K230fs, I860fs, and R899X have been reported by two or more independent groups.<sup>1,26-28</sup> The details of the analysis are pro-

vided in Supplementary Appendix 2, available with the full text of this article at <http://www.nejm.org>.

RESULTS

SCREENING

In the screening study, 4 of 15 microdissected plexiform lesions from patients with pulmonary hypertension were positive for ORF26 by PCR.

IMMUNOHISTOCHEMICAL FINDINGS

Ten of the 16 patients with primary pulmonary hypertension (62 percent), but none of the 14 patients with secondary pulmonary hypertension, had lung-tissue sections that showed the characteristic nuclear-staining pattern when probed with a monoclonal antibody directed against the LANA-1 of HHV-8 encoded by ORF73 (Table 1 and Fig. 1). The lymph node from one of the patients with Castleman's disease and primary pulmonary hypertension (Patient 17) was negative for LANA-1. We found that not only cells within the plexiform lesions but also bronchoepithelial cells, inflammatory cells (lymphocytes and macrophages), and endothelial cells lining patent lung vessels were positive for LANA-1. Smooth-muscle cells were consistently negative for LANA-1 (Fig. 1C). All specimens from the five controls were negative for LANA-1.

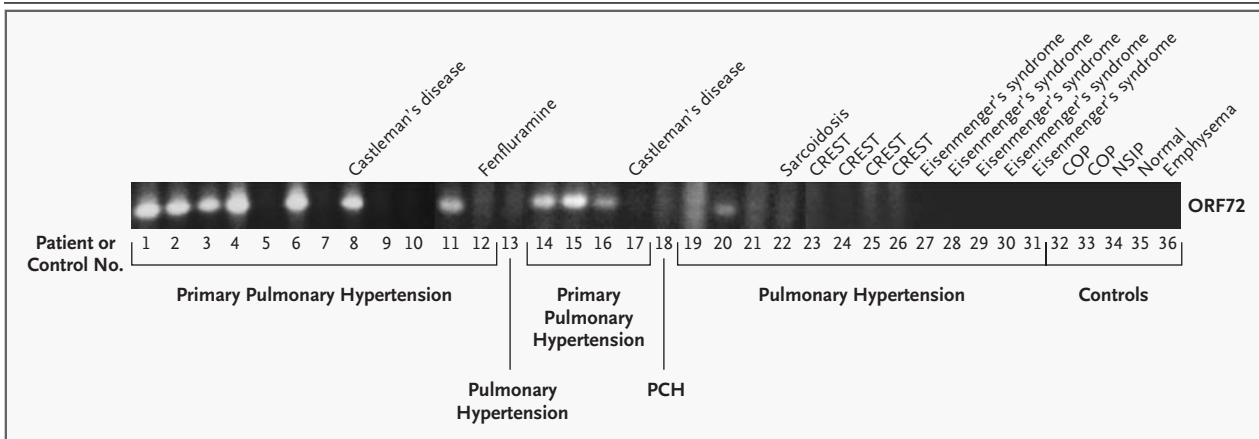
HHV-8 VIRAL CYCLIN

On PCR, 10 of the 16 patients with primary pulmonary hypertension (the same 10 who were positive for LANA-1 on immunohistochemical analysis) and 1 of 14 patients with secondary pulmonary hypertension had tissue sections that were positive for the viral cyclin gene of HHV-8 encoded by ORF72. Specimens from all five controls were negative for the viral cyclin gene on PCR (Table 1 and Fig. 2).

Sequence analysis of one skin lesion from a patient with Kaposi's sarcoma and four lung samples from patients with pulmonary hypertension who were positive for the HHV-8 viral cyclin gene on PCR analysis showed a high degree of sequence homology with the published full-length HHV-8 sequence. The Kaposi's sarcoma lesion was 91 percent homologous, as was the sample from Patient 2. The sample from Patient 4 was 97 percent homologous, that from Patient 6 was 86 percent homologous, and that from Patient 20 was 98 percent homologous (Fig. 3). Long stretches of conserved sequences were found toward the 5' end of the gene, whereas the sequences in the 3' region were more variable.

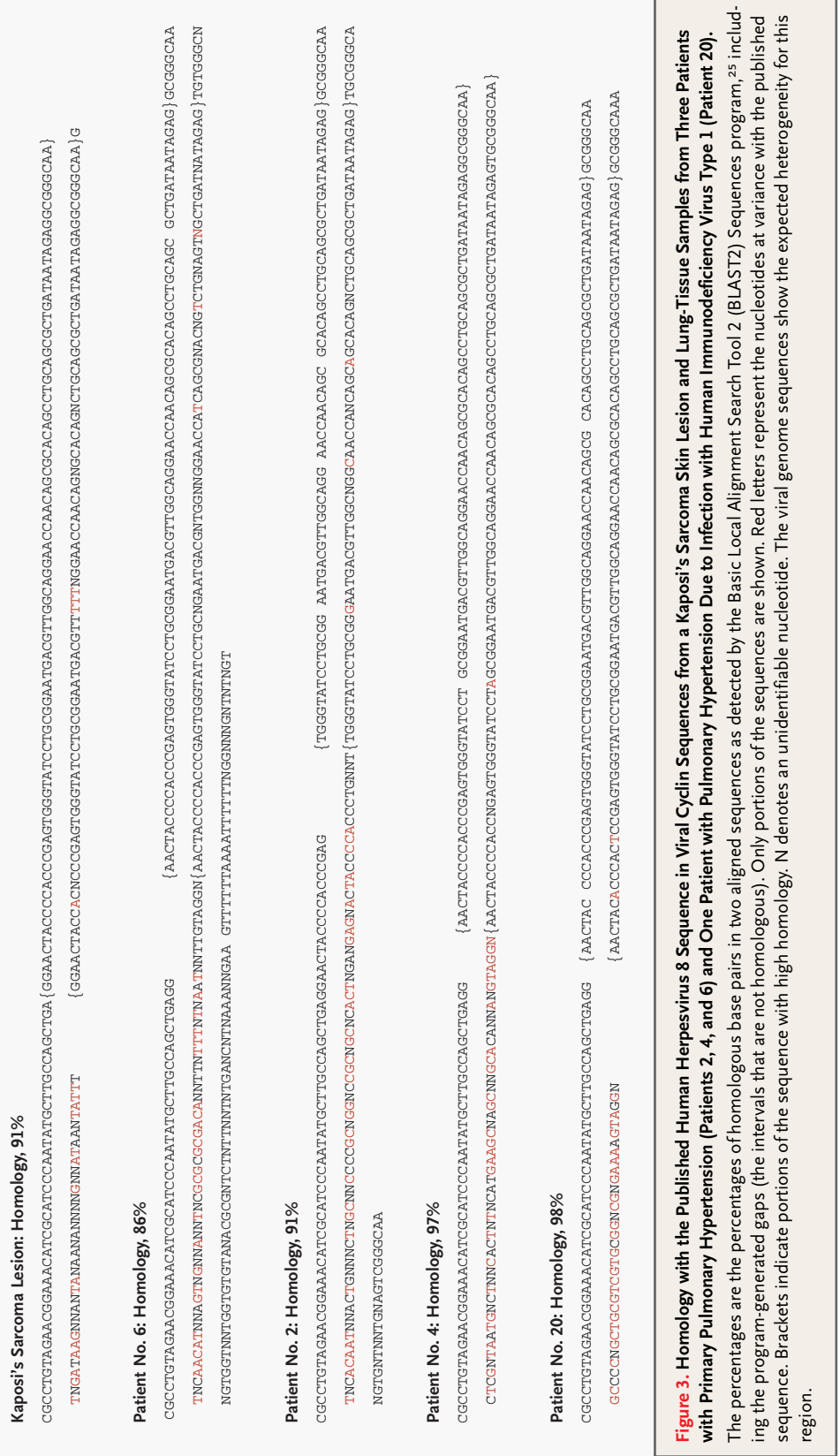
BMPR2 MUTATIONS

DNA from Patient 8 had an R899X mutation in BMPR2. DNA from Patients 7, 10, and 16 had a previously unreported restriction-fragment-length



**Figure 2.** Detection of a Human Herpesvirus 8 Gene by Means of Polymerase-Chain-Reaction Amplification of Viral Cyclin Encoded by Open Reading Frame 72 (ORF72).

Using primer pairs specific for ORF72, we subjected 200 ng of genomic DNA to one round (35 cycles) of PCR amplification. Genomic DNA was from patients with primary pulmonary hypertension (lanes 1 through 12 and 14, 15, 16, and 17), pulmonary capillary hemangiomas (PCH) (lane 18), secondary pulmonary hypertension (lanes 13 and 19 through 31), cryptogenic organizing pneumonia (COP) (lanes 32 and 33), nonspecific interstitial pneumonitis (NSIP) (lane 34), and emphysema (lane 36) and from a subject with normal lung tissue (lane 35). CREST denotes calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasis.



polymorphism (RFLP) pattern. Thus, 4 of the 16 patients with primary pulmonary hypertension who were screened (25 percent) had mutations. Two of these patients were positive for HHV-8 on immunohistochemical and PCR analysis (Patients 8 and 16), and two were negative (Patients 7 and 10) (Table 1).

#### DISCUSSION

Our studies provide evidence of HHV-8 infection in the vascular lesions and lung parenchymal cells of patients with nonfamilial primary pulmonary hypertension. HHV-8 infection of lung tissue was demonstrated with the use of two methods, immunohistochemical analysis for the expression of LANA-1 encoded by ORF73 and PCR for the detection of viral cyclin DNA encoded by ORF72. There was a high rate of concordance between staining for LANA-1 and the detection of viral cyclin DNA.

LANA-1 is constitutively expressed in lytic and latent HHV-8 infection<sup>29,30</sup> and is a reliable indicator of HHV-8 infection.<sup>31</sup> The pulmonary vascular lesions and parenchymal cells from 10 of 16 patients with nonfamilial primary pulmonary hypertension expressed LANA-1, whereas tissue samples from only 1 of 3 patients with HIV-1–associated severe pulmonary hypertension were positive. Kaposi's sarcoma was not present in this patient. These data suggest that HIV-1–associated pulmonary hypertension can occur independently of HHV-8 infection. In contrast to specimens from patients with primary pulmonary hypertension, the lung tissue from all patients with secondary pulmonary hypertension due to known causes, such as congenital cardiac abnormalities, collagen vascular disorders, anorexigen use,<sup>32</sup> and sarcoidosis, had no evidence of HHV-8 infection.

LANA-1, viral cyclin, and viral FLICE inhibitory protein (FLIP) are cotranscribed on two polycistronic messenger RNAs and are constitutively expressed in cells latently infected with HHV-8.<sup>31</sup> Consequently, the presence of LANA-1 in plexiform lesions from patients with primary pulmonary hypertension reliably predicts the coexpression of viral cyclin and viral FLIP gene products. Viral cyclin has both functional and sequence homology with D-type cyclins, which direct cyclin-dependent kinase 4 and 6 to phosphorylate the retinoblastoma protein.<sup>33,34</sup> Underphosphorylated retinoblastoma protein is sequestered by the E2F family of transcription factors, which regulates the expression of cell-cycle–entry genes. Therefore, viral cyclin induces cell-cycle pro-

gression by inhibiting the function of the retinoblastoma protein. The generation of free E2F also leads to the expression of p14<sup>ARF</sup>, ultimately increasing the expression of the p53 tumor-suppressor protein and inducing p53-dependent apoptosis.<sup>35</sup> LANA-1 inhibits the function of p53, thereby preventing apoptosis of latently infected cells.<sup>36</sup> The capacity of gene products expressed during latent infection (e.g., viral cyclin and LANA-1) to trigger unregulated cell-cycle progression while simultaneously blocking p53-dependent apoptosis is probably a major molecular mechanism contributing to the oncogenicity of HHV-8.<sup>30</sup> The pattern of inducing E2F-dependent transcription while inhibiting p53-dependent apoptosis is a common feature of several different DNA tumor viruses, such as human papillomaviruses, adenoviruses, and simian virus 40.<sup>37,38</sup>

The types of cells that HHV-8 may infect have not all been identified. We detected the expression of LANA-1 in a variety of cells in the lungs of patients with primary pulmonary hypertension, including bronchoepithelial cells, inflammatory cells (primarily lymphocytes and macrophages), and endothelial cells. Our data suggest that HHV-8 has the capacity to infect a wide variety of cells in lung tissue, and we hypothesize that HHV-8 infection may be responsible for a broader spectrum of diseases in humans than is currently known.

Although we demonstrated HHV-8 infection of the lungs in patients with primary pulmonary hypertension, we cannot conclude that infection alone causes this condition. It is possible that some unique aspect of primary pulmonary hypertension increases the risk of HHV-8 infection. Factors unique to the plexiform lesions (e.g., local production of inflammatory cytokines) may enhance the replication of HHV-8 in affected tissues. However, that HHV-8 infection has some role in the process is suggested by the number of features that are shared by Kaposi's sarcoma and the plexiform lesions of primary pulmonary hypertension (Table 2). In primary but not secondary pulmonary hypertension, many, but not all, of the endothelial-cell clusters, which make up a large portion of the plexiform lesions, are due to the monoclonal growth of endothelial cells.<sup>4</sup> In this context, it is of interest that early Kaposi's sarcoma lesions are characterized by polyclonal-cell hyperplasia, but they can develop into a true clonal cancer as the disease progresses to its nodular state.<sup>39</sup> Our findings show that Kaposi's sarcoma skin lesions and plexiform lesions in patients with primary pulmonary hypertension have

histologic similarities: slit-like vascular spaces and sheets of spindle cells that express factor VIII-related antigen (an endothelial-cell marker) and VEGF. HHV-8 may thus have a particular affinity for tissues and cells that have a high level of expression of VEGF, such as lung microvascular endothelial cells or Kaposi's sarcoma cells. The increased expression of VEGF may in turn increase the growth of endothelial cells.<sup>40-42</sup>

The resemblance between Kaposi's sarcoma lesions and the plexiform lesions of primary pulmonary hypertension suggests that the spectrum of molecular mechanisms leading to the development of severe pulmonary hypertension includes genetic and epigenetic mechanisms. Clearly, one or several genetic susceptibility factors are required to allow the proliferation of endothelial cells to occur in pulmonary precapillary arterioles. High shear stress (as occurs in tissues from patients with Eisenmenger's syndrome), anorexigen use, and perhaps HHV-8 infection may help trigger or amplify a process in which "the law of the endothelial cell monolayer has been broken"<sup>43</sup> and there is endothelial-cell dysfunction<sup>44</sup> and irreversible obliteration of the lumen by a disordered process of angiogenesis.<sup>42,43</sup>

We propose that HHV-8 infection and the expression of viral cyclin and LANA-1 in the lungs contribute to the growth of monoclonal endothelial cells and mutations in somatic endothelial cells in plexi-

**Table 2. Features Shared by Kaposi's Sarcoma Lesions and Plexiform Lesions from Patients with Primary Pulmonary Hypertension.\***

Expression of endothelial-cell and smooth-muscle-cell markers
Clusters of macrophages and lymphocytes
Increased expression of Bcl-2†
Reduced expression of p27
Up-regulation of VEGF
Expression of VEGF receptor 2

\* VEGF denotes vascular endothelial growth factor.

† Kaposi's sarcoma lesions express viral Bcl-2.

form lesions from patients with primary pulmonary hypertension. Our findings indicate that vasculotropic viruses such as HHV-8 can encourage the growth of endothelial cells by dysregulating cell growth or growth-factor signaling.

*Note added in proof:* While this article was being prepared, an additional patient with primary pulmonary hypertension and HHV-8-positive lung vascular lesions was identified.

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