Letters to the Editor

CORRESPONDENCE RE: BRYANT-GREENWOOD P, SORBARA L, FILIE AC, LITTLE R, YARCHOAN R, WILSON W, RAFFELD M, ABATI A. INFECTION OF MESOTHELIAL CELLS WITH HUMAN HERPES VIRUS 8 IN HUMAN IMMUNODEFICIENCY VIRUS-INFECTED PATIENTS WITH KAPOSI'S SARCOMA, CASTLEMAN'S DISEASE, AND RECURRENT PLEURAL EFFUSIONS. MOD PATHOL 2003;16:145–53.

To the Editor: In a recent issue of this journal, Bryant-Greenwood *et al.* (1) described the possible infection of mesothelial cells with human herpesvirus 8 (KSHV/HHV8) in patients with recurrent non-lymphomatous pleural effusions during Kaposi's sarcoma (KS) and Castleman's disease (CD). KSHV/HHV8 has been implicated in the pathogenesis of three cytokine-driven diseases: KS, multicentric CD, and primary effusion lymphoma (PEL), a peculiar lymphoma growing in liquid-phase within body cavities. KSHV/HHV8-infected individuals with or without overt KS or multicentric CD may indeed develop massive and recurrent intracavitary effusions other than PEL, as reported by Bryant-Greenwood *et al.*, our group (2), and other investigators (3). The tendency to form effusions in such patients has been recognized even before KSHV/HHV8 discovery (4–6). Altogether, these data point to a tropism of KSHV/HHV8-infected cells into the body cavities in non-neoplastic settings. How this contributes to the pathogenesis of recurrent effusions and, perhaps, to the development of PEL remains unknown. As well, the question of which cell type(s) harbor the virus within serous cavities remains somewhat unanswered. In my opinion, the most likely cell is of lympho-monocyte lineage.

The tropism of KSHV/HHV8 for mesothelial cells is theoretically possible. Viral DNA and transcripts have been detected in B cells, endothelial cells, macrophages, and epithelial cells; this has been explained by the ability of the virus to bind the ubiquitous cell surface heparan sulfate molecule (7). Furthermore, there is a close similarity between mesothelial cells and endothelial cells. However, a clear-cut proof that KSHV/HHV8 can infect mesothelial cells is lacking. Although Bryant-Greenwood and co-authors affirm that only mesothelial cells were microdissected and captured for PCR analysis for the search of KSHV/HHV8 DNA sequences, the depletion of virus-infected circulating cells was not ascertained. Also, immunohistochemistry against the latent nuclear antigen ORF-73 is not persuasive enough; the nuclear staining pattern is questionable, and the proof that ORF-73-positive cells are of mesothelial origin is not shown. Previous studies have indirectly excluded mesothelial cells as target of KSHV/HHV8 infection by PCR testing mesothelial rich effusions of non-neoplastic etiology (8) and of mesotheliomatous origin (8, 9) even with concomitant evidence of KSHV/HHV8 infection and KS (10). Nevertheless, the article of Bryant-Greenwood *et al.* renews the interest in the pathogenesis of KSHV/HHV8-positive effusions, and the need to perform additional studies to define the role of mesothelial cells and other resident cells in their interaction with KSHV/HHV8. Similarly to KS, a pathogenetic model could be that KSHV/HHV8-infected circulating cells are recruited into body cavities. Herein the virus finds an appropriate environment for establishing a persistent infection by stimulating mesothelial cell proliferation through the secretion of many cytokines, chemokines, and growth factors derived from inflammatory cells, particularly macrophages (11).

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In reply: In her thoughtful Letter to the Editor, Dr. Ascoli states that clear-cut proof that HHV8 can infect mesothelial cells is lacking. She questions the technique utilized for microdissection and isolation of mesothelial cells, asserting that the removal of the virus-containing cells in the background (*i.e.*, hematopoietic cells) was not established. In that vein, we would like to assure Dr. Ascoli that the mesothelial cells were indeed isolated from all other cells within the effusion before PCR analysis, as was detailed in our original publication (1). It is perhaps worth noting that the microdissection was performed by a cytopathologist who is trained to be able to use established morphologic criteria to distinguish mesothelial cells from background hematopoietic cells, thus the probability of contamination of the samples with background infected cells was min-

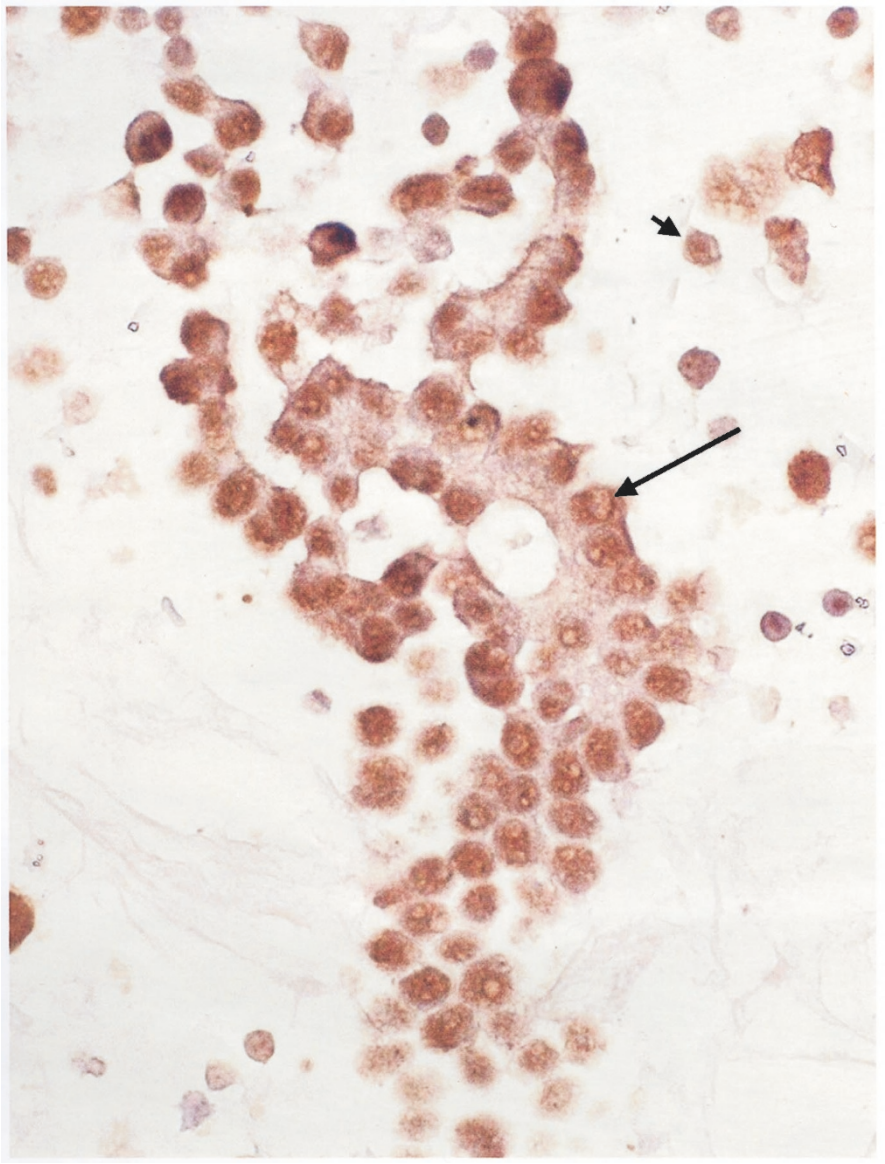

FIGURE 1. Primary effusion cavity lymphoma. Immunohistochemistry for HHV8 ORF73 reveals nuclear staining in chains of mesothelial cells (long arrow) and background lymphoma cells (short arrow) in this cell block preparation (hematoxylin and di-aminobenzidine).

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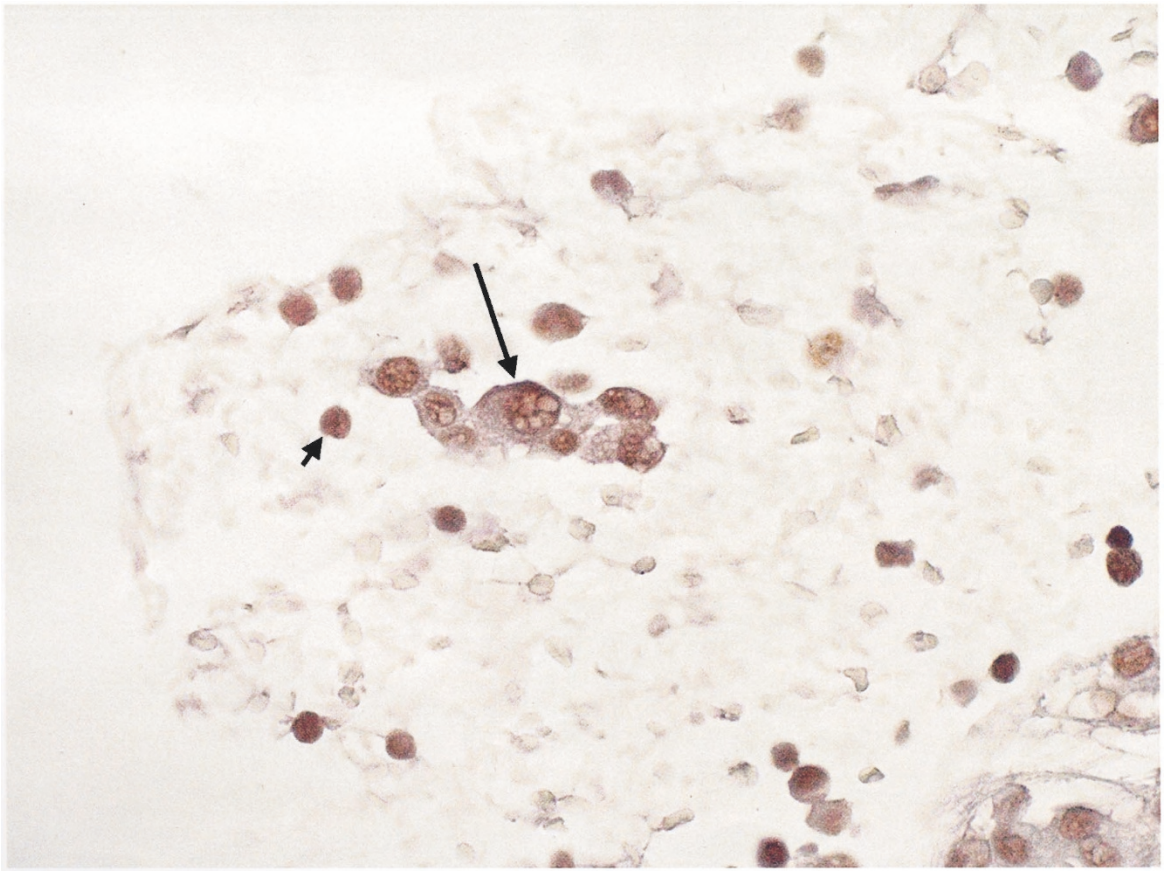

FIGURE 2. HHV8-related benign effusion. Immunohistochemistry for HHV8 ORF73 reveals nuclear staining in a small sheet of mesothelial cells (long arrow) and background benign lymphoid cells (short arrow) in this cell block preparation (hematoxylin and di-aminobenzidine).

imal (1). The isolation of unique groups of cells for further studies is the premise of microdissection, which has become a well-established and widely utilized technique.

She further questions the immunohistochemistry against HHV8 ORF-73, stating that it is not persuasive, *i.e.*, the nuclear staining pattern is questionable, and proof that ORF-73 staining in mesothelial cells is lacking. The question of mesothelial morphology and immunoreactivity is best demonstrated by Figures 1 and 2. In Figure 1, nuclear staining for ORF73 in the chains of mesothelial cells in this case of primary effusion cavity lymphoma are evident, as is nuclear staining in the background malignant lymphoma cells, which are known to be infected with HHV8. Juxtaposed to this is the original figure from our article (Fig. 2). This shows a small sheet of mesothelial cells exhibiting nuclear staining for ORF73, which also is seen in the background hematopoietic elements in this benign effusion associated with HHV8 infection. Mesothelial cells in cell blocks of effusions occur in sheets and strings and show a significant amount of intercellular adhesion, while hematopoietic elements occur as single cells. The morphologic distinction is made without difficulty.

Dr. Ascoli recapitulates an intriguing but unrelated prior area of study—the fact that HHV8 infection has not been directly associated with malignant mesothelioma (MM) (2–4). Due to the fact that malignant mesothelioma has been shown to secrete IL-6, a growth factor that has been associated with the growth of Kaposi's sarcoma, a potential association had been speculated. However, based on the studies of Dr. Ascoli and others, there has been consistent failure to locate HHV8 DNA sequences within the cells of malignant mesothelioma (2–4). Also, in a single case report of a patient with MM followed in a year by KS, the MM cells were negative for HHV8 by PCR (4). Nevertheless, the lack of association between HHV8 infection and mesothelioma is not germane to the question of whether mesothelial cells become infected with HHV8 in HHV8-infected patients. Her report that mesothelial cells were not found to be infected with HHV8 in a patient with mesothelioma who later developed KS may be at variance with the results in our article. However, this only shows that it is not found in all patients—not that it does not occur. Also, it is possible that mesothelioma cells are more resistant to HHV-8 infection than normal mesothelial cells. Finally, it is possible that the technique

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used in that article was not as sensitive or that there was sampling effect.

Additionally, as was seen in the aforementioned case report, MM can compromise lung function overall, with this unfortunate patient dying of respiratory failure. We have previously reported that **hypoxia** can actually activate HHV8 replication (5). Perhaps in this patient, respiratory insufficiency related to the MM activated HHV8 and contributed to the development of KS several months later.

In her letter, Dr. Ascoli theorizes that the recurrent effusions associated with HHV8 infection are due to the recruitment of infected circulating inflammatory cells into the body cavities with the emergence of persistent infection leading to effusions. This may certainly be true, but there is no rule limiting the HHV8 infection to one cell type. As noted above, our studies have provided evidence that the mesothelial cells contain viral DNA of HHV8 via immunohistochemistry and polymerase chain reaction. One also can speculate that persistent infection of the mesothelial cells by HHV8 contributes to the pathogenesis of the effusions.

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