CONCISE COMMUNICATIONS

Persistence of Human Herpesvirus 7 in Normal Tissues Detected by Expression of a Structural Antigen

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Human herpesvirus 7 (HHV-7) infection in histologically normal human tissues was investigated by immunohistochemical detection of the 85-kDa tegument phosphoprotein (pp85) encoded by the U14 gene. So far, two cell types were recognized as sites of HHV-7 infection in vivo: $CD4^+$ T lymphocytes, believed to be the site of latent infection, and epithelial cells of salivary glands, the site of productive infection and viral shedding. Unexpectedly, cells expressing the HHV-7 structural antigen were detectable in lungs, skin, and mammary glands. Morphologically and phenotypically, they were distinct from lymphocytes. Liver, kidney, and tonsils were positive, although the number of HHV-7–positive cells was low. Large intestine, spleen, and brain were negative. Different from the current notion of the state of HHV-7 in humans, the results show that a variety of tissues harbor cells at a late stage of infection and suggest that HHV-7 causes a persistent rather than a true latent infection.

Human herpesvirus 7 (HHV-7) was isolated from peripheral blood mononuclear cells (PBMC) and saliva of healthy individuals and of individuals affected by chronic fatigue syndrome [1–3]. It was considered an ''orphan virus,'' that is, without association with any known human disease. Discoveries of the past years are changing this perspective, as they led to the recognition that primary infection results sporadically in exanthem subitum or in febrile illnesses without rash [4, 5], that during primary infection the virus may have access to the central nervous system and induce neurologic complications [6], that infection or reactivation may take place in bone marrow and renal transplant recipients and complicate engraftment of transplanted organs [7, 8], and that viral DNA sequences are present in lesions and PBMC of individuals affected by pityriasis rosea [9]. These findings suggest possible associations between HHV-7 infection and diseases and also raise the possibility that HHV-7, similarly to other herpesviruses, may become a pathogen under conditions of immunosuppression.

Two cell types have been recognized as targets of HHV-7 infection in vivo, namely $CD4^+$ T lymphocytes and salivary glands. The initial observation that HHV-7 can be isolated from cultured PBMC following mitogenic or antigenic stimulation led to the idea that HHV-7 establishes a latent infection in $CD4^+$ T lymphocytes [1]. By contrast, in epithelial cells of salivary glands, the virus establishes a productive infection, which results in virus shedding in saliva [10]. Similarly to the in vivo tropism, even the in vitro tropism of HHV-7 appears to be very narrow, as $CD4^+$ T lymphocytes—either primary cultures or cell lines—are the only cell type known to support productive infection. In investigations focused on the detection of herpesviruses in Kaposi's sarcoma, some of us made the unexpected observation that, in the context of this tissue, HHV-7 can also infect cells expressing the CD68 antigen, a marker of monocyte/macrophage lineage [11]. This observation raised the possibility that HHV-7 infection in humans might not be limited to $CD4^+$ T lymphocytes and salivary glands and hence that the tissue and cell tropism of the virus may be broader than known so far.

While the entire HHV-7 genome sequence was reported recently [12], knowledge of the proteins specified by HHV-7 and of their biologic properties is scanty, mainly due to difficulties in growing the virus and consequently to few available specific reagents. One of our laboratories has characterized a highly immunogenic complex of proteins designated phosphoprotein 85 (pp85) [13, 14], which localizes to the outer layers of virion tegument [15]. pp85 is encoded by the U14 gene [15]. Monoclonal antibody (MAb) 5E1 reacts with pp85, does not cross-react with HHV-6, and hence recognizes in pp85 an HHV-7–specific epitope [13–15]. Comparison of the HHV-7 and HHV-6 U14 sequences shows regions at the C-terminus of the proteins with sufficient divergence to harbor potential HHV-7–specific epitopes [15].

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Table 1. Detection of HHV-7 in normal tissues by immunohistochemical staining (IHC) and polymerase chain reaction amplification (PCR).

Tissue	Positive/total		IHC^+ PCR^+	IHC^- PCR^-	Frequency of infected cells	Localization
	IHC	PCR				
Lung (4)*	7/8	2/5	1	1	+++	Alveoli, bronchial gland
Skin (11)*	7/11	4/6	2	1	++	Perivascular and periadnexal dermis
Liver	5/8	ND			+	Periportal areas
Salivary glands	6/10	8/8	6		+++	Clusters of acini [12]
Kidney	8/10	1/4	1	1	±	Pyelon, stromal tissue
Mammary glands (7)*	4/7	3/4	2	1	++	Periglandular stromal tissue, 1 glandular epithelial
Tonsils (6)*	2/6	ND			+	Connective
Skeletal muscle	2/3	0/2		1	±	
Gut						
Ileum, appendix	3/5	ND			±	Connective
Other	0/6	ND				
Spleen	0/4	ND				
Brain	0/8	0/5		5		

NOTE. ND, not done. Frequency of infected cells per positive field, evaluated subjectively, is expressed by increasing symbols.

* Bioptic specimens were obtained from individuals undergoing plastic surgery, tonsil resection or diagnostic lung biopsies (in parentheses, no. of bioptic samples of total). Rest were autoptic samples from patients who died of heart failure, lung embolism, or old age but not of cancer, viral (including human immunodeficiency virus) or bacterial infections, or organ transplantation.

To shed light on the natural history of HHV-7 infection, we investigated if the in vivo tropism of HHV-7 extends to cells and tissues other than $CD4^+$ T lymphocytes, salivary glands, and Kaposi's sarcoma–associated $CD68^+$ cells. We screened histologically normal bioptic or autoptic tissues for immunohistochemical detection of pp85 [11], since expression of a structural protein is indicative of late-stage infection.

Materials and Methods

Antibodies. The MAbs used in these studies were 5E1 specific for HHV-7 pp85 [11, 13–15], MAb 30 to herpes simplex virus glycoprotein D [16], a MAb (CD68 KP1, no. M848; Dako, Glostrup, Denmark) reacting with the KP1 epitope of CD68 as a specific marker of monocyte/macrophage lineage, MAb 0T01 05 Clotimmun to Factor XIIIa (Behring, Marburg, Germany), MAbs to CD3 (A452; Dako) and CD4 (NCL-CD4-1F6; Novocastra Laboratories, Newcastle upon Tyne, UK), and MAb to HHV-6 p100 (Chemicon, El Segundo, CA).

Tissue specimens. The bioptic tissue specimens taken from individuals undergoing plastic surgery, tonsil resection, or diagnostic lung biopsies and the autoptic specimens taken at necropsies were routinely fixed in 10% buffered formalin and embedded in paraffin.

Polymerase chain reaction (PCR) analyses. DNA was extracted from deparaffinized tissues by proteinase K digestion with standard procedures, as detailed previously [11]. Briefly, in order to avoid contamination and product carryover, sterile materials were used throughout the procedure, and the microtome blade was cleaned extensively with xylene after cutting of each specimen. Blank controls consisting of empty tubes containing sterile water were included with every 10th specimen and simultaneously subjected to DNA extraction and PCR amplification. In addition, in each set of specimens being analyzed, unrelated tissue specimens—previously found not to contain any HHV-7 amplifiable sequence—were included among the specimens being cut and processed for PCR; their results were consistently negative, confirming that there was no DNA carryover contamination. DNA extraction, PCR, and gel electrophoresis were done in separate laboratories. Successful amplification of a β-globin fragment indicated that the samples were adequate for PCR analysis and that no inhibitors were present [17].

HHV-7–specific sequences were amplified by nested PCR with two sets of primers. The first set was external primers HV7/HV8 and internal primers HV10/HV11, and PCR conditions were as detailed elsewhere [18]. About 50% of the specimens analyzed with this set of primers were also analyzed with a second set of primers specific for pp85. The external primers were H145E, 5′-CAAAGCGCTTAAATCAAGTGTC-3′, and H143E, 5′-GACACTTGATTTAAGCGCTTTG-3′; the internal primers were H145I, 5′-GCATTGGAATCCAAAGACAACC-3′, and H143I, 5′-GGTAACTGAAAGGCTGCAAGC-3′. PCR conditions were 35 cycles of 95°C for 30 s, 58°C for 30 s, and 72°C for 60 s and a final extension at 72°C for 10 min. The amplification products were subjected to electrophoresis in a 2% agarose gel and stained with ethidium bromide. Positive and negative controls were included routinely. The positive controls consisted of DNA extracted from HHV-7–infected cord blood mononuclear cells. The negative controls consisted of tubes containing all PCR reagents, but without DNA, every 5th or 10th analyzed sample. HHV-6 PCR was performed as described [11].

Immunohistochemistry (IHC). IHC was performed as detailed previously [11]. Tissue sections were deparaffinized with xylene and briefly digested with pronase (0.1% for 7 min). The endogenous peroxidase was blocked by incubation with 1% H_2O_2 in methanol. The sections were exposed to primary antibodies for 90 min or overnight at room temperature. After several rinses with PBS, the sections were reacted with an avidin-biotin-peroxidase coupled antibody (Vectastain ABC kit; Vector Laboratories, Burlingame, CA). The chromogen was 3-amino 9-ethyl carbazole or neufuchsin, used according to the manufacturer's protocol. Finally, sections were counter-stained with 1% hematoxylin. Serial sections with a maximum thickness of 4 μm were stained with different antibodies. This allowed identification of the same cell in at least two serial

sections. As a routine, each specimen was processed in parallel without primary antibody (i.e., with secondary antibody and substrates).

Computerized image analysis. Ektachrome slides of serial sections were scanned using a high-resolution film scanner (Kodak RFS 3570) and further processed with Photoshop software (Adobe, Seattle, WA) on a Macintosh Power PC 8100. An area representing ~60% related to the original image size was processed for image analysis. The images were artificially colored. Images of sections reacted with the anti–HHV-7 MAb were colored with a blue background and a red immunoreactive signal. Images of sections reacted with anti-CD68 antibody were artificially colored with a blue background and a brown immunoreactive product. The images were then overlaid in transparent layers, allowing the identification of co-localization of two immunohistochemical signals within the same cell in two serial sections.

Results

Detection of HHV-7 infection by IHC. Of the tissues examined, some lung, skin, and mammary gland samples were bioptic and some were autoptic. The remainder were autoptic samples. HHV-7 expression was detected by IHC analysis with the HHV-7–specific MAb 5E1 to pp85. The results summarized in table 1 and illustrated in part in figure 1 show that a number of human tissues, in addition to salivary glands, stain positively for pp85 and therefore harbor HHV-7–infected cells at a late stage of infection. The tissues that contained the highest relative number of infected cells were lungs, skin, and mammary glands (figure 1). Liver, kidney, tonsils, and appendix vermiformis contained a very low number of infected cells but could definitely be scored as positive. Skeletal muscle and ileum contained very few positive cells, while gut (other than ileum and appendix vermiformis), spleen, and brain were constantly negative.

To ascertain if the HHV-7–infected cells belonged to T lymphocytes, serial sections were stained with antibodies to CD3 and CD4. Most frequently, the HHV-7–positive cells did not belong to the T lymphocytes. To determine if the positive cells in lungs belonged to the monocyte/macrophage lineage, as is the case in Kaposi's sarcoma, and if the positive cells in skin belonged to dendritic cells, serial sections of the specimens were stained with MAb 5E1 to pp85 and with MAbs to CD68 or to factor XIIIa (markers of monocyte/macrophages and of dermal dendritic cells, respectively). The images were overlaid by computer analysis. In lung specimens, the cells expressing pp85 were not positive for CD68 staining and vice versa (figure 1A–C). In skin specimens, the cells expressing pp85 did not stain positively for factor XIIIa (data not shown). The results rule out that the infected cells in lungs and skins belonged to $CD68^+$ monocyte/macrophage or dendritic lineage, respectively. In lungs, the HHV-7–infected cells were epithelial-like and probably were pneumocytes and bronchial gland cells. In all other cases, the infected cells localized to stromal connective tissue.

The specificity of the immunohistochemical reaction was assessed as follows: First, lung specimens that stained positively for HHV-7 were reacted with a MAb to glycoprotein D of herpes simplex virus, a ubiquitous herpesvirus, and the specimens did not stain (figure 1G). They were also stained with a MAb to HHV-6 p100 (figure 1E). As the specimens contained amplifiable HHV-6 DNA sequences, there was a positive staining for HHV-6. The cells harboring the HHV-6 structural protein differed from those harboring HHV-7 pp85, supporting the conclusion that the IHC reactivity was not the result of unspecific binding of MAbs. As an additional criterion, we note that MAbs to cellular markers employed in this study did not stain the cells reactive to MAb 5E1, strengthening the conclusion that this latter reactivity was not the result of unspecific binding. Of relevance is the finding that coinfection of HHV-6 and -7 in the same cell seems to be specific for Kaposi's sarcoma [11], as it was not detected in normal tissues.

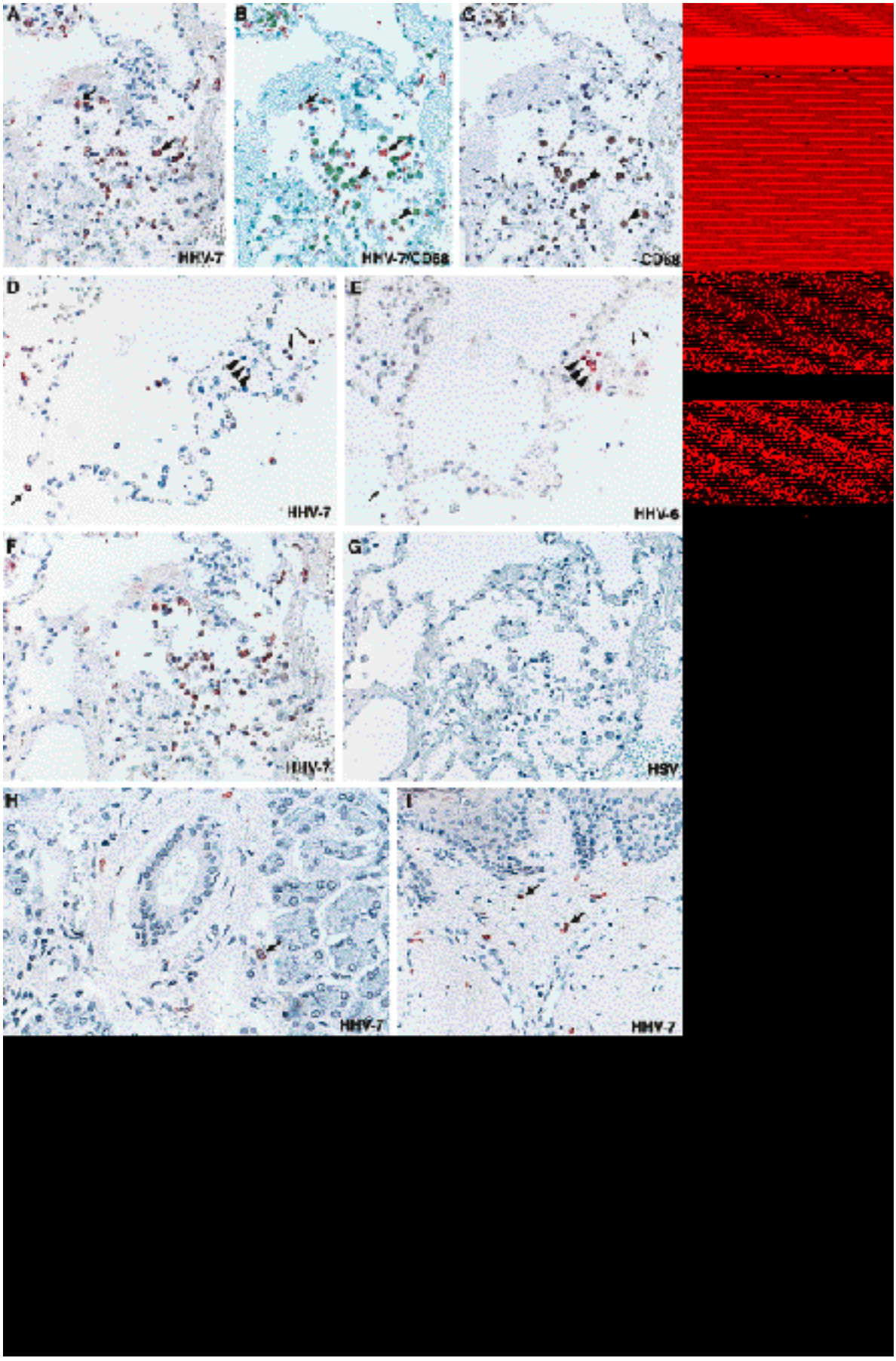
Detection of HHV-7 infection by PCR. To confirm the IHC results, a portion of the samples was analyzed by PCR. The IHC-positive samples included lungs, skin, and mammary and salivary glands; the IHC-negative samples included brain, skin, and skeletal muscle. As all samples were formalin-fixed and paraffin-embedded, preservation of DNA was controlled by PCR amplification of the β-globin gene [17]. Although this represents a good criterion for a eukaryotic gene present in two copies per cell, it is not necessarily good when applied to sequences that are present in much lower amounts, as was the case for HHV-7 in these samples. It was expected that the partial DNA degradation, inevitably present in paraffin-embedded archival samples, might yield a positive β-globin amplification reaction but result in a negative HHV-7 reaction, even if some viral sequences were indeed present. About 50% of PCR-suitable IHC-positive samples contained amplifiable HHV-7 sequences (table 1). The rest may have contained amounts of preserved HHV-7 sequences too low to yield detectable amplification. The IHC-negative samples were generally negative by PCR (table 1).

Discussion

In this study, HHV-7–infected cells, morphologically and immunophenotypically distinct from T lymphocytes, were present in a number of normal tissues. This finding changes our notions on the life of HHV-7 in humans and allows three major conclusions to be drawn.

First, the natural history of HHV-7 infection may best be defined as a steady-state low-level reactivation from latency or as a low-level persistence. On the basis that $CD4^+$ T lymphocytes are the site of latency and that salivary glands are the site of virus production, two scenarios are conceivable. Reactivation from latency would take place in $CD4^+$ T lymphocytes; the reactivated virus would then spread to other cells. In low-level persistence, the virus produced in salivary glands (or in as yet unidentified sites of replication) would represent the most likely source of virus that sustains the persistent infection.

Second, the range of HHV-7–susceptible cells in humans is broader than known so far. In addition to the known cellular


A
HHV-7
B
HHV-7/CD68
C
CD68
D
HHV-7
E
HHV-6
F
HHV-7
G
HSV
H
HHV-7
I
HHV-7

targets—$CD4^+$ T lymphocytes, salivary glands, and $CD68^+$ cells in the context of Kaposi's sarcoma—HHV-7 can also infect epithelial (lung) and mesenchymal cells. Of interest, cells with the $CD68^+$ marker were not infected by HHV-7 in tissues other than Kaposi's sarcoma. Therefore, infection of this cell lineage seems to be restricted to Kaposi's sarcoma tissue, as discussed previously [12].

Third, the major impact of the present finding is on future studies aimed to correlate HHV-7 infection or reactivation with specific diseases. For highly seroprevalent viruses that undergo latency, seroepidemiologic studies are of little help in identifying diseases associated with reactivation, and efforts to define correlations often rest on evidence of viral replication in pathologic specimens. Our data highlight that the mere presence of HHV-7–infected cells, even of cells expressing a structural antigen, does not necessarily imply an etiologic relationship between HHV-7 and the disease state.

References

1. Frenkel N, Schirmer EC, Wyatt LS, et al. Isolation of a new human herpesvirus from human CD4 T-cell. Proc Natl Acad Sci USA **1990**; 87:748–52.
2. Berneman ZN, Gallo RC, Ablashi DV, et al. Human herpesvirus 7 (HHV-7) strain JI: independent confirmation of HHV-7. J Infect Dis **1992**; 166:690–1.
3. Black JB, Inoue N, Kite-Powell K, Zaki S, Pellett PE. Frequent isolation of human herpesvirus 7 from saliva. Virus Res **1993**;29:91–8.
4. Ueda K, Kusuhara K, Okada K, et al. Primary human herpesvirus 7 infection and exanthema subitum. Pediatr Infect Dis J **1994**;13:167–8.
5. Portolani M, Cermelli C, Mirandola P, Di Luca D. Isolation of human herpesvirus 7 from an infant with febrile syndrome. J Med Virol **1995**; 45:282–3.
6. Torigoe S, Koide W, Yamada M, et al. Human herpesvirus 7 infection associated with central nervous system manifestations. J Pediatr **1996**; 129:301–5.
7. Chan PKS, Peiris JSM, Yuen KY, et al. Human herpesvirus 6 and human herpesvirus 7 infection in bone marrow transplant recipients. J Med Virol **1997**;53:295–305.
8. Osman HK, Peiris JS, Taylor CE, Karlberg JP, Madeley CR. Correlation between the detection of viral DNA by the polymerase chain reaction in peripheral blood leukocytes and serological responses to human herpesvirus 6, human herpesvirus 7, and cytomegalovirus in renal allograft recipients. J Med Virol **1997**;53:288–94
9. Drago F, Ranieri E, Malaguti F, Losi E, Rebora A. Human herpesvirus 7 in pityriasis rosea. Lancet **1997**;349:1367–8.
10. Di Luca D, Mirandola P, Ravaioli T, et al. HHV-6 and HHV-7 in salivary glands and shedding in saliva of healthy and HIV positive individuals. J Med Virol **1995**;45:462–8.
11. Kempf W, Adams V, Wey A, et al. $CD68^+$ cells of monocyte/macrophage lineage in the environment of AIDS-associated and classic-sporadic Kaposi's sarcoma are singly or doubly infected with human herpesvirus 7 and 6B. Proc Natl Acad Sci USA **1997**;94:7600–5.
12. Nicholas J. Determination and analysis of the complete nucleotide sequence of human herpesvirus 7. J Virol **1996**;70:5975–89.
13. Foà-Tomasi L, Avitabile E, Ke L, Campadelli-Fiume G. Polyvalent and monoclonal antibodies identify major immunogenic proteins specific for human herpesvirus 7 (HHV-7)–infected cells. Low cross reactivity with HHV-6. J Gen Virol **1994**;75:2719–27.
14. Foà-Tomasi L, Fiorilli MP, Avitabile E, Campadelli-Fiume G. Identification of a M_r 85,000 phosphoprotein as an immunodominant protein specific for human herpesvirus 7–infected cells. J Gen Virol **1996**;77: 511–8.
15. Stefan A, Secchiero P, Baechi T, Kempf W, Campadelli-Fiume G. The 85 KDa phosphoprotein of human herpesvirus 7 is encoded by U14 ORF and localizes to a tegument substructure in virion particles. J Virol **1997**;71:5758–63, 8084.
16. Brandimarti R, Huang T, Roizman B, Campadelli-Fiume G. Mapping of herpes simplex virus 1 genes with mutations which overcome host restrictions to infection. Proc Natl Acad Sci USA **1994**;9:5406–10.
17. Bauer H, Ting Y, Greer CE, et al. Genital human papilloma-virus infection in female university students as determined by a PCR-based method. JAMA **1991**;265:472–7.
18. Berneman ZN, Ablashi DV, Li G, et al. Human herpesvirus 7 is a T-lymphotropic virus and is related to, but significant different from, human herpesvirus 6 and human cytomegalovirus. Proc Natl Acad Sci USA **1992**;89:10552–6.

Figure 1. Immunohistochemical detection of HHV-7 pp85 in lung (**A–G**), mammary gland (**H**), and skin (**I**) specimens with monoclonal antibody (MAb) 5E1. **A–C** show lack of co-localization of HHV-7–infected cells (**A** and **B**, arrows) with cells bearing the CD68 marker (**B** and **C**, arrowheads) in lung tissue. Staining of HHV-7 pp85 antigen with MAb 5E1 (**A** and **B**, arrows). Staining with an anti-CD68 antibody (**C**, arrowheads). **B**, Overlaid serial sections from **A** and **C**. **D** and **E** show lack of co-localization of HHV-6–infected cells (**E**, arrowheads). Corresponding cells are indicated in **D** and **E** with arrows and arrowheads, respectively. **F** and **G**, Red immunostaining of HHV-7 pp85 in lung specimen (**F**) and lack of staining of serial section (**G**) with unrelated antibody: MAb directed to HSV glycoprotein D. **H** (mammary gland) and **I** (skin) show cells expressing HHV-7 pp85 (arrows).