JOURNAL OF VIROLOGY, Oct. 1997, p. 7963–7968 Vol. 71, No. 10
0022-538X/97/$04.00+0

Monocytes in Kaposi's Sarcoma Lesions Are Productively Infected by Human Herpesvirus 8

CORNELIA BLASIG,[1] CHRISTIAN ZIETZ,[2] BEATE HAAR,[2] FRANK NEIPEL,[3] STEFAN ESSER,[4] NORBERT H. BROCKMEYER,[4] ERWIN TSCHACHLER,[5] SANDRA COLOMBINI,[6] BARBARA ENSOLI,[7] AND MICHAEL STÜRZL[1]*

Abteilung Virusforschung, Max-Planck-Institut für Biochemie, 82152 Martinsried,[1] Pathologisches Institut der Ludwig-Maximilians-Universität München, 80337 Munich,[2] Institut für Klinische und Molekulare Virologie, Universität Erlangen-Nürnberg, 91054 Erlangen,[3] and Hautklinik, Universitätsklinik Essen, 45147 Essen,[4] Germany; Universitätshautklinik, Universität Wien, 1090 Vienna, Austria[5]; Institute of Human Virology, MBC, UMBI, Baltimore, Maryland 21201[6]; and Laboratory of Virology, Instituto Superiore di Sanitá, 00161 Rome, Italy[7]

Received 19 December 1996/Accepted 24 June 1997

PCR analysis and serological studies demonstrated a close association between Kaposi's sarcoma (KS)-associated herpesvirus, or human herpesvirus 8 (HHV-8), and the development of Kaposi's sarcoma (KS). The majority of the KS cells were shown to be latently infected by the virus. In this study we investigated which type of cell is productively infected in KS lesions. In situ hybridization was performed with strand-specific RNA probes complementary to the sequences coding for the minor capsid protein (VP23) of HHV-8. The VP23 gene is specifically expressed during the lytic or replicative period of the virus life cycle, and therefore it is a useful marker to detect productively infected cells. By in situ hybridization of KS lesions, a strong hybridization signal was detected only in a small subset of the KS cells of the lesions. Simultaneous application of immunohistochemical staining and in situ hybridization identified the virus-replicating cells to be of monocytic origin. Productively infected monocytes may be an important reservoir for transmission of the virus and for the increase and maintenance of the high load of HHV-8 generally observed in nodular KS lesions during late stages of infection.

Kaposi's sarcoma (KS) is described as a multifocal angioproliferative disease evident predominantly on the skin but also involving visceral organs and lymph nodes (15, 34). KS occurs in four different epidemiological forms: classical KS (16), African KS (4, 38), iatrogenic KS (21), and AIDS-associated KS (5, 12). Despite the different epidemiologies, the histological features of all forms of KS are similar (1). Proliferating spindle-shaped cells, called KS spindle cells, are characteristic and are considered to be the tumor cells of the lesions. Despite their morphological homogeneity, these KS spindle cells represent a heterogeneous population dominated by activated endothelial cells mixed with fibroblasts, smooth muscle cells, and cells of dendritic and monocytic origins (24, 26). In addition, a prominent vasculature and edema are peculiar features of KS, particularly in the early stage (for reviews, see references 26 and 34).

Recently, a novel human herpesvirus, referred to as either KS-associated herpesvirus or human herpesvirus 8 (HHV-8), has been detected in KS tissues (10). Subsequently, this agent has been found by PCR analysis in lesions from all forms of KS (2, 7, 10, 11, 14, 23, 27, 37). This finding suggested that HHV-8 may contribute to the development of this disease. In addition, HHV-8 has also been identified in primary effusion B-cell lymphomas (9), in Castleman's disease (31), in non-KS skin lesions (22), and in peripheral blood mononuclear cells from normal donors, particularly in geographical areas of high risk for KS (30). The close association between HHV-8 and KS has also been confirmed by seroepidemiological studies (13, 17, 19, 29). Moreover, in situ PCR analysis revealed that viral genomic DNA is localized in vascular endothelial cells of KS lesions as well as in KS spindle cells (6). The results of in situ PCR analysis were confirmed by in situ hybridization, in which a 0.7-kb transcript (T0.7 mRNA) coding for a membrane-associated protein (kaposin) was detected in most spindle cells of KS lesions (32, 36). However, the T0.7 transcript is expressed in all phases of the life cycle (lytic or productive and latent) of HHV-8 (5a). This expression of the T0.7 transcript led us to

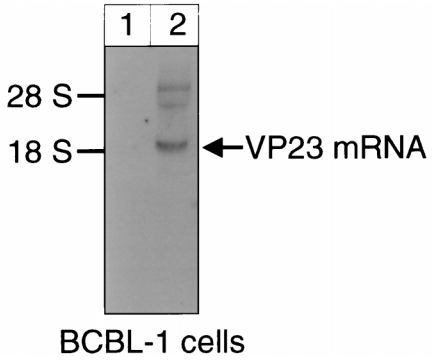
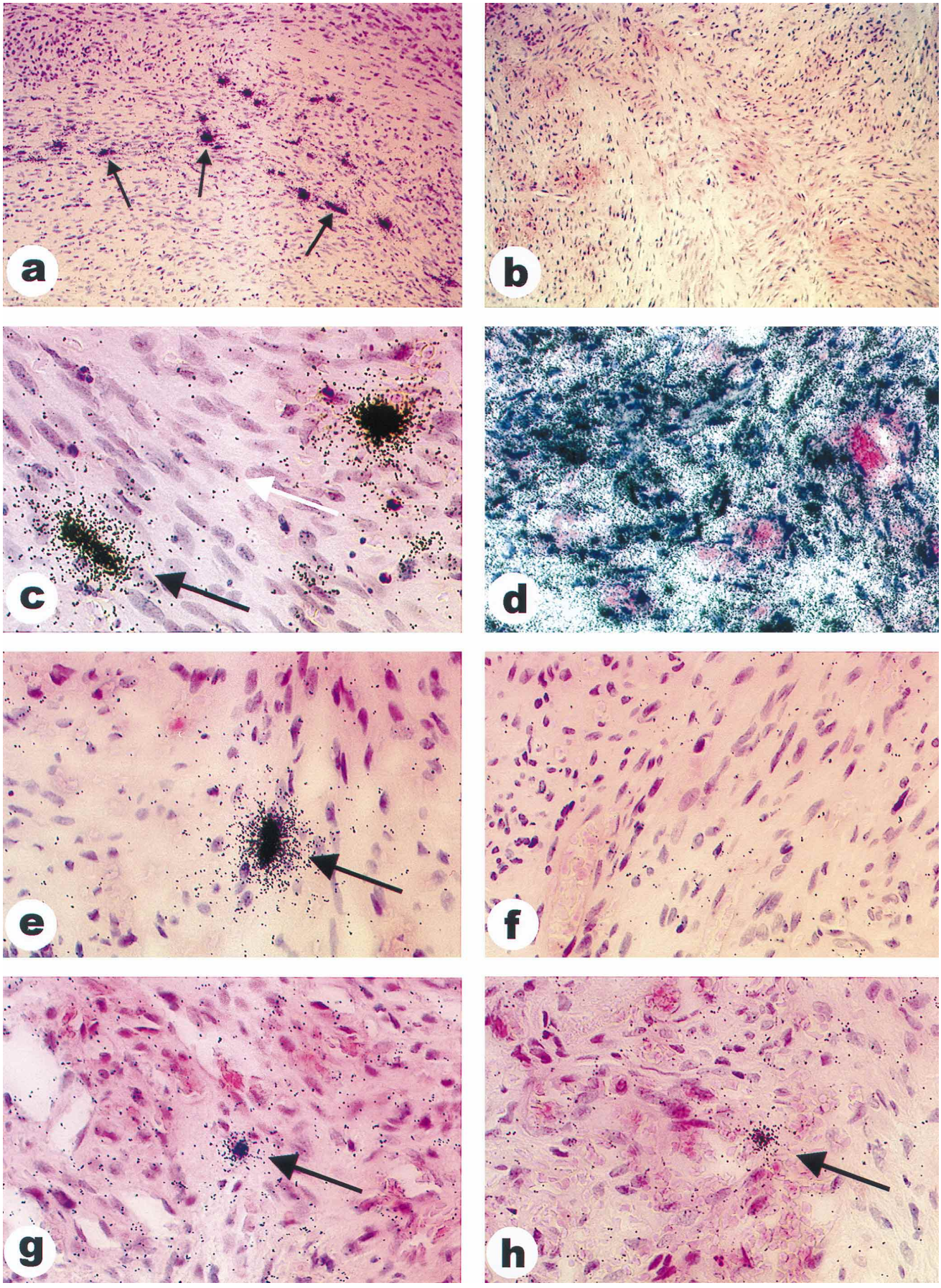


FIG. 1. HHV-8 VP23 is specifically expressed in lytically or productively infected BCBL-1 cells. Total RNA (5 μg) extracted from BCBL-1 cells either untreated (lane 1) or stimulated with TPA (20 ng/ml) (lane 2) was fractionated on a vertical 1% agarose–6% formaldehyde gel, transferred to a GeneScreen Plus nylon membrane, and hybridized with a 1,155-bp complementary DNA fragment coding for the VP23 gene which was radiolabeled by the random primer method (specific activity, 8×10^8 cpm/μg). Expression of VP23 mRNA could be observed only in TPA-treated BCBL-1 cells (lane 2).

* Corresponding author. Mailing address: Max-Planck-Institut für Biochemie, Abteilung Virusforschung, Am Klopferspitz 18a, 82152 Martinsried, Germany. Phone: 89-8578-2286. Fax: 89-8578-3777. E-mail: Stuerzl@Biochem.mpg.de.


a
b
c
d
e
f
g
h

question the types of cells in which HHV-8 may replicate in KS lesions. By electron microscopy and in situ hybridization techniques, Orenstein et al. have recently shown mature enveloped HHV-8 virions in vasoformative spindle cells and mononuclear cells (20). The morphological criteria have suggested that the mononuclear cells may be lymphocytes. Nevertheless, lymphocytes are present primarily in early-stage KS lesions, and in some cases, these cells cannot be detected by immunohistochemical analysis of tissue sections from late-stage KS (5a). Therefore, we investigated the origin of productively infected cells in KS lesions. Using a combination of cell-type-specific immunohistochemical staining techniques and in situ hybridization for detection of the mRNA coding for the HHV-8 minor capsid protein (VP23), we demonstrate that a subset of productively infected cells in the KS tumor tissue is of monocyte origin.

Expression of HHV-8 VP23 is a specific marker for productively infected cells. VP23 expression was used as a marker for productively infected cells. The specificity of this marker was demonstrated by Northern blot hybridization of RNA extracted from body cavity-based lymphoma (BCBL-1) cells which are latently infected by HHV-8 but can be activated to productive infection by treatment with tetradecanoyl phorbol acetate (TPA) (18, 25). This experiment demonstrated that VP23 mRNA is synthesized only in TPA-treated cells (Fig. 1, lane 2) and not in unstimulated BCBL-1 cells (Fig. 1, lane 1).

Detection of HHV-8 productively infected cells in KS lesions. In order to detect productively HHV-8-infected cells in KS lesions, in situ hybridization with strand-specific ^{35}S-radiolabeled RNA hybridization probes (specific activity, $\approx 10^9$ cpm/μg) was performed with nine biopsy samples of nodular KS skin lesions from HIV-infected homosexual male AIDS patients with stage IV level of infection, according to the Centers for Disease Control and Prevention classification system (8). All biopsy samples were positive for HHV-8 by PCR analysis (data not shown). For probe synthesis, transcription plasmid p557-19, which contains a 1,155-bp fragment of the HHV-8 gene coding for VP23 (open reading frame 26), was used. In situ hybridization was carried out under high-stringency conditions as described elsewhere (33, 35), with slight modifications that are detailed below.

In order to obtain the highest signal intensity, the in situ hybridization protocol was modified for the simultaneous detection of HHV-8 genomic DNA and VP23 mRNA. For this purpose, DNA in KS tissue sections was denaturated prior to the hybridization procedure. To achieve this denaturation, the tissue sections were incubated in 0.2 N HCl for 10 min, immersed in 75% formamide–2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) for 10 min, and denaturated in 75% formamide–2× SSC for 15 min at 70°C. Immediately after denaturation, the slides were dipped in ice-cold (4°C) 50% formamide–2× SSC until used in the hybridization protocol. Hybridization was carried out at 42°C. Under these conditions with the antisense RNA probe strong HHV-8-specific signals were obtained in numerous cells of all lesion specimens examined (Fig. 2a, arrows). HHV-8-positive cells either arranged in clusters (Fig. 2a) or scattered evenly through the lesions (data not shown) were intermingled with spindle cells. This pattern resembles the distribution of monocytic cells which are commonly present in KS lesions (34). HHV-8-specific signals were also found in a few cells with a spindle-shaped morphology (Fig. 2c, black arrow). However, the majority of the spindle cells were clearly negative (Fig. 2c, white arrow), although the mRNA of all cells in the tissue sections was accessible for hybridization, as shown by using a β-actin probe (Fig. 2d). The specificity of the in situ hybridization was also demonstrated by the simultaneous application of a radiolabeled antisense RNA probe and by a surplus of unlabeled sense strand decoy RNA. No signals were obtained in these control experiments (Fig. 2b).

In order to determine whether VP23 mRNA is expressed in HHV-8-positive cells, two other strategies that enabled discrimination between the detection of viral genomic DNA and VP23 mRNA were used. In the first, in situ hybridization was carried out without denaturation of the viral DNA, in which only single-stranded VP23 mRNA, and not double-stranded HHV-8 DNA, is accessible for hybridization with the antisense RNA probe. Strong signals were again detected in single cells intermingled with the KS spindle cell population in all the KS tissues examined (Fig. 2e, arrow). No signal was observed in control sections by using the radiolabeled sense strand RNA as a probe (Fig. 2f). This result indicated that signals obtained by this method were specific for VP23 mRNA and not for genomic DNA (Fig. 2e).

The second approach was directed exclusively to detection of genomic DNA. RNA was removed by RNase A treatment (500 μg/ml, 37°C, 30 min) of the sections prior to the proteinase K incubation step. In addition, double-stranded genomic HHV-8 DNA was denatured to be accessible for hybridization. With this protocol, signals were obtained by hybridization with both the antisense RNA probe (Fig. 2g, arrow) and the sense VP23 RNA probe (Fig. 2h, arrow). The signal intensities and distributions of HHV-8-positive cells in the KS tissues were comparable in both cases (Fig. 2g and 2h). This result indicated that in each case, specific hybridization with one strand of the double-stranded target DNA had occurred.

Equal periods of exposure in autoradiography were used for all experiments. Therefore, signal intensities obtained by the different methods represent the concentrations of the different target molecules (HHV-8 DNA or VP23 RNA) in HHV-8-positive cells. The comparison of signal intensities revealed that the amount of VP23 RNA exceeds the amount of HHV-8 DNA (compare Fig. 2e and g). This finding clearly demonstrates that VP23 is expressed and proves that HHV-8-positive cells detected by these methods (0.5 to 1% of the cells in the lesion) are productively infected by HHV-8.

FIG. 2. A subpopulation of cells in KS lesions is productively infected with HHV-8. Productively HHV-8-infected cells in KS tumors were identified by in situ hybridization with strand-specific radiolabeled RNA probes (specific activity, 10^9 cpm/μg of DNA) directed against the mRNA coding for the HHV-8 minor capsid protein (VP23). (a) Simultaneous detection of viral DNA and VP23 mRNA after denaturation of KS tissue DNA. Signals can be seen overlying single cells, which are arranged in clusters between the KS spindle cells. HHV-8-positive cells are marked by arrows. Magnification, ×500. (b) Control hybridization. No staining was observed when a surplus of the unlabeled sense strand RNA probe was applied to the tissue sections simultaneously with the radiolabeled antisense RNA probe. Magnification, ×500. (c) Signals for HHV-8 DNA and VP23 mRNA were detected on a few cells with a spindle-shaped morphology (black arrow), but the majority of the KS spindle cells were negative (white arrow). Magnification, ×5,000. (d) With an antisense RNA control probe specific for β-actin (specific activity, 10^9 cpm/μg), hybridization signals were found on each cell in the KS tissue. Magnification, ×3,500. (e) VP23 mRNA exclusively was detected when denaturation of the viral DNA in the KS tissue section was omitted. Strong signals were detected with the antisense RNA probe in single cells (arrow). Magnification, ×3,500. (f) Control hybridization by using a radiolabeled sense RNA probe. No signals were observed in KS sections. Magnification, ×3,500. (g and h) HHV-8 genomic DNA was detected only after denaturation of DNA in KS tissue sections and removal of RNA by RNase. The signal intensities in HHV-8-positive cells were comparable with both radiolabeled antisense (g) and sense (h) RNA probes (arrows). Magnification, ×3,500.

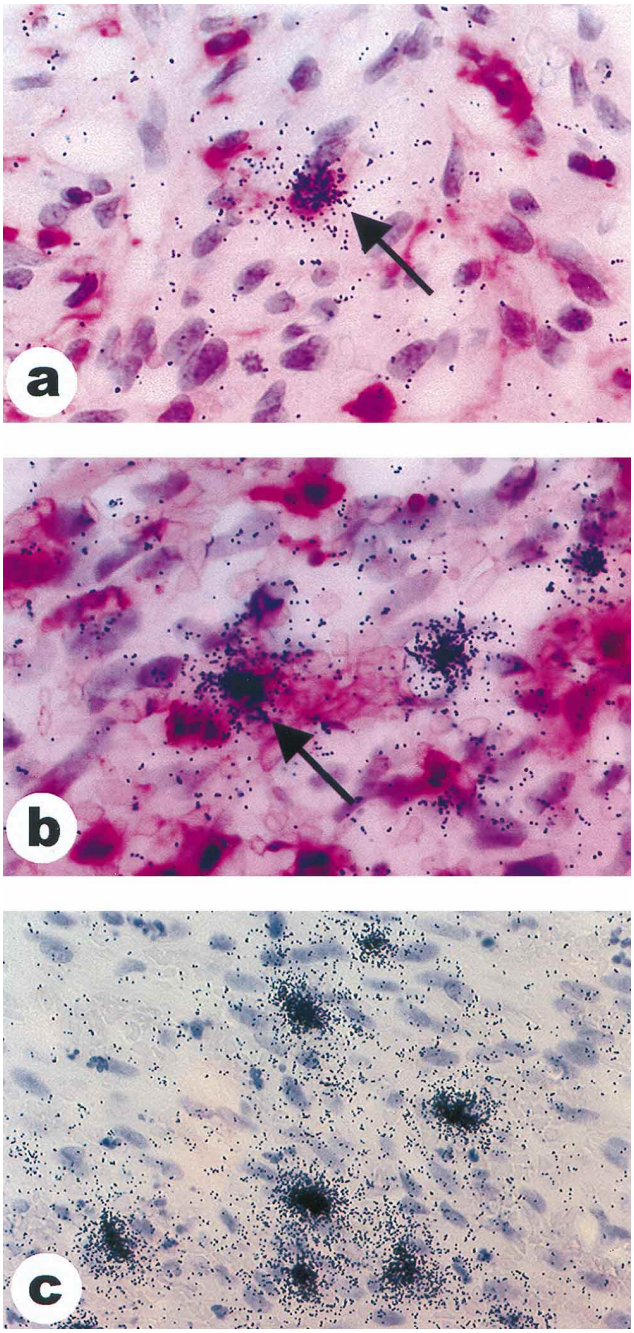


FIG. 3. Monocytes in KS lesions are productively infected by HHV-8. To determine whether the origin of productively HHV-8-infected cells in KS lesions is lymphocytic or monocytic, immunohistochemical stainings were done with antibodies specific for LCA and MAC 387. Stained tissues were then subjected to in situ hybridization with a radiolabeled antisense VP23 RNA probe (specific activity, 10^9 cpm/μg). (a) A clear colocalization of black silver particles specific for VP23 mRNA and LCA-positive cells (red staining) was observed. A doubly labeled cell is marked with an arrow. (b) A clear colocalization of black silver particles specific for VP23 mRNA and immunohistochemical signals specific for monocytes/macrophages (red staining) was observed. A doubly labeled cell is marked with an arrow. (c) Negative control. The primary antibody was replaced with bovine serum albumin. No cell type-specific staining was observed. Signals for VP23 mRNA are localized in single cells in the KS tissue sections. Magnification, ×3,500.

Monocytes in KS lesions are productively infected by HHV-8. To determine the type of the productively infected cells in KS lesions, in situ hybridization was combined with immunohistochemical staining with antibodies directed against leukocyte-associated antigens (antileukocyte common antigen [LCA], diluted 1:90 in 30 mM Tris-HCl [pH 7.4]–148 mM NaCl–5 mg of heparin per ml–180 U of RNasin per ml; DAKO, Glostrup, Denmark) and monocyte/macrophage-associated antigens [antimyeloid/histiocyte antigen [MAC 387], diluted 1:500 in 30 mM Tris-HCl [pH 7.4]–148 mM NaCl–5 mg of heparin per ml–180 U of RNasin per ml; DAKO) (Fig. 3). Because of the conditions required for the subsequent in situ hybridization, these were the only antibodies found to give optimal staining. Bound primary antibodies were detected by using the APAAP detection kit (DAKO) according to the supplier's instructions. Staining was done with the new fuchsin substrate system (DAKO). Stained tissue sections were then subjected to in situ hybridization in order to detect VP23 mRNA. A clear colocalization of HHV-8-specific signals was observed on single cells which expressed LCA (Fig. 3a, arrow) or MAC 387 (Fig. 3b, arrow). No staining was observed when the primary antibody was replaced with bovine serum albumin (Fig. 3c). Similar patterns of HHV-8-positive cells were observed with (Fig. 3a and b) or without (Fig. 3c) the addition of the antibodies. This result demonstrates that the sensitivity of the in situ hybridization was not impaired by the double-labeling method.

The in situ hybridization analysis showed that a subpopulation of productively HHV-8-infected cells in KS lesions is of monocyte origin. The observation that monocytic cells can be infected by HHV-8 was further substantiated by PCR detection of HHV-8 in the adherent monocytic cells derived from peripheral blood of AIDS-KS patients. Whole peripheral blood mononuclear cells from these patients were plated, and after 8 days of culture, the presence of HHV-8 DNA was determined in the floating and the adherent cell fractions as described previously (10). An HHV-8-positive signal was observed in the adherent monocytes but not in floating cells, which consisted mostly of B and T cells (data not shown). The negative results for the B cell-containing fraction, which has been previously shown to be infected by HHV-8 (3), are most likely due to the fact that B cells do not survive in culture and are lost within a few days, particularly in cultures from AIDS patients. Immunohistochemical characterization of the adherent cell population demonstrated that these cells were uniformly positive for monocyte-associated antigens, whereas CD19- and CD20-positive B cells were not detected (11a). In addition, these results are consistent with the finding of a recent publication that describes the isolation of HHV-8-positive cells with markers of macrophages from the blood of patients with all forms of KS (30).

Overall the data presented in this study demonstrate that monocytic cells in KS lesions are productively infected with HHV-8. With respect to the role of productively infected monocytes in the pathogenesis of KS, three different scenarios may be possible. (i) Monocytes become infected by HHV-8 after their arrival in KS lesions by the virus released from the KS spindle cells. (ii) Monocytes may phagocytize latently infected KS spindle cells, and during this process, lytic infection may be induced in incorporated KS spindle cells. (iii) HHV-8-infected monocytes from peripheral blood may serve as a vehicle for the recruitment of HHV-8 into tissues.

In all three scenarios, productively infected monocytes have to be regarded as an important cell type which increases the load of HHV-8 in KS lesions. However, scenario i is unlikely, because there are many noninfected monocytes present in KS

lesions and the KS spindle cells are most likely latently infected and may not produce virus (32). In addition, compared to the high number of HHV-8-infected KS spindle cells present in the lesions (32, 36), the number of productively infected monocytes is very low, suggesting that transmission of HHV-8 from infected spindle cells to monocytes is unlikely. Also, scenario ii is not very likely because the morphology of productively infected monocytes revealed no signs of phagocytosed spindle cells. By contrast, several points support the third hypothesis: HHV-8-infected monocytes can be detected in the peripheral blood of KS patients, chemokines such as MCP-1 are highly expressed in KS lesions (28), and recently we found increased adhesion of mononuclear cells to the endothelia in 32 HIV-infected patients compared to that in 17 non-infected controls (39). Increased adhesion of monocytes to the endothelium and locally increased concentrations of chemokines may support the extravasation of HHV-8-infected monocytes from the blood into the KS lesions.

Although the role of HHV-8 in KS is unknown, it is clear that productively infected monocytes are one type of cell in which HHV-8 replicates in KS lesions. Therefore, productively infected monocytes may contribute significantly to the transmission of the virus and to the increase and maintenance of the high load of HHV-8 generally observed in nodular KS lesions during the late stages of the disease.

We thank Peter Hans Hofschneider for his generous support and for the pleasant and inspirating time in his department, Thelma Coutts for linguistic revision of the paper, and Safiye Wurzelbauer for technical help (all from the Max-Planck-Institut für Biochemie, Martinsried, Germany). Furthermore, we acknowledge the kind support of Bernhard Fleckenstein (Institut für Klinische und Molekulare Virologie, Erlangen, Germany) and Don Ganem (University of California, San Francisco), who provided the BCBL-1 cell line.

This study was supported by the European Concerted Actions "Oncogenesis in HIV-Infection" and "Pathogenesis of AIDS-KS," by a grant of the Deutsche Forschungsgemeinschaft (DFG Sonderforschungsbereich 464), and by an award of the Münchner Medizinische Wochenschrift to M.S.

REFERENCES

1. **Ackerman, A. B., and G. J. Gottlieb.** 1988. Atlas of the gross and microscopic features, p. 29–33. *In* Kaposi's sarcoma: a text and atlas. Lea & Febinger, Philadelphia, Pa.
2. **Albini, A., M. G. Aluigi, R. Benelli, E. Berti, P. Biberfeld, C. Blasig, M. L. Calabro, F. Calvo, L. Chieco-Bianchi, M. Corbellino, A. Del Mistro, M. Ekman, A. Favero, P. H. Hofschneider, E. E. Kaaya, C. Lebbe, P. Morel, F. Neipel, D. M. Noonan, C. Parravicini, L. Repetto, M. Schalling, M. Stürzl, and E. Tschachler.** 1996. Oncogenesis in HIV-infection: KSHV and Kaposi's sarcoma. Int. J. Oncol. **9:**5–9.
3. **Ambroziac, J. A., D. J. Blackbourn, B. Herndier, R. Glogau, J. Gullett, A. McDonald, E. Lennette, and J. Levy.** 1995. Herpes-like sequences in HIV-infected and uninfected Kaposi's sarcoma patients. Science **268:**582–583.
4. **Bayley, A. C.** 1991. Occurrence, clinical behaviour and managment of Kaposi's sarcoma in Zambia. Cancer Surv. **10:**53–72.
5. **Beral, V., T. A. Peterman, R. L. Berkelman, and H. W. Jaffe.** 1990. Kaposi's sarcoma among persons with AIDS: a sexually transmitted infection? Lancet **335:**123–128.

5a. **Blasig, C., and M. Sturzl.** Unpublished observations.

6. **Boshoff, C., T. F. Schulz, M. M. Kennedy, A. K. Graham, C. Fisher, A. Thomas, J. O. Mcgee, R. A. Weiss, and J. J. O'Leary.** 1995. Kaposi's sarcoma-associated herpesvirus infects endothelial and spindle cells. Nat. Med. **1:**1274–1278.
7. **Buonaguro, F. M., M. L. Tornesello, E. Bethgiraldo, A. Hatzakis, N. Mueller, R. Downing, B. Biryamwaho, S. D. K. Sempala, and G. Giraldo.** 1996. Herpesvirus-like DNA sequences detected in endemic, classic, iatrogenic and epidemic Kaposi's sarcoma (KS) biopsies. Int. J. Cancer **65:**25–28.
8. **Centers for Disease Control.** 1993. Revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. Morbid. Mortal. Weekly Rep. **41:**1–19.
9. **Cesarman, E., Y. Chang, P. S. Moore, J. W. Said, and D. M. Knowles.** 1995. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in AIDS-related body-cavity-based lymphomas. N. Engl. J. Med. **332:**1186–1191.
10. **Chang, Y., E. Cesarman, M. S. Pessin, F. Lee, J. Culpepper, D. M. Knowles, and P. S. Moore.** 1994. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science **266:**1865–1869.
11. **Dupin, N., M. Grandadam, V. Calvez, I. Gorin, J. T. Aubin, S. Havard, F. Lamy, M. Leibowitch, J. M. Huraux, J. P. Escande, and H. Agut.** 1995. Herpesvirus-like DNA sequences in patients with Mediterranean Kaposi's sarcoma. Lancet **345:**761–762.

11a. **Ensoli, B.** Unpublished results.

12. **Friedman-Kien, A. E.** 1981. Disseminated Kaposi's sarcoma syndrome in young homosexual men. J. Am. Acad. Dermatol. **5:**468–471.
13. **Gao, S. J., L. Kingsley, M. Li, W. Zheng, C. Parravicini, J. Ziegler, R. Newton, C. R. Rinaldo, A. Saah, J. Phair, R. Detels, Y. Chang, and P. Moore.** 1996. KSHV antibodies among Americans, Italians and Ugandans with and without Kaposi's sarcoma. Nat. Med. **2:**925–928.
14. **Huang, Y. Q., J. J. Li, M. K. Kaplan, B. Poiesz, E. Katabira, W. C. Zhang, D. Feiner, and A. E. Friedman-Kien.** 1995. Human herpesvirus-like nucleic acid in various forms of Kaposi's sarcoma. Lancet **345:**759–761.
15. **Ioachim, H. L., V. Adsay, F. R. Giancotti, B. Dorsett, and J. Melamed.** 1995. Kaposi's sarcoma of internal organs. Cancer **75:**1376.
16. **Kaposi, M.** 1872. Idiopatisches multiples Pigmentsarkom der Haut. Arch. Dermatol. **4:**742–749.
17. **Kedes, D. H., E. Operskalski, M. Busch, R. Kohn, J. Flood, and D. Ganem.** 1996. The eroepidemiology of human herpesvirus-8 (Kaposi's sarcoma-associated herpesvirus): distribution of infection in KS risk groups and evidence for sexual transmission. Nat. Med. **2:**918–924.
18. **Komanduri, K. V., J. A. Luce, M. S. McGrath, B. G. Herndier, and V. L. Ng.** 1996. The natural history and molecular heterogeneity of HIV-associated primary malignant lymphomatous effusions. J. Acquired Immune Defic. Syndr. Hum. Retrovirol. **13:**215–226.
19. **Lennette, E. T., D. J. Blackbourn, and J. A. Levy.** 1996. Antibodies to human herpesvirus type-8 in the general population and in Kaposi's sarcoma patients. Lancet **348:**858–861.
20. **Orenstein, J. M., S. Alkan, A. Blauvelt, K.-T. Jeang, M. D. Weinstein, D. Ganem, and B. Herndier.** 1997. Visualization of human herpesvirus type 8 in Kaposi's sarcoma by light and transmission electron microscopy. AIDS **11:** F35–F45.
21. **Penn, I.** 1988. Secondary neoplasms as a consequence of transplantation and cancer therapy. Cancer Detect. Prevent. **12:**39–57.
22. **Rady, P. L., A. Yen, J. L. Rollefson, I. Orengo, S. Bruce, T. K. Hughes, and S. K. Tyring.** 1995. Herpesvirus-like DNA sequences in non-Kaposi's sarcoma skin lesions of transplant patients. Lancet **345:**1339–1340.
23. **Rady, P. L., A. Yen, R. W. Martin, I. Nedelcu, T. K. Hughes, and S. K. Tyring.** 1995. Herpesvirus-like DNA sequences in classic Kaposi's sarcoma. J. Med. Virol. **47:**179–183.
24. **Regezi, J. A., L. A. Mac Phail, T. E. Daniels, Y. G. DeSouza, J. S. Greenspan, and D. Greenspan.** 1993. Human immunodeficiency virus-associated oral Kaposi's sarcoma: a heterologous cell population dominated by spindle shaped endothelial cells. Am. J. Pathol. **143:**240–249.
25. **Renne, R., W. Zhong, B. Herndier, M. McGrath, N. Abbey, D. Keddes, and D. Ganem.** 1996. Lytic growth of Kaposi's sarcoma-associated herpesvirus (human herpesvirus b) in culture. Nat. Med. **2:**342–346.
26. **Roth, W. K., H. Brandstetter, and M. Stürzl.** 1992. Cellular and molecular features of HIV-associated Kaposi's sarcoma. AIDS **6:**895–913.
27. **Schalling, M., M. Ekman, E. E. Kaaya, A. Linde, and P. Biberfeld.** 1995. A role for a new herpes virus (KSHV) in different forms of Kaposi's sarcoma. Nat. Med. **1:**707–708.
28. **Sciacca, F. L., M. Stürzl, F. Bussolino, M. Sironi, H. Brandstetter, C. Zietz, D. Zhou, C. Matteucci, G. Peri, S. Sozzani, R. Bellini, M. Arese, A. Albini, F. Colotta, and A. Mantovani.** 1994. Expression on adhesion molecules, platelet-activating factor, and chemokines by Kaposi's sarcoma cells. J. Immunol. **153:**4816–4825.
29. **Simpson, G. R., T. F. Schulz, D. Whitby, P. M. Cook, C. Boshoff, L. Rainbow, M. R. Howard, S. J. Gao, R. A. Bohenzky, P. Simmonds, C. Lee, A. Ruiter, A. Hatzakies, R. S. Tedder, I. V. Weller, R. A. Weiss, and P. S. Moore.** 1996. Prevalence of Kaposi's sarcoma associated herpesvirus infection measured by antibodies to recombinant capsid protein and latent immunofluorescence antigen. Lancet **349:**1133–1138.
30. **Sirianni, M. C., S. Uccini, A. Angeloni, A. Faggioni, F. Cottoni, and B. Ensoli.** 1997. Circulating spindle cells: correlation with human herpesvirus-8 (HHV-8) infection and Kaposi's sarcoma. Lancet **349:**255.
31. **Soulier, J., L. Grollet, E. Oksenhendler, P. Cacoub, D. Cazales-Hatem, P. Babinet, M. F. d'Agay, J. P. Clauvel, M. Raphael, L. Degos, and F. Sigaux.** 1995. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in multicentric Castleman's disease. Blood **86:**1276–1280.
32. **Staskus, K. A., W. Zhong, K. Gebhard, B. Herndier, H. Wang, R. Renne, J. Benke, J. Pudney, D. J. Anderson, D. Ganem, and A. T. Haase.** 1997. Kaposi's sarcoma-associated herpesvirus gene expression in endothelial (spindle) tumor cells. J. Virol. **71:**715–719.
33. **Stürzl, M., W. K. Roth, N. H. Brockmeyer, C. Zietz, B. Speiser, and P. H. Hofschneider.** 1992. Expression of platelet-derived growth factor and its receptor in AIDS-related Kaposi's sarcoma in vivo suggests paracrine and autocrine mechanisms of tumor maintenance. Proc. Natl. Acad. Sci. USA **89:**7046–7050.

34. **Stürzl, M., H. Brandstetter, and W. K. Roth.** 1992. Kaposi's sarcoma: a review of gene expression and ultrastructure of KS spindle cells in vivo. AIDS Res. Hum. Retroviruses **8:**1753–1763.
35. **Stürzl, M., H. Brandstetter, C. Zietz, B. Eisenburg, G. Raivich, D. P. Gearing, N. H. Brockmeyer, and P. H. Hofschneider.** 1995. Identification of interleukin-1 and platelet-derived growth factor-B as major mitogens for the spindle cells of Kaposi's sarcoma: a combined in vitro and in vivo analysis. Oncogene **19:**2007–2016.
36. **Stürzl, M., C. Blasig, A. Schreier, F. Neipel, C. Hohenadl, E. Cornali, G. Ascherl, S. Esser, N. H. Brockmeyer, M. Ekman, E. E. Kaaya, E. Tschachler, and P. Biberfeld.** 1997. Expression of HHV-8 latency-associated T0.7 RNA in spindle cells and endothelial cells of AIDS-associated, classical and African Kaposi's sarcoma. Int. J. Cancer **72:**68–71.
37. **Su, I. J., Y. S. Hsu, Y. Chang, and I. W. Wang.** 1995. Herpesvirus-like DNA sequences in Kaposi's sarcoma from AIDS and non-AIDS patients in Taiwan. Lancet **345:**722–723.
38. **Taylor, J. F., A. C. Templeton, C. L. Vogel, J. L. Ziegler, and S. K. Kyalwazi.** 1971. Kaposi's sarcoma in Uganda: a clinico-pathological study. Int. J. Cancer **8:**122–135.
39. **Zietz, C., B. Holz, M. Stürzl, E. Rauch, R. Penning, and U. Löhrs.** 1996. Aortic endothelium in HIV-I infection: chronic injury, activation and increased leukocyte adherence. Am. J. Pathol. **149:**1887–1898.