VIROLOGY 228, 371–378 (1997)
ARTICLE NO. VY968386

SHORT COMMUNICATION

Characterization of a Chemokine Receptor-Related Gene in Human Herpesvirus 8 and Its Expression in Kaposi's Sarcoma

HONG-GUANG GUO,* PHILIP BROWNING,† JOHN NICHOLAS,‡ GARY S. HAYWARD,‡ ERWIN TSCHACHLER,§ YI-WEN JIANG,¶ MARIOLA SADOWSKA,* MARK RAFFELD,‖ SANDRA COLOMBINI,* ROBERT C. GALLO,* and MARVIN S. REITZ, JR.*,[1]

*Institute of Human Virology, Medical Biotechnology Center, University of Maryland Biotechnology Institute, Baltimore, Maryland 21201; †Vanderbilt Cancer Center, Vanderbilt University, Nashville, Tennessee 37232-6838; ‡Department of Oncology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205; §Department of Dermatology, University of Vienna Medical School, Vienna, Austria; ¶Advanced BioSciences Laboratories, Kensington, Maryland 20895; and ‖Laboratory of Pathology, National Cancer Institute, Bethesda, Maryland 20892

Received October 28, 1996; accepted December 5, 1996

Human herpesvirus 8 (HHV-8) is a recently discovered virus that is highly associated with Kaposi's sarcoma (KS) and AIDS-associated body cavity lymphomas, although it is also found in some normal individuals. HHV-8 is related by nucleotide sequence homology to herpesvirus saimiri (HVS), which causes T cell lymphomas in some New World monkeys, and to Epstein–Barr virus (EBV), a human herpesvirus linked etiologically with Burkitt's lymphoma and nasopharyngeal carcinoma. We report that, like HVS but unlike EBV, HHV-8 contains a gene (ORF74) with significant sequence homology to the high-affinity IL-8 receptor, a member of the α (CXC) chemokine receptor family of transmembrane G protein-coupled receptors. We also show by reverse transcription PCR that the chemokine receptor-related HHV-8 gene is detectable in some RNA samples from KS tissue, and that its expression varies independently from that of ORF26, a minor capsid protein. The presence of a potential chemokine receptor in HHV-8 and its expression in KS tissue suggests that it may be important in the regulation of viral gene expression and may play a role in the etiology of KS and AIDS-related body cavity lymphomas.

Kaposi's sarcoma (KS) was first reported as a rather indolent tumor associated with older men of Mediterranean origin. It has subsequently been reported to be endemic to parts of Africa in both sexes, and is also seen in transplant patients (for reviews see Penn, 1979; Ziegler *et al.,* 1984). It is a frequent feature in AIDS, particularly among homosexual men (for reviews see Friedman-Kien and Saltzman, 1990; Wahman *et al.,* 1991), and takes a quite aggressive course in this setting, contributing substantially to the morbidity and mortality of the disease. Human immunodeficiency virus type 1 (HIV-1) does not appear to be present in AIDS–KS tumors, and its causative role must therefore be indirect.

Recently, using a subtractive DNA hybridization technique called representation difference analysis (Lisitsyn and Wigler, 1993), two DNA fragments homologous to gamma herpesviruses, specifically herpesvirus saimiri (HVS) and Epstein–Barr virus (EBV), were isolated and characterized from biopsy tissue from KS (Chang *et al.,* 1994). These sequences appear to be represented in a large (~270 kb) DNA molecule which may represent the viral genome. Subsequent reports have characterized a 20-kb fragment containing these sequences (Moore *et al.,* 1996) and the fragment contains genes homologous to and colinear with those of HVS, strongly suggesting that the sequences indeed represent a human herpesvirus.

Using these sequences as a molecular probe, similar sequences have been found in DNA from almost all KS tissues from AIDS-associated KS, classical KS, African endemic KS, and iatrogenic KS (Chang *et al.,* 1994, 1996b; de Lellis *et al.,* 1995; Dupin *et al.,* 1995; Huang *et al.,* 1995; Moore and Chang, 1995; Whitby *et al.,* 1995; Buonaguro *et al.,* 1996; Luppi *et al.,* 1996; Noel *et al.,* 1996; O'Neill *et al.,* 1996). Seroepidemiologic studies have shown the presence of antibodies to apparent virus-specific antigens in KS patients but not in a majority of normal people (Gao *et al.,* 1996a,b; Kedes *et al.,* 1996; Miller *et al.,* 1996). Other reports have shown human herpesvirus 8 (HHV-8) DNA sequences to be present in normal people, although the prevalence appears to be lower (Rady *et al.,* 1995; Luppi *et al.,* 1996; Monini *et al.,* 1996).

[1] To whom correspondence and reprint requests should be addressed at the Institute of Human Virology, Medical Biotechnology Center, University of Maryland Biotechnology Institute, 725 West Lombard Street, Baltimore, MD 21201. Fax: (410) 706-4694. E-mail: reitz@umbi.umd.edu.

0042-6822/97 $25.00

In spite of the apparent close association of viral sequences with KS, cultured KS-derived cells do not contain them and virus-infected cells appear to be selectively and rapidly lost in culture. Several immortalized cell lines have been reported from KS, and these also lack HHV-8 sequences (Lunardi-Iskandar *et al.,* 1995). Recently, cell lines established from AIDS-associated body cavity lymphomas have been reported to contain the sequences and to express low but detectable amounts of extracellular transmissible virus (Cesarman *et al.,* 1995; Renne *et al.,* 1996).

Herpesviruses genomes have many genes and complex patterns of expression. The characterization of those viral genes expressed in KS and other diseases is required in order to understand how HHV-8 contributes to disease pathogenesis. We report here on the identification and sequence analysis of a part of the viral genome containing open reading frames (ORF) with homology to IL-8 chemokine receptors, which are G protein-coupled receptors (GCR) with seven transmembrane regions, and to part of the herpesvirus ORF75 virion protein. We also show that RNA for the chemokine receptor-related gene is expressed in some KS tissue, and that its expression does not coordinate with that of a viral minor capsid protein, a gene previously reported for HHV-8 (Moore *et al.,* 1996).

Total DNA was prepared from a body cavity lymphoma (BCBL) sample from an AIDS patient as described elsewhere (Sambrook *et al.,* 1989). DNA (100 mg) was partially digested with Sau3A (0.2 units, 37°, 25 min) and then size fractionated by sedimentation in a sucrose gradient by standard techniques (Sambrook *et al.,* 1989). A fraction containing DNA ranging from 13 to 20 kb was ligated into *Bam*HI arms of λ phage EMBL3 (Stratagene, La Jolla, CA). Phage DNA was packaged and the library was plated out and screened by standard methods (Sambrook *et al.,* 1989). The probe used was a ^{32}P-labeled 473-bp insert from a plasmid clone of a PCR fragment generated from the uncut lymphoma DNA using the published sequence (Chang *et al.,* 1994) of the HHV-8 homolog of ORF75 of HVS. The primers used for amplification of this region were 2B1 (5′-GCGATCTGTTTAGTCCGGA-3′) and 2B2 (5′-ATTCGCCAAGGACGTACAGCA-3′). Five positive clones were identified out of 10^5 total plaques. These were plaque purified and grown for further study. One of these, λ B6-1, was subcloned as *Sal*I fragments in pBluescript SK (Stratagene). The resultant subclones were screened with the same probe used for the λ library and a positive colony was picked. This plasmid (pB6-1) and λ B6-1 were sequenced directly by PCR cycle sequencing and primer walking.

RNA was extracted from KS biopsies or lymph node tissue from AIDS–KS patients by mincing the tissue finely with scissors or by grinding it in liquid N_2 in a mortar and pestle, homogenizing it in guanidinium thiocyanate and purifying the RNA by standard techniques (Sambrook *et al.,* 1989). Total RNA was purified from pleural effusions, BCBL samples, cultured cells or KS-derived spindle cells, prepared as described elsewhere (Browning *et al.,* 1994). Where noted, poly(A) RNA was prepared by oligo(dT)-cellulose chromatography. Total RNA (1 μg) was amplified by reverse transcriptase–PCR (RT–PCR) using Tth DNA polymerase (rTth reverse transcriptase RNA PCR Gene Amp Kit, Perkin-Elmer Applied Biosystems, Foster City, CA). Both the RT and amplification steps were performed under conditions recommended by the manufacturer. Thirty-five cycles of amplification were used, with 1 min at 95° and 1 min at 60° per cycle. Poly(A) RNA (200 ng) was amplified with random hexamer and MuLV Superscript RT (Gibco-BRL, Gaithersburg, MD) under conditions recommended by the manufacturer. Thirty-five cycles of amplification with recombinant *Taq* DNA polymerase (Perkin-Elmer) were then performed, with 1 min each at 94°, 58°, and 72° per cycle. Two regions were amplified, one representing a region of plasmid pB6-1 related by sequence homology to HVS ORF74, a chemokine receptor-related gene, and the other representing the HHV-8 homolog of HVS ORF26, a minor capsid protein (Chang *et al.,* 1994). The primer pair used for amplification of the HVS ORF74 homolog was 6L1R (5′-ACCTGCAGTAACTGACACGGTT-3′) and 6L4 (5′-GATTGGTCACCTACACCTTTCGCA-3′), which gives a fragment of 438 bp. Two primer pairs were used for the HVS minor capsid protein (ORF26) analog. One set of primers, used in the experiment with total RNA, was 2A1 (5′-CTCGGAGATTGCCACCGTTTAC-3′) and 2A2 (5′-GAGACTCTGAAGATAGGAGAAC-3′), which give a 732-bp fragment. The second pair, used for the amplification of poly(A) RNA, was based on primers used by Chang *et al.* (1994) and was 5′-AGCCGAAAGGATTCCACCAT-3′ and 5′-TCCGTGTTGTCTACGTCCAG-3′. These yielded a fragment of 233 bp. RT–PCR fragments were electrophoresed and analyzed by staining with ethidium bromide or by Southern blotting and hybridization to ^{32}P-labeled inserts from pB6-1 or a plasmid clone of the 732-bp fragment obtained by PCR amplification using primers 2A1 and 2A2.

Figure 1 shows the nucleic acid sequence and translation of the left-hand part of pB6-1 and the phage insert flanking region in λ B6-1. This sequence contains a long ORF coding for a potential 341-residue protein and starting with an ATG which is part of a Kozak consensus sequence (Kozak, 1983). There are several possible polyadenylation signals downstream of the termination codon, one of which is around position 1190, just beyond the position of the termination codon for the homolog of the HVS ORF75, the reading frame of which is in the opposite sense. This is the position in HVS of ORF74, a viral chemokine receptor homolog (Nicholas *et al.,* 1992). Analysis by the program SOAP (included in the PC/Gene sequence analysis package, Intelligenetics, Mountain View, CA), which calculates hydrophobicity by the

```
AAAGGAGTGGCTAAACAACACCTATACTACTTGTTATTGTAGCATGGCGGCCGAGGATTTCCTAACCATCTTCTT   75
                                                  M  A  A  E  D  F  L  T  I  F  L

AGATGATGATGAATCCTGGAATGAAACTCTAAATATGAGCGGATATGACTACTCTGGAAACTTCAGCCTAGAAGT  150
  D  D  D  E  S  W  N  E  T  L  N  M  S  G  Y  D  Y  S  G  N  F  S  L  E  V

GAGCGTGTGTGAGATGACCACCGTGGTGCCTTACACGTGGAACGTTGGAATACTCTCTCTGATTTTCCTCATAAA  225
  S  V  C  E  M  T  T  V  V  P  Y  T  W  N  V  G  I  L  S  L  I  F  L  I  N

TGTTCTTGGAAATGGATTGGTCACCTACACCTTTCGCAAGCACCGACCGCGGGCAGGAGCGATAGATATACTGCT  300
  V  L  G  N  G  L  V  T  Y  T  F  R  K  H  R  P  R  A  G  A  I  D  I  L  L

CCTGGGTATCTGCCTAAACTCGCTGTGTCTTAGCATATCTCTATTGGCAGAAGTGTTGATGTTTTTGTTTCCCAA  375
  L  G  I  C  L  N  S  L  C  L  S  I  S  L  L  A  E  V  L  M  F  L  F  P  N

TATCATCTCCACAGGCTTGTGCAGACTTGAAATTTTTTTTTACTATTTATATGTCTACTTGGATATCTTCAGTGT  450
  I  I  S  T  G  L  C  R  L  E  I  F  F  Y  Y  L  Y  V  Y  L  D  I  F  S  V

TGTGTGCGTCAGTCTAGTGAGGTACCTCCTGGTGGCATATTCTACGCGTTCCTGGCCCAAGAAGCAGTCCCTCGG  525
  V  C  V  S  L  V  R  Y  L  L  V  A  Y  S  T  R  S  W  P  K  K  Q  S  L  G

ATGGGTACTGACATCGCTGCACTGTCAATTGCATTGGTGCTGTCGGGGGATCTGTCGGCACAGGAGCAGGGTGGT  600
  W  V  L  T  S  L  H  C  Q  L  H  W  C  C  R  G  I  C  R  H  R  S  R  V  V

CGACCCGGTCAGCAAGCAGGCCATGTGTTATGAGAATGCGGGAAACATGACTGCAGACTGGCGACTGCATGTCAG  675
  D  P  V  S  K  Q  A  M  C  Y  E  N  A  G  N  M  T  A  D  W  R  L  H  V  R

AACCGTGTCAGTTACTGCAGGTTTCCTGTTACCCCTGGCCCTCCTTATTCTGTTTTATGCTCTCACCTGGTGTGT  750
  T  V  S  V  T  A  G  F  L  L  P  L  A  L  L  I  L  F  Y  A  L  T  W  C  V

GGTGAGGAGGACAAAGCTGCAAGCCAGGCGGAAGGTAAGGGGGGTGATTGTTGCTGTGGTGCTGCTGTTTTTTGT  825
  V  R  R  T  K  L  Q  A  R  R  K  V  R  G  V  I  V  A  V  V  L  L  F  F  V

GTTTTGCTTCCCTTACCACGTACTAAATCTACTGGACACTCTGCTAAGGCGACGCTGGATCCGGGACAGCTGCTA  900
  F  C  F  P  Y  H  V  L  N  L  L  D  T  L  L  R  R  R  W  I  R  D  S  C  Y

TACGCGGGGGTTGATAAACGTGGGTCTGGCAGTAACCTCGTTACTGCAGGCACTGTACAGCGCCGTTGTTCCCCT  975
  T  R  G  L  I  N  V  G  L  A  V  T  S  L  L  Q  A  L  Y  S  A  V  V  P  L

       970        980        990       1000       1010       1020
GATATACTCCTGCCTGGGATCCCTCTTTAGGCAGAGGATGTACGGTCTCTTCCAAAGCCTCAGGCAGTCTTTCAT 1050
  I  Y  S  C  L  G  S  L  F  R  Q  R  M  Y  G  L  F  Q  S  L  R  Q  S  F  M

GTCCGGCGCCACCACGTAGCCCGCGGATGTCTACGTGCCCTTCCCCCTTAATTTAATCTAGCCTCCCGTTCCCAT 1125
 S  G  A  T  T

GATGCAGAGAGGCGAATCTGGTTTGTACACAGATGTGACTATGTATTTGTTTTATTATGCGATTAAATGAGGGGT 1200

CTGATCCCAAAAGCAATGTTTAGTGGTGGTCGTTGATCTTCTTGACGCTCCATAGGTAGATTGACTGGAACGCCA 1275

TGGCCCACGGGGACATGACAGGGGTGTTAGGTCTGGTGGAACATGCTGCCACTGCCACGGATGGAACATCAGGGA 1350

TGGGTCTATGATCAGGGCAGCGTGTCGCCCGTCACTGGATGTAAGTCCGGCCACCGTGGAGTTGCCTGTGGGGTT 1425

TCTGGGATAGTGTCTGGCTGGCAGGGTCTCATCCGCGGCATTTCCATGGTAGGTGAGGGTTATCTCGCCTCGCTG 1500

TCTCAGTATGTACTCGAGGGCGTCCTGCTCGTACCGGACCCCCAGGTACTCGCCCTGGGCCCAGCTGGGCAGCAC 1575

CGTCCCCCGCAACACTCGGAGGAAAACGCTCTTAGTGTTCTGAGGGATCTGTATGTTTAGCCAGTGGCTGTCATA 1650

CAGCTTGGACACGTTGGTCTCCAGGTTTACCGCCCAGCGCTGGGGTGGTGTGGGTCCGTACGTGTATGGTGAGGA 1725

TTCCGACCGGCCCACTACACCCAGGGCCACCAGAAGCTGGAAGCCCACTTCGCCACAGCAGATGGAGAATGTGTC 1800

GGGCCTGTTTAGAAACTCTGTCAGGGTGGAGGCACAGGTAGGGTCGTTACACAGCGCCAGGACCCATCCCCTGGC 1875

GCTGGCGTAGCTGGCCTGGCAGCCTGTTCTGAGACATGTAATCAGACCAGAGAACCCCGACAAGGACTGTCCTCG 1950

TTTAAGCTCTTCCACAGTCACCGTGGCCACCTCAAAGCCCGTGTTCTGCA                        2000
```

FIG. 1. Sequence of HHV-8 chemokine receptor analog. Partial sequence of pB6-1, including homolog of HVS ORF74. The nucleotide sequence of a portion of pB6-1 and λ B6-1 including the ORF homologous to HVS ORF74 as well as its inferred amino acid sequence is shown. A probable polyadenylation signal is underlined.

TABLE 1

Relationship of HHV-8 GCR-like Sequence to Other gcr Genes

Gene	FASTA score (% identity/*n* residues)[a]
HVS GCR homolog	559 (36/308)
IL8R β CXC chemokine receptor	439 (28/323))
IL8R α CXC chemokine receptor	397 (28/314)
CMV US28 GCR homolog	326 (24/312)
Type 1A angiotensin receptor	297 (21/305)
EBV-induced GCR homolog	297 (23/308)
Burkitt's lymphoma GCR homolog	270 (22/285)
CKR1 C-C chemokine receptor	269 (20/312)
Bradykinin receptor	263 (24/294)
Anaphylatoxin chemotactic receptor	209 (24/324)
Fusin (HIV-1 fusion accessory)	205 (21/270)

[a] FASTA scores were derived as described in text.

method of Klein *et al.* (1984), predicts that the translated HHV-8 protein is an integral membrane protein that contains seven transmembrane regions, characteristic of GCRs, a family of proteins which includes chemokine receptors.

The inferred reading frame was used to screen the SwissProt database by a FASTA (Pearson and Lipman, 1988) search using the GCG sequence analysis package on the NIH mainframe computer and by a BEAUTY search (Worley *et al.,* 1995) using the Baylor University Molecular Biology server. The strongest match was indeed found with the HVS ORF74 GCR homolog (Nicholas *et al.,* 1992) (optimized FASTA score = 548, 36% amino acid identity in a 308-residue overlap). Significant optimized FASTA scores (>200) were also obtained with a variety of other GCR-related proteins. The closest matches were with various chemokine receptors, particularly those belonging to the high-affinity CXC chemokine receptor B chain (IL8RB) (Table 1). Other close matches include US28, a GCR homolog of human cytomegalovirus (HCMV), as well as fusin, a GCR-related protein recently identified as a cofactor for fusion of T cell line-tropic strains of the HIV (Feng *et al.,* 1996).

Figure 2 shows an alignment, performed using the CLUSTAL program of Higgins and Sharp (1988) (contained in the PC/Gene package), between the inferred amino acid sequence of the putative HHV-8 chemokine receptor and several other viral and cellular chemokine receptors. The putative transmembrane regions are indicated with brackets. Positions of amino acid identity between the HHV-8 chemokine receptor homolog and those of HVS or HCMV or between HHV-8 and IL8RB or fusin are shaded. Several other features found in most GCRs are conserved in the inferred HHV-8 protein, including several glycosylation signals in the N-terminal putative extracellular domain, proline residues in putative transmembrane regions V, VI, and VII, and cysteine residues in the putative second, third, and fourth extracellular domains.

RNA from KS tissue was analyzed by RT–PCR for expression of RNA from several HHV-8 genes, including the chemokine receptor-related gene. In one set of experiments, as shown in Fig. 3A, gene transcripts for the HVS minor capsid protein (ORF26) homolog were detected in one of four samples of total RNA at relatively high levels, in a second at low levels, and were not detectable in two other samples. RNA for the chemokine receptor homolog was detectable in three of four samples (Fig. 3B), including the one weakly positive for minor capsid protein RNA, but was not detectable in the sample strongly positive for minor capsid protein RNA.

In a separate set of experiments, using polyadenylated RNA (Fig. 3C), chemokine receptor transcripts were detected in a pleural effusion from AIDS–KS, but were negative in a second pleural effusion, a peripheral blood mononuclear cell (PBMC) sample from AIDS–KS, uncultured and early passage (p6) BCBL cells, two short-term cultures from KS tumors, and six samples of spindle cells purified from AIDS–KS patients. The positive AIDS–KS pleural effusion was also positive for RNA of the HVS minor capsid protein (ORF26), as were the uncultured BCBL sample and one short-term culture from a classical KS. The BCBL cell culture was also negative by PCR for HHV-8 DNA, suggesting that it did not contain HHV-8. Although the classical KS cell culture in lane 8 was negative for actin, the RNA otherwise appeared to be of sufficient quality for RT–PCR because it was positive for several HHV-8 transcripts other than the ORF75 or chemokine receptor homologs (see below). The cell line BCBL-1 (Renne *et al.,* 1996) was negative for chemokine receptor homolog RNA, but became positive after induction with sodium butyrate (not shown). None of the RNA samples shown in Fig. 3C were positive when the reverse transcription step was omitted, indicating that the amplified fragments indeed represented RNA transcripts.

These data indicate that although HHV-8 DNA sequences are almost always present in KS tissue, detectable expression of the different viral genes is variable. Consistent with this conclusion, we have shown elsewhere that, in contrast to the detection of RNA for the chemokine receptor and ORF74 homologs in a minority of the samples in Fig. 3C, RNA transcripts of a recently reported HHV-8 cyclin D homolog (Chang *et al.,* 1996a) were detectable in all of them except for the AIDS–KS PBMC, one AIDS–KS culture, and the BCBL short-term culture (Browning *et al.,* manuscript in preparation). PBSC-33 and -39, the two classical KS cultures, both pleural effusions, and the uncultured BCBL sample are also positive for RNA transcripts of one of the two chemokine homologs (MIP-1B) recently described by Nicholas *et al.* (submitted for publication).

We have identified and characterized a gene from HHV-8 that is closely related to the ORF74 chemokine

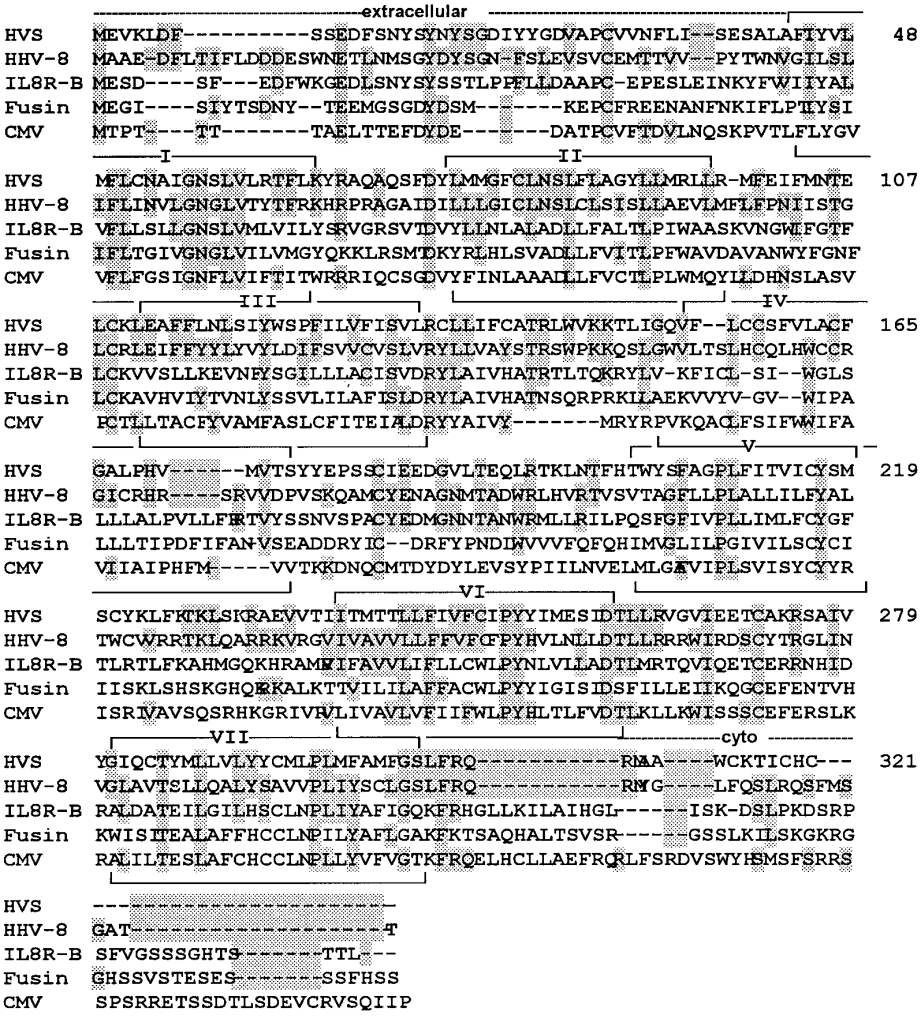

FIG. 2. HHV-8 G protein-coupled receptor homolog. Comparison of HHV-8 chemokine receptor homolog with cellular and viral chemokine receptors. Amino acid alignment was performed using the program CLUSTAL. Amino acid residues in the HHV-8 inferred amino acid sequence that are identical to those of the other proteins are shaded. Dashes indicate gaps in the amino acid sequences.

receptor homology of HVS. Among cellular genes, the predicted HHV-8 gene product is most closely related to the chemokine receptor family of GCR proteins, particularly receptors for the CXC chemokine IL-8. HSV ORF74 has in fact been shown to code for a functional IL-8 receptor (Ahuja and Murphy, 1993). The presence of a chemokine receptor in HHV-8 is of possible significance to KS pathogenesis, since the signals mediated by this kind of receptor are involved in chemotaxis and the inflammatory response, consistent with the mixed cellularity and inflammatory milieu of KS lesions. In this light, it is of interest that two genes homologous to the CC chemokine MIP-1 (Schall, 1991) have recently been identified in the HHV-8 genome (Nicholas *et al.,* submitted for publication), suggesting another mechanism by which HHV-8 might contribute to a chemotactic or inflammatory response in KS. It will obviously be of interest to determine whether either of the two chemokine homologs serves as a ligand for the HHV-8 ORF74 homolog. In view of the fact that the receptor homolog is most related to CXC chemokine receptors while both chemokines appear to be CC chemokines, however, these molecules seem more likely to form ligand–receptor pairs with cellular proteins than with each other. RNA transcripts of the chemokine receptor homolog are not universally detectable in KS tissue, suggesting that it is not necessary for the maintenance of KS lesions. Induction of its expression by butyrate in BCBL-1 cells suggests that it is expressed primarily during viral lytic replication. In view of its potential activity as a mediator of chemotaxis and inflammation, however, it may well be involved in pathogenesis *in vivo.*

It is plausible that HHV-8 could play a role in KS development, especially given the apparent high prevalence of viral antibodies and DNA in KS. Against this possibility, HHV-8 is not present in several neoplastic KS-derived cell

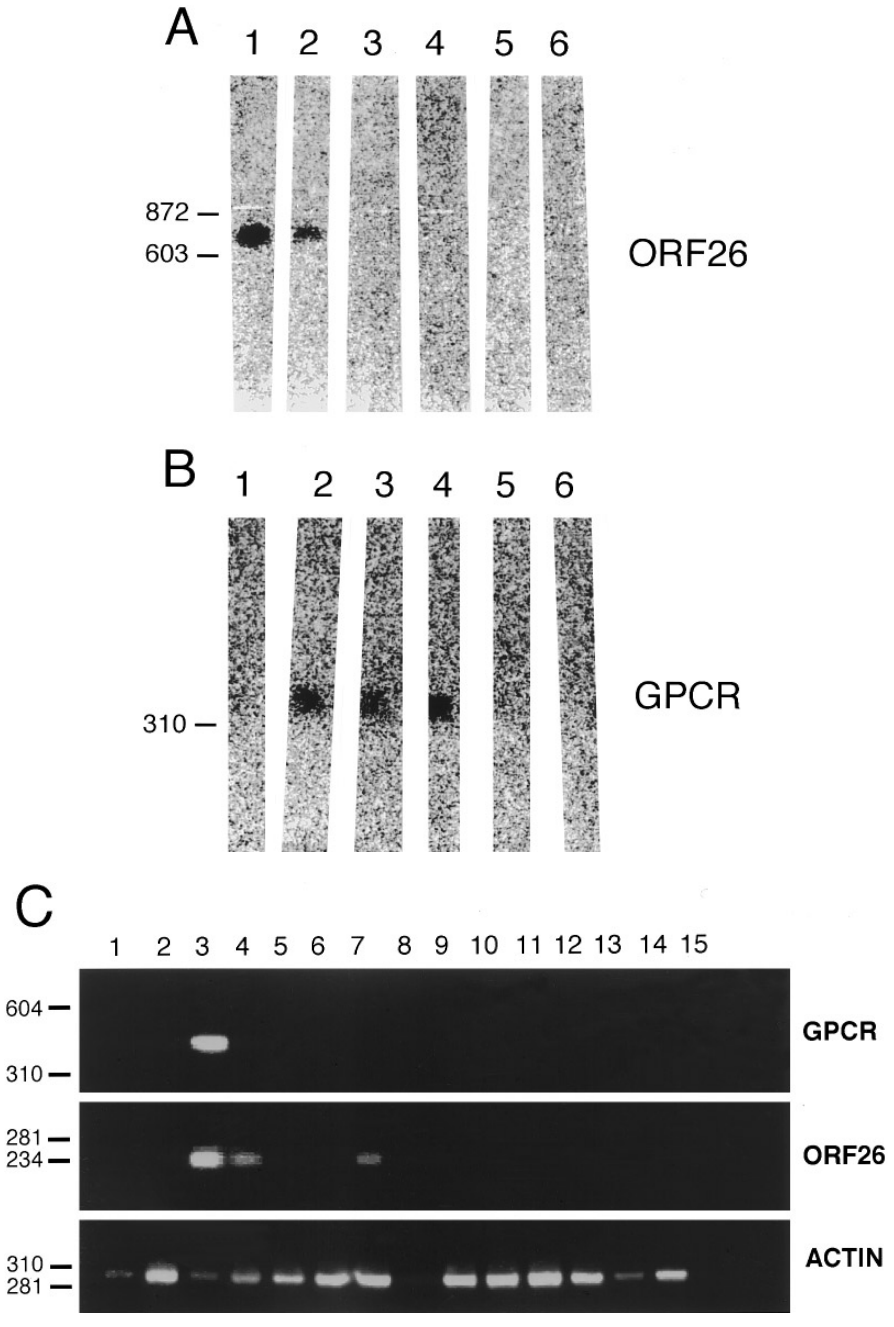

FIG. 3. Detection of HHV-8 RNA by RT–PCR. (A and B) Total RNA was subjected to reverse transcription and PCR amplification as described in the text and analyzed by Southern blot. Lanes 1–3 contain RNA samples from KS biopsies, lane 4 contains RNA from a lymph node biopsy from an AIDS–KS patient, lane 5 contains RNA from the Jurkat T cell line, and lane 6 contains pAW109 RNA from the RT–PCR kit, used here as a negative control. A used primers and a probe for the HHV-8 minor capsid protein (ORF26) homolog, and B used primers and a probe for the HHV-8 homolog to HVS ORF74. (C) Polyadenylated RNA was analyzed as described for A and B, except that the fragments were detected by staining with ethidium bromide. The top panel was analyzed for the ORF74 chemokine receptor homolog, the middle panel was analyzed for the minor capsid protein (ORF26) analog, and the bottom panel represents a control RT–PCR analysis for actin. Lane 1 was a PBMC AIDS–KS sample; lane 2 was an AIDS–KS cell line; lane 3 was a pleural effusion from an AIDS–KS patient; lane 4 was an uncultured BCBL sample from an AIDS–KS patient; lane 5 was a second pleural effusion from an AIDS–KS patient; lane 6 was a short-term culture (passage 6) from a BCBL sample from an AIDS–KS patient; lanes 7 and 8 were short term cultures (passage 2) from classical KS patients; lanes 9–14 were samples of spindle cells purified from peripheral blood of AIDS–KS patients; lane 15 was a negative control which did not contain any RNA. The numbers on the left give the position and size in kb of specific fragments from a molecular weight marker containing *Hin*dIII-digested λ phage and *Hae*III-digested ϕX174.

lines which bear many similarities to the spindle cells thought to be the tumor cell in KS (Lunardi-Iskandar *et al.,* 1995), and HHV-8 are not detectable in KS cells grown *in vitro* (Dictor *et al.,* 1996; Colombini, unpublished data). Although we have shown that HHV-8 chemokine receptor RNA is detectable in KS tissue, the only consistent expres-

sion of viral genes reported so far is of two small RNAs with little protein coding potential (Zhong *et al.,* 1996), which may represent latency genes. Furthermore, there are reports of a varying, but significant, incidence of HHV-8 detection in apparently healthy people (Rady *et al.,* 1995; Luppi *et al.,* 1996; Monini *et al.,* 1996). Obviously, with a virus as genetically complex as HHV-8, much work needs to be done on the expression of individual genes in order to properly assess its possible role in KS.

ACKNOWLEDGMENTS

We thank A. Mazzuca for expert editorial assistance. E.T. is supported by a grant (PO 1437-MED) from the Austrian Science Foundation. The DNA sequence has been deposited in GenBank under the accession number U82242.

REFERENCES

Ahuja, S. K., and Murphy, P. M. (1993). Molecular piracy of mammalian interleukin-8 receptor type B by herpesvirus saimiri. *J. Biol. Chem.* 268, 20691–20694.

Browning, P. J., Sechler, J. M. G., Kaplan, M., Washington, R. H., Gendelman, R., Yarchoan, R., Ensoli, B., and Gallo, R. C. (1994). Identification and culture of Kaposi's sarcoma-like spindle cells from the peripheral blood of human immunodeficiency virus-1-infected individuals and normal controls. *Blood* 84, 2711–2720.

Buonaguro, F. M., Tornesello, M. L., Beth-Giraldo, E., Hatzakis, A., Mueller, N., Downing, R., Biryamwaho, B., Sempala, S. D., and Giraldo, G. (1996). Herpesvirus-like DNA sequences detected in endemic, classic, iatrogenic and epidemic Kaposi's sarcoma (KS) biopsies. *Int. J. Cancer* 65, 25–28.

Cesarman, E., Moore, P. S., Rao, P. H., Inghirami, G., Knowles, D. M., and Chang, Y. (1995). In vitro establishment and characterization of two acquired immunodeficiency syndrome-related lymphoma cell lines (BC-1 and BC-2) containing Kaposi's sarcoma-associated herpesvirus-like (KSHV) DNA sequences. *Blood* 86, 2708–2714.

Chang, Y., Cesarman, E., Pessin, M. S., Lee, F., Culpepper, J., Knowles, D. M., and Moore, P. S. (1994). Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science* 266, 1865–1869.

Chang, Y., Moore, P. S., Talbot, S. J., Boshoff, C. H., Zarkowska, T., Godden-Kent, H., Paterson, H., Weiss, R. A., and Mittnacht, S. (1996a). Cyclin encoded by KS herpesvirus. *Nature* 382, 410.

Chang, Y., Ziegler, J., Wabinga, H., Katangole-Mbidde, E., Boshoff, C., Schulz, T., Whitby, D., Maddalena, D., Jaffe, H. W., Weiss, R. A., and Moore, P. S. (1996b). Kaposi's sarcoma-associated herpesvirus and Kaposi's sarcoma in Africa: Uganda Kaposi's Sarcoma Study Group. *Arch. Intern. Med.* 156, 202–204.

de Lellis, L., Fabris, M., Cassai, E., Corallini, A., Giraldo, G., Feo, C., and Monini, P. (1995). Herpesvirus-like DNA sequences in non-AIDS Kaposi's sarcoma. *J. Infect. Dis.* 172, 1605–1607.

Dictor, M., Rambech, E., Way, D., Witte, M., and Bendsoe, N. (1996). Human herpesvirus 8 (Kaposi's sarcoma-associated herpesvirus) DNA in Kaposi's sarcoma lesions, AIDS Kaposi's sarcoma cell lines, endothelial Kaposi's sarcoma simulators, and the skin of immunosuppressed patients. *Am. J. Pathol.* 148, 2009–2016.

Dupin, N., Grandadam, M., Calvez, V., Gorin, I., Aubin, J. T., Havard, S., Lamy, F., Leibowitch, M., Huraux, J. M., and Escande, J. P. (1995). Herpesvirus-like DNA sequences in patients with Mediterranean Kaposi's sarcoma. *Lancet* 345, 761–762.

Feng, Y., Broder, C. C., Kennedy, P. E., and Berger, E. A. (1996). HIV-1 entry cofactor: Functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science* 272, 872–877.

Friedman-Kien, A. E., and Saltzman, B. R. (1990). Clinical manifestations of classical, endemic African, and epidemic AIDS-associated Kaposi's sarcoma. *J. Am. Acad. Dermatol.* 22, 1237–1250.

Gao, S.-J., Kingsley, L., Li, M., Zheng, W., Parravicini, C., Ziegler, J., Newton, R., Rinaldo, C. R., Saah, A., Phair, J., Detels, R., Chang, Y., and Moore, P. S. (1996a). KSHV antibodies among Americans, Italians and Ugandans with and without Kaposi's sarcoma. *Nature Med.* 2, 925–928.

Gao, S. J., Kingsley, L., Hoover, D. R., Spira, T. J., Rinaldo, C. R., Saah, A., Phair, J., Detels, R., Parry, P., Chang, Y., and Moore, P. S. (1996b). Seroconversion to antibodies against Kaposi's sarcoma-associated herpesvirus-related latent nuclear antigens before the development of Kaposi's sarcoma. *N. Engl. J. Med.* 335, 233–241.

Higgins, D. G., and Sharp, P. M. (1988). CLUSTAL: A package for performing multiple sequence alignment on a microcomputer. *Gene* 73, 237–244.

Huang, Y. Q., Li, J. J., Kaplan, M. H., Poiesz, B., Katabira, E., Zhang, W. C., Feiner, D., and Friedman-Kien, A. E. (1995). Human herpesvirus-like nucleic acid in various forms of Kaposi's sarcoma. *Lancet* 345, 759–761.

Kedes, D. H., Operskalski, E., Busch, M., Kohn, R., Flood, J., and Ganem, D. (1996). The seroepidemiology of human herpesvirus 8 (Kaposi's sarcoma-associated virus): Distribution of infection in KS risk groups and evidence for sexual transmission. *Nature Med.* 2, 918–924.

Klein, P., Kanehisa, M., and DeLisi, C. (1984). Prediction of protein function from sequence properties. Discriminant analysis of a data base. *Biochim. Biophys. Acta* 787, 221–226.

Kozak, M. (1983). Comparison of initiation of protein synthesis in procaryotes, eucaryotes, and organelles. *Microbiol. Rev.* 47, 1–45.

Lisitsyn, N., and Wigler, M. (1993). Cloning the differences between two complex genomes. *Science* 259, 946–951.

Lunardi-Iskandar, Y., Gill, P., Lam, V. H., Zeman, R. A., Michaels, F., Mann, D. L., Reitz, M. S., Jr., Kaplan, M., Berneman, Z. N., and Carter, D. (1995). Isolation and characterization of an immortal neoplastic cell line (KS Y-1) from AIDS-associated Kaposi's sarcoma. *J. Natl. Cancer Inst.* 87, 974–981.

Luppi, M., Barozzi, P., Maiorana, A., Collina, G., Ferrari, M. G., Marasca, R., Morselli, M., Rossi, E., Ceccherini-Nelli, L., and Torelli, G. (1996). Frequency and distribution of herpesvirus-like DNA sequences (KSHV) in different stages of classic Kaposi's sarcoma and in normal tissues from an Italian population. *Int. J. Cancer* 66, 427–431.

Miller, G., Rigsby, M. O., Heston, L., Grogan, E., Sun, R., Metroka, C., Levy, J. A., Gao, S. J., Chang, Y., and Moore, P. (1996). Antibodies to butyrate-inducible antigens of Kaposi's sarcoma-associated herpesvirus in patients with HIV-1 infection. *N. Engl. J. Med.* 334, 1292–1297.

Monini, P., de Lellis, L., Fabris, M., Rigolin, F., and Cassai, E. (1996). Kaposi's sarcoma-associated herpesvirus DNA sequences in prostate tissue and human semen. *N. Engl. J. Med.* 334, 1168–1172.

Moore, P. S., and Chang, Y. (1995). Detection of herpesvirus-like DNA sequences in Kaposi's sarcoma in patients with and without HIV infection. *N. Engl. J. Med.* 332, 1181–1185.

Moore, P. S., Gao, S. J., Dominguez, G., Cesarman, E., Lungu, O., Knowles, D. M., Garber, R., Pellett, P. E., McGeoch, D. J., and Chang, Y. (1996). Primary characterization of a herpesvirus agent associated with Kaposi's sarcoma. *J. Virol.* 70, 549–558.

Nicholas, J., Cameron, K. R., and Honess, R. W. (1992). Herpesvirus saimiri encodes homologues of G protein-coupled receptors and cyclins. *Nature* 355, 362–365.

Noel, J. C., Hermans, P., Andre, J., Fayt, I., Simonart Th, Verhest, A., Haot, J., and Burny, A. (1996). Herpesvirus-like DNA sequences and Kaposi's sarcoma: Relationship with epidemiology, clinical spectrum, and histologic features. *Cancer* 77, 2132–2136.

O'Neill, E., Henson, T. H., Ghorbani, A. J., Land, M. A., Webber, B. L., and Garcia, J. V. (1996). Herpes virus-like sequences are specifically found in Kaposi sarcoma lesions. *J. Clin. Pathol.* 49, 306–308.

Pearson, W. R., and Lipman, D. J. (1988). Improved tools for biological sequence comparison. *Proc. Natl. Acad. Sci. USA* 85, 2444–2448.

Penn, I. (1979). Kaposi's sarcoma in organ transplant recipients: Report of 20 cases. *Transplantation* 27, 8–11.

Rady, P. L., Yen, A., Rollefson, J. L., Orengo, I., Bruce, S., Hughes, T. K., and Tyring, S. K. (1995). Herpesvirus-like DNA sequences in non-Kaposi's sarcoma skin lesions of transplant patients. *Lancet* 345, 1339–1340.

Renne, R., Zhong, W., Herndier, B., McGrath, M., Abbey, N., Kedes, D., and Ganem, D. (1996). Lytic growth of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) in culture. *Nat. Med.* 2, 342–346.

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). "Molecular Clonong: A Laboratory Manual," 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Schall, T. J. (1991). Biology of the RANTES/SIS cytokine family. *Cytokine* 3, 165–183.

Wahman, A., Melnick, S. L., Rhame, F. S., and Potter, J. D. (1991). The epidemiology of classic, African, and immunosuppressed Kaposi's sarcoma. *Epidemiol. Rev.* 13, 178–199.

Whitby, D., Howard, M. R., Tenant-Flowers, M., Brink, N. S., Copas, A., Boshoff, C., Hatzioannou, T., Suggett, F. E., Aldam, D. M., and Denton, A. S. (1995). Detection of Kaposi sarcoma associated herpesvirus in peripheral blood of HIV-infected individuals and progression to Kaposi's sarcoma. *Lancet* 346, 799–802.

Worley, K. C., Wiese, B. A., and Smith, R. A. (1995). BEAUTY; An enhanced BLAST-based search tool that integrates multiple biological information resources into sequence similarity search results. *Genome Res.* 5, 173–184.

Zhong, W., Wang, H., Herndier, B., and Ganem, D. (1996). Restricted expression of Kaposi sarcoma-associated herpesvirus (human herpesvirus 8) genes in Kaposi sarcoma. *Proc. Natl. Acad. Sci. USA* 93, 6641–6646.

Ziegler, J. L., Templeton, A. C., and Vogel, C. L. (1984). Kaposi's sarcoma: A comparison of classical, endemic, and epidemic forms. *Semin. Oncol.* 11, 47–52.