

Human Herpesvirus 7 Open Reading Frames U12 and U51 Encode Functional β -Chemokine Receptors

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Human herpesvirus 7 (HHV-7), which belongs to the betaherpesvirus subfamily and infects mainly CD4⁺ T cells in vitro, infects children during infancy. HHV-7 contains two genes, U12 and U51, that encode putative homologs of cellular G-protein-coupled receptors. To analyze the biological function of the U12 and U51 genes, we cloned these genes and expressed the proteins in cells. U12 and U51 encoded functional calcium-mobilizing receptors for β -chemokines, which include thymus and activation-regulated chemokine (TARC), macrophage-derived chemokine (MDC), EB11-ligand chemokine (ELC), and secondary lymphoid-tissue chemokine (SLC), but not for other chemokines, suggesting that the chemokine selectivities of the U12 and U51 products were distinct from those of the known mammalian chemokine receptors. ELC and SLC induced migration in Jurkat cells stably expressing U12, but TARC and MDC did not. In contrast, none of these chemokines induced migration in Jurkat cells stably expressing U51. Together, these data indicate that the products of U12 and U51 may play important and different roles in the pathogenesis of HHV-7 through transmembrane signaling.

Human herpesvirus 7 (HHV-7) was first isolated in 1990 from the peripheral blood lymphocytes of a healthy individual and also from a patient with chronic fatigue syndrome (16). HHV-7 is a ubiquitous virus that is similar to HHV-6. It is widespread in the general population, and seroconversion for HHV-7 occurs prior to age 4, which is somewhat later than for HHV-6 (44, 49, 50). Primary infection with HHV-7 causes exanthem subitum or high fever, as does HHV-6, and HHV-7 can reactivate HHV-6 in vivo and in vitro (6, 10, 45). HHV-7 can frequently be isolated from the saliva of healthy adults (49, 56).

HHV-7 belongs to the betaherpesvirus subfamily, which also includes HHV-6 and human cytomegalovirus (HCMV). The entire HHV-7 DNA sequences for two strains, JI and RK, have been reported (5, 36). HHV-7 is tropic for CD4⁺ T lymphocytes, utilizes the CD4⁺ molecule as at least part of its essential receptor for entry into target cells, and has an antagonistic effect on infection of CD4⁺ T cells by human immunodeficiency virus type 1 (28).

All members of the betaherpesviruses and most of the gammaherpesviruses encode chemokine receptor homologs (14, 17, 18, 24, 35, 41). HHV-7 contains two G-protein-coupled receptor (GPCR) homologs, which are encoded in open reading frames U12 and U51 (36). HHV-7 U12 and U51 are respective positional and structural homologs of HHV-6 U12 and U51 and of HCMV UL33 and UL78 (30, 31). In humans, chemokine receptors are classified into four groups, CC, CXC, CX3C, and C, and their ligand specificities have been defined (1, 11, 12, 32, 39, 40, 42, 43, 48, 51). We previously showed that HHV-6 U12 functions as a β -chemokine receptor linked to a

calcium-mobilizing signal transduction pathway for regulated upon activation, normal T expressed and secreted (RANTES), monocyte chemoattractant protein 1 (MCP-1), and macrophage inflammatory proteins 1 α and 1 β (MIP-1 α and MIP-1 β) (25). We showed that HHV-7 U12 also functions as a calcium-mobilizing receptor in response to the binding of MIP-3 β /EB11-ligand chemokine (ELC) (34). Furthermore, HHV-6 U51 has been shown to bind RANTES as well as to downregulate the transcription of this chemokine (31). Therefore, U12 and U51 could be functional β -chemokine receptors.

We report in this paper that HHV-7 U12 and U51, when produced in a stably transfected human erythroleukemia cell line, K562, are promiscuous high-affinity β -chemokine receptors that can potentially be linked to a calcium-mobilizing signal transduction pathway. Moreover, we found that HHV-7 U12 induced the migration of stably transfected cells toward ELC and secondary lymphoid-tissue chemokine (SLC), but not toward thymus and activation-regulated chemokine (TARC) or macrophage-derived chemokine (MDC), and that HHV-7 U51 did not induce the migration of stably transfected cells toward any of these chemokines.

MATERIALS AND METHODS

Cells and cell culture. SupT1 cells (National Institutes of Health AIDS Research and Reference Program, Rockville, MD), which are derived from a lymphoblastoid CD4⁺ T-cell line, were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (Life Technologies, Rockville, MD), 200 mM L-glutamine, 100 U penicillin, and 100 U streptomycin. The cell density was maintained between 2×10^5 and 1×10^6 cells/ml. The cultured SupT1 cells were collected by centrifugation at $400 \times g$ for 5 min and suspended in fresh medium at a density of either 2×10^5 cells/ml or 5×10^5 cells/ml. Nonadherent K562 cells, derived from human erythroleukemia cells (CCL 243), were from the American Type Culture Collection. The K562 cells and Jurkat cells were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum and 200 mM L-glutamine (complete medium).

Cloning of the HHV-7 U51 gene. A DNA fragment corresponding to U51 was amplified from HHV-7 cDNA-infected SupT1 cells by PCR using the following primers containing a KpnI site at the 5' end and a BamHI site at the 3' end, for subsequent cloning (34): 7U51-KpnI (5'-TGGGTACCTTTCTTAAGCTTTT GTA) and 7U51-BamHI (5'-TCGGATCCTTAAACTGTGTAGATTTTG).

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The design of these primers was based on the predicted genomic structure of HHV-7 U51. PCR was performed with approximately 0.5 ng of cDNA containing 0.2 mM deoxynucleoside triphosphates, 10 pmol of each primer, and 2.5 U *ExTaq* in a 50- μ l reaction volume (TAKARA, Kyoto, Japan). The PCR conditions were a 5-min preincubation at 94°C, followed by 25 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 2 min. The amplified products were subcloned directly into a pCR2.1 vector (Invitrogen, San Diego, CA) and sequenced using a SequiTherm Long-Read cycle sequencing kit and a 4000L DNA sequencer (Li-Cor, Inc., Lincoln, NE). After sequencing, the fragments were digested with *Kpn*I and *Bam*HI and subcloned into a pCEP4 vector (Invitrogen). pEFBOS-EX was digested with *Pvu*II and *Hind*III, the fragment was treated with T4 DNA polymerase, and the blunt-ended fragment was subsequently cloned into *Sal*I-digested pCEP4, generating the expression vector pCEP4-EF.

Construction of HHV-7 Flag-U12 and Flag-U51. A Flag tag was introduced at the N terminus of U12 or U51 by site-directed mutagenesis, and *Kpn*I and *Bam*HI restriction sites were added to facilitate cloning downstream of the EF-1 α promoter in the expression vector pCEP4-EF. The Flag tag was inserted after the second codon of the U12 or U51 gene. The following primers were used: 7U12F-*Kpn*I (5'-TGGGTACCATGGACTACAAAGACGATGACGAC AAGGACTCTAATTGATTTC), 7U12F-*Bam*HI (5'-TCGGATCCTCAAT TTTTATTGTCAG), 7U51F-*Kpn*I (5'-TGGGTACCATGGACTACAAAGA CGATGACGACAAGAAAAATATCGATTTAAC), and 7U51F-*Bam*HI (5'-T CGGATCCTTAAACTGTGTAGATTTTG). The amplification products were digested with *Kpn*I and *Bam*HI and inserted into the pCEP4-EF plasmid vector.

Cloning of the CCR1, CCR2, CCR4, CCR5, CCR7, CCR8, and CXCR1 genes. CCR1, CCR5, CCR7, CCR8, and CXCR1 were constructed previously (25, 34, 57). The CCR2 and CCR4 genes were amplified from the genomic DNA of the promyelocytic cell line HL-60. PCR was performed with the following primer sets: CCR2-Met-*Kpn*I (5'-GGGGTACCATCCACAACATGCTGTCC) and CCR2-Ter-*Bam*HI (5'-TCTGGATCCGGCTTCCGGCCTCGAG); CCR4-Met-*Xho*I (5'-GGGCTCGAGCCTGCTTGCCTGAGGAGC) and CCR4-Ter-*Bam*HI (5'-TGTGGATCCGTTACATGCTGTGC). The PCR conditions and sequencing analysis were as described above.

Creation of stably transfected cell lines. The genes for CCR2 and CCR4 were recloned from the plasmids described above and ligated between the *Kpn*I and *Bam*HI sites or between the *Xho*I and *Bam*HI sites of the hygromycin B-selectable, stable episomal vector pCEP4; the resulting constructs were designated pCECCR2 and pCECCR4, respectively. The genes for U12 and U51 were recloned from the plasmids described above (34) and ligated between the *Kpn*I and *Bam*HI sites of pCEP4-EF. K562 or Jurkat cells (10^7 cells) in log phase were electroporated in the presence of 20 μ g plasmid DNA with a Gene Pulser (Bio-Rad Laboratories). Electroporation conditions were a 300- μ l volume, 250 V, and 960 μ F, with a 0.4-cm-gap electroporation cuvette. Transfected cells were cultured in complete medium, and 48 h later the cells were seeded at 10^5 /ml in complete medium containing 250 or 75 μ g of hygromycin B per ml and then selected for 5 days. Subsequently, K562 cells or Jurkat cells were maintained in complete medium with 150 or 25 μ g of hygromycin B per ml. K562 and Jurkat cells were cultured as described above.

Flow cytometry. K562 cells transfected with U12 or U51 were resuspended in fluorescence-activated cell sorter (FACS) buffer (1 \times phosphate-buffered saline, 1% bovine serum albumin). When necessary, the cells were permeabilized by a 5-min incubation in acetone at 4°C and washed twice with FACS buffer. Both intact and permeabilized cells were resuspended in FACS buffer at 10^7 /ml. Cells were resuspended in 100 μ l of FACS buffer containing 40 μ g/ml anti-FLAG M2 monoclonal antibody (Sigma) and incubated for 30 min on ice. Cells were washed twice with 100 μ l FACS buffer, resuspended in 100 μ l FACS buffer containing the appropriate secondary antibody labeled with fluorescein isothiocyanate (Dako), diluted 1:50 in FACS buffer, and incubated for 30 min in the dark on ice. Cells were washed twice and resuspended in 500 μ l FACS buffer. Samples were analyzed on a FACSCalibur (CELLQuest software; BD Biosciences).

Intracellular [Ca²⁺] measurements. K562 cells transfected with U12, U51, CCR1, CCR2, CCR4, CCR5, CCR7, CCR8, or CXCR1 were used for intracellular Ca²⁺ measurements. The cells were washed twice in HEPES-buffered Krebs solution, which consisted of 124 mM NaCl, 5 mM KCl, 1.24 mM KH₂PO₄, 1.3 mM MgSO₄, 2.4 mM CaCl₂, 10 mM glucose, and 25 mM HEPES (pH 7.4) (HBKS). Next, 10^7 cells were incubated for 30 min at 37°C in the dark in 1 ml of HBKS containing 5 μ M Indo-1 AM (Dojin Chemical Co.). The cells were subsequently washed twice with HBKS and resuspended at 2.5×10^6 to 3×10^6 cells/ml. One milliliter of the cell suspension was placed in a continuously stirred cuvette at 37°C in a CAF-110 fluorometer (Jasco). Fluorescence was monitored at an excitation wavelength of 355 nm and emission wavelengths of 405 and 485 nm; the data are presented as the relative ratio of fluorescence detected at 405

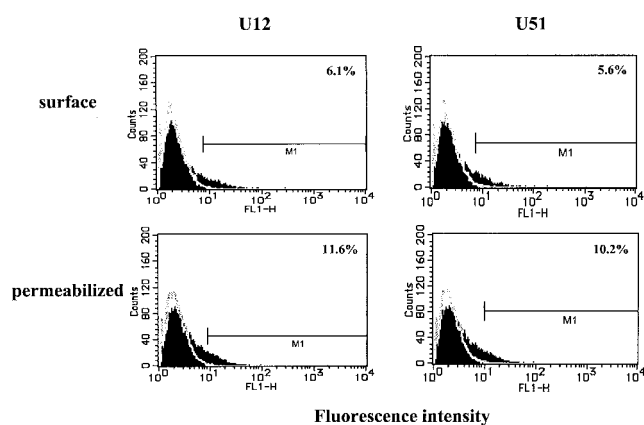


FIG. 1. Flow cytometric analysis with the anti-Flag monoclonal antibody of HHV-7 U12 and U51 expression in stably transfected K562 cells. The cells were either permeabilized or surface stained only. K562 cells were either transfected with the empty vector plasmid alone (open histograms) or with the U12 or U51 expression vector (shaded histograms). The gating strategy used to define U12 or U51 positivity is shown (horizontal bars). The results shown are representative of three independent experiments that yielded similar results.

and 485 nm. Data were collected every 10 ms. TARC, MDC, ELC, SLC, RANTES, I-309, MCP-1, and interleukin 8 (IL-8) were purchased from PeproTech EC (London, England).

Chemotaxis assay. The chemotaxis assays were performed in an assembled KK-chamber as described by Kanegasaki et al. (26), using RPMI 1640 medium supplemented with 20 mM HEPES and 0.1% bovine serum albumin (Nakarai Tesque, Kyoto, Japan). In brief, cells (10^3 in 1 μ l) suspended in medium were added to one compartment with a microsyringe. To adjust the position of the cells in the compartment and align them (70 to 100 cells) along the start line at the edge of the channel, the medium was drawn out from the other central hole provided for the opposite compartment immediately after injection of the cells. Next, a chemokine was injected into the compartment opposite the one containing the cells using a microsyringe. Chemokines used in the experiments were as follows: 10 μ M TARC, 10 μ M MDC, 10 μ M ELC, and 10 μ M SLC for Jurkat cells. To observe and record the migration of cells in the channel for 2 h, a charge-coupled device (CCD) camera or CCD video camera connected to a monitor was used. When chemotaxis of Jurkat cells was examined, the glass plate was coated with human fibronectin before assembly of the KK-chamber.

RESULTS

Expression patterns of Flag-U12 and Flag-U51. To examine the expression of HHV-7 U12 and U51 on the cell surface in K562 cells, we performed a fluorescence-activated flow cytometry analysis of their expression in K562 cells transfected with Flag-U12 and Flag-U51, before and after cell permeabilization, using an anti-FLAG monoclonal antibody. In agreement with our previous results, U12 and U51 could be detected on the surfaces of both intact and permeabilized cells, although the expression levels of Flag-U12 and Flag-U51 were uniformly weak (Fig. 1).

Signaling through the U12 and U51 GPCRs in response to CC chemokines. To test whether the U12 and U51 products were capable of signal transduction, intracellular Ca²⁺ levels were monitored by measuring the relative fluorescence of Indo-1-loaded cells stimulated with ELC/MIP-3 β , SLC, TARC, MDC, RANTES, MCP-1, I-309, or IL-8, as described previously (34) (Fig. 2A and B). To confirm the positive signaling by a specific ligand and receptor, we used as positive controls cells expressing CXCR1, CCR2, CCR8, or CCR5,

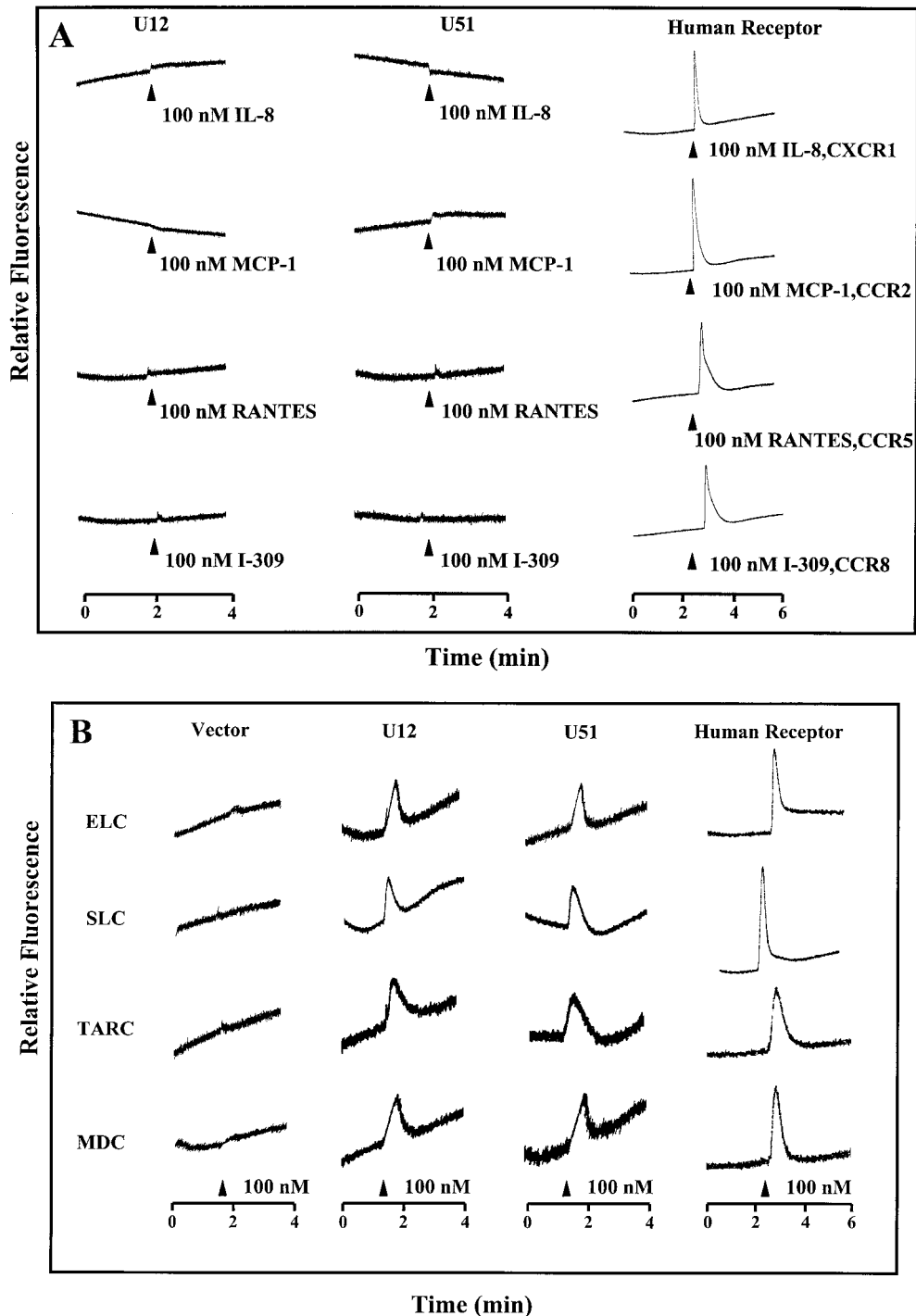


FIG. 2. (A) Ca^{2+} mobilization analysis in K562 cells transfected with U12 or U51 and treated with IL-8, MCP-1, RANTES, or I-309 and in K562 cells transfected with CXCR1, CCR2, CCR5, or CCR8 and treated with IL-8, MCP-1, RANTES, or I-309. (B) Ca^{2+} mobilization analysis in K562 cells transfected with vector alone, U12, U51, CCR7, or CCR4 and treated with ELC/MIP-3 β , SLC, TARC, or MDC. Indo-1 AM-loaded cells were suspended at 2.5×10^6 to $3 \times 10^6/\text{ml}$ and continuously monitored for fluorescence changes. Peptide agonists were added at the time points indicated by the arrowheads at a dilution of 1:25 (vol/vol), resulting in final concentrations of 100 nM. The tracings are from a single experiment that was representative of two separate experiments.

which responded to IL-8, MCP-1, I-309, or RANTES, respectively (Fig. 2A). Untransfected and pCEP4-transfected K562 cells did not respond to any of the chemokines tested, including IL-8, MCP-1, I-309, and RANTES (data not shown). K562

cells transfected with U12 or U51 responded to ELC, SLC, TARC, and MDC but not to the other chemokines (Fig. 2A and B). Treatment with buffer alone did not induce Ca^{2+} flux in the parental K562 cells or in K562 cells transfected with

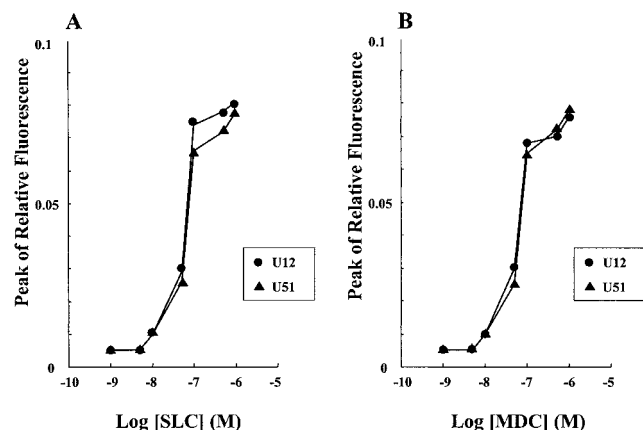


FIG. 3. Dose-response experiments. K562 cells expressing U12 and U51 were loaded with Indo-1 AM. The magnitudes of the peak of the calcium transient elicited by the indicated concentrations of SLC (A) and MDC (B) are shown.

CCR2, CCR8, CCR5, or CXCR1 (data not shown). Treatment of U12 and U51 gene transfectants with SLC and MDC induced transient elevations of the intracellular Ca^{2+} level, with a 50% effective concentration of about 30 nM (Fig. 3). ELC, SLC, TARC, and MDC showed activities similar to those of U12 and U51. ELC, SLC, TARC, or MDC at 100 nM fully desensitized the U12 and U51 transfectants against subsequent stimulation with an equal amount of ELC, SLC, TARC, or MDC (Fig. 4A). IL-8, MCP-1, I-309, and RANTES at 100 nM did not desensitize the U12 and U51 transfectants against subsequent stimulation with ELC, SLC, TARC, or MDC at 100 nM (Fig. 4B and data not shown). Furthermore, pretreatment with each of the active ligands at 100 nM did not desensitize these receptors to subsequent stimulation with an equal amount of the other active ligands for CCR4 or CCR7 (Fig. 4B and data not shown). However, pretreatment with MDC or ELC at 100 nM fully desensitized these receptors to subsequent stimulation with TARC or SLC at 100 nM (Fig. 4C). These results demonstrate that ELC, SLC, TARC, and MDC are ligands for U12 and U51.

Migration in a microchannel of Jurkat cells transfected with U12 or U51. We next examined the chemotactic responses of cells expressing U12 or U51 to the β -chemokines. Jurkat cells were stably transfected with CCR4, CCR7, U12, or U51, and the induction of migration of these cells toward TARC, MDC, ELC, or SLC was examined. After the Jurkat cells were aligned on the edge of a microchannel and the space was filled with medium, a chemokine was injected into the compartment opposite the one containing the cells. A CCD camera was used to make a digital time lapse recording of the cell migration in the channel (data not shown). As shown in Fig. 5A, Jurkat cells stably transfected with CCR7 or U12, but not with U51 or the vector alone, responded to SLC and ELC. Jurkat cells stably transfected with CCR4, but not with U12, U51, or the vector alone, responded to TARC and MDC. As shown in Fig. 5B, SLC induced vigorous migration of Jurkat cells stably transfected with CCR7 or U12, but not with U51 or the vector alone. SLC produced a dose-response curve (Fig. 5B). The results demonstrate that SLC and ELC are functional ligands for U12 to induce chemotaxis.

DISCUSSION

In this paper, we report that the U12 and U51 genes of HHV-7 encode chemokine receptors that were expressed in HHV-7-infected SupT1 cells, and we characterize the two proteins. In summary, we report in this paper the first characterization of the ability of HHV-7 U51 to function as a signal-transducing GPCR and of the ability of HHV-7 U12 to induce chemotaxis.

The U12 and U51 genes had the seven-transmembrane domains characteristic of chemokine receptors and highly conserved GPCR motifs at the junction of the transmembrane domain and the intracellular loop, although the N-terminal amino acid sequence of HHV-7 U51 was shorter than the N-terminal sequences of HHV-7 U12 and human chemokine receptors (7, 30). Flow cytometric data showed that the expression levels of U12 and U51 at the cell surface were low and that the total levels of protein expression (permeabilized cells) of these receptors were also low (Fig. 1). These findings are generally consistent with results previously reported (7, 30). However, treatment with ELC, SLC, TARC, or MDC induced Ca^{2+} flux in K562 cells transfected with Flag-U12 or Flag-U51, indicating that Flag-U12 and Flag-U51 were expressed on the cell surface (data not shown). The reasons for, and significance of, the poor expression of HHV-7 U12 and U51 are not clear and merit further examination, but they may involve protein stability, the type of cell, turnover, the promoter in the expression vector, or other properties (7, 30).

We previously showed that ELC is a high-affinity functional ligand for HHV-7 U12 (34). In this paper, we also showed that both the U12 and U51 genes could transduce specific chemokine signals into the cytoplasm, resulting in transient elevations of the intracellular Ca^{2+} concentration in response to ELC, SLC, TARC, and MDC (Fig. 2). Like other GPCRs, HHV-7 U12 and U51 were refractory to subsequent stimulation with the same concentration of ELC, SLC, TARC, or MDC (Fig. 4A). In addition, pretreatment with each of the active ligands ELC, SLC, TARC, and MDC did not desensitize these receptors to subsequent stimulation with the other active ligands (Fig. 4B and data not shown). However, pretreatment with MDC or ELC fully desensitized these receptors to subsequent stimulation with TARC or SLC (Fig. 4C). These results may indicate that U12 and U51 recognize the ligands for both CCR4 and CCR7, but they recognize the ligands for CCR4 via binding sites different from those for CCR7. Pretreatment with TARC did not desensitize CCR4 to subsequent stimulation with an equal amount of MDC (23). Pretreatment with SLC did not desensitize CCR7 expressed on cultured normal T cells to subsequent stimulation with an equal amount of ELC (54). These results may cause differences in binding affinity and efficiency of cross-desensitization between these chemokines depending on the cell type. The characterizations may be specific for HHV-7 U12 and U51.

ELC and SLC are constitutively expressed in various lymphoid tissues where lymphocytes expressing CCR7 are also abundant (51–53, 55). Thus, ELC and SLC may be involved in homeostatic lymphocyte recirculation and homing. TARC and MDC are expressed by cells of the dendritic lineage, which form a major component of the thymic architecture (21–23). TARC and MDC may function to attract or retain T cells in

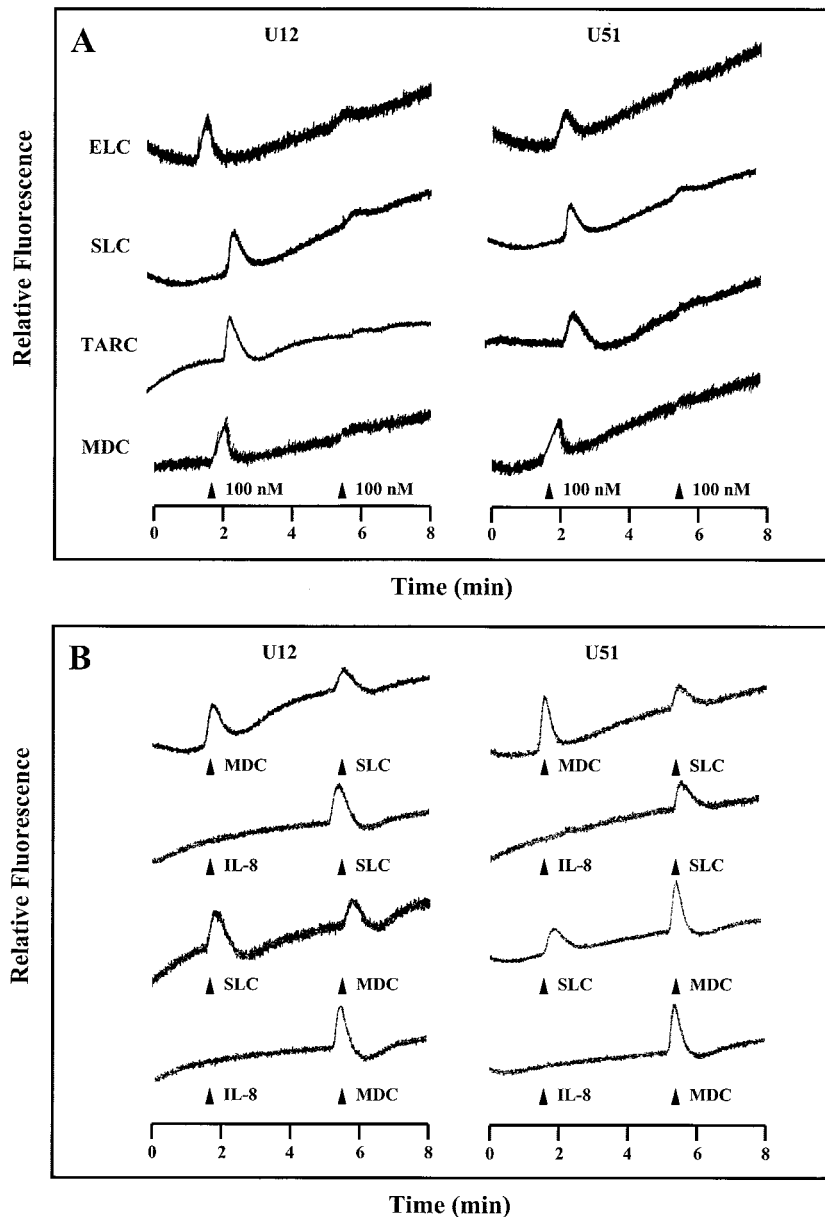


FIG. 4. Transmembrane signaling by HHV-7 U12 and U51: desensitization. (A, B, C). The relative fluorescence from Indo-1-loaded K562 cells stably transfected with U12 and U51 was monitored during the sequential addition of test substances at the times indicated by the arrowheads. The identity of each stimulus is given. The tracings are from a single experiment representative of two separate experiments.

the thymus and thereby mediate their trafficking and development.

The EB1/CCR7 gene was first reported as a lymphocyte-specific GPCR induced by Epstein-Barr virus infection. CCR7 is specific for ELC and SLC (33, 51) and acts to moderate cell migration and proliferation. The CCR4 gene was originally cloned from a human basophilic cell line, KU-812. CCR4 is a specific receptor for TARC and a selective receptor for MDC (21, 22). CCR4 is expressed in subsets of T cells at particular stages of differentiation and activation. The HHV-7 U12 and U51 products responded to ELC, SLC, TARC, and MDC in a calcium mobilization assay, indicating that U12 and U51 expressed in HHV-7-infected cells may act like CCR7 and

CCR4. Furthermore, HHV-6 and -7 upregulate CCR7 expression in CD4⁺ T cells, just as Epstein-Barr virus does in B cells upon cell entry (20).

Chemokine receptors form a subgroup of GPCRs, which are seven-transmembrane-domain proteins that couple extracellular stimuli to cellular responses through heterotrimeric G proteins. In humans, 6 CXC, 10 CC, 1 C, and 1 CX₃C chemokine receptor have been identified, and their ligand specificities have been defined (32). Furthermore, herpesviruses such as HCMV (2, 9, 29), herpesvirus saimiri (37), HHV-6 (18, 24), HHV-7 (30, 36), and HHV-8/Kaposi's sarcoma-associated herpesvirus (19) all encode GPCRs that are homologous to chemokine receptors. Although several of these viral chemokine

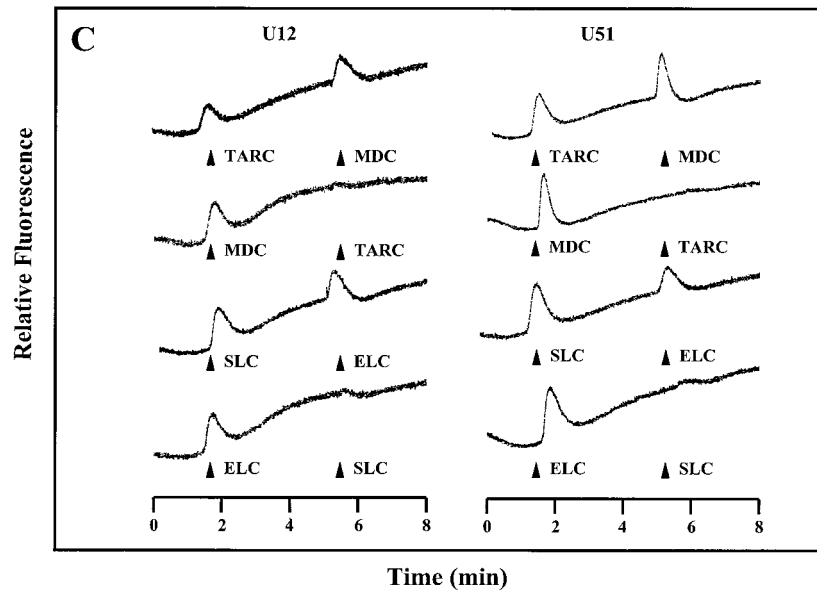


FIG. 4—Continued.

receptors exhibit high-affinity binding and their signaling properties are different from those of their cellular homologs, the function of these receptors in the viral life cycle is not clear.

HHV-7 U12 and U51 are structural and positional homologs of HCMV UL33 and UL78, respectively. There has been some progress in understanding the biological significance of some UL33-like genes, M33 (a GPCR encoded by murine CMV) and R33 (a GPCR encoded by rat CMV [RCMV]). A study with recombinant CMVs that carry either a disrupted M33 or a disrupted R33 gene in their genomes suggests that M33 and R33 are involved in the dissemination of the virus to, or its replication in, the salivary glands (4, 13). Like UL33 family

members, UL78 gene family members have important roles in the pathogenesis of infection (8, 38, 47). A significantly lower mortality rate was observed among rats infected with R78-deleted RCMV strains than among animals infected with wild-type RCMV (3, 27). HHV-6 U51, which belongs to the UL78 gene family, binds RANTES and downregulates the transcription of this chemokine (31). Here we showed that the HHV-7 U51 gene mediates the release of calcium from intracellular stores. However, to date, no signaling functions have been identified for other members of the UL78 family.

Our data show that ELC and SLC, but not TARC or MDC, induced chemotaxis in Jurkat cells transfected with HHV-7

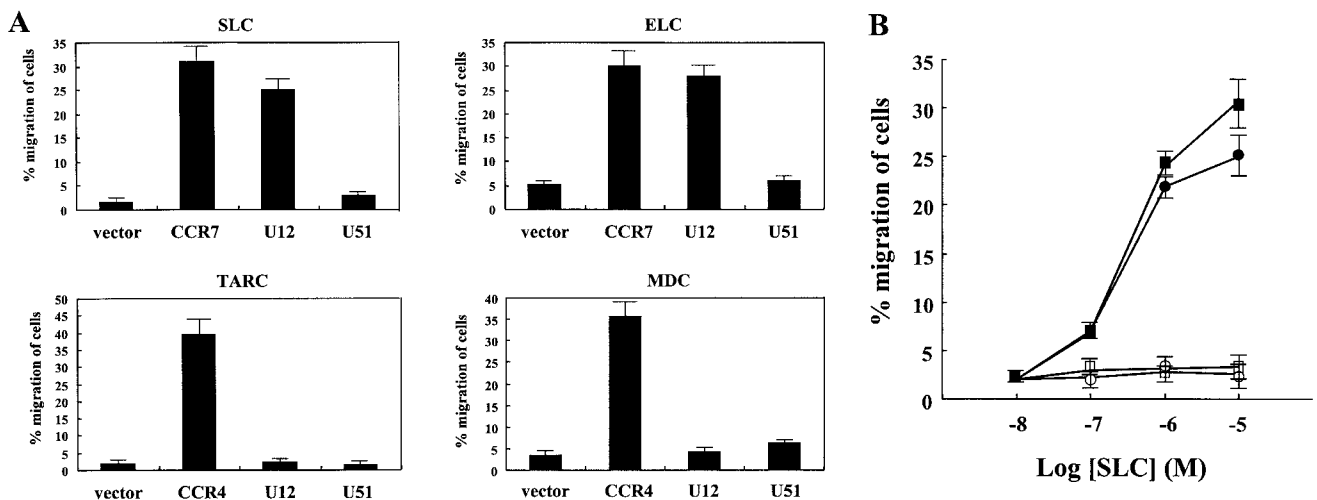


FIG. 5. Chemotactic responses of U12- or U51-expressing cells to chemokines. (A) Chemotactic responses of Jurkat cells stably transfected with vector alone, CCR7, U12, or U51 to ELC or SLC and of Jurkat cells stably transfected with vector alone, CCR4, U12, or U51 to TARC or MDC were assayed. (B) Dose-response of chemotaxis. Jurkat cells stably transfected with vector alone (open circles), CCR7 (closed squares), U12 (closed circles), or U51 (open squares) were applied to a compartment. The medium containing 10 μ M, 1 μ M, 100 nM, or 10 nM SLC was injected into the opposite compartment. Chemotactic responses were assayed as above. The assay was done in triplicate, and the number of migrating cells was counted for each well. Results representative of three separate experiments are shown.

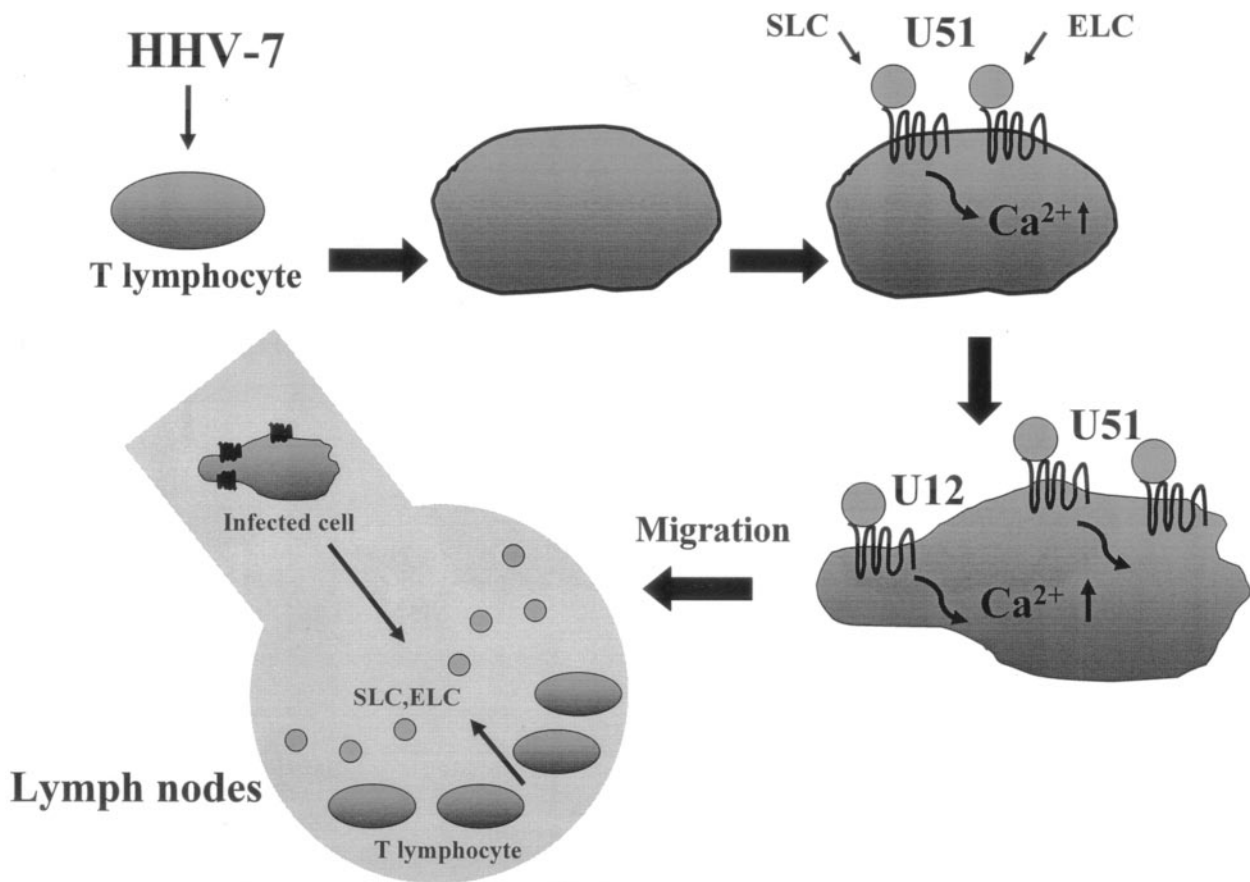


FIG. 6. Model for the role of HHV-7 U12 and U51 in infected cells. Our results suggest that the U12 gene product induces the migration of infected cells toward the lymph nodes for viral transmission and that the U12 and U51 gene products modulate a wide variety of intracellular signal transduction pathways in the infected cells.

U12 and that none of these chemokines induced chemotaxis in Jurkat cells transfected with HHV-7 U51 (Fig. 5A and B). To confirm this, we investigated whether ELC, SLC, TARC, or MDC could induce Ca^{2+} flux in Jurkat cells transfected with U12 or U51. Jurkat cells transfected with CCR7 or CCR4, which were used as positive controls, responded to ELC and SLC or TARC and MDC, respectively, and Jurkat cells transfected with U12 or U51 responded to ELC, SLC, TARC, and MDC (data not shown). Untransfected and pCEP4-transfected Jurkat cells, which were used as negative controls, did not respond to any chemokine tested (data not shown). CCR4 is expressed marginally on Jurkat cells (54), but Jurkat cells did not respond to TARC and MDC, and these chemokines did not induce chemotaxis in Jurkat cells transfected with CCR4 (Fig. 5A). Therefore, we have confirmed that ELC and SLC induced Ca^{2+} flux and chemotaxis in Jurkat cells transfected with U12. The reason only ELC and SLC induced chemotaxis in Jurkat cells transfected with HHV-7 U12 is unknown at present. Because HHV-6 U12 and U51 appear to have different regulatory mechanisms in that HHV-6 U12 is expressed at late times and U51 is expressed at early times postinfection (33), HHV-7 U12 and U51 also may have different mechanisms. At early times, through the action of U51, HHV-7 may regulate cellular processes to enhance viral replication or to inhibit an apoptotic response. At late times, through the action

of U12, lymphocytes infected with HHV-7 may migrate to lymph nodes. In general, entrance of T cells into lymph nodes from the blood is dependent on the expression of CCR7 (46). Considering the strong constitutive expression of ELC and also of SLC in various lymphoid tissues, where lymphocytes expressing CCR7 also exist abundantly, lymphocytes infected with HHV-7 may be attracted into various lymphoid tissues for viral transmission by these chemokines. Since HHV-7 U12 and U51 acted differently as chemokine receptors, our observations can be used to construct a model in which HHV-7 U12 and U51 regulate HHV-7-infected cells and mediate the migration of infected cells to the lymph nodes (Fig. 6).

Taken together, our results suggest that virally encoded putative chemokine receptors may play important roles in the infection and life cycle of herpesviruses, especially in vivo. The roles of chemokines such as ELC, SLC, TARC, and MDC and of U12 and U51 in HHV-7-infected T cells are not known at present, but all of these molecules may have biological activities in infected cells, such as promoting growth, protecting HHV-7-infected cells from apoptosis, and directing migration to specific anatomical locations to proliferate HHV-7-infected cells in vivo (Fig. 6). It is also possible that through the action of U12 and U51, HHV-7 can regulate cellular processes to enhance viral replication or to inhibit an apoptotic response, thereby allowing the establishment of a latent infection. Frail-

Ramos et al. showed that HHV-7 U12 and U51 may interact with salivary fractalkine to enhance viral entry or spread (15). Alternatively, U12 and U51 may act as molecular mimics to divert chemokines from their natural ligands and subvert a local immune response. The identification of ELC, SLC, TARC, and MDC as specific ligands for U12 and U51 now enables us to examine the possible roles of U12 and U51 in the infection process and life cycle of HHV-7. Such studies may lead to new strategies to prevent herpesvirus infection. We examined whether HHV-7-infected T cells responded to CCR4 or CCR7 ligands. HHV-7-infected SupT1 cells responded to TARC, MDC, ELC, and SLC, but mock-infected SupT1 cells did not respond to these ligands (unpublished data). The U12 and U51 expressed in HHV-7-infected SupT1 cells may act as chemokine receptors, or the cellular chemokine receptors induced by HHV-7 infection may respond to these ligands. Further studies exploring the functions of U12 and U51 in vivo may define new targets for drug development aimed at controlling various immune responses and suppressing persistent infections by lymphotropic herpesviruses.

ACKNOWLEDGMENTS

This paper was supported in part by a grant-in-aid for specially promoted research from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan.

We thank Yuttana Srinoulprasert and Masayuki Miyasaka (Osaka University Graduate School of Medicine) for advice on the KK-chamber and Takashi Nakayama, Osamu Yoshie (Kinki University School of Medicine), and Yasuko Mori (Osaka University Graduate School of Medicine) for valuable comments on this study. We also thank all of the members of our laboratory for helpful discussions.

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