Kaposi's Sarcoma-Associated Herpesvirus-G Protein-Coupled Receptor-Expressing Endothelial Cells Exhibit Reduced Migration and Stimulated Chemotaxis by Chemokine Inverse Agonists

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Received November 3, 2008; accepted February 26, 2009

ABSTRACT

A constitutively active G protein-coupled receptor (GPCR) encoded by Kaposi's sarcoma-associated herpesvirus (human herpesvirus-8) (KSHV) is expressed in endothelial (spindle) cells of Kaposi's sarcoma lesions. In this study, we report novel effects of basal signaling by this receptor and of inverse agonist chemokines on migration of KSHV-GPCR-expressing mouse lung endothelial cells. We show that basal signaling by KSHV-GPCR inhibits migration of endothelial cells in two systems, movement through porous filters and in vitro wound closure.

Naturally occurring chemokines, interferon γ -inducible protein-10 and stromal-derived factor-1, which act as inverse agonists at KSHV-GPCR, abrogate the inhibition of migration and stimulate directed migration (or chemotaxis) of these cells. Thus, the expression of KSHV-GPCR may allow infected endothelial cells in situ to remain in a localized environment or to directionally migrate along a gradient of specific chemokines that are inverse agonists at KSHV-GPCR.

Viruses have developed a variety of strategies to evade the host's immune system, including the piracy of cellular genes that are central to the host defense mechanism (Davis-Poynter and Farrell, 1996). Among these are genes encoding G protein-coupled receptors (GPCRs) that have been found in herpesviruses. The γ -herpesviruses encode homologs of CXC chemokine receptors, and β -herpesviruses generally encode homologs of both CC and CXC chemokine GPCRs. Because these viral GPCR homologs are present in many β - and γ -herpesviruses, it is thought they play an important role in the biology of the virus. Although their precise role(s) in the viral life cycle has not been delineated, a number of these receptors have been shown to be functional in in vitro and in vivo systems, and their possible roles in disease pathogenesis

have been suggested (Couty and Gershengorn, 2005; Vischer et al., 2006).

Kaposi's sarcoma-associated herpesvirus (KSHV), also called human herpesvirus-8, is a γ 2-herpesvirus that is the etiological agent of Kaposi's sarcoma (KS) and is also implicated in the pathogenesis of primary effusion lymphomas and some cases of multicentric Castleman's disease (Ganem, 1998; Neipel and Fleckenstein, 1999). KSHV encodes a chemokine-like GPCR (KSHV-GPCR) that is homologous to mammalian CXCR2 but binds a variety of CXC and CC chemokines (Cesarman et al., 1996). KSHV-GPCR signals in a chemokine-independent, constitutive manner and activates several signal transduction pathways (Arvanitakis et al., 1997; Geras-Raaka et al., 1998b; Munshi et al., 1999; Rosenkilde et al., 1999; Sodhi et al., 2000; Couty et al., 2001). KSHV-GPCR expression in NIH3T3 mouse fibroblasts induces cellular proliferation and oncogenic transformation, allows the development of KS-like tumors in nude mice, and acts as a potent angiogenic activator via stimulation of the production of vascular endothelial growth factor (Bais et al., 1998). Moreover, a transgenic mouse model in which KSHV-

This work was supported by the Intramural Research program of the National Institutes of Health National Institute of Diabetes and Digestive and Kidney Diseases [Grant 1Z01-DK047045-01 CEB].

Article, publication date, and citation information can be found at <http://jpet.aspetjournals.org>.
doi:10.1124/jpet.108.147686.

ABBREVIATIONS: GPCR, G protein-coupled receptor; KSHV, Kaposi's sarcoma-associated herpesvirus; KS, Kaposi's sarcoma; IP-10, interferon γ -inducible protein-10; SDF-1, stromal-derived factor-1; MLEC, microvascular lung endothelial cell; DMEM, Dulbecco's modified Eagle's medium; bpV, bisperoxo(pyridine-2-carboxyl)oxovanadate; Gro α , growth-related oncogene α ; MIG, monokine induced by interferon- γ ; ITAC, interferon-inducible T cell α chemoattractant; MAPK, mitogen-activated protein kinase; PTEN, phosphatase and tensin homolog.

GPCR expression was driven by the CD2 enhancer/promoter resulted in the development of angioproliferative, KS-like lesions (Yang et al., 2000).

Vascular cells are targets of KSHV infection (Boshoff et al., 1995) and KSHV-GPCRs are expressed in these cells (Cesarman et al., 1996; Guo et al., 1997), perhaps as early as within 24 h of infection (Paulose-Murphy et al., 2001). We (Couty et al., 2001) and others (Sodhi et al., 2000; Montaner et al., 2001) have studied the effects of KSHV-GPCR signaling in endothelial cells. Because the engagement of mammalian chemokine receptors usually stimulates cell chemotaxis (Baggiolini, 1998), and a GPCR (US28) encoded by human cytomegalovirus was shown to stimulate vascular smooth muscle cell migration (Streblov et al., 1999), we studied the effects of KSHV-GPCR signaling on endothelial cell migration. We report that the constitutively signaling chemokine-like KSHV-GPCR persistently inhibits mouse lung endothelial cell migration. Moreover, we demonstrate that naturally occurring chemokines acting as inverse agonists at KSHV-GPCR (Geras-Raaka et al., 1998a,b; Rosenkilde et al., 1999), such as interferon γ -inducible protein-10 (IP-10) and stromal-derived factor-1 (SDF-1), stimulate chemotaxis of KSHV-GPCR-expressing endothelial cells. This is the first evidence that a viral GPCR inhibits cell migration and that this inhibition can be reversed by endogenous chemokines.

Materials and Methods

Cell Lines. Microvascular endothelial cells were derived from lungs of newborn mice carrying an immortalizing transgene encoding SV40 large T antigen under the control of regulatory elements of the human vimentin gene (MLECs), and transfectants stably expressing KSHV-GPCRs or "mock-transfected" cells were generated by standard methods (Couty et al., 2001). A pool and a clone of KSHV-GPCR-expressing MLECs were studied and found to exhibit similar effects; however, the clonal cell line, which expressed a higher number of receptors (112,000 versus 82,000 per cell), exhibited greater responses than the pool and were used in these experiments. The cells were maintained in DMEM with 5% fetal calf serum (Invitrogen, Carlsbad, CA). All tissue culture dishes were coated with 0.2% gelatin.

Cell Migration Assay. Chemotaxis was measured using Transwell plates (6.5-mm diameter, 4- or 8- μ m pore size; Corning Life Sciences, Lowell, MA) in two protocols. 1) Cells (80,000/well) were added to the upper chamber of a Transwell in DMEM supplemented with 5% fetal calf serum. The next day, the filters (and upper chambers) were transferred to fresh 24-well plates coated with 0.1% gelatin. Medium was changed and replaced with DMEM containing 1% fetal calf serum. For chemotaxis, chemokines were added to the lower chamber in a final volume of 0.2 ml, cells that had migrated through the filter and become adherent to the bottom of the well during a 6-h incubation were fixed and stained using Wright's stain and counted using a microscope at 10 $\times$ magnification. 2) Cells (5000/well) were seeded directly onto the upper chamber of a Transwell in DMEM supplemented with 1% fetal calf serum, and chemokines were added to the lower chamber in a final volume of 0.2 ml. Cells that had migrated through the filter and become adherent to the undersurface of the filter during a 6-h incubation were fixed and stained using crystal violet stain and counted using a microscope at 10 $\times$ magnification. In each experiment, the number of cells that had migrated into the lower chamber or the undersurface of the filter in control incubations of KSHV-GPCR-expressing cells was set at 100%; this number ranged from 300 to 1000 cells in different experiments in protocol 1 and 100 to 300 cells in protocol 2. Each experiment was performed at least twice with three to six replicates.

Herbimycin (1 μ g/ml), C3-exoenzyme (10 μ M), or bisphosphoryl(pyridine-2-carboxyl)oxovanadate (bpV) (50 nM) (Calbiochem, La Jolla, CA) was added both in the upper and lower chambers before chemokine.

Measurement of Phosphorylation of Signaling Proteins. Changes in phosphorylation of selected proteins in signaling pathways were assayed with Proteome Profiler Array kit (ARY003; R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. We were able to use this kit to monitor phosphorylation status of several proteins, even though the antibodies were generated against human proteins as antigens as several of the antibodies cross-reacted with mouse proteins. In brief, KSHV-GPCR expressing MLECs (clone 16B) were grown to near confluence in DMEM supplemented with 10% fetal bovine serum. IP-10 (100 nM) was added, and samples were processed at $t = 0, 15, 30$, and 60 min. At the designated times, each dish was washed twice with phosphate-buffered saline and processed according to the kit protocol. Incubations with the array contained 300 μ g of lysate protein. Enhanced chemiluminescence was developed with SuperSignal West Pico Chemiluminescence substrate (Pierce Chemical, Rockford, IL). Net integrated pixel density for each spot (an average of duplicate spots after subtraction of average background density) was determined by densitometry and analyzed using Tina (ISBE, Sheffield, UK) software. Results were normalized to net integrated pixel density of kit-supplied internal positive controls.

Wound Closure Assay. Cells were grown in 24-well tissue culture plates to 80 to 90% confluence. A 0.2- to 0.5-mm straight gap in the cell layer was made with a blunt sterile glass pipette, avoiding scratching of the dish surface. The gap was then photographed with a black and white analog camera at video resolution connected to a port of an inverted microscope using a 4 $\times$ objective. The width of the gap was measured for each well in five equidistant locations at time 0 and then after 6 h. Wound closure was expressed as percentage of the original width occupied by migrating cells. Each experiment was performed in triplicate wells.

Statistics. Statistical analysis was performed by Student's t test; $p < 0.05$ was considered significant.

Results

KSHV-GPCR-Expressing MLECs Migrate More Slowly Than MLECs. KSHV-GPCRs were previously shown to signal via several transduction pathways in endothelial cells (Couty et al., 2001; Montaner et al., 2001) and to enhance their survival (Montaner et al., 2001). To assess the effect of KSHV-GPCR signaling on endothelial cell migration, we compared a mock-transfected cell line ("Mock") with mouse lung endothelial cells stably expressing KSHV-GPCRs. Without the addition of exogenous chemokines, KSHV-GPCR-expressing cells were found to form clusters during several days of culture, whereas mock cells were dispersed evenly on the bottom of the wells of the culture chamber (not shown). Although the basis for these differences was not apparent, one possibility was that the mock cells migrated more readily than KSHV-GPCR-expressing cells. We compared the migration of mock cells with KSHV-GPCR-expressing cells and found that 35% more mock cells had migrated than KSHV-GPCR-expressing cells under basal conditions ($p = 0.018$) (Fig. 1A, Control).

KSHV-GPCR Basal Signaling Inhibits MLEC Migration. Given that KSHV-GPCR signals basally in these cells (Couty et al., 2001) and that these cells exhibit decreased migration, we determined whether KSHV-GPCR signaling mediates inhibition of migration. We began by determining whether growth-related oncogene α (Gro α), an agonist that further activates KSHV-GPCR signaling (Gershengorn et al., 1998; Rosenkilde et al., 1999), and IP-10, an inverse agonist

Fig. 1. Effects of Gro α and IP-10 on migration (chemotaxis) of KSHV-GPCR-expressing MLECs. A, Gro α (100 nM) or IP-10 (100 nM) was added to the lower chambers and the number of cells that had migrated during a 6-h incubation was counted as described under *Materials and Methods*, protocol 1; the control was no added chemokine. Bars, mean $\pm$ S.E. of three to six replicates in four experiments. B, IP-10 (100 nM) was not added to either chamber (Control), to the lower chambers only (0/IP-10), to both chambers (IP-10/IP-10), or to the upper chamber only (IP-10/0), and the number of cells that had migrated during a 6-h incubation was counted as described under *Materials and Methods*, protocols 1 and 2. The number of KSHV-GPCR-expressing cells in the lower chamber or attached to the undersurface of the filter in the experimental control was set at 100%. The bars represent the mean $\pm$ S.E. of three replicates in three experiments.

that inhibits basal signaling by KSHV-GPCR (Geras-Raaka et al., 1998b), affected endothelial cell migration. We found that neither Gro α nor IP-10 had any effect on the migration of the mock-transfected cells (Fig. 1A). Gro α decreased the number of KSHV-GPCR-expressing cells that migrated by 41% ($p = 0.022$), and IP-10 increased the number of KSHV-GPCR-expressing cells to 237% of control ($p < 0.001$) (Fig. 1A). These data are consistent with the idea that basal signaling of KSHV-GPCR leads to inhibition of cell migration that may be further enhanced by the agonist Gro α and relieved by the inverse agonist IP-10.

Inhibition of KSHV-GPCR Signaling Stimulates Chemotaxis. To show whether the effects of the chemokine inverse agonist to increase migration of KSHV-GPCR-expressing cells exhibited directionality, that is, represented chemotaxis, or was a general increase in nondirectional chemokinesis, the effects of adding IP-10 to the upper and/or lower chambers of the Transwell apparatus were measured (Fig. 1B). When IP-10 was added to the upper chamber alone, migration into the lower chamber was not different from control. When IP-10 was added to both the upper and lower chambers, there was a smaller 33% increase in the number of cells that had migrated ($p = 0.015$ versus control) compared with adding IP-10 to the lower chamber alone. Thus, only

when IP-10 was added to the lower chamber was the number of cells migrating (into the lower chamber) significantly increased. Thus, because stimulated migration occurred only toward higher concentrations of IP-10, the increased migration caused by IP-10 represented chemotaxis rather than chemokinesis.

Inhibition of Chemotaxis Is Specifically Caused by KSHV-GPCR Signaling. To exclude the possibility that IP-10-induced migration in KSHV-GPCR-expressing cells was due to IP-10 acting as an agonist at an endogenous receptor rather than acting as an inverse agonist at KSHV-GPCR, we measured the effects of other chemokines in these cells (Fig. 2). One possibility was that CXCR3, for which IP-10 is an agonist (Luster, 1998), was expressed in KSHV-GPCR-expressing cells. Neither of the CXCR3 agonists, monokine induced by interferon- γ (MIG) or interferon-inducible T cell α chemoattractant (ITAC), had any effect on migration of these cells. Moreover, another naturally occurring chemokine that is an inverse agonist at KSHV-GPCR, SDF-1 (21), like IP-10, induced cell migration of KSHV-GPCR-expressing cells ($p < 0.001$). It was unexpected that SDF-1, a known chemoattractant for endothelial cells, did not stimulate migration of mock cells. These data provide pharmacological evidence that KSHV-GPCRs are mediating the effects on migration, and when KSHV-GPCR basal signaling is inhibited by inverse agonists, more endothelial cells migrated than in the absence of chemokine.

Further evidence in support of the idea that inhibition of KSHV-GPCR constitutive signaling by IP-10 was the stimulus for endothelial cell chemotaxis was obtained by showing that Gro α , which is an agonist at this receptor that inhibits chemotaxis in KSHV-GPCR-expressing cells but had no effect in the mock-transfected cells (Fig. 1), functioned as an inhibitor of IP-10-induced chemotaxis (Fig. 3). To our knowledge, the only receptor for which IP-10 is an inverse agonist and Gro α is an agonist, is KSHV-GPCR. Hence, these findings provide additional pharmacological evidence that KSHV-GPCR signaling inhibits migration of endothelial cells.

KSHV-GPCR-Regulated Chemotaxis Involves Activation of Protein Kinases, Rho and PTEN. Signaling through protein tyrosine kinases, small G proteins, and a dual-specificity protein/3-lipid phosphatase PTEN have been

Fig. 2. IP-10 and SDF-1 but not ITAC and MIG stimulate migration of KSHV-GPCR-expressing MLECs. IP-10, SDF-1, ITAC, or MIG (all at 100 nM) were added to the lower chambers, and the number of cells that had migrated during a 6-h incubation was counted as described under *Materials and Methods*, protocols 1 and 2; control was no added chemokine. The number of KSHV-GPCR-expressing cells in the lower chamber or attached to the undersurface of the filter in the experimental control was set at 100%. Bars, mean $\pm$ S.E. of three or four replicates in three to six experiments.

Fig. 3. Gro α inhibits IP-10-stimulated migration in KSHV-GPCR-expressing MLECs. No IP-10 (Control) or IP-10 (100 or 10 nM) without (No addition) or with 100 nM Gro α was added to the lower chambers, and the number of cells that had migrated during a 6-h incubation was counted as described under *Materials and Methods*, protocol 1. The number of KSHV-GPCR-expressing cells in the lower chamber in the experimental control was set at 100%. Bars, mean $\pm$ S.E. of two or three replicates in two experiments.

shown to be central to cell migration (Firtel and Chung, 2000). We hypothesized that KSHV-GPCR signaling also regulates chemotaxis through these pathways. Therefore, we studied the effect of a tyrosine kinase inhibitor, herbimycin, of C3-exoenzyme, an enzyme that specifically and irreversibly inactivates Rho protein, and of bpV, a specific PTEN inhibitor, on IP-10-induced chemotaxis in KSHV-GPCR-expressing cells. Herbimycin, C3-exoenzyme, and bpV pretreatment inhibited chemotaxis induced by IP-10 without having an effect on basal migration (Fig. 4). These data demonstrate that KSHV-GPCR-regulated chemotaxis involves protein tyrosine kinases, the Rho pathway and PTEN, similar to chemotaxis stimulated by mammalian chemokine receptors.

To determine whether IP-10 increased signaling through protein phosphorylation, we screened for phosphorylation of

Fig. 4. Herbimycin, C3-exoenzyme, and bpV pretreatment inhibits chemotaxis of KSHV-GPCR-expressing MLECs induced by IP-10. No IP-10 (Control) or 100 nM IP-10 was added to the lower chambers without (No addition) or with 1 μ g/ml herbimycin, 10 μ M C3-exoenzyme, or 50 nM bpV added to the upper and lower chambers. The number of cells that had migrated during a 6-h incubation was counted as described under *Materials and Methods*, protocol 1 or 2. The number of KSHV-GPCR-expressing cells in the lower chamber in the experimental control was set at 100%. Bars, mean $\pm$ S.E. of two or three replicates in three experiments.

proteins in several signaling pathways that mediate cell migration in endothelial cells using a phosphoprotein array. We found that several proteins within the p38 mitogen-activated protein kinase (MAPK) pathway, p38 MAPK α (Matsunaga et al., 2008) and downstream effectors cAMP response element-binding protein (Martorell et al., 2008) and mitogen- and stress-activated protein kinase 1/2 (Arthur, 2008), which have been associated with endothelial cell migration exhibited increased phosphorylation in MLECs expressing KSHV-GPCRs when treated with IP-10 (Fig. 5). These findings are consistent with IP-10 activating the p38 MAPK pathway that may mediate chemotaxis in these cells.

We previously demonstrated that KSHV-GPCR couples to pertussis toxin-sensitive and insensitive pathways in endothelial cells (Couty et al., 2001). To determine whether a pertussis toxin-sensitive G protein mediated IP-10-induced chemotaxis, we pretreated cells with toxin. We found that pertussis toxin pretreatment did not affect chemotaxis of KSHV-GPCR-expressing cells in response to IP-10 (data not shown). Thus, although chemotaxis stimulated by mammalian chemokine receptors is usually mediated by pertussis toxin-sensitive G proteins, migration inhibited by KSHV-GPCR is via coupling to a pertussis toxin-insensitive G protein(s). This may occur via activation of protein phosphorylation (Fig. 5).

IP-10 Inhibits Wound Healing by KSHV-GPCR-Expressing Cells. To confirm the effect of KSHV-GPCR signaling on migration in a different model system, we used an in vitro wound closure assay (Fig. 6). The extent of wound closure during a 6-h incubation was 45% greater for the mock-transfected compared with KSHV-GPCR-expressing cells ($p < 0.0001$). Although there was no effect of IP-10 in the mock-transfected cells, IP-10 increased wound closure by 34% in KSHV-GPCR-expressing cells ($p < 0.001$).

Discussion

Mammalian chemokine receptors do not usually exhibit basal signaling activity and do not affect cellular migration in the absence of chemokine agonists. When agonists bind, mammalian receptors initiate a signaling cascade(s) that leads to stimulated migration in the direction from lower to higher concentrations of chemokine, a response termed chemotaxis. It is noteworthy that the viral GPCR of cytomega-

Fig. 5. IP-10 increases phosphorylation of p38 MAPK, cAMP response element-binding protein, and mitogen- and stress-activated protein kinase 1/2 in KSHV-GPCR-expressing MLECs. MLECs expressing KSHV-GPCRs (clone 16B) were grown to near confluence in 75-cm² flasks. IP-10 (100 nM) was added to each flask, and the cells were lysed and processed at the desired time points as described under *Materials and Methods*. The results are presented as normalized net average integrated pixel density (see *Materials and Methods*).

Fig. 6. KSHV-GPCR-expressing MLECs migrate less rapidly than mock-transfected MLECs in a wound closure assay and are stimulated by IP-10. Wound closure assays were performed as described under *Materials and Methods*. **A**, photomicrographs of mock-transfected MLECs and KSHV-GPCR-expressing MLECs at time 0 (0 h) and after 6 h in the absence or presence of 100 nM IP-10. Bar, 100 μ m. **B**, quantitation of the degree of wound closure by mock-transfected (Mock) and KSHV-GPCR-expressing (KSHV-GPCR) cells in the absence (Control) or presence of 100 nM IP-10. Bars, mean $\pm$ S.E. of five measurements of three replicates in two experiments.

lovirus US28 exhibits these characteristics (Streblow et al., 1999). The molecular events involved in this response are beginning to be elucidated in several cell types. One of the best model systems for study of regulated cell migration is the amoeba, *Dictyostelium discoideum* (Parent and Devreotes, 1999; Firtel and Chung, 2000). From studies in yeast, it has been proposed that chemotaxis is dependent upon chemokine-induced polarization of signaling molecules within a complex at the cell surface membrane at the leading edge of migration. Evidence in support of this idea in mammalian cells has been obtained in chemotaxing neutrophils (Servant et al., 2000). Chemotaxis stops when the chemoattractant dissipates or when the concentration of chemoattractant is increased evenly around the cell, causing all receptors to be persistently activated. It is possible that receptor-desensitizing mechanisms, which dampen stimulated chemotaxis (Luster, 1998; Sherrill and Miller, 2008), may decrease the activity of these complexes. We propose that constitutively active KSHV-GPCRs may simulate a high concentration of chemoattractant distributed all around the cell, thereby leading to inhibition of migration. Inverse agonists may stimulate chemotaxis under these circumstances by inhibiting the constitutive signaling of KSHV-GPCRs.

Neutral antagonists inhibit agonist-induced responses, but only antagonists that act as inverse agonists can inhibit constitutively signaling receptors. Hence, inverse agonists can serve as tools to probe the role of constitutive signaling. We used two naturally occurring inverse agonists, IP-10 and SDF-1, which allowed us to suggest that the constitutive activity of KSHV-GPCR was the cause of inhibition of MLEC migration and that IP-10 may act to resensitize the signaling pathway and thereby allow it to mediate migration. However, we cannot formally exclude the possibility that IP-10 is acting as an agonist on a signaling pathway that mediates migration, such as the p38 MAPK pathway, even though it is an inverse agonist on other pathways. We think this is un-

likely. Nevertheless, expression of KSHV-GPCR in endothelial cells creates a situation in which cells chemotax in a gradient of a chemokine that has been thought of as an antagonist (inverse agonist) rather than an agonist.

We found that KSHV-GPCR-regulated chemotaxis involves protein kinases, the Rho pathway, and PTEN similar to chemotaxis stimulated by mammalian chemokine receptors. However, pertussis toxin treatment did not affect inhibition of migration caused by basal signaling by KSHV-GPCR, nor did it abolish chemotaxis of KSHV-GPCR-expressing cells stimulated by IP-10. These observations confirm our previous results (Couty et al., 2001) and those of others (Shepard et al., 2001) on the ability of this receptor to couple to multiple signaling pathways, notably to pertussis toxin-insensitive and -sensitive pathways.

In this study, we provide strong pharmacological evidence that KSHV-GPCR, at least in MLECs, behaves as an inhibitor of cell migration. Inhibition of migration of KSHV-infected cells may favor virus survival by, for example, limiting exposure of KSHV-infected cells to the host immune system. Montaner et al. (2001) reported that KSHV-GPCR expression caused an increase in human umbilical vein endothelial cell survival mediated by the Akt/PI3K pathway. We found that KSHV-GPCR expression in MLECs promoted cell survival through the Akt/PI3K pathway also (data not shown). These observations are consistent with the idea that KSHV-GPCR-expressing cells could represent a quiescent cell population with a prolonged lifetime in which the virus may survive longer. Moreover, several groups of investigators (Pati et al., 2001; Schwarz and Murphy, 2001; Shepard et al., 2001) have shown that KSHV-GPCR-expressing cells constitutively secrete proinflammatory cytokines that might attract noninfected endothelial cells. These noninfected cells could become infected and become new reservoirs if the virus were induced into a proliferative phase. In this way, several phases of the viral life cycle would be supported. It is noteworthy, there-

fore, that this idea is consistent with the pathological findings in KS lesions in which only a small fraction of cells harbor viruses. Moreover, it suggests a pathogenic mechanism in which poorly migratory cells serve as a nidus to recruit noninfected endothelial cells to form the KS lesion.

In conclusion, we demonstrated that expression of the chemokine-like KSHV-GPCR inhibits migration of MLECs. To our knowledge, this is the first evidence that a chemokine receptor negatively regulates endothelial cell migration. We used MLECs stably expressing KSHV-GPCRs because endothelial cells appear to be targets of KSHV infection. Therefore, a study of the effects of KSHV-GPCR signaling in this cell system may provide additional insights into the mechanisms by which the virus causes disease.

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