Human Interferon-γ-inducible Protein 10 (IP-10) Inhibits Constitutive Signaling of Kaposi’s Sarcoma-associated Herpesvirus G Protein-coupled Receptor

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Summary

A G protein–coupled receptor (GPCR) is encoded within the genome of Kaposi’s sarcoma-associated herpesvirus (KSHV)/human herpesvirus 8, a virus that may be involved in the pathogenesis of Kaposi’s sarcoma and primary effusion lymphomas. KSHV-GPCR exhibits constitutive signaling activity that causes oncogenic transformation. We report that human interferon (IFN)-γ-inducible protein 10 (HuIP-10), a C-X-C chemokine, specifically inhibits signaling of KSHV-GPCR. In contrast, monokine induced by IFN-γ (HuMig), which like HuIP-10 is an agonist of C-X-C chemokine receptor 3, does not inhibit KSHV-GPCR signaling. Moreover, HuIP-10, but not HuMig, inhibits KSHV-GPCR–induced proliferation of NIH 3T3 cells. These results show that HuIP-10 is an inverse agonist that converts KSHV-GPCR from an active to an inactive state. Thus, a human chemokine inhibits constitutive signaling and cellular proliferation that is mediated by a receptor encoded by a human disease-associated herpesvirus.

Key words: human monokine induced by interferon γ • C-X-C chemokine receptor 3 • inverse agonist • human herpesvirus 8 • tumorigenesis

Kaposi’s sarcoma-associated herpesvirus (KSHV)/human herpesvirus 8 is a gammaherpesvirus that may be involved in the pathogenesis of Kaposi’s sarcoma (KS; reference 1) and primary effusion (or body cavity–based) lymphomas (2). KSHV contains a gene encoding a G protein-coupled receptor (KSHV-GPCR; references 3 and 4), and mRNA transcripts for KSHV-GPCR have been found in tissues from patients with KS (5) and in cell lines derived from patients with primary effusion lymphomas (2, 3). We showed that KSHV-GPCR exhibits constitutive signaling, that is, signaling in the absence of agonist, via activation of phosphoinositide-specific phospholipase C (6), and that expression of KSHV-GPCR stimulates proliferation of rat fibroblasts (6) and causes transformation of mouse NIH 3T3 fibroblasts (7) in cell culture. Moreover, NIH 3T3 cells transformed by KSHV-GPCR form tumors in mice (7). Thus, KSHV-GPCR may be a mediator of KS-induced tumorigenesis.

KSHV-GPCR shows homology to human receptors, including C-X-C chemokine receptor (CXCR) 1, which binds IL-8, CXCR2, which binds IL-8 as well as several other C-X-C chemokines (8), and CXCR3, which binds human IFN-γ–inducible protein 10 (HuIP-10) and human monokine induced by IFN-γ (HuMig; references 9 and 10). However, CXCR1, CXCR2, and CXCR3 do not exhibit constitutive signaling, although like KSHV-GPCR they appear to signal via phospholipase C. For example, IL-8 activation of CXCR1 and CXCR2 stimulates formation of inositol phosphate second messenger molecules in a monkey kidney (COS) cell line (11), and HuIP-10 and HuMig activation of CXCR3 stimulates elevation of intracellular calcium, which is likely caused by phospholipase C–mediated generation of inositol 1,4,5-trisphosphate, in several cell types (9). In contrast, although several chemokines of the C-X-C and C-C families were shown to bind to KSHV-GPCR, none was found that would affect KSHV-GPCR signaling (6). In this report, we show that HuIP-10 (and two HuIP-10 analogues), but not HuMig, inhibits constitutive signaling of KSHV-GPCR. Thus, HuIP-10 is an inverse agonist of KSHV-GPCR signaling that converts the receptor from an active to an inactive state.

Materials and Methods

Inositol Phosphate Accumulation. KSHV-GPCRs were expressed in COS-1 and NIH 3T3 cells by transfection with pcKSHV-GPCR (6) or pCEFL-KSHV-GPCR (7) using the protocols described previously. The C-X-C chemokines HuIP-10 and HuMig, the mouse homologues MuIP-10 and MuMig, and the
HuIP-10 analogues were chemically synthesized using established procedures (12).

COS-1 cells transiently transfected with plasmid encoding KSHV-GPCR (5 μg/ml) or NIH 3T3 cells stably expressing KSHV-GPCR were labeled with myo-[3H]inositol, and the formation of [3H]inositol phosphates during a 90-min incubation was measured as described (6, 7).

Mutagenesis The full-length KSHV-GPCR cDNA in pcDNA3.1 (+) (pCKSHV-GPCR; reference 6) was used for mutagenesis. Mutants were prepared by PCR and were subcloned directly into pcKSHV-GPCR after digesting with EcoRI and EcoRV. Mutant KSHV-GPCR sequences were confirmed by the dideoxy chain termination method.

Results and Discussion

Fig. 1 illustrates that HuIP-10 specifically inhibits inositol phosphate second messenger formation in COS-1 cells expressing KSHV-GPCR. The dose of HuIP-10 that causes 50% inhibition of inositol phosphate formation is 15 nM. HuIP-10 had no effect on inositol phosphate production by COS-1 cells that did not express KSHV-GPCR (data not shown). These data are consistent with the idea that HuIP-10 is a specific inverse agonist (or negative antagonist; reference 13) of KSHV-GPCR.

As noted above, HuIP-10 is a ligand for CXCR3, which also binds HuMig (10). HuIP-10 and HuMig are CXCR3 antagonists (9, 14). To further characterize the effects of these chemokines on KSHV-GPCR signaling, the effects of HuMig and of the mouse homologues MuIP-10 and MuMig were determined. MuIP-10, like HuIP-10, inhibits signaling by KSHV-GPCR, whereas HuMig and MuMig have no effect on KSHV-GPCR signaling (Fig. 1). Thus, the IP-10 homologues are inverse agonists, whereas the Mig homologues exhibit no detectable activity at KSHV-GPCR.

HuIP-10 is a 77-amino acid polypeptide. To gain insight into the structural domain of HuIP-10 involved in inhibition of KSHV-GPCR signaling, two analogues truncated at the NH2 terminus were studied. HuIP-10(4–77) and HuIP-10(9–77) are lacking the first three or eight amino acid residues, respectively, of HuIP-10. Both HuIP-10(4–77) and HuIP-10(9–77) inhibited KSHV-GPCR signaling although with lower potencies than HuIP-10 (3- and 10-fold lower, respectively; Fig. 2 A). Thus, the NH2 terminus of HuIP-10 is not needed for inhibition of KSHV-GPCR signaling. This is an interesting finding because the agonist activity of several members of the C-X-C chemokine family, including HuIP-10, has been shown to be dependent on their NH2-terminal domains (8). For example, deletion of the NH2 terminus of IL-8 causes loss of agonist activity and forms peptides that act as competitive antagonists (15). The active domain (or pharmacophore) for inhibition of KSHV-GPCR signaling is not part of the NH2 terminus of HuIP-10.

It has been found that the putative extracellular NH2 termini of CXCR1 and CXCR2 are important for interacting with IL-8 (16). Therefore, we constructed a mutant KSHV-GPCR in which amino acid residues from positions 2–11 were deleted [KSHV-GPCR (Δ2–11)]. This mutant receptor exhibited constitutive signaling activity that was 70% (P > 0.005) of that of KSHV-GPCR (Fig. 2 B). Therefore, residues within the NH2 terminus may affect constitutive signaling by KSHV-GPCR. However, since signaling activity is directly related to receptor number (6), it is possible that the modest decrease in signaling is due to decreased expression. More importantly, the constitutive signaling activity exhibited by KSHV-GPCR (Δ2–11) was not inhibited by HuIP-10. As we have not been able to measure
KSHV-GPCR interacts with a much broader array of C-C chemokine receptors (22), but stimulates chemotaxis of eosinophils via chemokine receptor CCR3 (23). vMIP-I and vMIP-II block infection by HIV-1 by binding to chemokine receptors CCR3 and CCR5, and stimulate angiogenesis (23). It is not known whether any of these chemokines affects KSHV-GPCR signaling.

Evidence continues to accumulate supporting the idea that KSHV is involved in the pathogenesis of KS (1) and primary effusion lymphomas (2). More recently, it has been proposed that KSHV could also be involved in the development of multiple myeloma (24). From the perspective of demonstrating a pathogenic role for KSHV-GPCR in these diseases, it will be important to develop an animal model and to show that inhibition of KSHV-GPCR signaling inhibits tumorigenesis in KSHV-infected animals.

The discovery of an inhibitor of the constitutive signaling of KSHV-GPCR will allow direct testing in an animal model and to show that inhibition of KSHV-GPCR signaling results in stimulation of prolifer-ation of NIH 3T3 fibroblasts (7). Stimulation of the prolifer-ation of target cells by KSHV-GPCR could be a component of the pathogenesis of KS or primary effusion lymphomas (1, 2). To determine whether inhibition of KSHV-GPCR signaling would inhibit the proliferative response, the effects of the IP-10 homologues were measured in transfected NIH 3T3 cells stably expressing KSHV-GPCR.s. Fig. 3 (top) illustrates that HuIP-10 and MuIP-10 inhibit KSHV-GPCR signaling in NIH 3T3 cells, whereas Hu-Mig and MuMig have no effect.

Figure 3. Inhibition of constitutive KSHV-GPCR signaling and DNA synthesis in NIH 3T3 mouse fibroblasts. (A) Inositol phosphate second messenger formation, and 29 nM for inhibition of DNA synthesis. These results indicate that HuIP-10 required for 50% inhibition (IC50) of inositol phosphate second messenger formation is 39 nM (9–160 nM; 95% confidence interval), and for 50% inhibition of DNA synthesis is 29 nM (8–110 nM; 95% confidence interval). The data represent the mean ± SE of triplicate determinations in three experiments.

The concentrations required for 50% inhibition (IC50) of inositol phosphate second messenger formation is 39 nM (9–160 nM; 95% confidence interval), and for 50% inhibition of DNA synthesis is 29 nM (8–110 nM; 95% confidence interval). The data represent the mean ± SE of triplicate determinations in three experiments.

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