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Modulation of Host Gene Expression by the Constitutively Active G Protein-coupled Receptor of Kaposi's Sarcoma-associated Herpesvirus[1]

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ABSTRACT

Kaposi's sarcoma-associated herpes virus (KSHV) infects B cells and microvascular endothelium, and is linked to both lymphoid and endothelial neoplasms. KSHV encodes a G protein-coupled receptor (v-GPCR) that can bind several CC and CXC chemokines but is able to signal in the absence of known ligands. This signaling can transform cultured fibroblasts, promote angiogenesis *in vitro* and *in vivo*, and activate the mitogen-activated protein kinase, c-Jun-NH_2-terminal kinase, and p38 pathways. To assess the potential impact of v-GPCR signaling on host cell biology we have examined cellular gene expression in v-GPCR-transfected cells using DNA microarrays. v-GPCR expression up-regulated numerous cellular transcripts in both BJAB B cells and SLK endothelial cells, but with a remarkable degree of cell-type specificity. Among the most highly regulated genes in endothelial cells were the cytokines interleukin 6 and GROα; several genes affecting endothelial/vascular growth and remodeling were also induced, including plasminogen, thrombomodulin, the urokinase-type plasminogen activator receptor, and to a modest extent vascular endothelial growth factor C. By contrast, the most highly regulated genes in B cells were the CC chemokines macrophage inflammatory protein 1α and macrophage inflammatory protein 1β. No genes other than members of the dual-specificity phosphatase family were induced in both cell lines. The results indicate that the effects of KSHV GPCR expression in these two target cell types differ considerably and suggest that signaling by this molecule may make different contributions to the pathogenesis of KSHV-related endothelial and lymphoproliferative lesions.

INTRODUCTION

KSHV[3] (also called human herpesvirus 8) is a novel virus initially discovered because of its involvement in the endothelial neoplasm, KS (1). KS is a complex lesion characterized by: (*a*) proliferation of spindle-shaped cells of endothelial origin; (*b*) aberrant neoangiogenesis; and (*c*) persistent infiltration by inflammatory cells (mainly monocytes, T cells, and B cells). No one component of this lesion is fully malignant by standard laboratory criteria; KS spindle cells, for example, do not grow in soft agar or form tumors in nude mice, and the infiltrating T and B cells are cytologically normal (2). Evidence suggests that the KS neoplasm requires cross-talk between the inflammatory and endothelial elements of the tumor, with the inflammatory cells producing growth-promoting factors, and the endothelial cells generating angiogenic and proinflammatory mediators (3, 4). Despite this clear linkage to endothelial infection, sequence analysis of the KSHV genome reveals it to be a member of the lymphotropic (γ) subfamily of herpesviruses. In keeping with this, B cells are the primary reservoir of KSHV infection (5), and KSHV infection is linked to several lymphoproliferative diseases, including multicentric Castleman's disease and primary effusion lymphoma (6, 7).

As with other herpesviruses, KSHV can either persist in the host cell in a latent state, during which only a few viral genes are expressed, or it can initiate a lytic cycle of infection wherein many viral genes are transcribed, leading to production of new virions and ultimately death of the infected cell. In marked contrast to the well-established linkage between Burkitt's lymphoma and latent infection by Epstein-Barr virus, studies of KS have indicated important roles for both latent and lytic infection in KS pathogenesis. Latent infection is found within endothelial cells of KS tumors; in addition, a small subpopulation of the KS cells displays lytic KSHV replication in which most of the viral genes are expressed (8). The latency program contains several genes suggested to promote cell survival or proliferation, including the latency-associated nuclear antigen, FLICE-inhibitory protein, and kaposin A (9–13); it is proposed that they act in a cell-autonomous fashion to provide a growth advantage *in vivo*. However, KS tumorigenesis also requires ongoing lytic infection, because interruption of lytic replication aborts KS development at all stages of the natural history of KSHV infection (14). Because only a small percentage of KS cells are lytically infected, this suggests that products of lytic infection may act in a paracrine fashion to promote KS tumorigenesis. Consistent with this, the viral genome encodes in its lytic cycle several signaling proteins that can be exported from the cell, including homologues of IL-6 and CC chemokines (15–18).

In addition, KSHV encodes other, intracellular signaling molecules that can induce cellular gene expression and thereby potentially contribute paracrine mediators (19–21). The most important of these is the product of open reading frame 74, which encodes a v-GPCR that is a distant relative of the IL-8 receptor (22–26). Whereas this protein can interact with (and be stimulated by) a number of CC and CXC chemokines *in vitro* (27, 28), it displays a strong signaling activity in the absence of any known ligand and is thus considered to be constitutively active (29). v-GPCR is expressed during the mid-lytic cycle as the downstream gene of a bicistronic mRNA (26). Transfection of the gene into cultured fibroblasts can trigger growth deregulation (22), but *in vivo* this activity is unlikely to contribute to tumorigenesis because lytically infected cells do not survive viral replication. However v-GPCR expression induces expression of the potent angiogenic factor VEGF (22, 30), a mediator likely to play a role in KS neovascularizing action. GPCR expression activates several signaling cascades, including the mitogen-activated protein kinase, c-Jun-NH_2-terminal kinase, and p38 pathways (31–35). Stimulation of the p38 and mitogen-activated protein kinase pathways in fibroblasts can up-regulate the transcription factor HIF-1α (hypoxia-inducible factor 1α) and lead to enhanced release of VEGF (35). Indeed, expression of v-GPCR in the T-cell lineage of transgenic mice generates a peculiar lesion characterized by neoangiogenesis in areas surrounding GPCR-positive T-cell collections (36). KSHV GPCR expression also activates NFκB (37, 38), a transcription factor known to control expression of many cytokines and proinflammatory mediators, although the pathway by which this occurs is uncertain.

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[3] The abbreviations used are: KSHV, Kaposi's sarcoma-associated herpesvirus; KS, Kaposi's sarcoma; v-GPCR, viral G protein-coupled receptor; IL, interleukin; VEGF, vascular endothelial growth factor; TNF, tumor necrosis factor; NFκB, nuclear factor κB; poly(A), polyadenylic acid; MIP, macrophage inflammatory protein; EST, expressed sequence tag; PCBP, polycytidylic acid-binding protein; ICAM, intercellular adhesion molecule; uPAR, urokinase-type plasminogen activator receptor; DSP, dual specificity phosphatase.

To better understand the potential of the KSHV v-GPCR to influence host cell biology, we have examined the patterns of cellular gene expression in cells expressing this protein using DNA microarrays. Our results reveal that GPCR signaling triggers a variegated program of host gene expression that displays striking cell-type specificity.

MATERIALS AND METHODS

Plasmids. For control transfection experiments, pcDNA3.1 (Invitrogen) was used. The GPCR-expressing plasmid (pCR3.1-v-GPCR) has been described previously (39). Briefly, a genomic λ clone was PCR amplified with the following primers: GCR 1: 5′cgatcgcggccgcacctatactacttgtt; and GCR 2: 5′ cagcttgatcaccgcgggctacgtggtggc, and the PCR product was cloned into pCR 3.1 (a derivatized version of pcDNA3.1 for TA cloning; Invitrogen).

Tissue Culture and Transfections. BJAB cells were maintained in RPMI 1640 supplemented with 10% FCS and penicillin-streptomycin at 37°C in 5% CO_2. BJAB cells were transfected by washing the cells twice in serum-free medium, resuspending the cells at 4×10^7 per ml of serum-free medium, and electroporation of 500 μl of cells at 250 V, 950 μF. Cells were allowed to recover for 15 min and then transferred to 50 ml of complete medium. SLK cells were maintained in DMEM H-21 supplemented with 10% FCS and penicillin-streptomycin at 37°C in 5% CO_2. Cells were split to 60% confluence in T150 flasks 12 h before transfection and then transfected with 30 μg DNA using Fugene (Roche) as directed by the manufacturer. SLK and BJAB cell were transfected in parallel with pEGFP_C1 (Clontech) and monitored by fluorescence-activated cell sorter for GFP (green fluorescent protein) expression to determine transfection efficiency. In the experiments described here ~80% of the BJAB cells were transfected, and 20–40% of the SLK cells were transfected at time of harvest.

RNA Isolation. Total RNA was harvested using RNAzol B (Tel-Test, Inc.) as recommended by the manufacturer. Poly(A) RNA was isolated using Oligotex mRNA isolation kit (Qiagen) as described by the manufacturer.

Table 1 *Genes up-regulated in SLK cells 48 h after transfection with KSHV vGPCR*

Fold up-regulation[a]	Name	GenBank accession ID[b]
Cytokines		
3.4	GROα	W42723
2.8		W46900
3.3	IL-6	None[c]
2.6		N98591
1.8	VEGF C	H07991
Signaling proteins		
4.3	DSP 6	AA630374
4.0	Laminin, γ 2	AA677534
3.1	TNF receptor-associated factor (TRAF-1)	R71725
3.1	Plasminogen activator, tissue	AA453728
2.9	Thrombomodulin	H59861
2.5	uPAR	AA455222
2.3	Plasminogen activator, urokinase	AA284668
2.1	ICAM-1/CD58	R77293
2.1	Gem GTP-binding protein	AA418077
2.1	Inhibitor of apoptosis protein-2	H48706
2.1	20 kD interferon stimulated gene (ISG20)	AA150500
2.0	DSP 5	W65461
1.9	Plasminogen	T67549
1.9	Plasminogen activator inhibitor	N75719
Transcription factors		
2.4	Vitamin D receptor	AA485226
2.1	Suppressin	AA425806
2.1	Gut-enriched Kruppel-like factor (GKLF or KLF4)	H45711
Others		
3.6	Natural killer transcript 4	AA458965
2.8	Secretory granule proteoglycan-1	AA278759
2.5	Mercaptopyruvate sulfurtransferase	AA461065
2.4	Tissue factor pathway inhibitor-2 (TFPI-2)	AA399473
2.4	MHC, class II	AA486627
2.3	TNF superfamily member 13b	AA166695
2.3	5′ nucleotidase (CD73)	R60343
2.2	TNF-α induced protein 3	AA476272
2.2	Neuroendocrine polypeptide 7B2	AA670429
2.1	Major histocompatibility complex, class I	AA464246
2.1	Ig κ light chain, anti RhD	AA488070

[a] Average ratio calculated as described in "Materials and Methods."
[b] GenBank accession number of microarray EST clone.
[c] cDNA clone amplified with gene-specific PCR primers.

Northern Blotting. For Northern blots, 10 μg of total RNA or 3 μg of poly(A) RNA were separated on a 1% agarose-5% formaldehyde gel in 4-morpholinepropanesulfonic acid buffer [20 mM 4-morpholinepropanesulfonic acid (pH 7.0), 5 mM sodium acetate, and 1 mM EDTA] and transferred to Nytran SPC membrane (Schleicher and Schuell) for 12 h in 20× SSC (3.0 M NaCl and 0.3 M sodium citrate). Probes for Northern blotting were made by PCR amplification of the corresponding cDNA using primers used for microarray construction (see below) and labeled with [α-^{32}P]dCTP using Rediprime (Amersham). Blots were UV cross-linked and hybridized in Church buffer [1% BSA, 1 mM EDTA, 0.5 M sodium phosphate (pH 7.2), and 7% SDS] at 65°C overnight. Blots were washed twice for 45 min with 1× Church wash [0.04 M sodium phosphate (pH 7.2), 1 mM EDTA, and 7% SDS], and exposed to a phosphorimager screen for quantitation and subsequently to film.

ELISA Assays. ELISA assays for IL-6, GROα, and MIP-1α (R&D Systems) were performed as recommended by the manufacturer.

Micorarray Construction. The human cDNA microarray was constructed essentially as described (40). Arrays used in these experiments contained ~20,000 human cDNA elements derived from PCR amplification of a human EST clone set (Research Genetics) using the common primers 5′ctgcaaggcgattaagttgggtaac (forward) and 5′gtgagcggtaacaatttcacacaggaaacagc (reverse). Of these clones, approximately one-third were known genes and the rest were unannotated ESTs. Some genes were represented on the microarray by multiple independent EST clones. Clones of particular interest were resequenced to confirm their identity. In addition, the array contains ~200 additional cDNAs that were amplified using gene-specific primers for KSHV sequences as well as a few selected human genes.

Micorarray Hybridization and Data Analysis. Probes were prepared and hybridized essentially as described (41, 42).[4] Briefly, 2 μg poly(A) purified RNA was reverse transcribed using reverse transcriptase at 42 C for 2 h in 500 μM dATP, dCTP, dGTP, 300 μM amino-allyl dUTP, and 200 μM dTTP. RNA was hydrolyzed by incubating with 0.1 M NaOH at 65 C, and samples were desalted by centrifugation through μm-30 filters. Samples were coupled to either Cy3 or Cy5 (Amersham) in 50 mM NaBicarbonate buffer (pH 9.0) for 1 h. Probes were hybridized under coverslips in 3× SSC, 50 mM HEPES (pH 7.0), and 0.5% SDS at 65 C for 16–20 h.

Arrays were scanned using an Axon 4000B scanner (Axon Instruments) and analyzed using GenePix 3.0 software. Spots with obvious blemishes were flagged and excluded from analysis. For each array, a scalar normalization factor was applied to all of the ratios such that the overall ratio for all well measured spots was 1.0. Ratios from two independent transfection experiments were averaged, and genes were included in Tables 1 and 2 only if the fold up-regulation in each independent experiment was >1.6 and the average in both experiments was >2.0. Exceptions to this are VEGF-C and plasminogen, which were included because of their potential biological importance. ESTs with no annotation were excluded from the lists. The complete data sets and primary data are available.[5]

RESULTS AND DISCUSSION

Experimental Strategy. To explore the impact of v-GPCR expression on the host transcriptional program, we used cDNA microarray analysis, using RNA from cells transiently transfected with either a v-GPCR expression vector or a control plasmid. We used two cell lines (SLK and BJAB) representing the major targets for KSHV infection, endothelia, and B cells (respectively). The SLK cell line was established from an HIV-negative classical KS tumor in Israel; it displays endothelial markers (Ulex lectin binding; CD34) and forms vessel-like tubules when plated on Matrigel (43). Like all of the KS spindle cell lines, it lacks evidence of ongoing KSHV infection (latent viral genomes are reproducibly lost under these culture conditions; Refs. 44–47), so there is no endogenous source of v-GPCR. BJAB cells are derived from a human B-cell lymphoma. Both lines are fully immortalized, and the SLK cells grow well in 10% FCS alone,

[4] Internet address: http://www.microarrays.org.
[5] Internet address: http://derisilab.ucsf.edu/gpcr/gpcr.shtml.

without supplementation with VEGF, basic fibroblast growth factor, or other endothelial growth factors.

Transfection efficiencies (as judged by parallel transfection of GFP expression vector) were 20–40% for SLK cells after transfection with Fugene and ~80% for BJAB after electroporation (see "Materials and Methods"). We note that expression profiling of transiently transfected cells is not optimal for detection of down-regulation of gene expression because the background of untransfected cells obscures the reduction in signal. Accordingly, we have focused here on up-regulated mRNAs. At the single cell level, the actual fold induction is likely to be significantly higher than the values we observed, as analysis of the aggregate population provides a minimal estimate of transcript induction.

Polyadenylated RNA was prepared from v-GPCR- or vector-transfected cells at 48 h after transfection, and cDNA was prepared from each by reverse transcription, with the v-GPCR probe sample labeled with Cy5 and the control sample labeled with Cy3. Equal portions of the probes were then admixed and hybridized to the microarray. The microarray was composed of cDNAs from 20,000 human genes and ESTs, and ~200 spots corresponding to genomic open reading frames and intergenic regions of KSHV. In each experiment, hybridization to the spots corresponding to the genomic v-GPCR locus provided internal controls for viral transcript expression (see primary data).[5] The patterns of gene expression described below were unique to v-GPCR as other KSHV genes (*e.g.*, K3, K5, or K8) similarly transfected into SLK and BJAB gave distinct and nonoverlapping hybridization patterns (data not shown).

Gene Activation by v-GPCR in SLK Cells. Table 1 summarizes the principal genes that were observed to be up-regulated at least 2-fold in the presence of v-GPCR, grouped by broad functional category. To confirm that these observed up-regulations were authentic and not the result of array artifacts, we examined RNA from control and v-GPCR-transfected cells by Northern blotting for selected transcripts (Fig. 1). Each of the tested transcripts predicted by the array analysis to be induced by v-GPCR expression displayed up-regulation by this method, whereas a control RNA (for PCBP) that was unaffected by v-GPCR in the array analysis showed no change in transcript level (Fig. 1).

Among the strongest up-regulated host molecules on the array were cytokines, especially IL-6 and the chemokine GROα. ELISA assay of the growth medium of v-GPCR-transfected cells confirmed that elevated levels of immunoreactive IL-6 and GROα proteins were present (Fig. 2). IL-6 is a known stimulatory factor for the proliferation of KS spindle cells in culture (48), and up-regulation of IL-6 is also a prominent feature of multicentric Castleman's disease (49, 50). The up-regulation of GROα is also noteworthy, because this chemokine is the most potent activator of v-GPCR signaling (27, 28). This suggests the possibility of a positive feedback loop in which the constitutive activity of the receptor triggers production of a potent super-inducing ligand, amplifying the resulting signaling. The fact that both IL-6 and GROα are exported from cells is consistent with a contribution of v-GPCR to paracrine signaling. Interestingly, both IL-6 and GROα have been implicated in the stimulation of neoangiogenesis in certain situations (51).

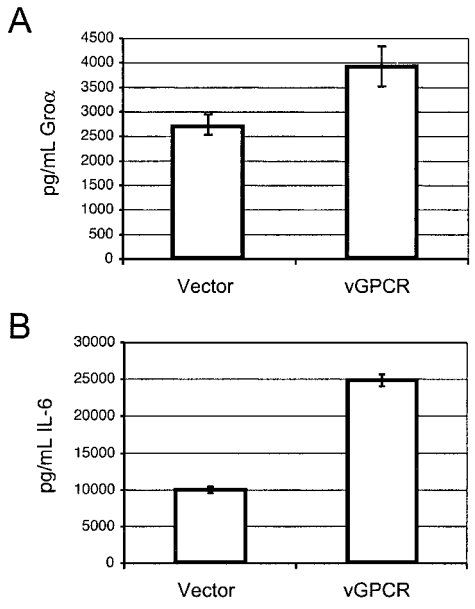

Fig. 2. v-GPCR increases the expression of GROα and IL-6 in SLK cells. Supernatants were collected from SLK cells 48 h after transfection with pcDNA3.1 (*vector*) or pCR3.13.1v-GPCR (*v-GPCR*) and assayed for the presence of GROα (*A*) or IL-6 (*B*) by ELISA; *bars*, ±SD.

Another important paracrine signaling factor induced by v-GPCR was VEGF-C. Although the observed induction was quite modest (1.8-fold), based on our average transfection efficiency of 30% for SLK, this induction may translate into approximately 3–4-fold up-regulation at the single cell level. This VEGF family member interacts weakly with VEGF receptor 2 and more strongly with VEGF receptor 3 (52, 53). The latter is localized primarily on endothelial cells of lymphatic lineage; its presence on KS spindle cells has suggested that these cells may in fact be derived from lymphatic endothelium (54), a notion that is supported by other pathologic features of KS (55). Therefore, it is particularly provocative that VEGF-C is up-regulated by endothelial expression of v-GPCR. Unlike other VEGF isoforms, this protein is not inducible by hypoxia (52, 53), so the induction is not because of the known ability of v-GPCR's to activate HIF-1α.

IL-6, GROα, and VEGF-C are all known to be regulated by NFκB, which in turn has been shown to be functionally activated by v-GPCR expression (31, 34, 37, 38). Also consistent with NFκB activation is the induction of expression of MHC class I and ICAM-1 (37). Our observations of IL-6 and ICAM-1 up-regulation in SLK have also been made by others in both primary endothelial cells (HUVEC) and in the endothelial cell line KSIMM (37). Moreover, two other genes moderately induced by GPCR expression, tissue factor pathway inhibitor 2 and TNF-α-inducible protein 3, are known to be TNF-inducible, and one of the major signaling activities of TNF is via NFκB activation. However, many other NFκB-inducible genes (*e.g.*, *ELAM*, *IL-1*) are not up-regulated, which may betoken the existence of counter-regulatory mechanisms or indicate that some of the aforementioned transcripts are being induced by other means.

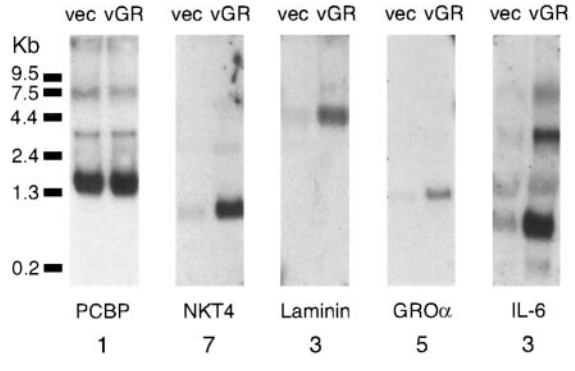

Fig. 1. Northern blot analysis of genes up-regulated in SLK by v-GPCR. Northern blot analysis was preformed on 20 μg of total RNA derived from SLK cells 48 h after transfection with pcDNA3.1 (*vec*) or pCR3.1v-GPCR (*vGR*). The blots were probed for natural killer transcript 4 (*NKT4*), Laminin, γ 2 (*laminin*), GROα, IL-6, and, as a control, PCBP, which is not affected by the expression of v-GPCR. The *numbers below the blot* show the fold up-regulation as quantitated by phosphorimager.

Table 2 *Genes up-regulated in BJAB cells 48 h after transfection with KSHV vGPCR*

Fold up-regulation[a]	Name	GeneBank, accession ID[b]
Cytokines		
7.2	MIP-1β	H62985
4.9	MIP-1α	AA677522
4.0		AA495985
2.5		R47893
Signaling proteins		
2.8	CD83	AA111969
2.4	DSP 2	AA759046
2.1	DSP 5	W65461
Transcription factors		
5.9	Early growth response 2 (Krox-20)	H98665
3.9	Nak-1/Nurr-1 transcription factor	N94487
Others		
5.8	Myoglobin	AA176581
2.0	Histone deacetylase 3	AA064973
2.0	PDGF receptor, β chain	R56211

[a] Average ratio calculated as described in "Materials and Methods."
[b] GenBank accession number of microarray EST clone.

In this connection, we note that several of the v-GPCR-up-regulated genes are transcription factors, including the vitamin D receptor (a member of the steroid-receptor superfamily of ligand-activated transcription factors). Up-regulation of the vitamin D receptor is noteworthy, as vitamin D agonists have shown modest therapeutic efficacy against KS in clinical trials (56). Suppressin, which is a widely expressed secretory protein linked to growth arrest in several tissues, shares partial homology with the *Drosophila* transcription factor DEAF-1. Nuclear isoforms of suppressin have been identified, but it is presently unclear if this protein, or an alternatively spliced isoform of same, also functions as a transcription factor (57). The up-regulation of multiple transcription factors is important, as it indicates the likelihood of secondary effects of v-GPCR signaling via the transcriptional activation of other, downstream groups of genes. It seems likely that many of the genes listed in Table 1 are in this class of indirectly activated transcripts.

A striking finding is the induction in endothelial cells of several proteins involved in the control of vascular remodeling and angiogenesis. Beyond VEGF-C, induction was observed for thrombomodulin, plasminogen, plasminogen activator, urokinase, and uPAR. During angiogenesis, local coagulation and fibrinolysis must be modulated in a controlled fashion. Urokinase-type plasminogen activator is an extracellular protease that activates plasminogen to stimulate fibrinolysis; it can be targeted to endothelial surfaces (and activated there) by interaction with its receptor, uPAR (58). Thrombomodulin binds and activates extracellular thrombin, which activates the anticoagulant protein C as well as the inhibitor of fibrinolysis TAFI (59). Fragments of plasminogen have also been described to modulate angiogenesis (60).

Many v-GPCR-induced proteins in SLK are involved in other aspects of cell signaling. Dual-specificity phosphatases 6 and 5 are up-regulated; these ser/thr and tyr-specific phosphatases are involved in down-regulating signaling via the c-Jun-NH_2-terminal kinase and p38 pathways, and are likely part of a counter-regulatory network triggered by constitutive activation of these pathways by the v-GPCR (61, 62). Also induced is TNF receptor-associated factor-1, a key intermediary in the TNF signaling pathway that is linked to NFκB induction and the generation of proinflammatory signals. Others, including human IAP-2 (inhibitor of apoptosis-2), are involved in the abrogation of apoptotic pathways and, thus, may also contribute to cell survival. Some but not all of the IFN-stimulated genes are also up-regulated by KSHV GPCR expression. However, the IFNs themselves are not induced, indicating that other factors are likely controlling the induction of these selected IFN-stimulated genes.

Finally, we note the seemingly paradoxical induction of MHC class I molecules, which would be expected to make KSHV-infected cells better targets for host CTL attack, leading to their elimination. However, we (63) and others (64–66) have earlier shown that the virus encodes two proteins, MIR1 and MIR2, that lead to the selective removal of MHC-I chains from the cell surface, thereby functionally negating this up-regulation.

v-GPCR-regulated Genes in BJAB B Cells. Table 2 shows the panel of genes up-regulated by at least 2-fold in BJAB cells 48 h after transfection. RNA isolated at two earlier time points after transfection (4 and 8 h) showed similar expression profiles.[5] As before, we confirmed by Northern blotting that several of the genes predicted to be induced by GPCR expression were indeed up-regulated. As shown in Fig. 3, transcripts for Egr-2, DSP5, and MIP-1α and β were all induced, whereas the abundance of the control PCBP mRNA was not affected.

Two features are immediately apparent from inspection of Table 2: (*a*) the list is much shorter that that generated by expression of v-GPCR in SLK cells (Table 1); and (*b*) it is virtually nonoverlapping with it. Only DSP5 is induced in both cell lines, although a second DSP, DSP2, is also up-regulated in transfected BJAB cells. As in SLK, cytokines were among the transcripts induced to the highest levels; however, in B cells the major induction involves the CC chemokines MIP-1α and MIP-1β (as opposed to IL-6 and GROα). Assay of the conditioned medium from GPCR-transfected cells for MIP-1α immunoreactive material confirms that elevated levels of MIP-1α protein are indeed present (Fig. 4), whereas ELISA assay for IL-6 revealed no change (data not shown). The logic of this induction is not altogether apparent. MIP-1α interacts with cells bearing CCR5 (and CCR1); these are chiefly T cells, macrophages, and dendritic

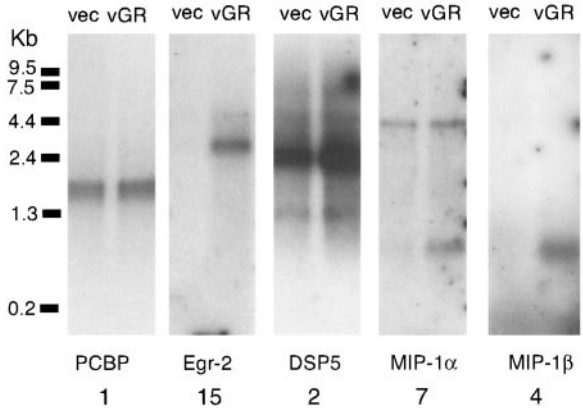

Fig. 3. Northern blot analysis of genes up-regulated in BJAB cells by v-GPCR. Northern blot analysis was performed on 3 μg of poly(A) RNA derived from BJAB cells 48 h after transfection with pcDNA3.1 (*vec*) or pCR3.1v-GPCR (*vGR*). The blots were probed for early growth reponse-2 (*Egr-2*), DSP5, MIP-1α, MIP-1β, and, as a control, the PCBP, which is not affected by the expression of v-GPCR. The *numbers below the blot* show the fold up-regulation of the RNA as quantitated by phosphorimager.

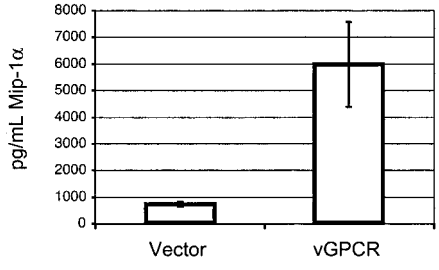

Fig. 4. v-GPCR increases the expression of MIP-1α in BJAB cells. Supernatants were collected from BJAB cells 48 h after transfection with pcDNA3.1 (*vector*) or pCR3.1v-GPCR (*v-GPCR*) and assayed for the presence of MIP-1α by ELISA; *bars*, $\pm$SD.

cells. These interactions promote chemotaxis, although recent studies suggest that they also affect T-cell differentiation (67, 68). It would seem to be disadvantageous to viral spread to attract T cells and antigen-presenting cells to sites of virus replication. Indeed, knockout mutations in these cytokines are associated with enhanced viral replication in influenza models (69). However, there is *in vitro* evidence that human B cells can migrate in response to low levels of MIP-1α (67), so perhaps the role of MIP-1 release is to recruit additional permissive cells to sites of lytic virus replication. Clearly, additional work will be required to more completely understand the meaning of this paracrine signaling pathway.

Both forms of MIP-1 are regulated transcriptionally by NFκB (70, 71), and it is likely that activation of this transcription factor contributes to the observed induction. Consistent with this is the induction of CD83, an immunoglobulin superfamily member normally expressed on mature dendritic cells. CD83 is also expressed on B and T cells in response to mitogenic signals, and this induction is known to require NFκB activation (72). Other transcription factors are also up-regulated in BJAB by v-GPCR expression, including Egr-2 and NAK-1. Egr-2 is a member of a zinc finger-containing family of proteins that are induced by growth factor signaling and that induce a variety of promitotic functions (73). Like NFκB, it is known to be up-regulated in B cells after B-cell receptor cross-linking, although this up-regulation is transcriptional for Egr proteins (as opposed to the post-transcriptional activation of NFκB). NAK-1 is a zinc finger-containing transcription factor that has been implicated in activation-induced cell death in B cells, although its exact contribution to this process remains unclear (74). It is possible that the induction of this factor is a downstream consequence of partial B-cell activation induced by GPCR signaling, because several of the other induced proteins of Table 2 (MIP-1, CD83, and Egr2) are known to be up-regulated after B-cell receptor cross-linking (73, 75). Indeed, triggering of activation-induced cell death might explain, at least in part, the brevity of the list of induced BJAB genes.

The most remarkable feature of the transcriptional programs induced by v-GPCR signaling is the striking degree of cell-type specificity observed. It seems likely that this results from differing patterns of transcription factor activation, differing tissue-specific coactivators, and possibly cell type-specific differences in chromatin structure. Irrespective of the underlying mechanisms, this remarkable cell-type specificity suggests that v-GPCR signaling will have different functional consequences in different cell types infected by KSHV. The corollary to this is that the potential contributions of this gene to KSHV pathogenesis will likely depend strongly on the cell type involved by KSHV in each of the different clinical entities with which it is associated.

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