

A Viral Conspiracy: Hijacking the Chemokine System Through Virally Encoded Pirated Chemokine Receptors

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Abstract Several herpesviruses and poxviruses contain genes encoding for G protein-coupled receptor (GPCR) proteins that are expressed on the surface of infected host cells and/or the viral envelope. Most of these membrane-associated proteins display highest homology to the subfamily of chemokine receptors known to play a key role in the immune system. Virally encoded chemokine receptors have been modified through evolutionary selection both in chemokine binding profile and signaling capacity, ultimately resulting in immune evasion and cellular reprogramming in favor of viral survival and replication. Insight in the role of virally encoded GPCRs during the viral lifecycle may reveal their potential as future drug targets.

1

Chemokine System

The chemokines and their cognate receptors play a key role in the immune system during homeostasis and inflammation by coordinating leukocyte migration, activation, degranulation, and differentiation. In addition, the chemokine system is involved in organogenesis, angiogenesis, and directing metastasis and growth of tumor cells (Murphy et al. 2000). The mammalian chemokine system (e.g., human, mouse, and rat) constitutes of approximately 45 chemokine ligands and 20 chemokine receptors (Murphy 2002).

Chemokines are a family of small proteins that adopt a similar tertiary folding, even in cases of low overall sequence identity (varying from 20% to 95%). They are characterized by a flexible amino-terminal domain, followed by a conserved core region consisting of a so-called N-loop, three anti-parallel β -strands, and a carboxyl-terminal α -helix, stabilized by disulfide bonds between four conserved cysteine residues (Mizoue et al. 1999). Four subclasses of chemokines (i.e., CC, CXC, CX3C, and XC) have been recognized on the basis of the number and sequential spacing of the first two conserved cysteine residues that are situated near the amino terminus (Zlotnik and Yoshie 2000). In addition, chemokines can be functionally classified into inducible (inflammatory) and homeostatic (constitutively expressed) chemokines, mediating inflammation-directed or basal (homing) leukocyte trafficking, respectively (Proudfoot 2002).

Recruitment of specific leukocyte populations by chemokines is essentially determined by the spatiotemporal expression of selected chemokine receptors, which belong to the membrane-associated G protein-coupled receptor (GPCR) family. Chemokine receptors are classified (i.e., CCR1–11, CXCR1–6, CX3CR1, and XCR1) according to their ability to bind a specific subclass of chemokines (Murphy 2002). Chemokine receptors are not only expressed on leukocytes but also on endothelial, smooth muscle, epithelial, stromal, and neural cells (Onuffer and Horuk 2002). Interestingly, most inflammatory chemokines display a high level of promiscuity by binding several chemokine receptor subtypes, and vice versa. In contrast, homeostatic chemokines are generally more specific, each interacting with a single chemokine receptor subtype (Proudfoot 2002). Given the prominent role of chemokine receptors in regulating intracellular signaling in response to chemokine ligands, these receptors are the most promising targets for immunomodulatory therapeutic interventions (Onuffer and Horuk 2002; Gao and Metz 2003). Interestingly, such receptors are also employed by several viruses in order to subvert the immune system and/or redirect intracellular signaling for their own benefit (Alcami 2003).

2

Viral Immune Evasion

Viruses are small, infectious, parasitic pathogens that (ab)use the host cell metabolism and “consume” cellular biomolecule resources for their replication. An important strategy which enables viruses to replicate efficiently in a host cell is to interfere with recognition and subsequent elimination of the infected cell by the immune system. To this end, distinct viruses have employed different strategies. For instance, large double-stranded DNA viruses, such as herpesviruses and poxviruses, encode viral mimics of host cytokines and chemokines, as well as their soluble binding proteins and/or membrane-associated receptors, to subvert the immune system (Alcami 2003). The viral genes encoding these proteins have probably been derived from the genomes of the viral host during evolution. Of particular interest are the viral genes that code for membrane-associated GPCRs, as these proteins are localized at the boundary of the extracellular and intracellular milieu, and transmit signals from the outside to the inside of the cell. The amino acid sequences of the virally encoded GPCRs (vGPCRs) are generally highly diverged between and within virus subfamilies. This suggests that these GPCRs have distinct and specialized functions that are optimized for different biological properties of each virus. Nonetheless, the majority of vGPCRs display highest sequence similarity to the subfamily of chemokine receptors (Fig. 1, Tables 1 and 2). Some vGPCRs are indeed responsive to chemokines, whereas for others no endogenous ligands have been identified and remain “orphan”. Importantly, in contrast to their cellular homologs, a number of vGPCRs signal ligand-independently (i.e., constitutively). Constitutive GPCR signaling is of major significance as revealed by several pathologies associated with activating GPCR mutations (Seifert and Wenzel-Seifert 2002). This constitutive activity of many vGPCRs, together with the current awareness that chemokines and their receptors play prominent roles in inflammatory pathologies and tumor metastases (Proudfoot 2002), suggests that vGPCRs may be key players in virus-associated diseases.

3

Herpesvirus-Encoded GPCRs

Herpesviruses have been isolated from a wide variety of vertebrates and are generally characterized by their strict specificity for a single host species (Davison 2002). Herpesviruses have been classified into three subfamilies, the α -, β -, and γ -herpesvirinae, on the basis of their biological properties,

Table 1 Herpesvirus-encoded GPCRs

Subfamily	Genus	Species		vGPCR	Cellular homolog ^a	% ^b
α-Herpesvirinae	Simplexvirus	<i>Human herpesvirus 1</i>	HHV-1	-	-	-
		<i>Human herpesvirus 2</i>	HHV-2	-	-	-
		<i>Human herpesvirus 3</i>	HHV-3	-	-	-
β-Herpesvirinae	Varicellovirus	<i>Cercopithecine herpesvirus 8</i> (Rhesus cytomegalovirus)	CeHV8	UL33	CCR10	20
				UL78	CXCR1	14
	Cytomegalovirus			Rh214	CCR5	22
				Rh215	CXCR1	22
				Rh216	CCR1	21
				Rh218	CXCR3	22
				Rh220	CX3CR1	34
			SCMV	ORF3	CCR4	22
				ORF4	CCR3	25
				ORF5	CCR2	22
				ORF6	CXCR1	21
			PoHV4	ORF7	CXCR6	21
				UL33	CCR3	20
				UL78	CXCR1	13
				US27	CXCR3	23
			HHV-5	US28	CX3CR1	38
				UL33	CCR10	21
				UL78	Somatostatin R3	12
				US27	CXCR3	23
				US28	CX3CR1	36

Table 1 (continued)

Subfamily	Genus	Species		vGPCR	Cellular homolog ^a	% ^b
γ-Herpesvirinae	<i>Lymphocryptovirus</i>	<i>Murid herpesvirus 1</i> (Mouse cytomegalovirus)	MCMV	M33	CCR10	21
		<i>Murid herpesvirus 2</i> (Rat cytomegalovirus)	RCMV	M78	Opiate R-like 1	13
		<i>Tupaia herpesvirus 1</i> (Tupaia herpesvirus)	TCMV	R33	CCR10	23
		<i>Human herpesvirus 6a</i>		R78	CCR10	15
		<i>Human herpesvirus 6b</i>		T33	CCR10	23
		<i>Human herpesvirus 7</i>		T78	Formyl peptide R-like	12
				U12	CCR10	19
				U51	Cysteinyl leukotriene R2	16
				U12	CCR10	19
				U51	Cysteinyl leukotriene R2	16
γ-Herpesvirinae	<i>Lymphocryptovirus</i>			U12	CX3CR1	20
				U51	CCR2	16
				ORF6	CXCR5	13
				BILF1	CXCR4	14
				BILF1	CXCR4	15
				A5	CCR3	15
				A5	CXCR2	14
				A5	CXCR2	15
				A5	CCR10	13
				-	-	-
γ-Herpesvirinae	<i>Rhadinovirus</i>	<i>Alcelaphine herpesvirus 1</i>	AHV1	ORF74	CXCR1	24
		<i>Porcine lymphotropic herpesvirus 1</i>	PLHV1	ORF74	CXCR2	26
		<i>Porcine lymphotropic herpesvirus 2</i>	PLHV2			
		<i>Porcine lymphotropic herpesvirus 3</i>	PLHV3			
		<i>Bovine herpesvirus 4</i>	BHV4			
		<i>Cercopithecine herpesvirus 17</i>	CeHV17			
		<i>Human herpesvirus 8</i>	HHV-8			
		(KS-associated herpesvirus)				

Table 1 (continued)

Subfamily	Genus	Species		vGPCR	Cellular homolog ^a	% ^b
		<i>Macaca fuscata rhadinovirus</i>	MFRV	ORF74	CXCR1	24
		<i>Murid herpesvirus 4</i>	MuHV4	ORF74	CCR4	20
		<i>Saimiriine herpesvirus 2</i>	HVS2	ORF74	CXCR2	24
		(<i>Herpesvirus saimiri</i>)				
		<i>Equid herpesvirus 2</i>	EHV2	ORF E1	CCR3	51
				ORF E6	CCR10	16
				ORF74	CXCR5	22
				ORF E1	CCR3	51

^a Nearest cellular homologs are identified by basic local alignment search tool (BLAST) analysis of each vGPCR on the human protein sequence reference database at NCBI, subsequently followed by ClustalW analysis

^b Percentage amino acid identity

Table 2 Chordopoxvirus-encoded GPCRs

Genus	Species		vGPCR	Cellular homolog ^a	% ^b
<i>Avipoxvirus</i>	<i>Fowlpox virus</i>	FPV	FPV021	GPCR1	32
			FPV027	GPCR1	29
	<i>Canarypox virus</i>	CNPV	FPV206	EBV-induced GPCR2	35
			CNPV039	GPCR1	34
<i>Capripoxvirus</i>			CNPV045	GPCR1	70
			CNPV277	EBV-induced GPCR2	36
			CNPV315	Chemokine-like R1	28
			-	-	-
<i>Capripoxvirus</i>	<i>Goatpox virus</i>	GTPV	-	-	-
	<i>Sheeppox virus</i>	SPPV	SSPV09	CCR8	40
	<i>Lumpy skin disease virus</i>	LSDV	LSDV011	CCR8	39
<i>Molluscipoxvirus</i>	<i>Molluscum contagiosum virus</i>	MOCV	-	-	-
<i>Orthopoxvirus</i>	<i>Camelpox virus</i>	CMPV	-	-	-
	<i>Cowpox virus</i>	CPV	-	-	-
	<i>Ectromelia virus</i>	ECT	-	-	-
	<i>Monkeypox virus</i>	MPV	-	-	-
	<i>Vaccinia virus</i>	VV	-	-	-
	<i>Variola virus</i>	VAR	-	-	-
	<i>Bovine papular stomatitis virus</i>	BPSV	-	-	-
<i>Parapoxvirus</i>	<i>Orf virus</i>	ORFV	-	-	-
<i>Suipoxvirus</i>	<i>Swinepox virus</i>	SPV	SPV146	CCR8	34
	<i>Yaba monkey tumor virus</i>	YMTV	7L, 145R	CCR8, CCR8	51, 39
<i>Yatapoxvirus</i>	<i>Yaba-like disease virus</i>	YLDV	7L, 145R	CCR8, CCR8	53, 44
Unclassified	<i>Mulle deer pox</i>	DPV83	gp013, gp162	CCR8, CCR4	42, 32

^a Nearest cellular homologs are identified by basic local alignment search tool (BLAST) analysis of each vGPCR on the human protein sequence reference database at NCBI, subsequently followed by ClustalW analysis

^b Percentage amino acid identity

genome organization, and deduced amino acid sequence similarity between conserved gene orthologs (McGeoch et al. 2000). About 43 genes are shared between most members of the three herpesvirus subfamilies (Davison et al. 2002). These so-called core genes encode proteins that contribute to universal processes such as viral DNA replication and packaging into the viral capsid. During the course of coevolution with their host, individual herpesvirus subtypes acquired unique genes through pirating or gene duplication. Among these so-called accessory genes are genes that allow the virus to subvert the host immune response.

3.1

β -Herpesvirinae

The β -Herpesvirinae subfamily comprises two genera, namely *Roseolovirus* and *Cytomegalovirus* (CMV). Hitherto, four members of the *Roseolovirus* genus have been isolated, three of which are found in man. In contrast, host-specific cytomegaloviruses have been isolated from a wide variety of mammals from the orders Rodentia (e.g., mouse and rat), Scandentia (e.g., tree shrew), and Primates (e.g., rhesus macaque, African green monkey, chimpanzee, and human). CMV genomes are the largest of all herpesviruses (195–241 kb), whereas genomes of roseoloviruses are somewhat smaller (153–162 kb). The genomes of roseoloviruses and CMVs, share extensive characteristics, including position and orientation of large blocks of genes (Neipel et al. 1991; Gompels et al. 1995; Nicholas 1996; Weir 1998).

3.1.1

Roseoloviruses

Three distinct species of *Roseolovirus* have been isolated from peripheral blood of humans. Human herpesvirus (HHV)-6A was first isolated from peripheral blood mononuclear cells derived from adults with acquired immunodeficiency syndrome (AIDS) and displaying lymphoproliferative disorders (Salahuddin et al. 1986). In addition, a second highly related variant of HHV-6, sharing an overall nucleotide sequence identity of 90% (Dominguez et al. 1999), but displaying distinct biological properties, was formally recognized and named HHV-6B. The third human roseolovirus, HHV-7, is highly related to the HHV-6 variants with respect to genome organization as well as sequence, with HHV-6 and HHV-7 genes sharing deduced amino acid identities between 22% and 75% (Nicholas 1996; Dominguez et al. 1999).

Primary infection with HHV-6B occurs between 6 and 12 months of age, whereas infection with HHV-7 occurs at a later time, though often within

the first 4 years of childhood (De Bolle et al. 2005). The time of HHV-6A infection is still unknown, but is thought to occur following infection with HHV-6B. As a consequence, roseoloviruses are ubiquitously spread in the general adult population, usually reaching a seroprevalence of greater than 95%. Primary infection with HHV-6b or HHV-7 results in an acute febrile illness that is in some cases followed by the appearance of a mild skin rash on the face and trunk (i.e., exanthem subitum or roseola infantum; Yamanishi et al. 1988; Tanaka et al. 1994). Interestingly, infection with HHV-6A is usually asymptomatic (Dewhurst et al. 1993; Stodberg et al. 2002; Freitas et al. 2003). Clinical complications of (primary) HHV-6 and -7 infections include febrile seizure, but also meningoencephalitis, encephalopathy, and multiple sclerosis (for a review see De Bolle et al. 2005). Importantly, primary HHV-7 infection can reactivate HHV-6 (Frenkel and Wyatt 1992; Katsafanas et al. 1996; Tanaka-Taya et al. 2000). In contrast, reactivation of HHV-6 in healthy children has been reported to occur usually without clinical consequences (Caserta et al. 2004).

Roseoloviruses are (T-)lymotropic and replicates most efficiently in vitro CD4⁺ T lymphocytes (Takahashi et al. 1989), but can also infect various other cell types in vitro (De Bolle et al. 2005). HHV-6B and HHV-7 replicate predominantly in salivary glands, with viral shedding into saliva being the major route of virus transmission (Harnett et al. 1990). After primary infection, roseoloviruses persist latently in the host in monocytes and early bone marrow progenitor cells (De Bolle et al. 2005). In healthy individuals, the pathogenic potential of roseoloviruses is kept under control by the immune system. However, both HHV-6 and HHV-7 can reactivate under immunosuppressive conditions (e.g., in AIDS patients and transplant recipients).

The genome of roseoloviruses contains two GPCR-encoding genes, i.e., *U12* and *U51*. The *U12* and *U51* genes are situated on similar positions and have a similar orientation as the *UL33* and *UL78* genes of CMVs, respectively. The *U12* and *U51* genes of HHV-6 are expressed with similar late and early kinetics (Isegawa et al. 1998; Menotti et al. 1999). Temporal expression profiles of the HHV-7-encoded GPCRs have not been reported yet, but are presumably similar to those observed for the HHV-6- and CMV-encoded receptors.

U12 and *U51* display the highest amino acid sequence identity to human chemokine receptors. Although the shared sequence identity between these virally encoded receptors and the cellular receptors is rather limited (<20%), both *U12* and *U51* are highly responsive to a variety of CC chemokines. HHV-6B *U12* displays high binding affinity for CCL5, CCL4, and CCL2, and lower affinity for CCL3 (Fig. 2). Moreover, these CC chemokines induce *U12*-mediated increases in intracellular Ca²⁺ levels in stably transfected K562 cells, via pertussis toxin-insensitive signaling pathways (Isegawa et al. 1998). In-

terestingly, the HHV-7-encoded U12 displays a different chemokine binding profile than HHV-6B U12 and induces intracellular Ca^{2+} signaling in stably transfected K562 cells in response to CCL17, CCL19, CCL21, and CCL22 (Fig. 2), but not CCL1, CCL2, and CCL5 (Nakano et al. 2003; Tadagaki et al. 2005). In addition, expression of HHV-7 U12 in Jurkat cells induces chemotaxis of these cells towards CCL19 and CCL21 (Tadagaki et al. 2005). Interestingly, CCL17 and CCL22 are unable to attract U12-expressing Jurkat cells. Hence, besides CCR7 and its cognate ligands, U12 expression on the surface of HHV-

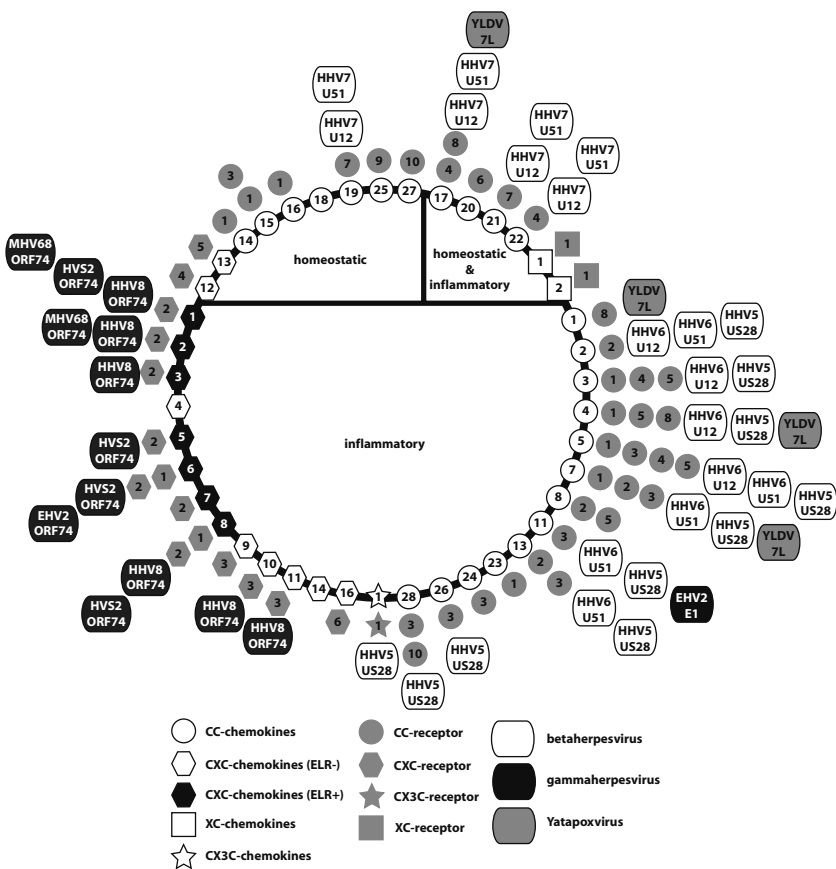


Fig. 2 Chemokine binding of vGPCRs. Homeostatic as well as inflammatory chemokines bind a specific subset of herpesvirus-encoded GPCRs. Chemokines are depicted on the *inner circle*. Their cognate chemokine receptors are displayed next to them, followed by vGPCRs from the β -herpesvirus, γ -herpesvirus, and yatapoxvirus families that were shown to bind the respective chemokines

7-infected T lymphocytes may also support homing of these cells into lymph nodes, which may contribute to viral dissemination.

Despite its low sequence similarity with chemokine receptors, the HHV-6-encoded U51 displays an overlapping chemokine binding profile with HHV-6 U12. This protein binds CCL5 and CCL2 with high affinity, but is unable to bind CCL3. In addition, HHV-6 U51 efficiently binds CCL7, CCL11, CCL13, and HHV-8-encoded viral macrophage inflammatory protein (vMIP)-II (Milne et al. 2000). Interestingly, when transfected into adherent cells (HEK293 or 143tk cells), HHV-6 U51 accumulates predominantly intracellularly in the endoplasmic reticulum and cannot be detected on the cell surface. In contrast, U51 is readily detectable on the cell surface of transfected T lymphocytic cell lines as well as on HHV-6-infected cord blood mononuclear cells *in vitro* (Menotti et al. 1999). Hence, expression of U51 at the cell surface appears to be cell type-specific, and requires trafficking functions that are apparently present in activated T cells and monocytes, but not in adherent cell types. Interestingly, stable expression of HHV-6 U51 in epithelial cells results in morphological alterations consisting of increased spreading and flattening of the cells and downregulation of CCL5 expression and secretion (Milne et al. 2000). The latter may contribute to immune evasion of the infected cells. The mechanism(s) by which U51 modulates epithelial cell functioning remains to be elucidated, but may include constitutive and/or autocrine U51-mediated signaling as observed for other vGPCRs (Milne et al. 2000). Moreover, in view of the impaired trafficking of U51 to the cell membrane as observed in some adherent cell lines, U51 cell membrane expression needs to be confirmed in epithelial cells.

Despite the low amino acid sequence identity between HHV-7 U51 and U12, these proteins induce intracellular Ca^{2+} elevation in response to the same chemokines (Tadagaki et al. 2005). In contrast, however, U51-expressing Jurkat cells were not able to migrate towards any of the tested chemokines (Tadagaki et al. 2005).

3.1.2

Cytomegaloviruses

Human CMV (HCMV) or HHV-5 is a widely spread virus, with a seroprevalence ranging from 50% to 80%, and it is able to persist lifelong in a latent form. Primary infection of immunocompetent hosts is usually asymptomatic. In contrast, primary infection or reactivation of the virus in immunocompromised hosts, such as the developing fetus, transplant recipients, or AIDS patients, can have severe implications and be fatal (Zhou et al. 1996). Common complications after HCMV infection include damage of liver, brain, retina,

and lung (interstitial pneumonitis; Landolfo et al. 2003). Coinfection of HCMV with *human immunodeficiency virus* (HIV) has been shown to accelerate progression to AIDS and dementia in HIV patients (Webster 1991; Kovacs et al. 1999). Increasing evidence suggests that HCMV may also contribute to the development of vascular diseases, e.g., atherosclerosis, restenosis, and vascular allograft rejection (Zhou et al. 1995; Burnett 2001).

CMV primarily infects monocytes, smooth muscle cells, and endothelial and epithelial cells of the upper gastrointestinal, respiratory, or urogenital tracts (Landolfo et al. 2003), and disseminates throughout the body by latently infected monocytes in the blood (Streblow and Nelson 2003). Allogenic stimulation of these monocytes induces differentiation into macrophages, which in latently infected cells is accompanied by reactivation of HCMV leading to the release of infectious virions (Streblow and Nelson 2003). CMV, like other β - and γ -herpesvirus subfamilies, appears to have “pirated” genes encoding key regulatory cellular proteins, showing highest homology to chemokine receptors (Murphy 2001; Sodhi et al. 2004b). HCMV encodes four GPCRs referred to as US27, US28, UL33, and UL78 (Chee et al. 1990). Two GPCR-encoding genes, i.e., *UL33* and *UL78*, are conserved with respect to position and orientation in the genomes of all sequenced β -herpesviruses. Possibly, these genes have been captured from an ancient host species by an ancestral β -herpesvirus. Interestingly, except for the intronless *UL33* genes of rhesus macaque CMV (RhCMV or *cercopithecine herpesvirus 8*) and rat CMV (RCMV), all *UL33* genes consist of two exons interrupted by a single intron. CMV-encoded *UL33* orthologs are fairly well conserved and display deduced amino acid sequence identities varying from 35% to 68% (Fig. 1). In contrast, amino acid sequences have diverged considerably between the *UL78* orthologs of individual CMV species (12%–54% sequence identity), suggesting a reduced selective pressure on this protein during the course of evolution.

Intriguingly, CMVs infecting host species of the primates (i.e., human, chimpanzee, African green monkey, and rhesus macaque) have pirated an additional GPCR-encoding gene cluster compared with CMVs that infect species from the Glires (i.e., mouse and rat) or Scandentia (i.e., tree shrew) orders. This gene cluster is located in the unique short (US) region of the CMV genome, which is not present in nonprimate CMV genomes, and consists of two adjacent genes in HCMV and chimpanzee CMV (CCMV) (*US27* and *US28*), and five juxtaposed genes in RhCMV and simian CMV (SCMV) (Table 1). Interestingly, the divergence in sequence and number of genes in this gene cluster parallels the coevolution of primate CMVs with two different host species families, with HCMV and CCMV infecting members of the Hominidae family (human and chimpanzee, respectively) and RhCMV and SCMV infecting Old World monkeys (rhesus macaque and African green

monkey, respectively). In addition, the highest amino acid sequence identities within proteins encoded by this gene cluster as well as common chemokine binding profiles are observed between rh220, cUS28, and hUS28 (34%–71%), suggesting that these genes have emerged through duplication and rapid diversification of an ancestral US28-like gene.

The genes encoding US28 and UL78 are expressed with early kinetics, whereas US27 and UL33 genes are transcribed with late kinetics (Mocarski 1996). The late and early expression kinetics of UL33 and UL78, respectively, are similar to those of their corresponding U12 and U51 counterparts in *Rose-olovirus*. In addition, CMV-encoded GPCRs are constituents of the virion, with UL33 (Margulies et al. 1996), UL78 (Oliveira and Shenk 2001), US28, rhUS28.5 (Penfold et al. 2003), and presumably US27 being expressed on the viral envelope. Colocalization of US28 (Fraile-Ramos et al. 2001), US27, and UL33 (Fraile-Ramos et al. 2002) with two major HCMV envelope glycoproteins, i.e., glycoprotein B and H, on virus-wrapping membranes of endocytotic vesicles in transfected or HCMV-infected cells, indicates that these GPCRs are incorporated in the viral envelope.

Although expression of CMV-encoded receptors seems not to be essential for infection of permissive cells in vitro, deletion of either R33/M33 (Davis-Poynter et al. 1997; Beisser et al. 1998) or R78/M78 (Oliveira and Shenk 2001; Kaptein et al. 2003) has significant impact on viral dissemination in vivo. A reduced replication in salivary glands and a lower mortality in infected animals is apparent in in vivo studies using recombinant RCMV and mouse CMV (MCMV) strains, lacking the corresponding *UL33* and *UL78* genes (see Vink et al. 2001 for references), underlining the importance of these receptors in the pathogenesis of infection.

The HCMV-encoded receptor US28 is so far the best characterized HCMV-encoded GPCR. It possesses a large spectrum chemokine-binding profile, including binding of a number of inflammatory CC as well as CX3CL chemokines (Gao and Murphy 1994; Kuhn et al. 1995; Kledal et al. 1998; Penfold et al. 2003; Fig. 2). This broad-spectrum binding profile suggests that US28 could act as a chemokine scavenger and thereby aid in subversion of the immune system (Kuhn et al. 1995; Kledal et al. 1998). CC chemokines, which are shown to bind to US28, induce increasing levels of intracellular calcium, activation of mitogen-activated protein kinase (MAPK) and focal adhesion kinase (FAK). Interestingly, infection of smooth muscle cells with CMV leads a US28-dependent migration. Hence, activation of the former signaling pathways by US28 may provide a molecular basis for the involvement of HCMV in the progression of atherosclerosis. These effects appear to be primarily $G\alpha_{12/13}$ mediated and involve activation of tyrosine kinase-linked signaling pathways (Streblow et al. 2003).

Despite the sequence similarity to chemokine receptors and US28, US27 does not seem to interact with chemokines. Therefore, this receptor is still classified as an orphan receptor. Interestingly, US28 is able to alter cellular signaling in a constitutive manner when expressed in COS-7 cells and after HCMV infection (Casarosa et al. 2001; Casarosa et al. 2003a). US28 is considered a versatile signaling device since it is able to activate multiple signaling networks in a constitutively active manner via activation of effectors and transcription factors within infected cells [i.e., InsP production, nuclear factor (NF)- κ B, cAMP-response element (CRE), and nuclear factor activated T cell (NFAT)]. US28 shows promiscuous G protein coupling, through primarily G_q , G_s , and G_{12} proteins (Casarosa et al. 2001; Waldhoer et al. 2002; Casarosa et al. 2003a; Minisini et al. 2003). The chemokine receptor homologs, on the other hand, do not display—or display only limited—ligand-independent signaling and activate primarily $G_{i/o}$ proteins (Offermanns 2003). Interestingly, US28-mediated, constitutive signaling potentiates chemokine-induced signaling of the G_i -coupled CCR1 (Bakker et al. 2004). Since HCMV primarily infects leukocytes, smooth muscle, and endothelial cells—in which chemokine receptors play prominent roles—HCMV-encoded receptor expression may alter ligand-induced signaling via these receptors and contribute to the CMV-induced pathology.

US28-mediated signaling (constitutive or ligand-dependent) is accompanied by G protein receptor kinases (GRK)-mediated phosphorylation of the C-terminal tail, which is followed by a rapid constitutive, agonist-independent endocytosis into perinuclear endosomes and recycling of the receptor US28. The observed internalization of US28 is not dependent on the constitutive activity but involves the C-terminal tail, which serves as a docking site for β -arrestins as well as other scaffolding proteins (Brady and Limbird 2002; Heydorn et al. 2004; Lefkowitz and Shenoy 2005). Binding of these proteins appears important for intracellular signaling and/or receptor trafficking. Interestingly, however, US28 internalization via clathrin-coated pits or lipid rafts is independent of β -arrestins but requires AP-2 adaptor complex proteins and dynamin (Fraile-Ramos et al. 2003; Droese et al. 2004).

CC chemokines do not modulate the constitutive signaling of US28 in the InsP, NF- κ B, and CRE assays (Casarosa et al. 2001; McLean et al. 2004), while CX3CL1 acts as inverse agonist in these assays (Casarosa et al. 2001). When US28 loses its capacity to constitutively internalize upon deletion of its C-terminal tail, the CC chemokines, as well as CX3CL1, activate these signaling pathways instead (Waldhoer et al. 2003). Thus, differential modulation of constitutive US28 internalization kinetics and the cellular context in which US28 is expressed determine the efficacy of chemokines acting at this receptor.

The broad chemokine binding profile in combination with rapid and constitutive internalization kinetics (Fraile-Ramos et al. 2001) allows US28 to sequester inflammatory chemokines efficiently from the environment of HCMV-infected cells. As a consequence, the recruitment of leukocytes—and therefore the inflammatory response—may be hampered (Fig. 3; Bodaghi et al. 1998; Billstrom et al. 1999; Randolph-Habecker et al. 2002).

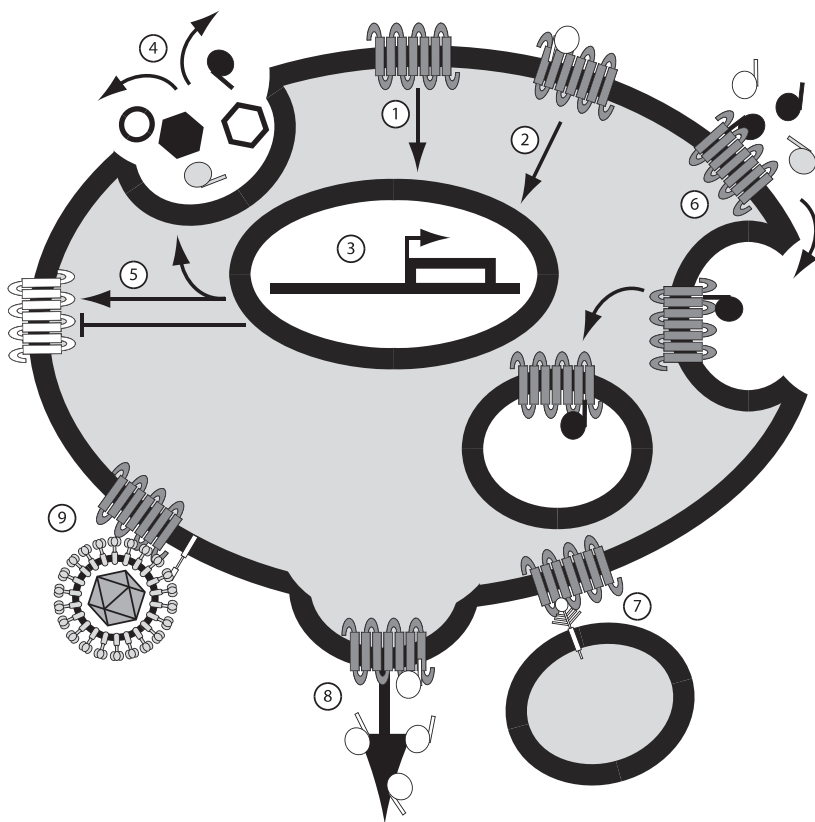


Fig. 3 Suggested roles for vGPCRs. Constitutive (1) or ligand-dependent signaling (2) of a vGPCR results in up-/downregulation of gene expression (3), including autocrine and/or paracrine (angiogenic) factors (4) as well as cellular GPCR proteins (5). Due to their broad-spectrum chemokine binding profile, vGPCRs may serve as chemokine decoy receptors by internalizing bound chemokine, thereby limiting the immune response (6). Binding of vGPCRs (US28) to membrane-associated CX3CL1 facilitates cell-cell adhesion (7). vGPCR-mediated chemotaxis in response to chemokine stimulation may increase viral dissemination and other pathogenesis (8). In addition, vGPCRs may act as HIV coreceptor (9)

The constitutive activity of US28 can be inhibited by a small nonpeptidergic inverse agonist VUF2274 (Casarosa et al. 2003b) derived from a CCR1 antagonist (Hesseltgesser et al. 1998). VUF2274 dose-dependently inhibits US28-mediated constitutive activation of phospholipase C in both transfected and HCMV-infected cells, and US28-mediated HIV entry. Importantly, VUF2274 inhibits CCL5 binding in a noncompetitive manner, thus acting as an allosteric modulator. Although a gain in affinity is required, these inverse agonists will serve as valuable tools to further determine the role of (constitutive activity of) US28 in CMV infection.

For UL33, like US28, constitutive signaling has been reported in transfected and infected cells, while no signaling has been detected for UL78 (Casarosa et al. 2003a). Both UL33 and UL78 still remain orphan. In addition, R33 and M33 are able to signal in a constitutively active manner (Gruijthuijsen et al. 2002; Waldhoer et al. 2002). The constitutive signaling of R33 differs from that of UL33 in that R33 is only able to couple to $G_{i/o}$ and G_q , while UL33 shows activation of the G_q , $G_{i/o}$, and G_s classes.

Taken together, CMVs may effectively use their virally encoded receptors to orchestrate multiple signaling networks within infected cells. Importantly, the immediate-early (IE) promoter of HCMV, constituting the genetic switch for progression of viral infection and reactivation, contains four consensus CRE and four NF- κ B binding sites. Binding of cognate transcription factors to these sites is required for efficient transactivation of the immediate early promoter (Hunninghake et al. 1989; Keller et al. 2003; DeMeritt et al. 2004; Lee et al. 2004). Moreover, NF- κ B is a ubiquitously expressed transcription factor that plays a critical role in the regulation of inducible genes in immune response and inflammatory events associated with e.g., atherosclerosis (Chen et al. 1999). NFAT is an important regulator of immune responses, developmental processes, and angiogenesis (Horsley and Pavlath 2002). It is suggestive to propose that US28 and UL33, through constitutive activation of these transcription factors, induces expression of viral IE proteins and cellular proteins, leading to alteration of the immune response in favor of viral survival and spreading and may contribute by this means to the onset, progression, or enhancement of inflammatory disorders. Further studies in cellular systems more relevant to HCMV infection are required to elucidate the role of these receptors in CMV pathology.

3.2

γ -Herpesvirineae

The γ -Herpesvirineae family is subdivided into the *Lymphocryptovirus* and *Rhadinovirus* genera (Table 1). Although γ -herpesviruses have cell transform-

ing potential, their associated malignancies are mostly seen in the context of immune suppression, such as HIV coinfection or iatrogenic immune suppression, suggesting these viruses are normally “controlled” by the immune system.

3.2.1

Rhadinoviruses

Hitherto, about 48 species of the *Rhadinovirus* genus have been isolated from a wide variety of mammals, of which 8 genomes have been fully sequenced. HHV-8, also known as Kaposi’s sarcoma-associated herpesvirus (KSHV), is the only human rhadinovirus identified to date, and was first discovered in Kaposi’s sarcoma (KS) skin lesion of an AIDS patient (Chang et al. 1994; Cesarman et al. 1995; Renne et al. 1996). In contrast to the ubiquitous (and infectious) dissemination of most other herpesviruses within their natural host populations, HHV-8 displays a rather low infectivity rate and is unevenly distributed among geographically disparate human populations (Hayward 1999). HHV-8 seroprevalence is low (<5%) in the general population of most European, Asian, and American countries, but can range from 10% to 40% in some Mediterranean countries (Hayward 1999) and 40% to 100% in African countries (Dedicoat and Newton 2003). In addition, HHV-8 seropositivity is highly prevalent among homosexual men (Verbeek et al. 1998). HHV-8 establishes lifelong, latent infections in pre- and post-germinal center B cells and endothelial precursor cells (Dupin et al. 1999), which is characterized by the expression of only a limited number of viral genes (Jenner et al. 2001).

While HHV-8 infection of healthy individuals is usually without severe pathogenic consequences, immune suppression (e.g., in AIDS or transplant recipients) can result in impaired control of HHV-8, leading to multifocal angioproliferative KS lesions (Sturzl et al. 1997) and/or lymphoproliferative diseases (primary effusion lymphomas, multimeric Castleman’s disease).

Like other herpesviruses, HHV-8 has captured a cellular gene from its hosts, *ORF74*, which resembles chemokine receptors. The *ORF74* receptor shows significant similarity with the human chemokine receptor CXCR2, known to play an important role in angiogenesis, embryonic development, wound healing, and tissue regeneration. Importantly, constitutive expression and signaling of *ORF74* induce focus formation in stably transfected NIH3T3 cells, which is accompanied by an increased production and secretion of vascular endothelial growth factor (VEGF), a major angiogenesis activator (Bais et al. 1998). Moreover, these *ORF74*-expressing cells form highly vascularized tumors that resemble KS when injected into nude mice (Bais et al. 1998). Likewise, transgenic mice expressing HHV-8-encoded *ORF74* in hematopoietic or

vascular endothelial cells develop angioproliferative KS-like lesions (Yang et al. 2000; Guo et al. 2003; Sodhi et al. 2004c).

ORF74 can constitutively couple to G proteins of the $G_{q/11}$, $G_{i/o}$, and G_{13} classes, thereby modulating a multitude of intracellular signaling pathways, including phospholipases, adenylyl cyclases, kinases, and small G proteins (Arvanitakis et al. 1997; Rosenkilde et al. 1999; Couty et al. 2001; Montaner et al. 2001; Shepard et al. 2001; Smit et al. 2002). Importantly, HHV-8 ORF74-mediated (constitutive) signaling is (partially) increased by angiogenic chemokines [containing a Glu-Leu-Arg (ELR) amino acid motif in the N-terminus]. CXCL8 (ligand for CXCR2) acts as a low-potency partial/neutral agonist (Rosenkilde et al. 1999; Rosenkilde and Schwartz 2000; Couty et al. 2001; Smit et al. 2002; McLean et al. 2004), while the non-ELR, angiostatic chemokines CXCL10 and CXCL12 (ligands of CXCR3 and CXCR4, respectively) and HHV-8-encoded vMIP-II decrease constitutive ORF74 signaling, thus acting as inverse agonists (Fig. 2). Importantly, constitutive signaling by HHV-8 ORF74 as well as chemokine modulation of this constitutive activity are prerequisites for the oncogenic potential of ORF74 in vivo (Holst et al. 2001; Sodhi et al. 2004c). ORF74-mediated modulation of intracellular signaling networks leads to increased transcription of cellular gene and paracrine factors regulating a range of cellular processes including transformation, proliferation, and immortalization (Bais et al. 1998, 2003). HHV-8-ORF74-mediated upregulation and secretion of proangiogenic growth factors and chemokines by lytic cells recruits neighboring cells that can be subsequently infected by released virions (Sodhi et al. 2004a).

Examination of biopsies of KS lesions from AIDS patients revealed high phosphorylated (activated) Akt (PKB) levels, suggesting a critical role for this antiapoptotic serine-threonine kinase in the onset and progression of KS pathology (Sodhi et al. 2004c). Moreover, inhibition of the PI3K/PDK1/Akt pathway prevented proliferation and survival of ORF74-expressing endothelial cells in vitro, and inhibited their tumorigenic potential upon allografting into nude mice (Sodhi et al. 2004c). ORF74 activates Akt by stimulating PI3K through $G\beta\gamma$ -subunits of both pertussis toxin-sensitive and -insensitive G proteins (Montaner et al. 2001), but also via phospholipase C (PLC)-dependent protein kinase C and p44/42 MAPK activation (Smit et al. 2002). In addition, ORF74 activates Akt indirectly in an autocrine manner by upregulating both the expression of VEGF (Bais et al. 1998; Sodhi et al. 2000) and its cognate receptor KDR2 (Bais et al. 2003). Upregulation of growth factors, chemokines, and cytokines in even a few ORF74-expressing cells drives angioproliferative tumor formation by paracrine stimulation of neighboring cells that are latently infected with HHV-8 (Montaner et al. 2003; Jensen et al. 2005). Despite its oncogenic potential, ORF74 is not sufficient for inducing KS in immuno-

competent individuals as indicated by one case of KS in every 17,000 HHV-8 infections. ORF74 is primarily expressed during (early) lytic replication, which occurs in about 3% of the spindle-shaped tumor cells within KS lesions (Kirshner et al. 1999; Sun et al. 1999), whereas the majority of cells in KS lesions are latently infected with HHV-8 (Staskus et al. 1997). Moreover, continuous expression of HHV-8-ORF74 appears to be essential for the progression of KS (Jensen et al. 2005). In this respect, it is puzzling how transient expression of HHV-8-ORF74 in lytic cells can cause KS. However, dysregulated expression of ORF74 under certain circumstances—such as HIV-1 coinfection, inflammation, or aborted lytic cycle progression—has been hypothesized to result in non-lytic (continuous) expression of ORF74 in a fraction of KS tumor cells (Sodhi et al. 2004a). In fact, the KS incidence increases about 10,000-fold in HIV-1-infected man, and 100,000-fold in HIV-1-infected homosexual men (Gallo 1998; Reitz et al. 1999), whereas HHV-8-infected transplant recipients have a 500-fold increased risk in developing KS (Cathomas 2003).

In contrast to the ability of HHV-8-ORF74 to constitutively activate a multitude of signaling pathways by coupling to G_q , G_i , and $G_{12/13}$ proteins (Bais et al. 1998; Munshi et al. 1999; Rosenkilde et al. 1999; Sodhi et al. 2000), the ORF74 proteins encoded by nonhuman rhadinoviruses activate a narrower range of G proteins in a ligand-independent manner. Herpesvirus saimiri-encoded ORF74 (i.e., HVS-ORF74 or ECRF3) signals constitutively via G_i and $G_{12/13}$ proteins, but not through coupling to G_q (Rosenkilde et al. 2004). The ORF74 protein encoded by equid herpesvirus 2 (i.e., EHV2-ORF74) only activates G_i -mediated pathways in a constitutive manner (Rosenkilde et al. 2005), whereas ORF74 of murine γ -herpesvirus 68 (i.e., MHV68-ORF74), also known as *murine herpesvirus 4* (MuHV4), is devoid of any constitutive activity (Verzijl et al. 2004). Both constitutive (G_i and $G_{12/13}$) and non-constitutive (G_q) HVS-ORF74-mediated signaling pathways can be stimulated by CXCL1 and CXCL6, whereas CXCL5 and CXCL8 act as neutral antagonists (Rosenkilde and Schwartz 2004). Interestingly, the non-ELR CXC chemokines that act as inverse agonists on the HHV-8-ORF74, do not bind the HVS-ORF74. Likewise, both human and mouse CXCL1 and CXCL2 stimulates MHV68-ORF78-mediated activation PLC, NF- κ B, p44/p42 MAPK, and Akt, as well as the inhibition of cAMP formation, whereas non-ELR CXC, CC, and CX3C chemokines were ineffective (Verzijl et al. 2004). In contrast to the broad chemokine binding profile of HHV-8-, EHV2-, and MHV68-ORF74, only a single chemokine (CXCL6) binds to EHV2-ORF74, resulting in a further increase of its constitutive G_i -mediated signaling (Rosenkilde et al. 2005).

Despite the apparent lack of constitutive activity, MHV68-ORF74 expression in NIH3T3 cells induces focus formation by these cells (Wakeling et al. 2001). This transforming potency of MHV68-ORF74 may result from consti-

tutively signaling through yet-unidentified signaling pathways (Verzijl et al. 2004). Alternatively, autocrine secretion of mouse CXCL1 (i.e., KC) by NIH3T3 cells (Bosio et al. 2002) may activate the MHV68–ORF74 that is expressed on the cell surface of these cells. In fact, MHV68–ORF74-mediated signaling in response to mouse CXCL1 enhances in vitro viral replication in permissive NIH3T3 cells (Lee et al. 2003). In contrast, disruption of the MHV68–ORF74 gene did not affect in vitro replication of MHV68 in infected NIH3T12 cells, or in vivo replication in spleen and lungs (Moorman et al. 2003). Interestingly, MHV68–ORF74 appeared to be essential for efficient reactivation of MHV68 from latency (Lee et al. 2003; Moorman et al. 2003). Like other ORF74 genes, *MHV68–ORF74* is an early lytic gene but is also expressed in latently infected cells (Kirshner et al. 1999; Sun et al. 1999; Wakeling et al. 2001).

Interestingly, ORF74-encoding genes are absent in two rhadinoviruses: the *bovine herpesvirus 4* and *alcelaphine herpesvirus 1*. In contrast to other members of the *Rhadinovirus* genus that are sequenced to date, the EHV2 genome contains three additional vGPCR-encoding ORFs adjacent to the conserved ORF74 (Telford et al. 1995). Interestingly, the hitherto uncharacterized ORF E6 displays highest sequence identity to the A5 receptors of *alcelaphine herpesvirus 1* and the lymphotropic porcine herpesviruses 1–3. Moreover, this subfamily of rhadinovirus-encoded GPCRs is homologous to the lymphocryptovirus-encoded BILF1 receptors (see the next section). The other two ORFs are gene duplicates that encode the E1 protein. These ORFs are located in the terminal direct repeat elements on both ends of the genome. E1 displays highest sequence identity to members of the cellular CC chemokine receptor family (30%–51%) and poxvirus-encoded CC chemokine receptors (25%–30%). Despite the relatively high sequence identity with a variety of CC chemokine receptors, only the CCR3-specific chemokine CCL11 was able to induce an E1-mediated increase in intracellular Ca^{2+} levels and chemotaxis (Camarda et al. 1999; Fig. 2).

3.2.2

Lymphocryptoviruses

In contrast to other herpesviruses, lymphocryptoviruses (LCV) have only been isolated from “higher primate” species of the infraorder Simiiformes. Hitherto, about 44 distinct LCVs have been identified (Ehlers et al. 2003). The LCV genomes which have been sequenced include those infecting man [i.e., HHV-4 or Epstein-Barr virus (EBV)], common marmoset [(i.e., *calitrichine herpesvirus 3* (CalHV3)], and rhesus macaques [i.e., *cercopithecine herpesvirus 15* (CeHV15)] have been fully sequenced (Table 1).

LCVs are ubiquitous (>90%) B lymphotropic viruses that establish life-long, generally asymptomatic, persistent infections in memory B lymphocytes (Wang et al. 2001). However, the potency of LCV to transform B lymphocytes can result in acute infectious mononucleosis, as well as malignant lymphomas, such as Hodgkin's and Burkitt's lymphomas, and post-transplant/AIDS-associated lymphomas (Middeldorp et al. 2003; Thorley-Lawson and Gross 2004). In addition, EBV has been directly associated with nasal natural killer (NK)-T cell lymphoma, nasopharyngeal and gastric carcinoma, oral hairy leukoplakia, and leiomyosarcoma (Middeldorp et al. 2003; Thompson and Kurzrock 2004). Such lymphomas are thought to arise from proliferating, infected B cells that are blocked in the transition from naïve to memory B cells, and/or are not efficiently eliminated by cytotoxic T cells. Hence, individuals with deficiencies in T cell-mediated immunity (e.g., post-transplant immunosuppression and AIDS) are in particular risk of developing EBV-associated lymphoproliferative diseases (Rivailler et al. 2004; Thorley-Lawson and Gross 2004).

The genome of LCVs contains one gene coding for a vGPCR, which is transcribed in various EBV-positive tumor cells (Beisser et al. 2005). LCV-encoded GPCRs show very limited amino acid sequence identity (<15%) to any cellular GPCR (Table 1). Nevertheless, functional analysis revealed that the EBV BILF1 protein is a functional membrane-associated GPCR that constitutively activates NF- κ B and CRE signaling pathways—both implicated in cell proliferation—in a G_i -dependent manner (Beisser et al. 2005; Paulsen et al. 2005). In addition, BILF1 constitutively inhibits phosphorylation of the RNA-dependent protein kinase, which is important for antiviral responses (Beisser et al. 2005). Hitherto, BILF1 is still considered an “orphan” receptor, and information on its biological relevance is yet unknown.

4

Poxvirus-Encoded GPCRs

The Poxviridae is a family of large, brick-shaped, double-stranded DNA viruses. A characteristic of these viruses is that they replicate in the cytoplasm of infected cells, independent of the host nuclear machinery. Poxvirus infections are characterized by acute febrile illness accompanied by skin lesions that blister and form pockmarks. Infections are often self-limiting. Some species of poxvirus, however, can cause life-threatening infections in certain hosts (e.g., *variola virus* or smallpox infections in human). Most poxviruses are epitheliotropic and transmitted by direct contact or via the respiratory tract (Diven 2001). Many poxviruses are able to infect a range of host species.

Poxviruses may reside in a reservoir host in which viral infection results in mild, subclinical conditions. However, transfer of the virus to a zoonotic host often causes more severe pathologies (McFadden 2005).

The poxvirus family is divided into the Entomopoxvirinae and Chordopoxvirinae subfamilies, which infect insects or vertebrates, respectively (Table 2). Genome analysis and phylogenetic analysis of multiple deduced amino acid sequences divide the *Chordopox* genera in four (to five) main subgroups (Gubser et al. 2004; see Table 2). Interestingly, the genomes of avipoxviruses, capripoxviruses, suipoxvirus, and yatapoxviruses contain one or more putative GPCR-encoding genes (see Table 2).

4.1

Yatapoxviruses, Suipoxviruses, and Capripoxviruses

The *Yaba-like disease virus* (YLDV) contains two genes, *7L* and *145R*, encoding for membrane-associated proteins that display 53% and 44% amino acid sequence identity with CCR8 (Lee et al. 2001). YLDV-encoded 7L protein, but not 145R, displays a similar chemokine binding profile to CCR8, and binds hCCL1, hCCL7, hCCL4, hCCL17, vMIPI, and vMIPII, but not by mCCL1 (Najarro et al. 2003). In addition, 7L couples to G proteins and induces p44/p42 MAPK phosphorylation in response to CCL1 stimulation. Protein expression analyses of YLDV-infected cells revealed that 7L is expressed as early as 2 h postinfection and its expression increases with time. Blocking late gene expression using a viral DNA replication inhibitor resulted in a 26% decrease in 7L protein expression, suggesting that 7L displays both early and late gene expression kinetics.

The mechanism by which 7L exactly interferes with the CCR8-mediated adaptive and innate immune response has not yet been determined. However, considering the upregulation of CCL1 secretion by dendritic cells, mast cells, and dermal endothelial cells in certain skin inflammations (Gombert et al. 2005), resulting in the recruitment of CCR8-expressing T cells and Langerhans-type dendritic cells, it is tempting to speculate that 7L may sequester CCL1 from the environment of infected cells to impair the immune response. In fact, CCR8 appears to be a vulnerable target for viral hijacking, as several viruses specifically target this receptor by mimicking its ligands (e.g., HHV-8-encoded vMIP-I and vMIP-II, and the molluscum contagiosum virus-encoded vMCC-1) or expressing membrane-associated CCR8 mimics. Alternatively, 7L-mediated signaling in response to CCL1 may also activate anti-apoptotic as well as migratory signaling pathways, as observed for CCR8 (Haque et al. 2001; Louahed et al. 2003; Spinetti et al. 2003; Haque et al. 2004), thereby increasing cell survival and viral dissemination.

Genomes of sui- and capripoxviruses contain a single GPCR gene, of which the deduced amino acid sequences display highest sequence identity to CCR8 (Table 2). However, no pharmacological data are yet available for these receptors.

4.2

Avipoxviruses

The genomes of the fowlpox and canarypox viruses of the Avipoxvirus genus contain 3 and 4 ORFs encoding for vGPCRs. These vGPCRs share about 24% sequence identity with some members of CXC chemokine receptor family, but share more identity with GPCR1 and EBV-induced GPCR2. Nevertheless, this unique cluster of avipoxvirus-encoded GPCRs still awaits functional characterization.

5

Conclusions

Exploitation of the chemokine receptor system through molecular mimicry appears to be an effective means to assist viruses in evading immune surveillance, thus contributing to viral dissemination and virus-induced pathology (Fig. 3). Infection of cells and consequent expression of viral chemokine receptors enables them to respond to a broad spectrum of chemokines, evading the immune response or facilitating viral dissemination to areas with increased chemokine expression (Figs. 2 and 3). The ability of the viral chemokine receptors to signal in a constitutively active manner via promiscuous G protein coupling turns them into versatile signaling devices that modulate cellular signaling networks, thereby reprogramming the cellular machinery to modulate cellular function after infection.

Although many attractive roles have been attributed to this class of receptors, little is known about their (patho)physiological potential. The biological significance of ORF74 and the members of the UL33 and UL78 family in the pathogenesis of HHV-8 and CMV infections has been demonstrated *in vivo*. Mouse models and studies using recombinant rodent CMVs that carry a disrupted gene or lack the respective gene (Davis-Poynter et al. 1997; Bais et al. 1998; Beisser et al. 1998, 1999; Yang et al. 2000; Oliveira and Shenk 2001; Guo et al. 2003; Kaptein et al. 2003; Sodhi et al. 2004c; Streblow et al. 2005) indicate a role for these viral receptors in pathophysiology. GPCRs constitute a highly drugable class of membrane-associated proteins, accounting for about 50% of protein targets for therapeutic interventions. In addition, the

awareness that chemokines and their cognate receptors play a prominent role in numerous pathophysiological processes urges the quest for bioavailable small-molecule antagonists that specifically block viral chemokine receptor functioning (Onuffer and Horuk 2002). Small nonpeptidergic compounds inhibiting US28 constitutive signaling can be considered as tools to investigate the role of US28 in CMV pathology and may serve as promising therapeutics for clinical antiviral intervention. Also for the other viral chemokine receptors, however, specific (pharmacological or RNA interference) inhibitors or antibodies targeting these viral chemokine receptors is essential to elucidate the contribution of viral chemokine receptors to viral pathogenesis and reveal their potential as a future drug target.

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