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REVIEW

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Structure, function and physiological consequences of virally encoded chemokine seven transmembrane receptors

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A number of human and animal herpes viruses encode G-protein coupled receptors with seven transmembrane (7TM) segments—most of which are clearly related to human chemokine receptors. It appears, that these receptors are used by the virus for immune evasion, cellular transformation, tissue targeting, and possibly for cell entry. In addition, many virally-encoded chemokine 7TM receptors have been suggested to be causally involved in pathogenic phenotypes like Kaposi sarcoma, atherosclerosis, HIV-infection and tumour development. The role of these receptors during the viral life cycle and in viral pathogenesis is still poorly understood. Here we focus on the current knowledge of structure, function and trafficking patterns of virally encoded chemokine receptors and further address the putative roles of these receptors in virus survival and host -cell and/or -immune system modulation. Finally, we highlight the emerging impact of these receptor on virus-mediated diseases.
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Abbreviations: KSHV, Kaposi's sarcoma-associated herpesvirus; ORF, open reading frame; UL, unique long region; US, unique short region

Introduction

Herpesviruses appear to have taken advantage of the sophisticated endogenous chemokine system of their hosts (Rosenkilde *et al*., 2001; Vischer *et al*., 2006), which comprises over 40 chemokines (*chemo*tactic cyto*kines*, that is, CC and CXC chemokines, one C and one CX_3CL1 chemokine) and more than 16 chemokine receptors (CXCRs, CCRs, CX_3CR and the Duffy antigen) (Murphy *et al*., 2000). During evolution, the viruses may have adopted such chemokine genes from their host and modified them as to benefit their own survival and propagation (Ahuja and Murphy, 1999; Rosenkilde *et al*., 2001; Smit *et al*., 2003; Michelson, 2004). Hence, herpesviruses have been found to not only encode chemokine receptors, chemokines and chemokine-binding proteins but also to modulate the expression of chemokines and their receptors in the host organism (Rosenkilde, 2005). Thus, studying these 'pirated' receptors and how they have been shaped by the virus during evolution provides essential knowledge about (i) their role in viral survival strategies and (ii) some basic principles of seven *T*rans*M*embrane (7TM) receptor function within the rhodopsin-like (class A) receptors.

Pirating the endogenous chemokine receptor system

The majority of virally encoded 7TM receptors are closely related to the family of endogenous human chemokine receptors. Many of these receptors bind endogenous chemokines and subsequently activate downstream signalling pathways. Few receptors, however, do not seem to resemble any of the endogenous 7TM receptor families and are therefore difficult to classify. For these 'orphan' virally encoded 7TM receptors, no ligand has (yet) been identified. Nevertheless, such orphan receptors may still have a functional role, as exemplified by the BILF1 receptor from human and rhesus Epstein–Barr virus (EBV) (Beisser *et al*.,

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2005; Paulsen *et al*., 2005) or the human cytomegalovirus (HCMV)-encoded receptor unique long region 33 (UL33) (Waldhoer *et al*., 2002), which both signal in a constitutive—ligand independent—manner.

Rhodopsin-like 7TM receptors: structure and function

Despite the fact that around 50% of all drugs on the market target 7TM receptors (Drews, 2000; Hopkins and Groom, 2002), we still know very little about the structure of these receptors. In fact, until recently, only the crystal structure of bovine rhodopsin in its inactive, dark state, was available (Palczewski *et al*., 2000; Li *et al*., 2004; Okada *et al*., 2004). However, last month the long awaited crystal structure of a non-rhodopsin 7TM receptor—the beta adrenergic receptor—was published by several groups, also in its inactive state (Cherezov *et al*., 2007; Rasmussen *et al*., 2007; Rosenbaum *et al*., 2007). Despite the lack of a crystal structure of the active form(s) of 7TM receptors, a plethora of functional data—based on biophysical (for example, site-directed spin labelling; Farrens *et al*., 1996; Hubbell *et al*., 2003), chemical (for example, cross-linking studies; Jacobsen *et al*., 2006) or molecular pharmacological approaches (for example, metal-ion site engineering; Elling *et al*., 1997, 2006; Rosenkilde *et al*., 1999, 2006b)—have resulted in a generally accepted model for 7TM receptor activation (Schwartz *et al*., 2006). According to this model, the transmembrane helices 3 (TM-3), TM-6 and TM-7 move away from each other at the intracellular side during receptor activation, creating space for heterotrimeric G proteins, arrestins and/or other adaptor/scaffolding proteins to interfere with the active receptors (Farrens *et al*., 1996; Shi *et al*., 2002; Hubbell *et al*., 2003; Schwartz *et al*., 2006). On the extracellular side, these three helices approach each other, constrained by either intramolecular bonds within the different helices or intermolecular bonds between the ligand (agonist in this case) and the receptor. A highly conserved Pro in TM-6 serves as a pivot for these helical movements during receptor activation. A tryptophane TrpVI:13/6.48 located two residues upstream of ProVI:15/6.50 in the so-called CWxP motif (see Figure 1) has been dedicated a special role in receptor activation. It functions as a rotamer, such as pointing towards TM-7 in the inactive receptor state, while changing conformation and pointing towards TM-5 in the active receptor state (Lin and Sakmar, 1996; Ballesteros *et al*., 2001; Elling *et al*., 2006; Schwartz *et al*., 2006).

Another region of importance for 7TM receptor activation is the ionic lock formed by interactions between the Arg in the highly conserved D/ERY motif at the intracellular end of TM-3 (ArgIII:26/3.50) and the adjacent Asp/Glu and an additional Asp/Glu in the intracellular loop III (IC-3) (see Figure 1; Ballesteros *et al*., 2001). However, 30% of rhodopsin-like 7TM receptors—including all chemokine receptors—contain a positively charged residue at the corresponding position in IC-3, which does not support an interaction with the Arg in the DRY motif. In accordance with this, it was recently shown that the introduction of an acidic residue in substitution with the positively charged residue in IC-3 resulted in a highly impaired CCR5 receptor function (Springael *et al*., 2007). Thus, the molecular interactions locking the inactive state in receptors devoid of this acidic residue in IC-3 must be different.

The highly conserved NPxxY motif in TM-7 (see Figure 1) is involved in receptor regulation through several mechanisms. One is the interaction between the TyrVII:20/7.53 (in the NPxxY motif) with the conserved Phe in helix 8 (located perpendicular to the membrane at the intracellular side) (Fritze *et al*., 2003). Another mechanism is through the highly conserved Asn (in the NPxxY motif) that is oriented towards TM-6 in the inactive state(s), whereas it is oriented towards the middle of TM-2 (towards Asp in position II:10/2.50) in the active state(s) (Govaerts *et al*., 2001; Urizar *et al*., 2005).

Virally encoded 7TM receptors: structure and function

Ligand binding. About 50% of all known virally encoded chemokine receptors have been 'de-orphanized', that is, their chemokine-binding profile is known. Some of these receptors bind a rich repertoire of endogenous chemokines. For instance, open reading frame74 (ORF74) from HHV8 can be activated by ELR^+ CXC chemokines (CXCL1-3; ELR^+ CXC chemokines have a Glu-Leu-Arg (ELR) motif preceding the first Cys in the N terminus of the protein and are considered to promote cell proliferation/angiogenesis), and inhibited by ELR^- CXC chemokines (CXCL10 and CXCL12, ELR^- CXC chemokines lack this motif and are angiostatic chemokines), whereas a third group of ELR^+ CXC chemokines have a Glu-Leu-Arg (ELR) motif preceding the first Cys in the N terminus of the protein and are considered to promote cell proliferation/angiogenesis, whereas a third group of ELR^+ CXC chemokines (represented by CXCL5, −7 and −8) binds to the receptors with no change in receptor activity (Gershengorn *et al*., 1998; Rosenkilde *et al*., 1999). Another example is unique short region 28 (US28) from HCMV, which predominantly binds CC chemokines and the membrane-bound chemokine fractalkine CX_3CL (Kledal *et al*., 1998; Casarosa *et al*., 2001; Waldhoer *et al*., 2002). For both of these receptors, chemokine binding was shown to be dependent upon the receptor N terminus (Ho *et al*., 1999; Rosenkilde *et al*., 2000; Casarosa *et al*., 2005). The C terminus, including helix 8, has been shown to be involved in ligand-binding recognition and signalling in ORF74 from HHV8/Kaposi's sarcoma-associated herpesvirus (KSHV) (Schwarz and Murphy, 2001; Liu *et al*., 2004; Verzijl *et al*., 2006). In contrast to the broad chemokine-binding pattern of ORF74 and US28, other virally encoded receptors such as the U51 and UL12 receptor families (HHV6 and HHV7) interact with only a few chemokines (Isegawa *et al*., 1998; Menotti *et al*., 1999; Bradel-Tretheway *et al*., 2003; Nakano *et al*., 2003; Tadagaki *et al*., 2005, 2007).

The nature of chemokine binding to their cognate receptor(s) is determined by the relatively large size of the chemokines (~65–95 amino acids). Thus, in contrast to the small endogenous 7TM receptor ligands (for example, monoamines) that activate their cognate receptors by binding deep in the main receptor-binding pocket, chemokines more likely interfere with residues located in the extracellular receptor loops (Jarnagin *et al*., 1999; Schwarz and Wells, 2002) and the N terminus of the receptor

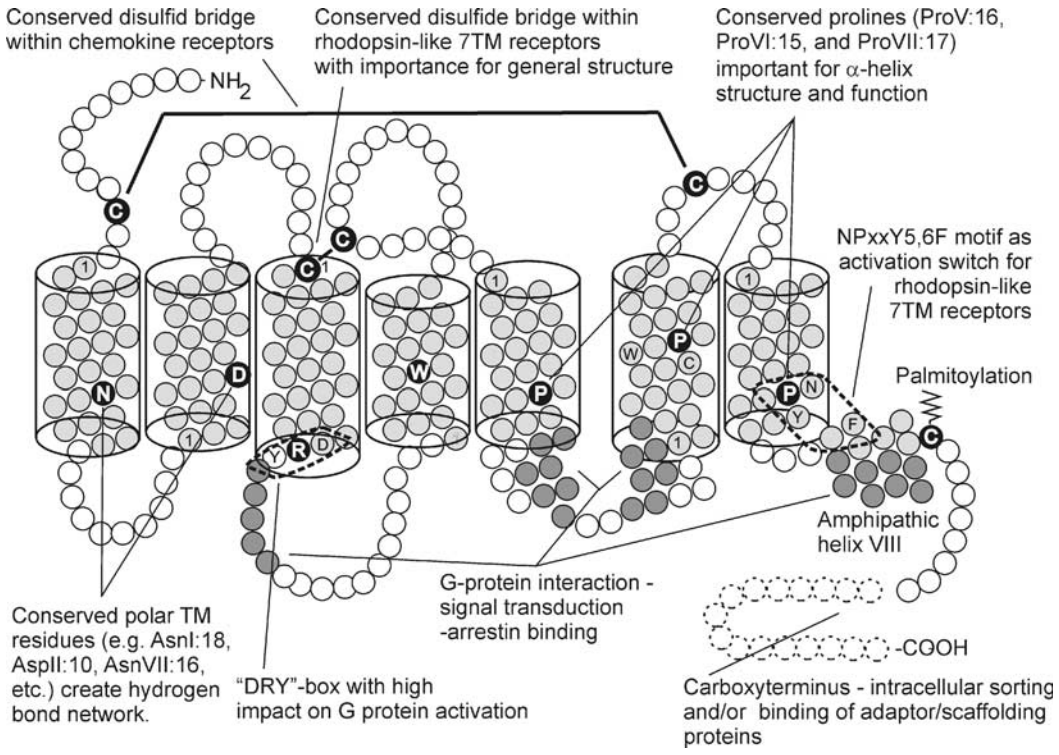

Figure 1 Serpentine model of a rhodopsin-like 7TM chemokine receptor. Black circles with a white letter represent conserved residues in each helix. Residues important for binding of intracellular adaptor/scaffolding or signalling molecules are indicated in grey. Two generally accepted numbering systems for transmembrane-located residues in 7TM receptors are used: (i) the Schwartz-Baldwin (Baldwin, 1993; Schwartz, 1994) numbering system, where each residue is labelled according to its actual position within a transmembrane helix and (ii) the Ballesteros–Weinstein system, which is based on labelling a highly conserved position in each helix with the number 50 (Ballesteros and Weinstein, 1995). For instance, the conserved arginine in the 'DRY' motif at the bottom of TM-3 is given the number III:26/3.50, according to the Schwartz/Ballesteros numbering systems, respectively. This figure was adapted from Schwartz and Holst (2003) with permission.

(Schwarz and Wells, 2002; Rosenkilde and Schwartz, 2006). Upon chemokine binding, one of the main signalling pathways activated by these receptors is that of the pertussis toxin-sensitive Gαi/o proteins, resulting in intracellular calcium release and cell migration towards chemokine gradients (Fitzsimons *et al*., 2006).

Receptor activation. Virally encoded chemokine receptors essentially bear the same structural hallmarks important for receptor activation as endogenous 7TM receptors. However, detailed mutational studies of the ORF74 and US28 receptor families have greatly expanded our understanding of the impact of these different structural motifs on receptor activation. For instance, ORF74 encoded by the equine herpesvirus 2 possesses a 'DTW' (Asp, Thr, Trp) instead of the well-conserved 'DRY' motif at the end of TM-3. Here, this motif is dispensable for ligand-induced as well as constitutive receptor activity (Rosenkilde *et al*., 2005). Nevertheless, other virally encoded receptors—for example, US28 and the UL33-like receptors—strongly depend upon the Arg for proper signalling (Gruijthuijsen *et al*., 2002; Waldhoer *et al*., 2003). Furthermore, mutational and depletion studies of the C-terminal tail—including the 'helix 8' motif (see Figure 1)—of these receptors have revealed its general importance in the regulation of ligand-binding selectivity, putatively through changes in receptor conformation as such. In addition, the C-terminal tail is also implicated in regulating receptor activation, G-protein recruitment and trafficking patterns of the receptors (Schwarz and Murphy, 2001; Mokros *et al*., 2002; Waldhoer *et al*., 2003; Liu *et al*., 2004; Verzijl *et al*., 2006).

Signalling. The constitutive signalling activity observed for ~60% of virally encoded 7TMs stands in stark contrast to endogenous 7TM receptors, where constitutive activity may occur in some instances (for example, the ghrelin, the somatostatin and the EBI2 receptor; Seifert and Wenzel-Seifert, 2002; Rosenkilde *et al*., 2006a). In addition, the constitutive activity displayed by these roughly one-third of rhodopsin-like 7TM receptors seems to be highly dependent on assay conditions (for example, variations in receptor expression, availability of accessory G proteins and the choice of cell type; Seifert and Wenzel-Seifert, 2002). However, constitutive signalling activity is not a common trait for endogenous chemokine receptors (Rosenkilde *et al*., 2001; Thelen, 2001; Smit *et al*., 2003). Most of the virally encoded receptors activate a plethora of different pathways in a constitutive manner, that is, at the level of G proteins, MAP kinases and transcription factors (Waldhoer *et al*., 2002; Rosenkilde, 2005; Smit *et al*., 2007). One such example is the ORF74 receptor encoded by HHV8. This receptor signals constitutively through multiple pathways, including the activation of various G proteins, like, Gαq, Gαi and Gα12/13 (Bais *et al*., 1998; Munshi *et al*., 1999; Rosenkilde *et al*., 1999;

Sodhi *et al*., 2000), MAP kinases including p38, p44/p42 and JNK/SAPK and transcription factors nuclear factor-κB, CREB and NFAT (McLean *et al*., 2004). The multitude of these actions ultimately leads to cellular transformation and production of angiogenic and inflammatory factors (Bais *et al*., 1998; Munshi *et al*., 1999; Sodhi *et al*., 2000; Couty *et al*., 2001; Montaner *et al*., 2001; Schwarz and Murphy, 2001; Smit *et al*., 2002). In addition, vascularized tumours develop when ORF74-expressing cells are injected into nude mice (Bais *et al*., 1998) and transgenic expression of ORF74 results in Kaposi's sarcoma (KS)-like lesions in mice (Yang *et al*., 2000; Guo *et al*., 2003; Montaner *et al*., 2003). Other examples of such promiscuously and constitutively signalling receptors are the UL33 receptor family (UL33, M33 and R33 from human, murine and rat CMV, respectively) and US28 from HCMV (Kledal *et al*., 1998; Casarosa *et al*., 2001, 2003; Waldhoer *et al*., 2002; McLean *et al*., 2004; Sherrill and Miller, 2006).

Trafficking of virally encoded chemokine receptors

Expression–Endocytosis–Recycling–Degradation of 7TM receptors: to live or to die

7TM receptor-mediated signalling is extensively regulated to guarantee an appropriate cell surface receptor density in a given physiological setting. First, receptors have to be properly expressed on the cell surface, hence, a quality control system monitoring the proper folding of these proteins and their transport from the endoplasmic reticulum to the cell surface needs to be in place (Duvernay *et al*., 2005). Once expressed on the cellular surface, receptors bind to ligands and set off intracellular signalling cascades, whereupon they are subjected to the process of endocytosis/internalization. Many 7TMs are endocytosed by a mechanism involving receptor phosphorylation, interaction with β-arrestins and concentration in clathrin-coated pits (Ferguson *et al*., 1998). However, the functional consequences of 7TM receptor endocytosis through this conserved cellular mechanism are diverse. Trafficking of internalized 7TMs by a rapid recycling pathway restores the complement of functional receptors in the plasma membrane and promotes resensitization of receptor-mediated signal transduction. In contrast, the sorting of internalized 7TMs to lysosomes promotes proteolytic downregulation of receptors, leading to a prolonged attenuation of cellular signal transduction (Tsao *et al*., 2001; Bartlett *et al*., 2005). Thus, the sorting of individual receptors between recycling and degradative fates is a fundamental mechanism and highly regulated process that controls the signalling capacity of 7TM receptors. Disturbance of such a tightly controlled system can manifest itself in the onset of diseases and has been described for constitutively active mutant signalling receptors (Parnot *et al*., 2002). Most of these constitutively active receptors are generally also constitutively internalized. To date, for most ligands targeting 7TM receptors, their effects on the trafficking properties of their cognate receptor are unknown. For virally encoded receptors, however, a few studies have addressed this issue in detail (see below; Waldhoer *et al*., 2003).

Several proteins have been identified that specifically target 7TM receptors—typically by interaction with their C-terminal domains—to either recycling (Cao *et al*., 1999; Gage *et al*., 2001; Hanyaloglu *et al*., 2005; Hanyaloglu and von, 2007; Wang *et al*., 2007) or degradative pathways (for reviews see also, Brady and Limbird, 2002; Bockaert *et al*., 2004). One such sorting protein is the *G*PCR-*a*ssociated *s*orting *p*rotein-1 (GASP-1), which targets 7TM receptors to the degradative pathway (Whistler *et al*., 2002; Bartlett *et al*., 2005; Enquist *et al*., 2007; Martini *et al*., 2007; Tappe-Theodor *et al*., 2007; Thompson *et al*., 2007), whereas receptors that do not interact with GASP-1 recycle to the cell surface. In addition, whereas sorting nexin 1—such as GASP-1—has been reported to be involved in targeting 7TM receptors to the lysosomal pathway (Gullapalli *et al*., 2006), the *N*-ethylmaleimide-sensitive factor induces recycling of receptors back to the membrane (Gage *et al*., 2005).

Structural determinants involved in viral 7TM receptor trafficking

It has been shown for many 7TM receptors that their cytoplasmic tail is involved in specifying a receptor's cellular localization, scaffolding and/or trafficking through a variety of mechanisms. For proper surface expression, endoplasmic reticulum export signals have been identified in the C termini of many 7TM receptors (Duvernay *et al*., 2005). For instance, the lack of C-terminal residues R327 and R328 in the rat CMV-encoded receptor R33 results in an intracellularly retained receptor. These RR residues in the so-called RxxxxCxxGxLxxRRxxL motif are conserved among UL33 family members (Gruijthuijsen *et al*., 2002). It has been suggested that these positively charged basic residues are involved in the interaction of 7TMs with negatively charged phospholipid heads in the cellular membrane and/or these motifs may play a role in the interaction between 7TMs and proteins that guide correct 7TM receptor expression.

In addition, the N terminus and extracellular loops of 7TM receptors may require glycosylation to be exported to the cell surface (Duvernay *et al*., 2005). For the murine CMV-encoded receptor M33, it has been demonstrated that a 10 amino-acid stretch at the far N terminus of the receptor needs to be glycosylated to be expressed properly and perform its constitutive signalling properties (Sherrill and Miller, 2006).

Typically, ligand-induced receptor activation results in the phosphorylation of the C terminus, and in most cases, the subsequent recruitment of β-arrestins initiates receptor endocytosis (Ferguson *et al*., 1998). As for the US28 receptor, the C terminus has a high number of serine/threonine residues, providing a broad range of putative phosphorylation sites. Indeed, US28 is a constitutively and highly phosphorylated protein (Mokros *et al*., 2002; Miller *et al*., 2003) and its cytoplasmic tail *per se* is able to confer constitutive receptor endocytosis to even other 7TM receptors (Waldhoer *et al*., 2003; Huttenrauch *et al*., 2005). Interestingly, C-terminal-deficient mutants of US28 display higher constitutive activity than the wild-type receptor and are—in contrast to the wild-type receptor—predominantly expressed on the cell surface (Waldhoer *et al*., 2003). These findings suggest that the cellular localization of US28

(membrane vs intracellular) dictates the degree of its signalling capacity.

Taking a detour: virally encoded 7TMs traffic beyond 'classical' pathways

Despite their similar signalling properties (that is, constitutive activity), virally encoded 7TM receptors can differ dramatically in their cellular localization and trafficking patterns. For instance, whereas ORF74 from HHV8 and murine herpesvirus 68 seem to be located predominantly on the cell surface (Wakeling *et al*., 2001; Waldhoer *et al*., 2003), other viral receptors, such as US28, US27 and UL33, from HCMV can be found in up to 80% in intracellular compartments (Fraile-Ramos *et al*., 2001; Waldhoer *et al*., 2003). The majority of these receptors are found in the membranes of intracellular organelles that are components of the endocytotic pathway, in particular multivesicular late endosomes/lysosomes or multivesicular bodies (Fraile-Ramos *et al*., 2001, 2002). For US28, numerous pathways have been suggested to be involved in its lysosomal targeting. For instance, although US28 is able to recruit arrestins (Miller *et al*., 2003), it has been shown to be constitutively endocytosed completely independent of β-arrestins (Fraile-Ramos *et al*., 2003; Droese *et al*., 2004). Moreover, US28 seems to be able to utilize caveolae/lipid rafts instead of a solely clathrin-dependent pathway (Droese *et al*., 2004) and recent data suggest that GASP-1 might be involved in the lysosomal targeting of this receptor (Heydorn *et al*., 2004 and personal observation).

Functional consequences of viral receptor trafficking

The multiple routes of internalization utilized by a virally encoded 7TM receptor highlight yet again the profound adaptational strategies of viruses: taking such a 'cellular detour'—that is, skipping the most generally regulated processes of receptor internalization by β-arrestins/clathrin-dependent pathways—has not been demonstrated for many 7TM receptors. It was recently demonstrated that HCMV virions were budding into the membranes of these multivesicular endosomes, and it was suggested that this is the place where the viral receptors are incorporated into the viral membranes during the final stages of HCMV assembly (Fraile-Ramos *et al*., 2002). Hence, one might speculate that it poses an advantage for the virus to employ multiple pathways for its proteins to be targeted to places of virion assembly.

In addition, the cellular localization of a virally encoded 7TM receptor can influence the receptor-activating properties of a ligand. More specifically, depending on the receptor's cellular localization, the chemokine fractalkine/CX_3CL1 acts either as an inverse agonist or an agonist on US28. Whereas CX_3CL1 is a partial inverse agonist on the wild-type receptor, it acts as a partial agonist on C-terminal-deficient mutants of US28. This suggests that CX_3CL1 is in fact an agonist on US28, but its agonism is masked by the constitutive endocytosis of the wild-type receptor. By blocking receptor endocytosis (that is, truncation of the C terminus) and consequently increasing the number of receptors on the cell surface at a given time, the true agonist properties of CX_3CL1 are revealed (Waldhoer *et al*., 2003). Indeed, it is tempting to speculate that it might be equally advantageous for a viral protein to be expressed predominantly on the cell surface. For instance, Streblow *et al*. (1999) suggested that US28 mediates the migration of SMCs in response to the chemokines RANTES/CCL5 and MCP-1/CCL2. In this study, US28 was concentrated at the leading edge on the cell surface of SMCs when exposed to a chemokine gradient.

Thus, specific cellular environments could determine the expression and sorting of viral 7TM receptors and ultimately elucidate the functional relevance of this sorting process in viral infection.

Virally encoded 7TM receptors and their link to disease

As outlined in the previous sections, virally encoded 7TM receptors have acquired 'skills' that might contribute significantly to virus-induced pathology, including the following: (i) binding of a broad spectrum of chemokines, (ii) displaying high constitutive receptor activity through promiscuous G-protein coupling or (iii) constitutively trafficking to intracellular compartments favouring virus assembly. Although our knowledge about the structural, signalling and trafficking capacities of these receptors *in vitro* accumulates, their actual role in virus-induced pathology *in vivo* remains vastly unknown (Table 1).

Viruses 'turn' on their hosts

Herpesviruses are widespread pathogens, which establish a life-long latent and persistent infection after primary infection. Although primary infection in immunocompetent hosts is often asymptomatic, reactivation can lead to serious pathological conditions and in some cases to fatal diseases (Vischer *et al*., 2006). For instance, HCMV can cause febrile illness (mononucleosis-like symptoms in seldom cases) (Mocarski, 1995), but in immunocompromised recipients, such as HIV patients, bone marrow and organ transplant recipients, reactivation of HCMV may have a severe impact on lung (interstitial pneumonitis), brain and retinal physiology (Mocarski, 1995; Gandhi and Khanna, 2004). HCMV has also been associated with chronic diseases, including for example, vascular diseases (Stassen *et al*., 2006) and malignancies (Cobbs *et al*., 2002; Harkins *et al*., 2002). Whereas the primary infection of KSHV is asymptomatic, it is associated with three proliferative diseases, that is, KS, primary effusion lymphomas and some forms of multicentric Castleman's disease.

As for EBV, the primary infection generally occurs at an early age and is usually asymptomatic (for references see, Vischer *et al*., 2006). EBV infection acquired during adolescence or later may cause severe—but benign—infectious mononucleosis. The presence of EBV in various stages of B-cell development and its ability to infect certain epithelial cells have been shown to contribute to the development of diverse lymphomas (Hodgkin's disease, Burkitt's lymphoma)

Table 1 Overview of virus-encoded chemokine (and 'orphan') 7TM receptors

Family of virus	*Virus*	*Gene name*	*Receptor type*	*Pharmacology*	*Known or presumed function*	*References*
β-herpesviruses	Human CMV	US27	Receptor (CCR-like)	Constitutively internalizing, no constitutive signalling, no ligands identified	Located on viral envelope	Fraile-Ramos *et al*. (2002); Waldhoer *et al*. (2002); Margulies and Gibson (2007)
		US28	CC and CX3C receptor	Constitutively signalling and internalizing CC and CX3C receptor	Chemokine scavenger function, virus attachment and cell fusion, smooth muscle cell migration, possible oncogene	Kledal *et al*. (1998); Streblow *et al*. (1999); Casarosa *et al*. (2001); Waldhoer *et al*. (2003); Maussang *et al*. (2006)
		UL33	Receptor (CCR-like)	Constitutively signalling and internalizing, no ligands identified	Expressed in viral particles and virus-infected cells	Margulies *et al*. (1996); Waldhoer *et al*. (2002)
		UL78	Putative receptor	Unknown	Unknown	Bankier DNA Seq. 1991 2:1
	Mouse CMV	M33	Receptor (CCR-like)	Constitutively signalling, no ligands identified	Virulence factor, important for viral replication in salivary glands	Davis-Poynter *et al*. (1997); Waldhoer *et al*. (2002); Sherill *et al*. (2006)
		M78	Putative receptor	Unknown	Virulence and replication	Oliveira and Shenk (2001)
	Rat CMV	R33	Receptor (CCR-like)	Constitutively signalling, no ligands identified	Virulence factor, important for viral replication in salivary glands	Beisser *et al*. (1998); Gruijthuijsen *et al*. (2002); Casarosa *et al*. (2003)
		R78	Putative receptor	Unknown	Virulence and replication	Beisser *et al*. (1999)
	HHV6	U12	CCR	Binds CC chemokines, not constitutively active	Unknown	Gompels *et al*. (1995); Isegawa *et al*. (1998)
		U51	CCR	Binds CC chemokines, constitutively signalling	Downregulation of CCL5/RANTES expression	Milne *et al*. (2000); Fitzsimons *et al*. (2006)
	HHV7	U12	Receptor (CCR-like)	Binds CC chemokines	Unknown	Nicholas *et al*. (1996); Tadagaki *et al*. (2005, 2007)
		U51	Receptor (CCR-like)	Binds CC chemokines, not constitutively active	Unknown	Nicholas *et al*. (1996) Tadagaki *et al*. (2005, 2007)
γ1-herpesviruses	Epstein–Barr virus	BILF/A5	Rhodopsin-like 7TM receptor	Constitutively signalling, no ligands identified	Expressed during lytic infection	Paulsen *et al*. (2005); Beisser *et al*. (2005)
γ2-herpesviruses	HHV8 (KSHV) (Kaposi's sarcoma virus)	ORF74-HHV8	CXC receptor	Binding of and regulation by CXC chemokines Constitutively signalling, weakly constitutively internalizing	Lytic expression, possibly involved in Kaposi's sarcoma formation, antiapoptotic, angiogenic, tumorigenic	Bais *et al*. (1998); Rosenkilde *et al*. (1999); Yang *et al*. (2000)
	Herpesvirus Saimiri (HVS)	ECRF3	CXC-receptor	Broad-spectrum CXC chemokine binding, constitutively signalling	Unknown	Ahuja *et al*. (1993); Rosenkilde *et al*. (2004)
	Ateles herpesvirus (AtHV)	ORF74-AtHV	Receptor (CXC-like)	Unknown	Unknown	Albrecht *et al*. (2000)
	Mouse HV68 (MHV68)	ORF74-MHV68	CXC receptor	Broad-spectrum CXC chemokine binding, not constitutively active	Lytic expression, reactivation from latency	Rochford *et al*. (2001); Lee *et al*. (2003); Moorman *et al*. (2003); Verzilj *et al*. (2004)
	Equine HV2 (EHV2)	E1	CC receptor	Binds CCL11/eotaxin, not constitutively active	Unknown	Camarda *et al*. (1999)
		E6	Receptor	Unknown	Unknown	Telford *et al*. (1995)
		ORF74-EHV2	Receptor (CXC-like)	Constitutively signalling, binds CXCL6	Unknown	Rosenkilde *et al*. (2005)

Abbreviations: CMV, cytomegalovirus; ORF, open reading frame; UL, unique long region.
Systematic description of virus family, virus, name of gene, receptor type, pharmacology, known or presumed function and selected references.

and carcinomas (nasopharyngeal and gastric carcinoma) in immunocompetent individuals (Middeldorp *et al*., 2003).

Primary infections with HHV6b and HHV7 often occur in early years of childhood and result in acute febrile illness, which—in some cases—is followed by the appearance of a mild rash. Clinical complications include febrile seizures, but may also cause meningo-encephalitis, encephalopathy and multiple sclerosis (De Bolle *et al*., 2005).

Cellular chemokine receptors not only control differentiation and trafficking of leukocytes but also appear to be implicated in various chronic inflammatory and vascular diseases and oncogenesis (Muller *et al*., 2001; Balkwill, 2004). In view of their crucial role in regulation of the immune system and pathological conditions, a role for the virus-encoded receptors in virus-induced pathology is anticipated.

A delicate balance between host and virus

To establish a persistent infection, the maintenance of a delicate balance between the host's immune system, limiting production of virus particles, and the virus is essential. A disturbed balance, for example, induced by inflammatory processes, can lead to reactivation and propagation of herpesviruses and the initiation and/or progression of, in some cases, fatal diseases (for references, see Rosenkilde *et al*., 2001; Vischer *et al*., 2006). Interestingly, most herpesvirus-encoded receptors bind a broad spectrum of chemokines, and some are able to internalize and deplete chemokines from the cellular surroundings (Billstrom *et al*., 1999). This suggests that these receptors may facilitate immune evasion. In some cases, the expression of viral receptors even leads to the migration of cells towards a chemokine gradient, thus likely contributing to viral dissemination (Streblow *et al*., 1999; Tadagaki *et al*., 2005). Moreover, by means of their constitutive activity and ability to bind chemokines, these receptors may also enhance inflammatory processes, including vascular disease and cancer.

Vascular diseases

Atherosclerosis is no longer considered a disorder of lipid accumulation, but is instead an inflammatory disease accompanied with the infiltration of macrophages, T lymphocytes and increased expression of adhesion molecules and cytokines (Paoletti *et al*., 2004). Increasing evidence indicates an HCMV link in vascular diseases, such as atherosclerosis, restenosis and vascular allograft rejection (Zhou *et al*., 1996; Hsich *et al*., 2001). HCMV is believed to

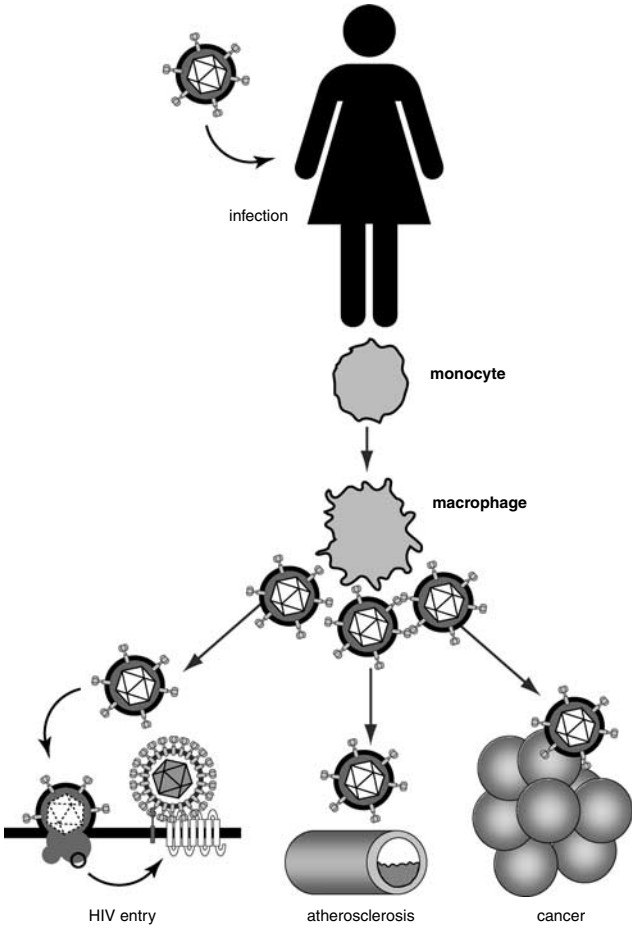

Figure 2 Herpesviruses encode one or more 7TM receptors that are expressed in host cells after viral infection. Some of these receptors act as a HIV co-receptor, whereas others might play a role in the progression of cardiovascular diseases or cancer.

exert its influence on vascular onset of the disease through activation of inflammatory mechanisms.

Vascular SMC migration is of critical importance for the development of atherosclerosis and other vascular diseases. Interestingly, HCMV infection of primary arterial SMCs results in significant cellular migration, which is dependent on US28 expression and CC chemokines (Streblow *et al*., 1999). This might provide a molecular basis for the correlative evidence that links HCMV to the acceleration of vascular disease. Likewise, the murine CMV-encoded M33 receptor appears to be essential for vascular SMC migration. Using siRNAs to eliminate M33 in infected cells leads to an effective attenuation of cell migration (Melnychuk *et al*., 2005). Moreover, the rat CMV-encoded R33 receptor seems to be critical in rat CMV-accelerated transplant vascular sclerosis and chronic rejection, as well as vascular SMC migration (Streblow *et al*., 2005).

HIV

Various epidemiological studies have shown a positive synergism between HIV and CMV infection (Kovacs *et al*., 1999). In early stages of HIV infection, the endogenous chemokine receptor CCR5 is used as co-entry factor, whereas CXCR4 is required in later stages (Ray and Doms, 2006). Interestingly, US28 also exhibits HIV co-receptor activity for both R5 and X4 HIV strains when co-expressed with CD4 (Pleskoff *et al*., 1997).

Oncogenic herpesviruses

It is well established that γ herpesviruses possess oncogenic potential, as they are able to transform cells upon infection (Flore *et al*., 1998). KSHV is the aetiological agent of KS (Ganem, 1997), primary effusion lymphomas and multicentric Casle's disease. EBV is associated with Burkitt's lymphoma and Hodgkin's disease (Young and Murray, 2003). Both γ herpesviruses encode for 7TM receptors, which display constitutively active signalling properties (Arvanitakis *et al*., 1997; Beisser *et al*., 2005). ORF74 is believed to act as a viral oncogene and considered a key determinant in the pathology of KS, as it possesses proliferative, angiogenic and antiapoptotic properties, recognized to drive the cell-transforming properties of KSHV. Vascularized tumours are formed when NIH-3T3 cells expressing ORF74 are injected into nude mice (Bais *et al*., 1998). Furthermore, transgenic mice expressing ORF74 within haematopoietic cells develop KS-like lesions in multiple organs (Yang *et al*., 2000; Holst *et al*., 2001; Guo *et al*., 2003; Montaner *et al*., 2003; Jensen *et al*., 2005). ORF74 was found to trigger a complex angiogenic programme *in vivo* (for example, placental growth factor, platelet-derived growth factor B and inducible NO synthase) contributing to formation of KS lesions in a paracrine manner (Grisotto *et al*., 2006).

Although a strong causative link between ORF74 and KS has been identified, expression of ORF74 is only limited in a subset of lytically infected cells in KS lesions (Kirshner *et al*., 1999; Sun *et al*., 1999). A so-called 'hit and run' mechanism has been proposed, involving ORF74-induced autocrine immortalization of lytically infected endothelial cells, followed by a loss of ORF74 expression and paracrine vascular endothelial growth factor receptor2 activation (Bais *et al*., 2003). Alternatively, dysregulated expression of the viral programme may lead to non-lytic ORF74 expression (Sodhi *et al*., 2004). Recent data suggest that paracrine secretion of angiogenic factors from ORF74-expressing cells is required for sustained tumour growth (Montaner *et al*., 2006). Downregulation of ORF74 expression or targeting its downstream signalling components—such as Akt/PKB or haem oxygenase-1 (Marinissen *et al*., 2006)—in transgenic ORF74 mice results in the regression of KS lesions (Grisotto *et al*., 2006). Hence, it is feasible to target ORF74 or linked signalling pathways for potential treatment of KS.

The EBV-encoded receptor BILF1 has only recently been identified. Its ability to constitutively activate signalling pathways linked to proliferation (nuclear factor-κB), as well as its ability to downmodulate cellular antiviral responses, suggests oncogenic potential (Beisser *et al*., 2005; Paulsen *et al*., 2005). However, detailed analysis of this receptor in, for example, various B-cell stages and preferably transgenic animal models expressing BILF1 is yet required to evaluate its contribution to EBV-associated lymphomas and carcinomas.

Unlike these oncogenic viruses, HCMV infection fails to transform susceptible cells. Nevertheless, HCMV has been suggested to possess onco-modulatory properties (Soderberg *et al*., 1996; Cinatl *et al*., 2004). Although the causative role for HCMV in the development of malignancies remains to be established, various HCMV proteins have been detected in tumour tissues with high frequency (Cobbs *et al*., 2002; Harkins *et al*., 2002). HCMV enhances the malignant behaviour of tumour cells by upregulating different growth factors and cytokines, resulting in increased cell survival, proliferation and angiogenesis (Cinatl *et al*., 2004). Hence, HCMV is considered an onco-modulatory rather than an oncogenic virus.

Recent studies revealed that the HCMV-encoded receptor US28 induces a pro-angiogenic and transformed cellular phenotype in NIH-3T3 fibroblasts by upregulating the expression of the vascular endothelial growth factor. *In vivo*, US28-expressing fibroblasts promote tumorigenesis in a nude mouse model (Maussang *et al*., 2006). However, the constitutively inactive mutant R129A of US28 (Waldhoer *et al*., 2003) induces delayed and attenuated tumour formation, indicating the importance of constitutive receptor signalling activity in the early onset of tumour development (Maussang *et al*., 2006). In addition, US28 expression resulted in an angiogenic phenotype in HCMV-infected glioblastoma cells (Maussang *et al*., 2006). The constitutive activity of US28 and its ability to bind a variety of CC chemokines might facilitate tumour progression after infection, as chemokine levels have been shown to be markedly increased in certain types of cancer (Balkwill, 2004). However, the expression of US28 does not induce or enhance transformation in all cell types. For instance, US28 induces rapid apoptosis in a variety of cell types (Pleskoff *et al*., 2005), indicating that the cellular context dictates the behaviour of this viral receptor. Moreover, US28—when expressed in two different melanoma cell lines—prevents (Seidl *et al*., 2006; Schaider *et al*., 2007) rather then promotes (Maussang *et al*., 2006) tumour formation in mice.

Translational studies—spanning from detailed characterization of virus receptor signalling and ligand binding to the putative roles of these receptors in virus-induced pathology—are required to gain further insights into this intriguing class of receptors. Given the huge knowledge—and the many successes—in drug discovery and development within the rhodopsin-like 7TM receptors, it is tempting to suggest that the receptors encoded by human herpesviruses may serve as innovative and novel drug targets.

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Conflict of interest

The authors state no conflict of interest.

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