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REVIEW

Emerging roles of cytomegalovirus-encoded G protein-coupled receptors during lytic and latent infection

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Abstract

Cytomegaloviruses (CMVs) have developed multiple diverse strategies to ensure their replicative success and to evade immune recognition. Given the fact that G protein-coupled receptors (GPCRs) are key regulators of numerous cellular processes and modify a variety of signaling pathways, it is not surprising that CMVs and other herpesviruses have hijacked mammalian GPCRs during their coevolution. Human cytomegalovirus (HCMV) encodes for four viral GPCR homologues (vGPCRs), termed US27, US28, UL33, and UL78. Although HCMV-encoded GPCRs were first described in 1990, the pivotal functions of these viral receptor proteins were detected only recently. Here, we summarize seminal knowledge on the functions of herpesviral vGPCRs with a focus on novel roles of cytomegalovirus-encoded vGPCRs for viral spread and the regulation of latency.

Keywords Cytomegalovirus · G protein-coupled receptor (GPCR) · Chemokine receptor · Cell signaling · US28

Introduction

G protein-coupled receptors (GPCRs), also termed seven-transmembrane receptors, constitute the largest and most diverse family of eukaryotic membrane receptors involved in signal transduction. Noteworthy, a tremendous diversity of physiological processes is substantially regulated by GPCRs, such as neuro-transmission, angiogenesis, cell proliferation, and death, as well as activation and suppression of the immune system [1–5]. Hence, it is not surprising that GPCRs have emerged as crucial players in viral entry and spread, modulation of the host immune system, and assurance of cell survival after infection. Moreover, various herpes- and poxviruses encode homologous versions of cellular GPCRs (vGPCRs), which contribute to the dysregulation of cellular signaling processes [6–11]. Whereas most of these viruses express one or two vGPCRs, human cytomegalovirus (HCMV) encodes four vGPCRs, termed US27, US28, UL33, and UL78 [7, 12]. Although vGPCRs are phylogenetically related to human seven-transmembrane receptors, they exhibit considerable differences in characteristics including their sorting and signaling capacities. Since vGPCRs emerge as important determinants of viral pathogenicity, there is growing interest to utilize the pharmacologic targeting of vGPCRs as a novel antiviral principle.

Structure and function of cellular G protein-coupled receptors

With more than 800 members, G protein-coupled receptors (GPCRs) constitute the largest and most diverse family of membrane receptors [13]. Due to their role as key regulators of numerous cellular processes, GPCRs exhibit central relevance to the current clinical practice of medicine. As highly valued drug targets, 50–60% of all currently available pharmaceuticals modulate GPCR functions, directly or indirectly. The wide range of therapeutic effects thereby counteracts various symptoms including pain, allergic rhinitis, schizophrenia, or hypertension [14–16].

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GPCRs are located at the cytoplasmic membrane where they mediate cellular responses to a multitude of signals, such as neurotransmitters, hormones, calcium ions, chemokines, sensory stimuli, or nucleotides [17, 18]. Interestingly, for some GPCRs, the so-called orphan receptors, no ligands have been identified so far, however, they often modulate activities of other receptors through the formation of heteromers [19, 20]. Although it was long believed that GPCRs function as monomers, recent reports have highlighted that GPCR activation is not restricted to ligands, but can also occur via interaction with another receptor [21]. The activation of a GPCR by extracellular stimuli or multimerization induces a conformational change of the receptor, which in turn activates an intracellular signaling cascade [22]. Even though GPCRs regulate an enormous diversity of cellular mechanisms, they share a common structure, which is highly conserved in many eukaryotes. GPCRs, also termed seven-transmembrane-spanning receptors, consist of seven-transmembrane-spanning α-helical segments, which are connected via three intracellular loops (i1, i2, i3) and three extracellular loops (e1, e2, e3) [23]. Disulfide bonds between conserved cysteine residues in the extracellular loops e1 and e2 stabilize the structure of a GPCR [24]. Whereas the C-terminal domain is localized intracellularly to interact with effector molecules, the N-terminal tail, which is usually glycosylated, faces the extracellular environment and possesses an important function in ligand binding [24]. Due to the presence of conserved amino acid residues, the superfamily of GPCRs is subdivided into five main families, named glutamate, rhodopsin, adhesion, frizzled/taste2, and secretin GPCRs [1].

Heterotrimeric G proteins are the common factor to transmit signals of GPCRs across the plasma membrane to activate intracellular signaling pathways [18, 25]. A highly conserved Asp-Arg-Tyr (DRY) motif in the C-terminal region of the third transmembrane domain of GPCRs is thought to be the crucial site of G protein binding [26]. G proteins consist of three subunits termed Gα, Gβ, and Gγ. The Gα subunit binds either guanosine diphosphate (GDP) in its resting state or guanosine triphosphate (GTP) when activated. Upon agonist binding, a conformational change of the 7TM occurs, which is followed by an exchange of GDP to GTP and the dissociation of the Gα-subunit from the Gβ and Gγ subunits, which are closely bound to one another, referred to as Gβγ [27]. The Gβγ and Gα subunits of the G protein stimulate a vast number of effector molecules, whose nature highly depends on the Gα subunit specificity of the respective GPCR [28]. Gα proteins are classified into four subfamilies, termed $G\alpha_s$, $G\alpha_i$, $G\alpha_q$, and $G\alpha_{12}$. Stimulation of $G\alpha_s$ is known to activate the adenylyl cyclase (AC) and to increase levels of cyclic adenosine monophosphate (cAMP). In contrast, activated $G\alpha_i$ proteins inhibit the induction of AC. Moreover, $G\alpha_{12}$ leads to an activation of the Rho family of GTPases and through $G\alpha_q$ coupling, GPCRs induce phospholipase C (PLC) activity and, consequently, cleavage of phosphatidylinositol biphosphate (PIP_2) into diacylglycerol (DAC) and inositol triphosphate (IP_3) as well as the NF-κB pathway [29–31]. Notably, although the exact mechanism of NF-κB activation by GPCRs remains largely elusive, recent reports have shown that the signaling cascade involves the scaffold protein CARMA3 together with the BCL10/MALT1 complex [32, 33]. Besides, signaling can result from activated Gβγ, which induces the activity of phospholipases, ion channels, or lipid kinases [27]. Interestingly, in addition to this "classical" transduction pathway, GPCRs have been demonstrated to activate G protein-independent signals via further scaffold proteins including arrestins [34].

Herpesviral vGPCRs

The appearance of viral G protein-coupled receptors (vGPCRs) in genomes of herpesviruses is probably a result of viral hijacking during coevolution with the respective hosts. While α-herpesviruses do not encode GPCR homologues, the β-herpesviral genomes contain several vGPCRs and the γ-herpesviral genomes contain at least one vGPCR (Fig. 1). The respective proteins resemble human chemokine receptors in structure and function and are highly suspected and reported to promote immune evasion and viral dissemination [35]. Chemokine receptors are classified according to the specific subclass of chemokines that they respond to in CXC receptors (CXCR1-6), CC receptors (CCR1-10) as well as the CX3CR1 and XCR1 receptors. Chemokines are chemotactic cytokines and comprise the largest family of cytokines, consisting of 43 so far identified endogenous chemokine ligands in humans. They are divided into four families: C, CC, CXC and CX3C chemokines, which is based on the number and arrangement of conserved cysteine residues in the N-terminus of chemokines that form disulfide bonds for stabilization of the biologically important tertiary structure. The γ-herpesvirus Kaposi's sarcoma-associated herpesvirus (KSHV) infects mainly endothelial cells but also B cells, monocytes, macrophages and DCs [36, 37]. Latent infection is mainly asymptomatic, but may manifest as Kaposi's sarcoma (KS) lesions, which are highly vascularized neoplasms often visible as red patches on the skin. KSHV encodes a single vGPCR, named ORF74, which shows highest sequence homology to human CXCR2. Interestingly, ORF74 binds a broad range of human chemokines including CXCL1-8, CXCL10, CXCL12, CCL1 and CCL5 [9] (Fig. 1). ORF74 is expressed in KS lesions and was identified as an important factor for the initiation and development of KS as suggested by experiments in transgenic mice [38]. Also, the genome of Epstein–Barr virus (EBV) encodes for one GPCR homologue, referred to as BILF1,

Subfamily	α-herpesvirus	β-herpesvirus								γ-herpesvirus	
Virus	HSV1, HSV2, VZV	HCMV				HHV6		HHV7		EBV	KSHV
vGPCR	none	US27	US28	UL33	UL78	U12	U51	U12	U51	BILF1	ORF74
CKR homolog		CXCR3 (21%)	CX3CR1 (35%)	CCR10 (21%)	CXCR1 (5%)	CCR10 (19%)	CCR7 (4%)	CX3CR1 (20%)	CCR2 (2%)	CXCR4 (15%)	CXCR2 (27%)
Ligands (endogenous agonists and inverse agonists)		not known	CCL2 CCL3 CCL4 CCL5 CCL7 CCL11 CCL13 CCL26 CCL29 CX3CL1	not known	not known	not known	not known	not known	not known	not known	CXCL1-8 CXCL10 CXCL12 CCL1 CCL5
Signaling: constitutive		?	yes	yes	?	?	?	?	?	yes	yes
ligand-induced		?	yes	?	?	?	?	?	?	?	yes

Fig. 1 Viral GPCRs encoded by human herpesviruses. While herpesviruses of the β- and γ-subfamilies encode at least one viral GPCR (vGPCR), the genomes of α-herpesviruses do not contain genes with homology to cellular GPCRs. The vGPRS show a variable degree of sequence identity to cellular chemokine receptors (CKR). The CKR homolog with closest sequence identity is indicated (percentage amino acid identity is given in brackets, data taken from [35]). The most important ligands of the respective vGPCRs are also listed. Furthermore, the scheme summarizes whether a vGPCR signals in a constitutive and/or a ligand-induced manner

which is transcribed as an early gene. EBV infects mainly B cells and epithelial cells and was one of the first discovered human tumor viruses. BILF1 shares highest sequence homology with CXCR4 and constitutively couples to $G\alpha_{i/o}$ proteins but no ligands have been identified to target BILF1 hitherto (Fig. 1). Several reports suggest that BILF1 was evolved by EBV as versatile immunomodulatory protein to promote viral persistence. BILF1 reduces the levels of MHC class I at the cell surface to inhibit CD8(+) T-cell recognition of infected cells [39]. Furthermore, BILF1 inhibits the phosphorylation of RNA-dependent protein kinase R (PKR), thus antagonizing this important innate defense mechanism [40]. The roseoloviruses HHV-6A, HHV-6B and HHV-7 also encode vGPCRs: the early gene products U12 and U51, which are expressed late during infection [41, 42]. HHV-6A/B-encoded U12 shares highest homology with CCR10, while HHV-7-encoded U12 is most similar to CX3CR1. U51 of HHV-6 shares the highest homology with CCR7, while U51 from HHV-7 is closest to CCR2 (Fig. 1). However, their functions during lytic infection or latency are largely unknown [35].

Functions of cytomegalovirus-encoded GPCRs

HCMV encodes four GPCR homologues, termed US27, US28, UL33, and UL78 [7, 12, 43] (Fig. 1). While UL33 and UL78 exhibit a high conservation among all cytomegaloviruses, US27 and US28 are restricted to primate CMVs. Many reports have demonstrated that expression of these vGPCRs is not essential for viral replication in vitro [44, 45]. Furthermore, due to the strict specificity of CMVs for their respective hosts, the in vivo characterization of the role of HCMV-encoded vGPCRs is hampered. Thus, initial studies concentrated on the definition of ligand binding and signaling activities of the HCMV vGPCRs using cell culture models.

US28 is the most intensely studied HCMV-encoded vGPCR and seems to be a versatile viral tool (recently reviewed in [46]). US28 is the only HCMV-encoded vGPCR that responds to different human chemokines like CCL2 (MCP1; monocyte chemoattractant protein 1), CCL3 (MIP-1α; macrophage inflammatory protein 1-alpha), CCL4 (MIP-1β; macrophage inflammatory protein 1-beta), CCL5 (RANTES; regulated on activation, normal T cell expressed and secreted) or CX3CL1 (fractalkine) and induces various signaling pathways in response to promiscuous G protein-dependent signaling (Figs. 1, 2) [47–50]. Responses to its chemokine ligands include induction of a calcium flux due to PLCβ or ERK2 activation and induction of SMC migration via SCR- or RhoA-dependent signaling [48, 51–53]. However, US28 was also described to signal constitutively in a ligand-independent manner which activates PLCβ and NF-κB, STAT3, NFAT and CREB via interaction with $G\alpha_q$ or $G\alpha_i$ as well as Gβ/Gγ subunits [54–57]. Parts of these signaling events seem to promote proliferative signals during lytic HCMV infection thereby associating US28 with vascular diseases and potential oncomodulatory effects [8, 51, 52]. US28 undergoes fast and constant endocytosis and

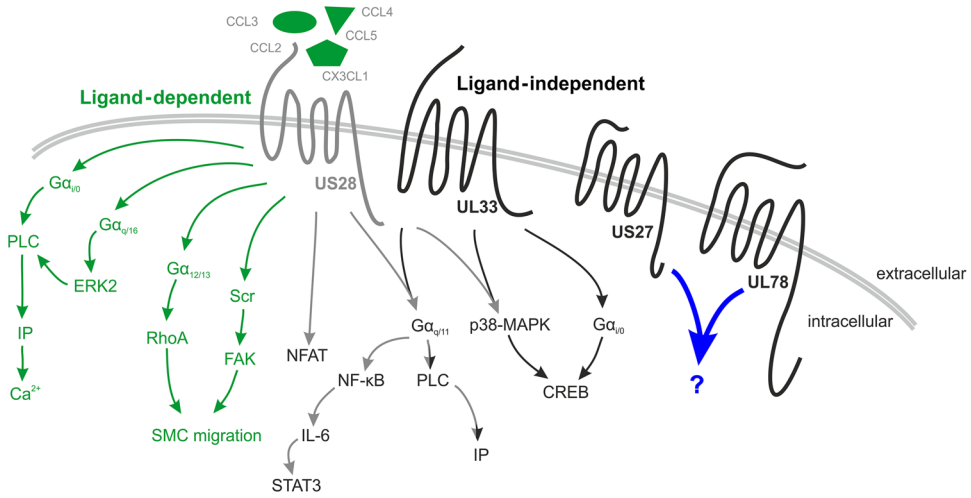

Fig. 2 Schematic representation of the ligand-dependent and ligand-independent signaling activities of US28 as well as the ligand-independent signaling activities of UL33. Stimulatory effects are indicated by arrows, green for ligand-dependent signaling of US28, gray for the ligand-independent signaling of US28, and black for ligand-independent signaling of UL33. Gray/black arrows display similar activities of US28 and UL33. Blue color displays unknown signaling capacities of US27 and UL78. Modified according to [64–66]

recycling and is therefore mostly found in intracellular vesicles in vitro [58, 59]. In this context, US28 was also identified to act as a chemokine sink by binding and internalizing chemokines thereby withdrawing them from the environment of infected cells [45, 53, 60, 61]. Meanwhile, structural details for chemokine recognition are available, since the crystal structure of US28 in complex with the chemokine domain of human CX3CL1 could recently be solved [62]. This is an important step forward since it allows for the structure-based discovery of US28 small molecule ligands that could be used for pharmacological modulation of this vGPCR [63].

So far, no ligands for the vGPCRs US27, UL78, and UL33 could be identified. Thus, these proteins are still orphan receptors (Figs. 1, 2). UL33 and its homologues in mouse (M33) and rat CMV (R33) are able to activate several signaling pathways in a constitutive manner [67–70]. For the rodent homologues M33 and R33, a critical role for infection of the host could be demonstrated [71–73]. Gene-knockout viruses were used to show that deletion of M33 or R33 resulted in less virulent CMV variants which no longer replicated in salivary glands. Intriguingly, the in vivo replication defect of an M33-deficient virus was partially complemented by UL33 and US28. This strongly suggests that the respective vGPCRs share biological functions [74].

In contrast to US28 and UL33, the signaling activities of US27 and UL78 are not well characterized. US27 and US28 lie directly adjacent to each other in the viral genome and share 31% sequence identity. The results of a recent phylogenetic analysis indicate that the human chemokine receptor CX3CR1 served as the common ancestor and subsequent gene duplication gave rise to two vGPCRs which evolved distinct functions during virus infection [75]. Since US27 is heavily glycosylated and possesses two conserved cysteine residues in the second and third extracellular loop, a potential involvement of US27 in chemokine binding appears possible, however, no ligands could be identified so far [76]. Expression of US27 in multiple cell types is described to result in two remarkable phenotypes. First, US27 expression enhances cell proliferation and survival, which was linked to suppression of negative growth regulators [77]. Second, in contrast to UL33 and UL78 that are described to reduce CXCR4 receptor functions, US27 increases CXCR4 expression levels and CXCL12-promoted signaling of CXCR4 [78, 79]. Alterations of CXCR4 internalization dynamics in the presence of US27 resulting in prolonged intracellularly located CXCR4 with delayed recycling kinetics were suggested as the mechanism for this observation [80]. Regarding its subcellular localization, US27 is mainly associated with the endosomal machinery and undergoes constitutive endocytosis in transient and stable transfection systems [81]. Interestingly, the C-terminal domain of US27 was suggested to confer the primarily intracellular localization of US27 [82]. However, the function of US27 during infection is poorly understood. The *US27* gene is highly conserved among HCMV strains including clinical isolates and laboratory strains indicating an important role during infection [75, 83]. Viral mutants lacking the *US27* gene are replication competent but are limited to spreading from cell-to-cell rather than by the extracellular route [84]. The localization of US27 in the viral envelope is consistent with this finding and suggests a role during entry or egress [81]. Upon de novo synthesis, US27 is expressed with late expression kinetics. Starting at 48 hpi, US27 is mainly found in the perinuclear structures associated with the cVAC [81, 85]. A recent approach to identify novel signaling capabilities of the HCMV-encoded vGPCRs with special interest in the poorly characterized US27 and UL78, revealed a strong and so far undescribed NF-κB activation by US27 which is assumed to be relevant for viral dissemination [86].

Knowledge about the role of UL78 during viral infection is limited. Studies using UL78-deficient viral strains

indicated a role of this vGPCR for viral entry and efficient replication in epithelial cells but not in fibroblasts [87]. Furthermore, the rodent homologues M78, encoded by murine CMV and R78, encoded by rat CMV, were utilized to investigate the relevance of this vGPCR for viral pathogenesis in vivo [88–90]. Deletion of R78 from rat cytomegalovirus (RCMV) strains resulted in considerably higher survival compared to wild-type (wt)-infected animals [88]. In addition, studies using the murine cytomegalovirus (MCMV) homologue M78 revealed that UL78 might be involved in tissue tropism, since deletion of M78 did not affect dissemination in general but attenuated the replication of MCMV in the salivary glands of infected mice [89]. Similar to the three other vGPCRs of HCMV, UL78 undergoes constitutive internalization [91]. Wagner et al. reported that UL78 can be detected at the plasma membrane but it mainly localizes to the endoplasmic reticulum. Furthermore, using several in vitro assays, they were able to demonstrate that UL78 forms heteromers with US28. This appears to be important for a silencing of US28-mediated activation of NF-κB-dependent gene expression by UL78 [92]. Besides, UL78, together with UL33, has been shown to disturb the cell surface expression, ligand-induced internalization, and signal transduction of the cellular chemokine receptors CCR5 and CXCR4, which was caused by receptor heteromerizations [79]. Consequently, UL78, similar to US27, seems to be more than just an orphan receptor and requires further investigation to unravel its main purpose for efficient HCMV replication.

Role of CMV-encoded vGPCRs for viral spread via the regulation of DC migration

One recently emerging role of vGPCRs with high importance for pathogenesis pertains to the regulation of dendritic cell migration during viral dissemination [93]. Already in 2008, it was detected that mutations of the murine CMV vGPCR M33 which abrogate constitutive signaling result in significantly diminished MCMV infection of the salivary glands [73]. UL33 of HCMV was shown to be able to functionally substitute for M33 in vivo suggesting conserved biological roles of these vGPCRs. A further characterization of the phenotype of M33 knockout viruses revealed an additional attenuation for infection of the spleen and pancreas as well as a severe defect in reactivation from latency indicating tissue-specific functions of M33 during infection [71]. Although infection experiments conducted in immunocompromised mice suggested that M33 only plays a role for viral amplification once the virus reaches the salivary gland, recent studies indicate a more profound role of M33 dictating efficient viral dissemination [94, 95]. Farrell and colleagues reported that following intranasal infection, MCMV migrates via lung $CD11c^+$ dendritic cells (DC) to lymph nodes (LN), blood and then salivary glands. This route requires that infected dendritic cells do not only enter LNs but they also need to recirculate into blood to mediate dissemination. However, although traffic to draining LNs is well recognized, DCs usually do not leave LNs but are thought to die locally following arrival in LNs [96]. This suggested that MCMV has evolved specific mechanisms to stimulate the exit of infected DCs from LNs thus fostering viral dissemination. It could be shown that MCMV-infected DCs exited LNs not via efferent lymph but via specialized vessels, the so-called high endothelial venules (HEV) which represent major sites of extravasation of lymphocytes from the blood into the LN via a multistep adhesion process (Fig. 2) [97]. As cell migration is controlled by chemokines, viral chemokine receptor homologues were suspected to potentially mediate the LN traverse of infected DCs. Indeed, infection experiments with MCMV harboring a knockout of the chemokine receptor M33 resulted in an accumulation of infected DCs in LNs.

Consequently, viral spread was greatly reduced [97]. Since an M33 point mutant lacking G_q signaling exhibited an identical phenotype, the constitutive signaling activity of M33 appears to be required to promote the exit of infected DCs from LNs via HEVs (Fig. 3). Furthermore, US28 could substitute for M33 in promoting DC recircularization while signaling-deficient US28 was inactive [97]. This suggests that a similar mechanism of vGPCR-driven viral dissemination via DC recircularization which may function through the downregulation of DC retention signals could also be true for HCMV infection. In summary, these experiments indicate a key role for cytomegalovirus vGPCRs in systemic viral spread.

Regulation of HCMV latency by the vGPCR US28

Several studies reported that the HCMV-encoded vGPCR US28 is expressed during lytic infection and latency [98, 99]. However, its function during latency has remained undefined for a long time and is only recently beginning to be elucidated. Humby and O'Connor were the first to demonstrate that US28 was required during latency either in an in vitro latency model or using primary ex vivo-cultured CD34(+) hematopoietic progenitor cells (HPCs) [100]. They observed that infection of CD34(+) HPCs with viruses lacking the *US28* gene resulted in transcription from the major immediate early promoter and the production of infectious virus. Mechanistically, it is suggested that US28 attenuates mitogen-activated protein kinase (MAPK) and NF-κB signaling as well as c-fos expression in latently infected cells, which is required for suppression of the HCMV major

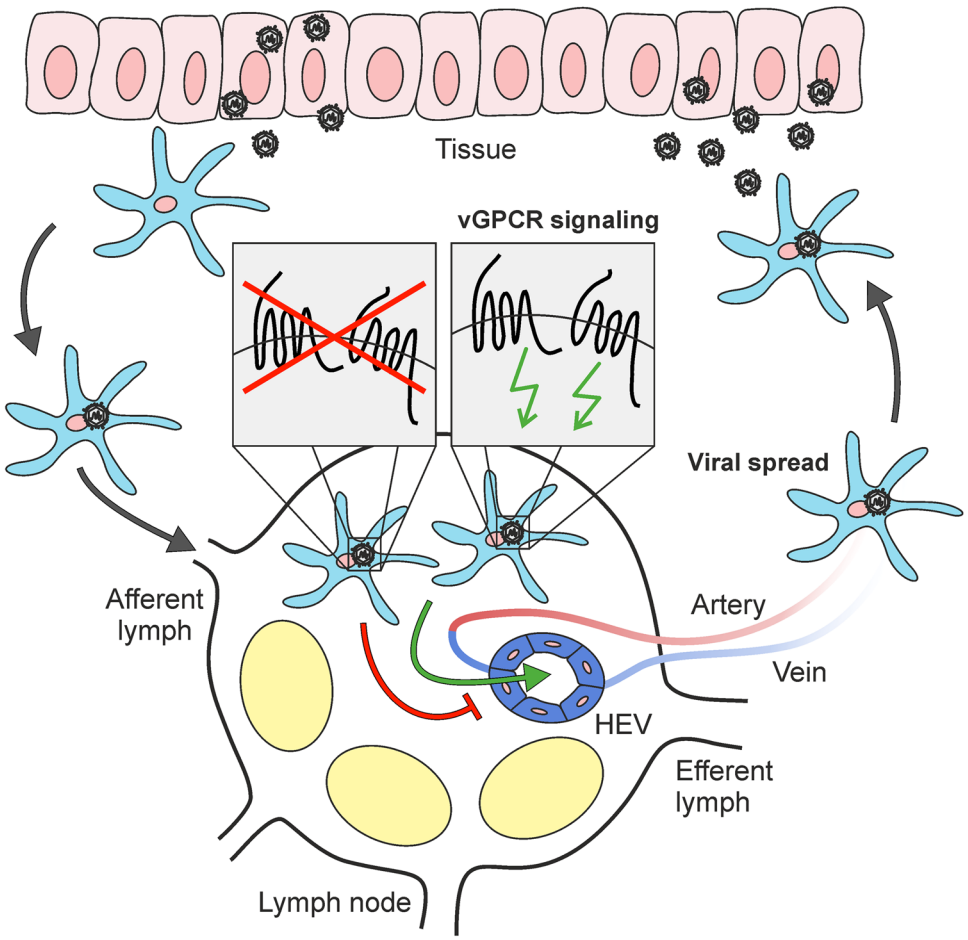

Fig. 3 Model summarizing a novel role of vGPCRs for viral spread via DC recirculation. CMV-infected DCs migrate to draining lymph nodes. In the absence of vGPCR signaling, DCs are retained in lymph nodes. In the presence of vGPCR signaling, infected DCs can enter high endothelial venules (HEV) to reach the blood and to mediate viral spread to other tissues. Modified according to [95]

immediate early promoter (MIEP) to prevent lytic infection [101, 102]. This is unexpected since previous studies reported that a similar set of signal transduction pathways is utilized by US28 to activate the MIEP upon infection of permissive cell types [103]. This strongly stresses the cell-context dependency of vGPCR signaling. An alternative mechanism was proposed by Zhu et al.: they described a requirement for US28 to activate the STAT3-iNOS-NO axis which reprograms HPCs into a unique monocyte subset to achieve latency [104]. Thus, although different studies concordantly demonstrate a requirement for US28 signaling to maintain HCMV latency in CD34(+) progenitor cells and CD14(+) monocytes, the exact way how US28 generates a host environment conducive to latency requires further investigation.

Importantly, however, those studies strongly support the notion that US28 might serve as an appealing drug target during latency. On the one hand, inverse agonists may be used to interfere with US28 signaling. This induces viral reactivation in latently infected cells [101]. Consequently, cytotoxic T-cell killing of these normally immunologically undetectable cells may help to purge the latent viral reservoir [105]. Although the potency of the presently available inverse agonists of US28 (e.g. VUF2274) is limited, there is an ongoing intense search for optimized drugs for pharmacological modulation of this vGPCR [106]. An alternative strategy makes use of the fact that US28 is a membrane protein exhibiting a high rate of constitutive internalization thus efficiently sequestering chemokines from the environment [45]. This inspired the concept of using chemokine-based immunotoxins to target cytomegalovirus-infected cells. Spiess et al. designed a synthetic CX3CL1 variant with increased affinity and specificity for US28 that was fused with the cytotoxic domain of Pseudomonas Exotoxin A [107]. This immunotoxin not only caused a direct killing of lytically infected cells but the specific targeting of latently infected cells could also robustly reduce virus reactivation [107, 108]. Although concerns regarding potential side effects of such an immunotoxin-based antiviral approach may delay its use in vivo, the ex vivo depletion of latently infected HPCs before stem cell transplantation may constitute an alternative feasible approach to decrease the burden of latent HCMV.

Concluding remarks

Although cytomegalovirus-encoded vGPCRs have first been detected approximately 30 years ago, only recently novel functions of these signal transduction molecules with utmost importance for viral pathogenesis could be unraveled. On the one hand, there is accumulating evidence that vGPCR signaling is required for systemic viral spread via DC recircularization. On the other hand, interference with vGPCR signaling interrupts latency fostering viral reactivation. Thus, cytomegalovirus-encoded vGPCRs emerge as an Achilles heel of cytomegaloviruses, since pharmacological modulation of these receptors in combination with conventional drugs like ganciclovir may not only arrest active infection but could also help to reduce the latent viral reservoir.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Fredriksson R, Lagerstrom MC, Lundin LG, Schioth HB (2003) The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. Mol Pharmacol 63(6):1256–1272
2. Xu HE, Xiao RP (2012) A new era for GPCR research: structures, biology and drug discovery. Acta Pharmacol Sin 33(3):289–290
3. Esche C, Stellato C, Beck LA (2005) Chemokines: key players in innate and adaptive immunity. J Invest Dermatol 125(4):615–628
4. Lattin J, Zidar DA, Schroder K, Kellie S, Hume DA, Sweet MJ (2007) G-protein-coupled receptor expression, function, and signaling in macrophages. J Leukoc Biol 82(1):16–32
5. Newton K, Dixit VM (2012) Signaling in innate immunity and inflammation. Cold Spring Harb Perspect Biol 4(3):a006049
6. Sodhi A, Montaner S, Gutkind JS (2004) Viral hijacking of G-protein-coupled-receptor signalling networks. Nat Rev Mol Cell Biol 5(12):998–1012
7. Montaner S, Kufareva I, Abagyan R, Gutkind JS (2013) Molecular mechanisms deployed by virally encoded G protein-coupled receptors in human diseases. Annu Rev Pharmacol Toxicol 53:331–354
8. Vischer HF, Siderius M, Leurs R, Smit MJ (2014) Herpesvirus-encoded GPCRs: neglected players in inflammatory and proliferative diseases? Nat Rev Drug Discov 13(2):123–139
9. Rosenkilde MM, Waldhoer M, Luttichau HR, Schwartz TW (2001) Virally encoded 7TM receptors. Oncogene 20(13):1582–1593
10. Alcami A (2007) New insights into the subversion of the chemokine system by poxviruses. Eur J Immunol 37(4):880–883
11. Seet BT, McFadden G (2002) Viral chemokine-binding proteins. J Leukoc Biol 72(1):24–34
12. Chee MS, Satchwell SC, Preddie E, Weston KM, Barrell BG (1990) Human cytomegalovirus encodes three G protein-coupled receptor homologues. Nature 344(6268):774–777
13. Takeda S, Kadowaki S, Haga T, Takaesu H, Mitaku S (2002) Identification of G protein-coupled receptor genes from the human genome sequence. FEBS Lett 520(1–3):97–101
14. Lundstrom K (2009) An overview on GPCRs and drug discovery: structure-based drug design and structural biology on GPCRs. Methods Mol Biol 552:51–66
15. Hill SJ (2006) G-protein-coupled receptors: past, present and future. Br J Pharmacol 147(Suppl 1):S27–S37
16. Wang CI, Lewis RJ (2013) Emerging opportunities for allosteric modulation of G-protein coupled receptors. Biochem Pharmacol 85(2):153–162
17. Kristiansen K (2004) Molecular mechanisms of ligand binding, signaling, and regulation within the superfamily of G-protein-coupled receptors: molecular modeling and mutagenesis approaches to receptor structure and function. Pharmacol Ther 103(1):21–80
18. Marinissen MJ, Gutkind JS (2001) G-protein-coupled receptors and signaling networks: emerging paradigms. Trends Pharmacol Sci 22(7):368–376
19. Chakraborty H, Chattopadhyay A (2015) Excitements and challenges in GPCR oligomerization: molecular insight from FRET. ACS Chem Neurosci 6:199–206
20. Tang XL, Wang Y, Li DL, Luo J, Liu MY (2012) Orphan G protein-coupled receptors (GPCRs): biological functions and potential drug targets. Acta Pharmacol Sin 33(3):363–371
21. Park PS, Filipek S, Wells JW, Palczewski K (2004) Oligomerization of G protein-coupled receptors: past, present, and future. Biochemistry 43(50):15643–15656
22. Kobilka BK, Deupi X (2007) Conformational complexity of G-protein-coupled receptors. Trends Pharmacol Sci 28(8):397–406
23. Kobilka BK (2007) G protein coupled receptor structure and activation. Biochim Biophys Acta 1768(4):794–807
24. Davidson FF, Loewen PC, Khorana HG (1994) Structure and function in rhodopsin: replacement by alanine of cysteine residues 110 and 187, components of a conserved disulfide bond in rhodopsin, affects the light-activated metarhodopsin II state. Proc Natl Acad Sci USA 91(9):4029–4033
25. Oldham WM, Hamm HE (2008) Heterotrimeric G protein activation by G-protein-coupled receptors. Nat Rev Mol Cell Biol 9(1):60–71
26. Rovati GE, Capra V, Neubig RR (2007) The highly conserved DRY motif of class A G protein-coupled receptors: beyond the ground state. Mol Pharmacol 71(4):959–964
27. Smrcka AV (2008) G protein betagamma subunits: central mediators of G protein-coupled receptor signaling. Cell Mol Life Sci 65(14):2191–2214
28. Hamm HE (1998) The many faces of G protein signaling. J Biol Chem 273(2):669–672
29. McCudden CR, Hains MD, Kimple RJ, Siderovski DP, Willard FS (2005) G-protein signaling: back to the future. Cell Mol Life Sci 62(5):551–577
30. Sato M, Blumer JB, Simon V, Lanier SM (2006) Accessory proteins for G proteins: partners in signaling. Annu Rev Pharmacol Toxicol 46:151–187
31. Nobles M, Benians A, Tinker A (2005) Heterotrimeric G proteins precouple with G protein-coupled receptors in living cells. Proc Natl Acad Sci USA 102(51):18706–18711
32. Grabiner BC, Blonska M, Lin PC, You Y, Wang D, Sun J, Darnay BG, Dong C, Lin X (2007) CARMA3 deficiency abrogates G protein-coupled receptor-induced NF-κB activation. Genes Dev 21(8):984–996

33. Sun L, Deng L, Ea CK, Xia ZP, Chen ZJ (2004) The TRAF6 ubiquitin ligase and TAK1 kinase mediate IKK activation by BCL10 and MALT1 in T lymphocytes. Mol Cell 14(3):289–301
34. Latek D, Modzelewska A, Trzaskowski B, Palczewski K, Filipek S (2012) G protein-coupled receptors—recent advances. Acta Biochim Pol 59(4):515–529
35. de Munnik SM, Smit MJ, Leurs R, Vischer HF (2015) Modulation of cellular signaling by herpesvirus-encoded G protein-coupled receptors. Front Pharmacol 6:40. https://doi.org/10.3389/fphar.2015.00040
36. Blackbourn DJ, Lennette E, Klencke B, Moses A, Chandran B, Weinstein M, Glogau RG, Witte MH, Way DL, Kutzkey T, Herndier B, Levy JA (2000) The restricted cellular host range of human herpesvirus 8. AIDS (London England) 14(9):1123–1133
37. Cesarman E, Damania B, Krown SE, Martin J, Bower M, Whitby D (2019) Kaposi sarcoma. Nat Rev Dis Primers 5(1):9. https://doi.org/10.1038/s41572-019-0060-9
38. Guo HG, Sadowska M, Reid W, Tschachler E, Hayward G, Reitz M (2003) Kaposi's sarcoma-like tumors in a human herpesvirus 8 ORF74 transgenic mouse. J Virol 77(4):2631–2639
39. Zuo J, Currin A, Griffin BD, Shannon-Lowe C, Thomas WA, Ressing ME, Wiertz EJ, Rowe M (2009) The Epstein–Barr virus G-protein-coupled receptor contributes to immune evasion by targeting MHC class I molecules for degradation. PLoS Pathog 5(1):e1000255. https://doi.org/10.1371/journal.ppat.1000255
40. Beisser PS, Verzijl D, Gruijthuijsen YK, Beuken E, Smit MJ, Leurs R, Bruggeman CA, Vink C (2005) The Epstein–Barr virus BILF1 gene encodes a G protein-coupled receptor that inhibits phosphorylation of RNA-dependent protein kinase. J Virol 79(1):441–449
41. Isegawa Y, Ping Z, Nakano K, Sugimoto N, Yamanishi K (1998) Human herpesvirus 6 open reading frame U12 encodes a functional beta-chemokine receptor. J Virol 72(7):6104–6112
42. Menotti L, Mirandola P, Locati M, Campadelli-Fiume G (1999) Trafficking to the plasma membrane of the seven-transmembrane protein encoded by human herpesvirus 6 U51 gene involves a cell-specific function present in T lymphocytes. J Virol 73(1):325–333
43. Attwood TK, Findlay JB (1994) Fingerprinting G-protein-coupled receptors. Protein Eng 7(2):195–203
44. Margulies BJ, Browne H, Gibson W (1996) Identification of the human cytomegalovirus G protein-coupled receptor homologue encoded by UL33 in infected cells and enveloped virus particles. Virology 225(1):111–125
45. Bodaghi B, Jones TR, Zipeto D, Vita C, Sun L, Laurent L, renzana-Seisdedos F, Virelizier JL, Michelson S (1998) Chemokine sequestration by viral chemoreceptors as a novel viral escape strategy: withdrawal of chemokines from the environment of cytomegalovirus-infected cells. J Exp Med 188(5):855–866
46. Krishna BA, Miller WE, O'Connor CM (2018) US28: HCMV's Swiss Army Knife. Viruses 10(8):445. https://doi.org/10.3390/v10080445
47. Casarosa P, Waldhoer M, LiWang PJ, Vischer HF, Kledal T, Timmerman H, Schwartz TW, Smit MJ, Leurs R (2005) CC and CX3C chemokines differentially interact with the N terminus of the human cytomegalovirus-encoded US28 receptor. J Biol Chem 280(5):3275–3285
48. Gao JL, Murphy PM (1994) Human cytomegalovirus open reading frame US28 encodes a functional beta chemokine receptor. J Biol Chem 269(46):28539–28542
49. Kledal TN, Rosenkilde MM, Schwartz TW (1998) Selective recognition of the membrane-bound CX3C chemokine, fractalkine, by the human cytomegalovirus-encoded broad-spectrum receptor US28. FEBS Lett 441(2):209–214
50. Kuhn DE, Beall CJ, Kolattukudy PE (1995) The cytomegalovirus US28 protein binds multiple CC chemokines with high affinity. Biochem Biophys Res Commun 211(1):325–330
51. Streblow DN, Soderberg-Naucler C, Vieira J, Smith P, Wakabayashi E, Ruchti F, Mattison K, Altschuler Y, Nelson JA (1999) The human cytomegalovirus chemokine receptor US28 mediates vascular smooth muscle cell migration. Cell 99(5):511–520
52. Streblow DN, Vomaske J, Smith P, Melnychuk R, Hall L, Pancheva D, Smit M, Casarosa P, Schlaepfer DD, Nelson JA (2003) Human cytomegalovirus chemokine receptor US28-induced smooth muscle cell migration is mediated by focal adhesion kinase and Src. J Biol Chem 278(50):50456–50465
53. Vieira J, Schall TJ, Corey L, Geballe AP (1998) Functional analysis of the human cytomegalovirus US28 gene by insertion mutagenesis with the green fluorescent protein gene. J Virol 72(10):8158–8165
54. Casarosa P, Bakker RA, Verzijl D, Navis M, Timmerman H, Leurs R, Smit MJ (2001) Constitutive signaling of the human cytomegalovirus-encoded chemokine receptor US28. J Biol Chem 276(2):1133–1137
55. McLean KA, Holst PJ, Martini L, Schwartz TW, Rosenkilde MM (2004) Similar activation of signal transduction pathways by the herpesvirus-encoded chemokine receptors US28 and ORF74. Virology 325(2):241–251
56. Slinger E, Maussang D, Schreiber A, Siderius M, Rahbar A, Fraile-Ramos A, Lira SA, Soderberg-Naucler C, Smit MJ (2010) HCMV-encoded chemokine receptor US28 mediates proliferative signaling through the IL-6-STAT3 axis. Sci Signal 3(133):ra58
57. Vischer HF, Leurs R, Smit MJ (2006) HCMV-encoded G-protein-coupled receptors as constitutively active modulators of cellular signaling networks. Trends Pharmacol Sci 27(1):56–63
58. Fraile-Ramos A, Kledal TN, Pelchen-Matthews A, Bowers K, Schwartz TW, Marsh M (2001) The human cytomegalovirus US28 protein is located in endocytic vesicles and undergoes constitutive endocytosis and recycling. Mol Biol Cell 12(6):1737–1749
59. Waldhoer M, Casarosa P, Rosenkilde MM, Smit MJ, Leurs R, Whistler JL, Schwartz TW (2003) The carboxyl terminus of human cytomegalovirus-encoded 7 transmembrane receptor US28 camouflages agonism by mediating constitutive endocytosis. J Biol Chem 278(21):19473–19482
60. Billstrom MA, Lehman LA, Scott WG (1999) Depletion of extracellular RANTES during human cytomegalovirus infection of endothelial cells. Am J Respir Cell Mol Biol 21(2):163–167
61. Randolph-Habecker JR, Rahill B, Torok-Storb B, Vieira J, Kolattukudy PE, Rovin BH, Sedmak DD (2002) The expression of the cytomegalovirus chemokine receptor homolog US28 sequesters biologically active CC chemokines and alters IL-8 production. Cytokine 19(1):37–46
62. Burg JS, Ingram JR, Venkatakrishnan AJ, Jude KM, Dukkipati A, Feinberg EN, Angelini A, Waghray D, Dror RO, Ploegh HL, Garcia KC (2015) Structural biology. Structural basis for chemokine recognition and activation of a viral G protein-coupled receptor. Science 347(6226):1113–1117. https://doi.org/10.1126/science.aaa5026
63. Luckmann M, Amarandi RM, Papargyri N, Jakobsen MH, Christiansen E, Jensen LJ, Pui A, Schwartz TW, Rosenkilde MM, Frimurer TM (2017) Structure-based discovery of novel US28 small molecule ligands with different modes of action. Chem Biol Drug Des 89(3):289–296. https://doi.org/10.1111/cbdd.12848
64. van Cleef KW, Smit MJ, Bruggeman CA, Vink C (2006) Cytomegalovirus-encoded homologs of G protein-coupled receptors and chemokines. J Clin Virol 35(3):343–348

65. Slinger E, Langemeijer E, Siderius M, Vischer HF, Smit MJ (2011) Herpesvirus-encoded GPCRs rewire cellular signaling. Mol Cell Endocrinol 331(2):179–184
66. Langemeijer EV, Slinger E, de MS, Schreiber, Maussang A, Vischer D, Verkaar H, Leurs F, Siderius R, Smit M MJ (2012) Constitutive beta-catenin signaling by the viral chemokine receptor US28. PLoS One 7(11):e48935
67. Waldhoer M, Kledal TN, Farrell H, Schwartz TW (2002) Murine cytomegalovirus (CMV) M33 and human CMV US28 receptors exhibit similar constitutive signaling activities. J Virol 76(16):8161–8168
68. Farrell HE, Abraham AM, Cardin RD, Molleskov-Jensen AS, Rosenkilde MM, vis-Poynter N (2013) Identification of common mechanisms by which human and mouse cytomegalovirus seven-transmembrane receptor homologues contribute to in vivo phenotypes in a mouse model. J Virol 87(7):4112–4117
69. Casarosa P, Gruijthuijsen YK, Michel D, Beisser PS, Holl J, Fitzsimons CP, Verzijl D, Bruggeman CA, Mertens T, Leurs R, Vink C, Smit MJ (2003) Constitutive signaling of the human cytomegalovirus-encoded receptor UL33 differs from that of its rat cytomegalovirus homolog R33 by promiscuous activation of G proteins of the Gq, Gi, and Gs classes. J Biol Chem 278(50):50010–50023
70. Gruijthuijsen YK, Casarosa P, Kaptein SJ, Broers JL, Leurs R, Bruggeman CA, Smit MJ, Vink C (2002) The rat cytomegalovirus R33-encoded G protein-coupled receptor signals in a constitutive fashion. J Virol 76(3):1328–1338
71. Cardin RD, Schaefer GC, Allen JR, vis-Poynter NJ, Farrell HE (2009) The M33 chemokine receptor homolog of murine cytomegalovirus exhibits a differential tissue-specific role during in vivo replication and latency. J Virol 83(15):7590–7601
72. Beisser PS, Vink C, Van Dam JG, Grauls G, Vanherle SJ, Bruggeman CA (1998) The R33 G protein-coupled receptor gene of rat cytomegalovirus plays an essential role in the pathogenesis of viral infection. J Virol 72(3):2352–2363
73. Case R, Sharp E, ned-Jensen T, Rosenkilde MM, vis-Poynter N, Farrell HE (2008) Functional analysis of the murine cytomegalovirus chemokine receptor homologue M33: ablation of constitutive signaling is associated with an attenuated phenotype in vivo. J Virol 82(4):1884–1898
74. Farrell HE, Abraham AM, Cardin RD, Sparre-Ulrich AH, Rosenkilde MM, Spiess K, Jensen TH, Kledal TN, vis-Poynter N (2011) Partial functional complementation between human and mouse cytomegalovirus chemokine receptor homologues. J Virol 85(12):6091–6095
75. Scarborough JA, Paul JR, Spencer JV (2017) Evolution of the ability to modulate host chemokine networks via gene duplication in human cytomegalovirus (HCMV). Infect Genet Evolut 51:46–53. https://doi.org/10.1016/j.meegid.2017.03.013
76. Margulies BJ, Gibson W (2007) The chemokine receptor homologue encoded by US27 of human cytomegalovirus is heavily glycosylated and is present in infected human foreskin fibroblasts and enveloped virus particles. Virus Res 123(1):57–71
77. Lares AP, Tu CC, Spencer JV (2013) The human cytomegalovirus US27 gene product enhances cell proliferation and alters cellular gene expression. Virus Res 176(1–2):312–320
78. Arnolds KL, Lares AP, Spencer JV (2013) The US27 gene product of human cytomegalovirus enhances signaling of host chemokine receptor CXCR4. Virology 439(2):122–131
79. Tadagaki K, Tudor D, Gbahou F, Tschische P, Waldhoer M, Bomsel M, Jockers R, Kamal M (2012) Human cytomegalovirus-encoded UL33 and UL78 heteromerize with host CCR5 and CXCR4 impairing their HIV coreceptor activity. Blood 119(21):4908–4918
80. Boeck JM, Spencer JV (2017) Effect of human cytomegalovirus (HCMV) US27 on CXCR4 receptor internalization measured by fluorogen-activating protein (FAP) biosensors. PLoS One 12(2):e0172042. https://doi.org/10.1371/journal.pone.0172042
81. Fraile-Ramos A, Pelchen-Matthews A, Kledal TN, Browne H, Schwartz TW, Marsh M (2002) Localization of HCMV UL33 and US27 in endocytic compartments and viral membranes. Traffic 3(3):218–232
82. Stapleton LK, Arnolds KL, Lares AP, Devito TM, Spencer JV (2012) Receptor chimeras demonstrate that the C-terminal domain of the human cytomegalovirus US27 gene product is necessary and sufficient for intracellular receptor localization. Virol J 9:42
83. Murphy E, Yu D, Grimwood J, Schmutz J, Dickson M, Jarvis MA, Hahn G, Nelson JA, Myers RM, Shenk TE (2003) Coding potential of laboratory and clinical strains of human cytomegalovirus. Proc Natl Acad Sci USA 100(25):14976–14981
84. O'Connor CM, Shenk T (2011) Human cytomegalovirus pUS27 G protein-coupled receptor homologue is required for efficient spread by the extracellular route but not for direct cell-to-cell spread. J Virol 85(8):3700–3707
85. Niemann I, Reichel A, Stamminger T (2014) Intracellular trafficking of the human cytomegalovirus-encoded 7-trans-membrane protein homologs pUS27 and pUL78 during viral infection: a comparative analysis. Viruses 6(2):661–682
86. Hutterer C, Niemann I, Milbradt J, Frohlich T, Reiter C, Kadioglu O, Bahsi H, Zeittrager I, Wagner S, Einsiedel J, Gmeiner P, Vogel N, Wandinger S, Godl K, Stamminger T, Efferth T, Tsogoeva SB, Marschall M (2015) The broad-spectrum antiinfective drug artesunate interferes with the canonical nuclear factor kappa B (NF-kappaB) pathway by targeting RelA/p65. Antivir Res 124:101–109. https://doi.org/10.1016/j.antiviral.2015.10.003
87. O'Connor CM, Shenk T (2012) Human cytomegalovirus pUL78 G protein-coupled receptor homologue is required for timely cell entry in epithelial cells but not fibroblasts. J Virol 86(21):11425–11433
88. Beisser PS, Grauls G, Bruggeman CA, Vink C (1999) Deletion of the R78 G protein-coupled receptor gene from rat cytomegalovirus results in an attenuated, syncytium-inducing mutant strain. J Virol 73(9):7218–7230
89. Kaptein SJ, Beisser PS, Gruijthuijsen YK, Savelkouls KG, van Cleef KW, Beuken E, Grauls GE, Bruggeman CA, Vink C (2003) The rat cytomegalovirus R78 G protein-coupled receptor gene is required for production of infectious virus in the spleen. J Gen Virol 84(Pt 9):2517–2530
90. Oliveira SA, Shenk TE (2001) Murine cytomegalovirus M78 protein, a G protein-coupled receptor homologue, is a constituent of the virion and facilitates accumulation of immediate-early viral mRNA. Proc Natl Acad Sci USA 98(6):3237–3242
91. Wagner S, Arnold F, Wu Z, Schubert A, Walliser C, Tadagaki K, Jockers R, Mertens T, Michel D (2012) The 7-transmembrane protein homologue UL78 of the human cytomegalovirus forms oligomers and traffics between the plasma membrane and different intracellular compartments. Arch Virol 157(5):935–949
92. Tschische P, Tadagaki K, Kamal M, Jockers R, Waldhoer M (2011) Heteromerization of human cytomegalovirus encoded chemokine receptors. Biochem Pharmacol 82(6):610–619
93. Farrell HE, Stevenson PG (2019) Cytomegalovirus host entry and spread. J Gen Virol. https://doi.org/10.1099/jgv.0.001230
94. Bittencourt FM, Wu SE, Bridges JP, Miller WE (2014) The M33 G protein-coupled receptor encoded by murine cytomegalovirus is dispensable for hematogenous dissemination but is required for growth within the salivary gland. J Virol 88(20):11811–11824. https://doi.org/10.1128/jvi.01006-14
95. Farrell HE, Bruce K, Lawler C, Oliveira M, Cardin R, Davis-Poynter N, Stevenson PG (2017) Murine cytomegalovirus spreads by dendritic cell recirculation. mBio 8(5):e01264-17. https://doi.org/10.1128/mBio.01264-17

96. Randolph GJ, Angeli V, Swartz MA (2005) Dendritic-cell trafficking to lymph nodes through lymphatic vessels. Nat Rev Immunol 5(8):617–628. https://doi.org/10.1038/nri1670
97. Farrell HE, Bruce K, Ma J, Davis-Poynter N, Stevenson PG (2018) Human cytomegalovirus US28 allows dendritic cell exit from lymph nodes. J Gen Virol 99(11):1509–1514. https://doi.org/10.1099/jgv.0.001154
98. Beisser PS, Laurent L, Virelizier JL, Michelson S (2001) Human cytomegalovirus chemokine receptor gene US28 is transcribed in latently infected THP-1 monocytes. J Virol 75(13):5949–5957
99. Hargett D, Shenk TE (2010) Experimental human cytomegalovirus latency in CD14+ monocytes. Proc Natl Acad Sci USA 107(46):20039–20044. https://doi.org/10.1073/pnas.1014509107
100. Humby MS, O'Connor CM (2015) Human cytomegalovirus US28 is important for latent infection of hematopoietic progenitor cells. J Virol 90(6):2959–2970. https://doi.org/10.1128/jvi.02507-15
101. Krishna BA, Poole EL, Jackson SE, Smit MJ, Wills MR, Sinclair JH (2017) Latency-associated expression of human cytomegalovirus US28 attenuates cell signaling pathways to maintain latent infection. mBio 8(6):e01754-17. https://doi.org/10.1128/mBio.01754-17
102. Krishna BA, Humby MS, Miller WE, O'Connor CM (2019) Human cytomegalovirus G protein-coupled receptor US28 promotes latency by attenuating c-fos. Proc Natl Acad Sci USA. https://doi.org/10.1073/pnas.1816933116
103. Boomker JM, The TH, de Leij LF, Harmsen MC (2006) The human cytomegalovirus-encoded receptor US28 increases the activity of the major immediate-early promoter/enhancer. Virus Res 118(1–2):196–200
104. Zhu D, Pan C, Sheng J, Liang H, Bian Z, Liu Y, Trang P, Wu J, Liu F, Zhang CY, Zen K (2018) Human cytomegalovirus reprogrammes haematopoietic progenitor cells into immunosuppressive monocytes to achieve latency. Nat Microbiol 3(4):503–513. https://doi.org/10.1038/s41564-018-0131-9
105. Krishna BA, Lau B, Jackson SE, Wills MR, Sinclair JH, Poole E (2016) Transient activation of human cytomegalovirus lytic gene expression during latency allows cytotoxic T cell killing of latently infected cells. Sci Rep 6:24674. https://doi.org/10.1038/srep24674
106. Lee S, Chung YH, Lee C (2017) US28, a virally-encoded GPCR as an antiviral target for human cytomegalovirus infection. Biomol Ther 25(1):69–79. https://doi.org/10.4062/biomolther.2016.208
107. Spiess K, Jeppesen MG, Malmgaard-Clausen M, Krzywkowski K, Dulal K, Cheng T, Hjorto GM, Larsen O, Burg JS, Jarvis MA, Garcia KC, Zhu H, Kledal TN, Rosenkilde MM (2015) Rationally designed chemokine-based toxin targeting the viral G protein-coupled receptor US28 potently inhibits cytomegalovirus infection in vivo. Proc Natl Acad Sci USA 112(27):8427–8432. https://doi.org/10.1073/pnas.1509392112
108. Krishna BA, Spiess K, Poole EL, Lau B, Voigt S, Kledal TN, Rosenkilde MM, Sinclair JH (2017) Targeting the latent cytomegalovirus reservoir with an antiviral fusion toxin protein. Nat Commun 8:14321. https://doi.org/10.1038/ncomms14321