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Cytomegalovirus Encoded G Protein-Coupled Receptors

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ABSTRACT

Cytomegalovirus **(CMV)** is a widespread pathogen that infects a variety of mammals, including humans, rats, mice, guinea pigs, chimpanzees, and rhesus macaques. Each strain of CMV is specifically adapted to the mammalian species they infect. As members of the *Herpesviridae* family, each CMV is capable of establishing life-long infection via latency, and most healthy individuals remain asymptomatic. Besides virion structure and latency, an additional feature of most CMV strains is that they encode multiple G protein-coupled receptors **(GPCRs)**, which are homologous to cellular chemokine receptors. As membrane-bound proteins, GPCRs transmit extracellular signals intracellularly and activate signal transduction pathways governing cellular processes such as chemotaxis, proliferation, and transcription. Viral GPCRs (**vGPCRs**) may modulate cell signaling to the advantage of the virus, for example, to promote virus dissemination and evasion of immune surveillance. Although vGPCRs are not typically essential for viral replication, their importance during infection has been highlighted by several elegant studies demonstrating that deletion of vGPCRs can reduce viral growth kinetics and impair the establishment of infection in various tissues. Given that GPCRs have historically been successful drug targets, the roles of vGPCRs during CMV infection are worth investigating further. Here, we provide a summary of the current findings on CMV GPCRs.

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INTRODUCTION

Human cytomegalovirus (**HCMV**) is a member of the beta-herpesvirus subfamily and a widespread pathogen that infects a large percentage of the population worldwide [1]. Typical of herpesviruses, HCMV establishes life-long persistent and latent infection. During latency, the viral genome continues to exist within the host with little to no viral replication or protein synthesis. HCMV latency is generally asymptomatic among the immunocompetent, but infection has been linked to development of autoimmune, cardiovascular, and proliferative diseases [2-5]. Upon reactivation, transcription of multiple genes involved in lytic infection begins, leading to cell death and release of newly produced virions. HCMV reactivation can cause several severe inflammatory diseases including pneumonitis, hepatitis, and retinitis [6]. Moreover, primary infection of a fetus *in utero* can result in significant sensorineural birth defects, making HCMV a particularly dangerous pathogen for the immune compromised.

THE VIRUS PARTICLE

The HCMV genome is a double-stranded linear DNA molecule, and it is the largest among human herpesviruses, encoding over 236 kb and 192 open reading frames (**ORFs**) [7]. Multiple strains of HCMV exist, with high levels of conservation in certain genes and high levels of variation among others, particularly genes encoding membrane associated or secreted proteins [7-9]. This hyper variation is hypothesized to be a result of immune selection over the evolutionary course of humans and their ancestors, as well as recombination of viral genomes during infection with multiple strains [8,10]. HCMV strains are separated into two categories: laboratory and clinical. Clinical strains, such as Merlin, TR, and TB40/E are more genetically similar to HCMV isolated from patients than laboratory strains, such as AD169 and Towne, which accumulate mutations or lose virulence genes in culture [11,12].

The viral DNA genome is housed within an icosahedral protein capsid, which is then surrounded by the tegument [13,14] (Figure 1). Covering the capsid and tegument is the membrane envelope, a lipid bilayer derived from host cell membranes. Within the viral envelope are proteins originating from the host and virus, including three glycoprotein complexes (**gCI-III**) necessary for virus adsorption into cells. Complex gCI is a homodimer of glycoprotein B (**gB**) which facilities fusion of the viral envelope with the cell membrane [15,16]. The gCII conmplex, consisting of heterodimers of gM and gN, is the most abundant glycoprotein complex of the HCMV envelope and also the least studied complex [17]. The gH, gL, and gO proteins form gCIII and use platelet-derived growth factor-α (**PDGFR-α**) as an entry receptor [18]. In addition to these glycoprotein complexes, the virus particle also contains several virally encoded G protein-coupled receptors (**GPCRs**).

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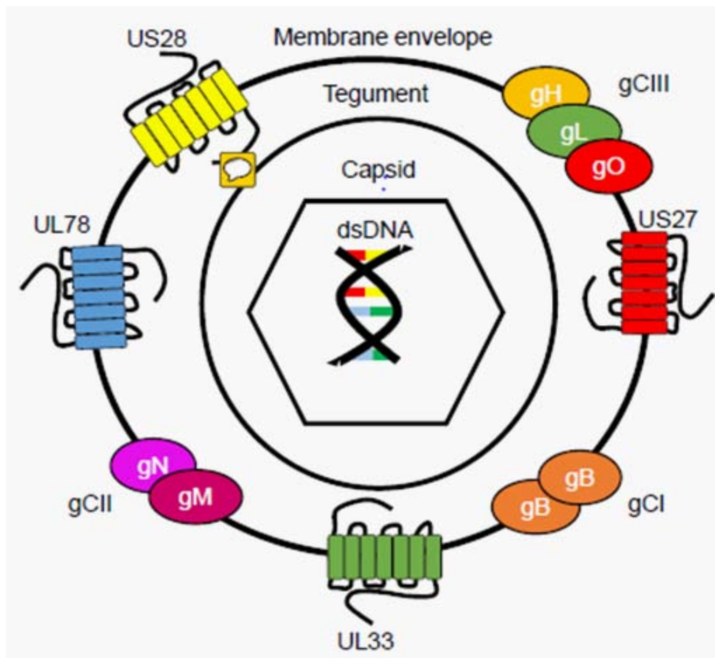

Figure 1: Schematic representation of the CMV particle. The double-stranded DNA genome lies within an icosahedral protein capsid. The capsid is surrounded by the proteinaceous tegument layer. The membrane envelope is derived from the host cell and includes receptors necessary for virus adsorption into the cell. In addition to viral glycoproteins complexes gCI, gCII, and gCIII, the virion also contains virally encoded G protein-coupled receptors US27, US28, UL33 and UL78.

G PROTEIN COUPLED RECEPTORS (GPCRS)

GPCRs constitute the largest family of membrane-bound proteins involved in transducing intracellular signaling from extracellular stimuli, such as hormones, neurotransmitters, and in the immune system, chemokines [19]. Structurally, GPCRs typically have seven hydrophobic transmembrane α-helices (**7TMs**) connected by alternating intracellular and extracellular loops, with an extracellular amino-terminus and an intracellular carboxy-terminus (Figure 2). The intracellular domains of the GPCR associate with heterotrimeric G proteins, typically through a DRY motif in the third helix region (second intracellular loop) [20]. In the resting state, a GDP molecule is bound to the Gα-subunit, rendering it inactive [21,22]. Upon ligand binding, the GPCR undergoes a conformation change causing release of the bound GDP from the Gα-subunit and exchange for GTP [21,22]. The Gα and Gβγ subunits then disassociate from the GPCR to activate downstream effector proteins and induce the production of second messenger molecules. The Gα-subunit remains active until it hydrolyzes the GTP back to GDP [21,22]. A significant difference between viral GPCRs (**vGPCR**) and their cellular homologs is that they can constitutively activate G protein signaling independent of ligand binding. The crystal structure of

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US28, solved to 2.9-Angstrom resolution, suggests that this constitutive activation is property is due to destabilization of the inactive conformation of the amino acids surrounding the DRY motif [23]. This configuration enables vGPCRs to continuously activate G protein signaling pathways that are normally induced via ligand binding.

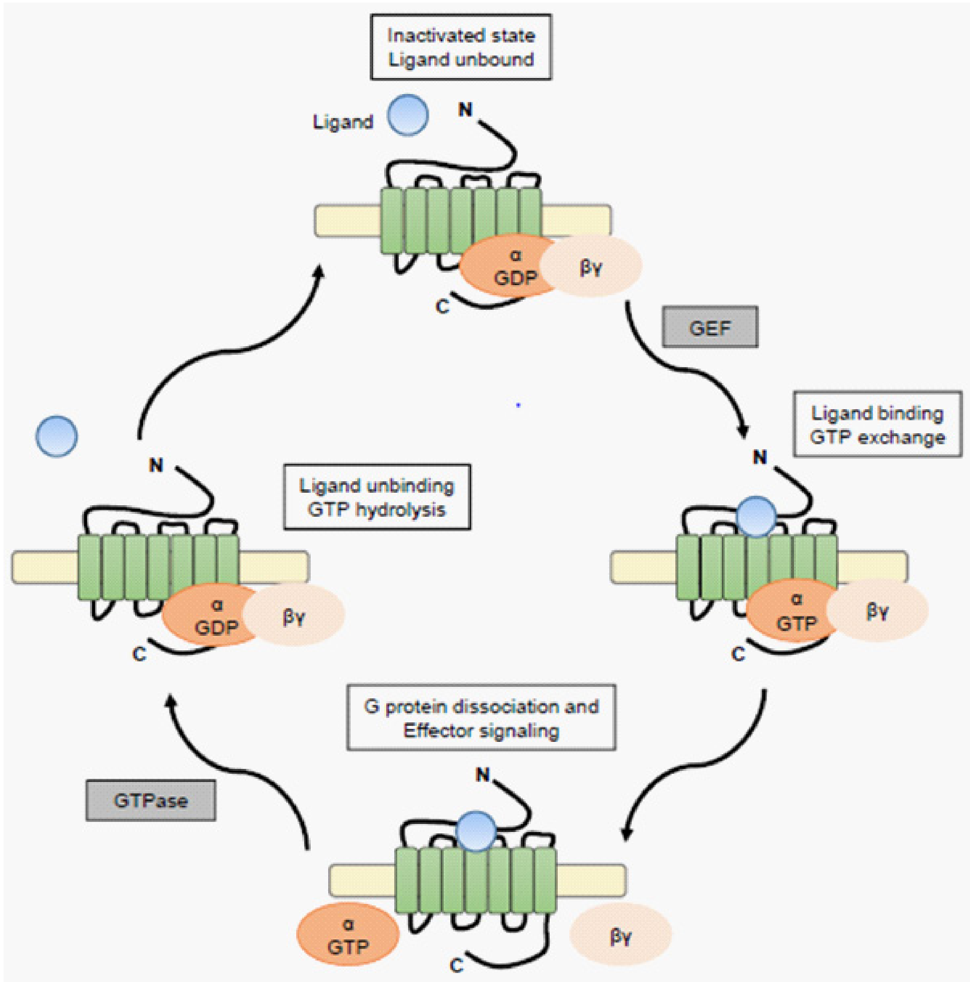

Figure 2: Typical G protein-coupled receptor (**GPCR**) activation and signaling scheme. In its inactive, ligand-unbound state, the Gα subunit of a typical GPCR is GDP-bound and attached to the Gβγ subunit. Upon extracellular ligand binding, guanine nucleotide exchange factors (**GEFs**) exchange GDP for GTP on the Gα subunit. The Gα and Gβγ subunits then disassociate from each other and activate several signaling cascades. GTPases rapidly hydrolyze GTP to GDP, the ligand disassociates from the GPCR, and the receptor complex is returned to its inactive state. While many cellular GPCR are activated in a ligand-dependent manner as depicted here, many viral GPCR are constitutively active and transmit signals through a variety of cellular cascades in the absence of any ligand binding.

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There are four main subfamilies of Gα subunits: Gαs, Gαi/o, G q/11, and Gα12/13, and each activates different intracellular signal pathways. The Gαs protein activates adenylyl cyclase, the first enzyme of the cAMP-dependent pathway. Adenylyl cyclase catalyzes the conversion of ATP to cyclic-AMP (**cAMP**) and pyrophosphate, and the cAMP activates cAMP-dependent proteins kinases, including protein kinase A (**PKA**). PKA is a serine/threonine kinase that then phosphorylates other proteins involved in energy metabolism or transcription. The Gαi/o protein is mainly involved in inhibiting adenylyl cyclase, thereby decreasing cAMP production.

The Gαq/11 protein is mainly involved in activating PLC (**phospholipase C**). PLC cleaves phospholipid phosphatidylinositol 4,5-bisphosphate (**PIP2**) into diacylglycerol (**DAG**) and inositol 1,4,5-trisphosphate (**IP3**), both of which act as second messengers. IP3 causes release of calcium ions (**Ca^{2+})** from the smooth endoplasmic reticulum, initiating a cascade of cellular activity. Ca^{2+} and DAG are involved in activating protein kinase C (**PKC**), an enzyme involved in phosphorylating other proteins in the signal transduction cascade.

Activation of Gα12/13 leads to rearrangement of actin microfilaments and promotes chemotaxis via interaction with Rho and focal adhesion kinase (**FAK**) [24]. Finally, the Gβγ subunits are also able to activate PLC, several isoforms of AC, and different ion channels [24,25]. The amplification and diversification of GPCR-mediated signal transduction involves activation of several cellular processes, including cytoskeletal rearrangement, transcription, cell proliferation, and chemotaxis.

VIRAL GPCR

While HCMV remains the most studied, multiple species of CMV that infect a variety of mammals have been discovered. Considerable effort has also been spent studying the mouse and rat homologs, murine CMV (**MCMV**) or rat CMV (**RCMV**), and more recently the guinea pig (**GPCMV**) model has been successfully developed for the study of congenital transmission. The closest relatives to HCMV that infect primates are chimpanzee CMV (**CCMV**) and rhesus macaque CMV (**RhCMV**) [26,27].

Most CMVs that have been sequenced encode multiple vGPCRs. The HCMV genome encodes four vGPCRs: US27, US28, UL33, and UL78 [28,29]. UL33 and UL78 homologs are found in MCMV, RCMV, and GPCMV [30], while RhCMV encodes five tandem homologs of US28, designated RhUS28.1, RhUS28.2, RhUS28.3, RhUS28.4, and RhUS28.5 [31]. Similarly, US27 is the result of a gene duplication within the HCMV genome, and both US27 and US28 share a common ancestor with human CX3CR1 [32], suggesting that HCMV may be experimenting with manipulation of host chemokine networks. Interestingly, M33, the MCMV GPCR homolog of UL33, shares approximately 47% nucleotide sequence with its HCMV counterpart, yet signals more similarly to US28 [33-35]. Encoding more than one GPCR may enable the virus to develop multiple survival strategies via complex interactions among the viral receptors themselves or host proteins. Indeed, three viral GPCRs-US28, UL33 and M33-activate signaling cascades using more than one Gα protein subunit

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[36] (Table 1). The end result of manipulating the host cell machinery may be virus dissemination for propagation purposes or evading host immune responses.

Table 1: Properties of Cytomegalovirus-encoded G protein-coupled receptors.

Virus	GPCR	G proteins	Signaling	Ligands	Expression	Dimers
HCMV	US27	X[1]	X	X	Late	US28
	US28	Gαq , Gαi/o, Gα12/13	PLC, NF-κB, FAK, ERK1/2, CREB	CC- and CX_3Cchemokines	Early; latency	US27, UL33, UL78
	UL33	Gαq, Gαi, Gαs	PLC, CREB	X	Late	US28, UL33, UL78, CXCR4, CCR5
	UL78	X	X	X	Early	US28, UL33, UL78, CXCR4, CCR5
MCMV	M33	Gαq/11	CREB, NF-κB, PLCβ	mRANTES	Early	X
	M78	X	X	X	Early	X
RCMV	R33	Gαq/11, Gαi/o, Gβγ	PLC	X	Early	X
	R78	X	X	X	Early	X
RhCMV	RhUS28.5	X	X	CC- and CX_3Cchemokines	Immediate-early to early	X

Note: X indicates the information is not known or has not been evaluated.

TEMPORAL GENE EXPRESSION

Viral gene expression is tightly regulated during infection in a temporal cascade pattern: immediate early (**IE**) genes are essential for viral replication and activate the expression of early (**E**) genes, which are involved in DNA replication. Finally, late (**L**) viral genes are turned on and these encode virion structural proteins that are incorporated into progeny virions [37]. US28, UL78, M33, M78, R33, and R78 are expressed with early kinetics and most likely into the late phase [33,38-42]. In contrast, US27 and UL33 are expressed with late kinetics [43]. Only US28 is expressed during latency, and indeed has been shown to be essential for the establishment of latency, at least in experimental *in vitro* model systems [44,45]. Coordinating which vGPCR is expressed during certain points of infection may allow for more fine control of the host cell machinery, virion assembly, and other cellular activities than if all vGPCRs were expressed simultaneously.

CELLULAR LOCALIZATION

Whereas most cellular GPCRs are present at the exterior surface of the cellular membrane, many viral GPCRs are predominantly localized to intracellular compartments [46]. In transfected cells, US27, US28, and UL33 were observed mainly in late endosomes and lysosomes [43,47-49]. In infected fibroblasts, US27 was found in the perinuclear assembly zone, as well as on the cell surface [50]. Relatively low surface levels are consistent with the rapid, constitutive internalization that is characteristic of these vGPCR [48,51]. Intracellular localization of viral GPCRs may facilitate their incorporation in the viral membrane.

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CHEMOKINE BINDING

Of the four GPCRs encoded by HCMV, only US28 is classified as a true chemokine receptor and has high affinity for several cellular ligands. US28 binds to CX3CL1/Fractalkine, CCL2/MCP-1, CCL5/RANTES, CCL7/MCP-3 [29,52-54]. US28 has been labeled as a 'chemokine sink' because in HCMV-infected cells, US28 was found to sequester CCL2/MCP-1 and CCL5/RANTES from the supernatant [55,56]. This property was exclusively attributed to US28, as infection with a virus lacking US27 continued to down-regulate chemokines from culture media [56]. Actively sequestering chemokines from the extracellular milieu may serve as an immune evasion strategy employed by HCMV to decrease extracellular inflammatory cytokines around the infected cell.

In contrast to US28, the remaining HCMV GPCR (US27, UL33, and UL78) are currently considered orphan receptors, exhibiting no affinity or ligand-dependent activation of signaling to cellular ligands [57,58]. Even in the absence of ligand binding, these viral GPCRs could impact the activity of other GPCR receptors through heterodimerization [59]. For example, in transfected cells, co-expression of US28 with either UL33 or UL78 reduced US28's ability to activate NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), whereas complex formation with US27 had no effect on NF-κB signaling [60]. Deciphering interactions among vGPCR during virus infection has proven challenging, and vGPCR dimerization and potential effects on signaling have not yet been evaluated in infected cells.

Of the rodent vGPCRs, only M33 exhibits affinity for a ligand. M78, R33, and R78 are currently considered orphan receptors, and GP33 and GP78 have not yet been evaluated [39]. Murine RANTES (**mRANTES**) enhanced SMC migration in cells infected with wild-type MCMV or adenovirus vector expressing M33 [39]. Of the RhCMV US28 homologs, only RhUS28.5 exhibited affinity for human CX3CL1/Fractalkine [31]. In transfected HEK293 cells, CX3CL1/Fractalkine induced low levels of RhUS28.5 internalization, but did not elicit calcium mobilization [31]. Further analysis revealed RhUS28.5 binds to a range of human CC chemokines, including MCP-1/CCL2, MIP-1a/CCL3, MCP-3/CCL7, MCP-4/CCL13, and Eotaxin-3/CCL26 [31].

Chemokines serve as important messengers in GPCR activation and signaling, and many chemokines bind multiple receptors inducing different cellular responses. Conversely, a vGPCR may be an orphan receptor, exhibit no response to any endogenous or viral ligand, and yet still serve in some capacity to the virus. Excluding the olfactory GPCRs, approximately 140 human GPCRs are currently classified as orphan receptors, but are nonetheless important for immunology, proliferation, and development purposes [61].

G PROTEIN SIGNALING

Human chemokine receptors mainly couple to the Gαi/o subunit, but as mentioned above, virus receptors signal with multiple Gα subunits via ligand-dependent and -independent methods [36]. Common tools for identifying the G protein signaling pathway activated by GPCRs involves

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the use of reporter plasmids and chemical inhibitors of signaling proteins, typically in cells that are transfected to express the indicated GPCR.

US28 can constitutively activate PLC in several types of transfected and HCMV-infected cells [49,62-64]. Additionally, US28 constitutively signals through Gαi and Gαq to activate several transcription factors, including NFAT (nuclear factor of activated T cells), NF-κB, and SRF (serum response factor) [65]. Furthermore, the effect US28 produces in response to ligand stimulation is cell-type dependent. CX3CL1/Fractalkine activates FAK and ERK in fibroblasts via Gαq, but the same chemokine acted as an inverse agonist in Cos-7 and Hek293 cells as PLC and NFAT constitutive activation were decreased [65-67]. The mechanism of how US28 tunes G protein signaling between chemokine-dependent or -independent stimulation may have been revealed by studying its crystal structure in complex with or without CX3CL1/Fractalkine [23]. As mentioned earlier, the DRY motif has an important role in switching between active and inactive states of GPCRs [20,68]. The amino acid environment of the aspartic acid (**Asp**) residue of the DRY motif of US28 is likely to destabilize the inactivate state of the GPCR, thereby favoring constitutive activation [23].

UL33 couples to Gαq, Gαi, and Gαs and constitutively activates PLC, p38, and CREB [69]. US27 appears to have some constitutive signaling activity because when expressed, cells exhibited an increase in proliferation, as well as higher levels of ATP, DNA synthesis, and apoptosis resistance than control, US28-expressing, or cells expressing a US27 DRY box mutant (R128A) [70]. Additionally, the presence of wild-type and mutant versions of US27 or US28 modulated expression levels of several cellular genes in the JAK-STAT or p53 pathways; however, the G proteins mediating these effects were not determined in these assays [70,71]. Finally, UL78 is not yet known to couple to any particular G protein, but it likely activates different signaling pathways not shared by the others to expand the options for manipulation of host responses. Combined with the possibility of promiscuously binding to multiple G proteins and being constitutively activated, diversifying roles enables multiple vGPCRs to hijack the host cell machinery and modulate numerous signaling pathways and outcomes.

CHEMOTAXIS

The binding of chemokine ligands commonly incudes cell movement along a chemokine gradient. After ligand binding, calcium is rapidly released from the ER, triggering the activation of kinases that promote reorganization of the actin cytoskeleton and ultimately, chemotaxis. Modulation of cellular migration poses serious implications for HCMV pathogenesis and dissemination.

HCMV infection induced arterial smooth muscle cell (**SMC**) migration, but not migration of venous SMCs, normal human dermal fibroblasts (**NHDFs**) nor human umbilical vein endothelial cells (**HUVECs**) [72]. This migration was dependent on US28 expression as HCMV infection of

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SMCs with a US28 deletion (ΔUS28) decreased migration rate to near mock infection levels [72]. Coinfection of adenovirus encoding US28 and HCMV lacking US28 was able to restore SMC migration to wild-type HCMV infection levels [72].

US28-mediated migration of SMCs would suggests that the vGPCR could be the underlying factor that associates HCMV with acceleration of several vascular diseases, including restenosis, atherosclerosis, and chronic rejection [73-75]. Additionally, in glioblastoma multiforme tumors, either HCMV infection or US28 over expression promoted glioma growth and invasion, with CCL5/RANTES treatment further enhancing the invasive phenotype [76]. Additional evidence exists of cell type-specific and ligand specific US28-mediated cell migration. In US28-expressing cells, CCL5/RANTES induced migration of SMCs but not macrophages, while CX3CL1/Fractalkine promoted macrophage migration but also inhibited CCL5/RANTES-mediated SMC migration [77].

Additionally, US27, UL33, and UL78 are orphan receptors, but have been shown to modify the signaling of CXCR4 in transfected cells. In HEK293 cells, wild-type US27 expression up-regulated CXCR4 transcription and augmented calcium mobilization and chemotactic response towards its cognate ligand CXCL12/SDF-1 [78]. In contrast, transfection of UL33 or UL78 in monocytes resulted in a decrease of CXCR4 and CCR5 surface levels and chemotaxis towards CXCL12/SDF-1 and CCL5/RANTES [79]. Additionally, HCMV infection of HUVECs led to downregulation of the surface levels of CXCR4 at late time periods compared to mock or US28-deletion mutant infected cells [80].

These findings suggest that US27 and UL33/UL78/US28 possess opposite functions as they positively or negatively affect CXCR4 activity, respectively. As chemotaxis activated by CXCL12/SDF-1 can induce CXCR4-expressing cells to migrate towards the bone marrow, HCMV may manipulate the CXCR4/CXCL12 axis during infection for viral dissemination or immune evasion. Upregulation of CXCR4 activity would encourage infected cells to migrate to the stromal tissues where CXCL12/SDF-1 is highly expressed, while down regulation would induce cells to egress from tissues and reenter circulation. Heterodimerization between CXCR4 and HCMV's GPCR may facilitate this process as accumulating evidence shows that each pairing encompasses distinct properties than its individual components [81]. US28 was shown to form homodimers with itself and heterodimers with US27, UL33, and UL78 [60]. Also, UL33 and UL78 formed heterodimers with CXCR4 [79], while US27 was in close proximity to CXCR4 at the cell surface and in internalized vesicles [51]. When an infected cell is in circulation, US28 may physically associate with UL33 or UL78, thereby freeing US27 to be constitutively active, increasing CXCR4 surface levels, and inducing chemotaxis of the cell towards stromal tissues to spread the infection. In these newly infected cells, US28 may associate with US27, freeing UL33 and UL78 to down regulate CXCR4 surface levels, encouraging the cells to leave the stromal tissues and enter circulation. This manipulation of a host cell receptor could aid HCMV in establishing a systemic infection throughout the host.

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Similarly, in murine SMCs, MCMV infection increased cell migration independent of any exogenous source of ligand, and as mentioned above, M33 enhanced migration via mRANTES stimulation [39]. As SMC migration is important in the development of atherosclerosis, M33, similar to US28, may assist in the pathogenesis of this and other inflammatory vascular diseases.

As an enveloped virus, the outer layer of HCMV is derived from the host cellular membrane; therefore, surface membrane proteins of virus and host origin may be incorporated into viral particles during the assembly phase. US27, US28, and UL33 were discovered in HCMV particles, dense bodies, and non-infectious enveloped particles (**NIEPs**) [17,43,82]. Upon fusion of the newly infected cell's membrane and the virion envelope, the presence of these GPCRs could activate G protein signaling before *de novo* protein synthesis, rapidly inducing signaling and potentially, chemotaxis, immediately following infection [49].

VIRUS DISSEMINATION

US27, US28, UL33, and UL78 of HCMV are not essential for viral replication, but they may have important roles in virus persistence and dissemination as demonstrated by studies using virus with deletions for viral GPCRs, including mouse and rat CMVs [40]. US27 is required for dissemination via the extracellular route but not direct cell-to-cell spread [50]. Infection of fibroblasts and HUVECs at a low MOI with virus encoding an abrogated version of US27 resulted in reduced titer levels of extracellular virus [50].

In contrast, compared to wild-type virus, fibroblast infection using a virus with a US28 deletion (ΔUS28) resulted in a 10-fold or 100-fold increase in extracellular virus accumulation at high and low MOIs, respectively, but displayed a growth defect in epithelial cells, where spread of the virus is exclusively cell-to-cell [50,83]. Culturing in the presence with a neutralizing antibody that binds to glycoprotein gH significantly reduced the viral spread of the US28 knockout, while the wild-type strain experienced slightly decreased levels. This suggests US28 may contribute to cell-to-cell virus dissemination during infection.

The role of HCMV UL33 in virus dissemination is not currently known, but studies involving its murine and rat homologs may suggest some involvement. Deletion of M33 in MCMV resulted in a weakened ability to reactivate from the spleen and lungs in mice, and reduced viral DNA levels in latently infected spleens, lungs, and bone marrow [84]. Additionally, M33 is essential for replication in salivary glands, a site of horizontal host transmission, and has important role in regulating latency [33,84]. This requirement was dependent on M33 signaling, as a point mutation in the signaling motif, M33(R131Q), failed to replicate in the salivary glands [85]. While most cellular GPCRs have a conserved DRY motif, an Asp-Arg-Tyr (**DRY**) sequence, M33 has modified motif, an Asn-Arg-Tyr (**NRY**) sequence, which appears to be important for signaling.

Additionally, in a separate *in vivo* study using a mouse model, recombinant versions of MCMV were engineered, including an M33 deletion (ΔM33), M33 signaling deficient (M33(R131Q)) and

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M33 deletion plus insertion of either HCMV US28 (ΔM33/US28) or UL33 (ΔM33/UL33) [86]. Regarding infectivity of the salivary gland, wild-type MCMV attained high titer levels, ΔM33/US28 and ΔM33/UL33 reached low levels at later time points tested, and ΔM33 and M33(R131Q) failed to establish MCMV infection [86]. This data indicates efficient MCMV replication in the salivary glands is highly specific to M33 signaling [86]. Furthermore, in an assay to measure reactivation from latency, ΔM33/US28 and ΔM33/UL33 were able to restore, to some extent, reactivation from spleen and lung explant cultures compared to wild-type MCMV, while M33(R131Q) infection exhibited greatly reduced reactivation rates [86]. Although US28 and UL33 could not effectively establish infection in mouse salivary glands, this data suggests they may signal similarly to M33 in other tissues. Together, this data could support using MCMV as a model in functional studies of HCMV-encoded GPCRs *in vivo*.

Virus infection with a UL78 deletion in endothelial and epithelial cells, two important cell types for *in vivo* virus replication and spread, resulted in reduced titer levels compared to wild-type virus that was not observed in fibroblasts [38]. This indicates UL78 is required for efficient replication in epithelial and endothelial cells, and the cell-type difference was attributed UL78's role in virion entry [38]. Infection of fibroblasts and macrophage *in vitro* using MCMV with a mutant version of M78 caused reduced growth kinetics, as well as a lower titer yield in several organs [87]. Additionally, mice infected with MCMV lacking M78 exhibited a greater survival rate than wild-type virus-infected mice [87].

R33 is not essential for virus replication *in vitro*, but has an important role in RCMV pathogenesis [40]. Compared to wild-type virus, infection of immunosuppressed rats with R33-deficient RCMV (ΔR33) resulted in a higher survival rate, and presence of the recombinant virus in the salivary glands could not be detected, indicating R33 is important in the active replication of RCMV in the salivary glands [40]. Together, these findings indicate that R33 may be involved in RCMV replication in other organs and tissues.

Lastly, R78 of RCMV aids in systemic virus dissemination and in efficient virus production in specific cell types [88,89]. In RCMV-infected rats, R78 gene expression was detected in all the tissues tested, including the spleen, lung, liver, kidney, salivary glands, and heart, although a different study revealed R78 was not required for replication in the salivary glands [88,89]. Moreover, in fibroblast and smooth muscle cells, and not monocytes or endothelial cells, efficient RCMV growth kinetics required R78: infection with a R78 deletion yielded lower titer levels in fibroblast and smooth muscle cells compared to wild-type virus [88]. Additionally, *in vivo* studies revealed the mortality rate was lower among RCMV ΔR78, indicating R78 contributes to overall RCMV pathogenesis [88]. Collectively, these studies using HCMV, MCMV, and RCMV indicate viral GPCRs play important roles in virus dissemination and tissue-specific infection: deletion of just one would render a virus inefficient in using multiple routes of intercellular and inter-host transmission.

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HCMV AND HIV

As mentioned above, HCMV infection poses life-threatening risks to the immunocompromised, especially HIV-infected patients. HCMV infection is frequent among HIV patients, and may be involved in HIV pathogenesis [90] . Progressive loss of a robust immune system permits HCMV reactivation and replication. HCMV's broad cell tropism includes endothelial and epithelial cells, smooth muscle cells, fibroblast, neuronal cells, hepatocytes, and cells of the myeloid lineage [91]. This broad cell tropism can lead to tissue necrosis, followed by subsequent end-organ disease. Complications of HIV and HCMV co- infection include chorioretinitis, enterocolitis, esophagitis, pneumonitis, and encephalitis [92].

Using its envelope glycoprotein, gp120, HIV gains entry into host cell by engaging CD4 and a co-receptor, CXCR4 and CCR5 [93-95]. Interestingly, US28 has also been shown to be a co-receptor for HIV entry and promote cell fusion [96,97]. Having US28 as a co-receptor for HIV entry would further complicate co-infection with HCMV. Additionally, in THP-1 monocytes, transfection of UL33 or UL78 resulted in blocking HIV entry by modulating CCR5 and CXCR4 co-receptor activity via heterodimerization [79]. How US28, UL33, and UL78 affect HIV entry during co-infection is unclear, but aiding or blocking HIV entry into HCMV- infected cells may depend on factors such as timing as these three vGPCRs are expressed at different points post infection.

CONCLUSION

Cytomegaloviruses have co-evolved with their mammalian hosts for thousands of years. They establish life-long infection through latency, and these viruses have developed multiple strategies to avoiding immune system eradication and enhancing viral replication. Latency and productive infection are generally asymptomatic to the host, but cause serious illness in the immunocompromised. These vGPCRs were incorporated into the viral genome most likely during a capture event of an ancestral mammalian host receptor. Many vGPCRs are constitutively active, with some known to active multiple signaling pathways; therefore, they can significantly affect cellular activity and phenotype. Their importance to their respective species is highlighted by several studies revealing deletion or mutation can have a measurable effect on growth kinetics, establishing tissue-specific infection, and mortality rate.

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