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The US27 gene product of human cytomegalovirus enhances signaling of host chemokine receptor CXCR4

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ABSTRACT

Human cytomegalovirus (HCMV) is a member of the *Herpesviridae* family that manipulates host immune responses and establishes life-long latent infection, in part through mimicry of cytokines, chemokines, and chemokine receptors. The HCMV US27 gene product is a putative chemokine receptor with no known ligands. We generated a stable US27 cell line to screen for chemokine ligands but unexpectedly found that US27 potentiated the activity of an endogenous human chemokine receptor, CXCR4. Cells expressing both US27 and CXCR4 exhibited greater calcium mobilization and enhanced chemotaxis in response to CXCL12/SDF-1α than controls. Quantitative RT-PCR revealed a significant increase in CXCR4 expression when US27 was present, and elevated CXCR4 receptor levels were detected via flow cytometry, western blot, and immunofluorescence microscopy. Potentiation of CXCR4 signaling by US27 could represent a novel strategy by which HCMV targets virus-infected cells to the bone marrow in order to expand the reservoir of latently infected cells.

Introduction

Human cytomegalovirus (HCMV) is a member of the β-herpesvirus subgroup and a prevalent pathogen infecting a vast majority of the human population. Although immune-competent hosts are generally asymptomatic upon infection, HCMV poses a serious health risk for immune-compromised hosts (Kesson and Kakakios, 2007). HCMV is the primary infectious cause of birth defects, including deafness and mental retardation (Revello et al., 2008).

Despite a vigorous host immune response to HCMV infection, the virus is able to avoid immune clearance and establish lifelong latency (Jackson et al., 2011). HCMV has evolved tactics to suppress inflammatory cytokines (Spencer et al., 2002), avoid elimination by natural killer cells (Beck and Barrell, 1988; Tomasec et al., 2000), and prevent apoptosis of infected cells (McCormick, 2008). Another immune evasion technique is the codification of proteins with structural similarity to the G-protein coupled receptor (GPCR) superfamily (Chee et al., 1990). Chemokine receptors are a subset of GPCR that play a pivotal role in the immune response by receiving and reacting to chemokine signals, which attract and direct immune cells to sites of injury and infection (Charo and Ransohoff, 2006). Viral mimicry of chemokine receptors provides an effective mechanism for manipulation of human chemokine signaling that could facilitate both virus dissemination and avoidance of harmful host immune cells (Holst and Rosenkilde, 2003; Vischer et al., 2006).

Chemokine receptor homologs encoded by HCMV include US27, US28, UL33, and UL78. Of these, US28 is the most extensively researched and was found to bind to several human CC chemokines (Gao and Murphy, 1994; Neote et al., 1993; Stropes et al., 2009). This binding can lead to calcium mobilization, IP_3 production, and activation of transcription factors such as NFAT, CREB, and NF-κB (Billstrom et al., 1998; Casarosa et al., 2001; Vieira et al., 1998). US28 has also been shown to act as a co-receptor for HIV entry (Pleskoff et al., 1997), promotes fusion between cells (Pleskoff et al., 1998), triggers migration of smooth muscle cells (Streblow et al., 1999), and contributes to the characteristic angiogenic and invasive phenotype of glioblastoma cells (Soroceanu et al., 2011). Most recently, US28 has been shown to form heteromers with the other HCMV chemokine receptors US27, UL33 and UL78 (Tschische et al., 2011). While no functional changes were observed with the US28:US27 dimer, the US28:UL33 dimer and the US28:UL78 dimer both ablated activation of NF-κB by US28, suggesting a complex level of regulation in which these viral receptors may act in concert to either promote or block signaling through particular pathways during the course of virus infection.

US27 and US28 are adjacent in the HCMV genome and share 31% sequence identity. Both proteins are expressed in infected cells (Fraile-Ramos et al., 2002), although is US28 expressed throughout lytic and latent infection, while US27 is expressed late in lytic infection only, indicating the two genes are independently

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regulated and function at different stages of the virus infection cycle. To date, US27 remains an orphan receptor that binds no known cellular ligands (Bodaghi et al., 1998; Stapleton et al., 2012). US27 exhibits many structural features of chemokine receptors, including seven transmembrane domains with conserved cysteine residues in the extracellular loops, and extensive glycosylation of the extracellular domain (Margulies and Gibson, 2007), a common characteristic for receptors with small peptide or chemokine ligands (Katritch et al., 2012). US27 also contains a DRY (aspartic acid, arginine, tyrosine) motif in the second intracellular loop that is critical for activation of associated G proteins following ligand engagement (Flanagan, 2005), and a di-leucine motif in the carboxy-terminal intracellular domain that mediates receptor endocytosis (Stapleton et al., 2012).

US27 is present in the envelope of the virus particle (Margulies and Gibson, 2007), but in virus-infected cells, the receptor is rapidly internalized with the majority of the US27 protein found in endosomes, the Golgi apparatus, and perinuclear compartments (Fraile-Ramos et al., 2002). Intracellular localization was found to be mediated by the carboxy-terminal domain of the US27 protein product (Stapleton et al., 2012). While viral mutants that lack US27 are replication competent (Bodaghi et al., 1998), these viruses are incapable of spreading via the extracellular route (O'Connor and Shenk, 2011), leading to speculation that US27 may play a role in virion assembly or egress.

Here, we set out to "de-orphanize" HCMV US27 and instead report the surprising discovery that US27 can directly enhance the calcium signaling activity of a human chemokine receptor, CXCR4. This finding is in direct contrast to the effects of other viral chemokine receptors, which have been found to impair CXCR4 function. Moreover, we found elevated levels of CXCR4 receptor in cells expressing US27, which resulted in increased cell migration in vitro and could have profound implications for immune cell trafficking in HCMV patients. Potentiation of CXCR4 activity by US27 demonstrates yet another highly sophisticated method of immune modulation employed by HCMV.

Results

CXCR4 induces greater calcium mobilization in response to CXCL12/SDF-1α in the presence of HCMV US27

We previously attempted to investigate the functional activity of US27 by performing a chemokine ligand screen (Stapleton et al., 2012). HEK293 cells stably expressing US27 (293-US27) were loaded with a calcium sensitive dye and exposed to more than 100 different individual human chemokines. Only one chemokine elicited a calcium flux response: CXCL12/SDF-1α. The response to CXCL12/SDF-1α was expected due to the presence of CXCR4 endogenously expressed on HEK293 cells (Hoffmann et al., 2012). However, the magnitude of the calcium response to CXCL12/SDF-1α in 293-US27 cells was consistently 2–3 times greater than the response in HEK293 cells, which express only CXCR4 (Fig. 1).

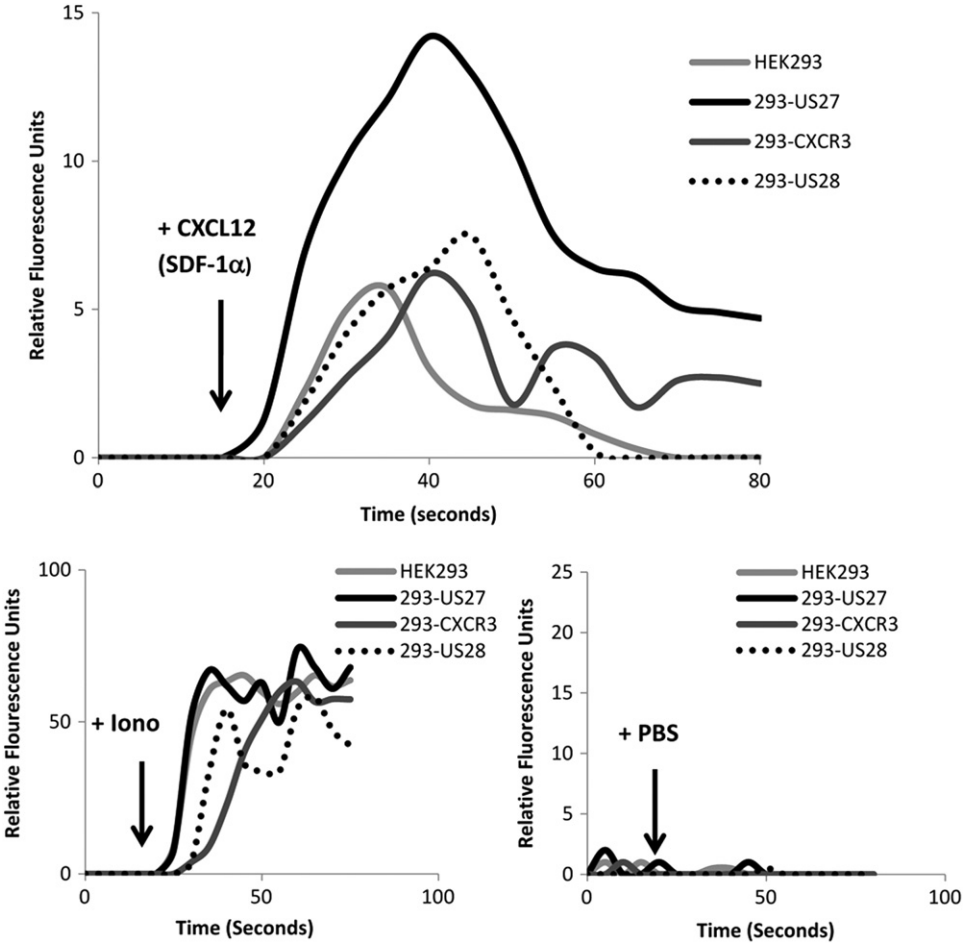

Fig. 1. Increased calcium mobilization in cells expressing CXCR4 and HCMV US27. HEK293 cells and stable 293-US27, 293-US28, and 293-CXCR3 cell lines were loaded with Fluo-4 dye, treated with 100 μg/ml CXCL12/SDF-1α in a volume of 10 μl, and fluorescence intensity monitored over time. An equal volume of PBS was added as a negative control and 1 mg/ml ionomycin in a volume of 5 μl served as a positive control. These results are representative of four independent experiments.