Characterization of Signaling Properties of the HCMV-Encoded G Protein Coupled Receptor US28 and Analysis of its Contribution to Viral Induced Atherosclerosis

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The herpesvirus, human cytomegalovirus (HCMV), has been shown to induce vascular smooth muscle migration, and therefore, is a likely contributor to the development of vascular diseases such as atherosclerosis. Atherosclerosis involves a sequence of inflammatory events that ultimately result in a narrowing of the walls of arterial vessels by the formation of fibrous plaques. The release of chemoattractant molecules (such as RANTES) by LDL engorged macrophages and the subsequent migration of smooth muscle cells from surrounding areas into the vessel intima is associated with the formation of an advanced atherosclerotic lesion. It has been demonstrated that this process is augmented by infection of surrounding smooth muscle cells with HCMV, and therefore, the expression of viral gene products is implicated in the formation of vascular disease. HCMV encodes a G protein-coupled receptor (GPCR), US28, which binds to the chemokine RANTES and demonstrates various modalities of cell signaling, including activation of phospholipase C-â (PLC- â), release of calcium from intracellular stores, and activation of numerous transcription factors. The US28 gene product is the suspected culprit of HCMV-associated atherosclerosis, as US28 activity has been demonstrated to result in arterial smooth muscle cell migration. In this study we have investigated the signaling properties of various US28 mutants in smooth muscle cells infected with HCMV. Unexpectedly, the N-terminal chemokine binding domain is not required for PLC- â signaling and RANTES has no effect. Conversely, this chemokine binding domain and stimulation with RANTES is required for calcium release from intracellular stores. We have also investigated the effects of US28 on signaling via endogenous lysophosphatidic acid (LPA) receptors. LPA is a component of oxidized LDL and like other chemoattractant molecules has been demonstrated to play a role in the development of atherosclerotic plaques. Interestingly, the data indicate that US28 can enhance signaling via endogenous LPA receptors. Taken together, our results indicate that US28 induces a variety of signaling events in smooth muscle cells; some dependent on agonist and some independent of agonist. We are currently investigating how these US28 signaling events contribute to the pathogenesis of HCMV induced atherosclerosis.