Summary

The ubiquitous human cytomegalovirus encodes four putative G-protein coupled receptors with sequence homology to human chemokine receptors: US27, US28, UL33 and UL78. To date, only the US28 gene product has been characterized as a functional receptor for the inflammatory chemokines RANTES (CCL5), MIP-1α (CCL3), MIP-1β (CCL4), MCP-1 (CCL2) and MCP-3 (CCL7). Upon ligand binding US28 mediates the elevation of intracellular calcium levels, the activation of mitogen-activated protein kinase (MAPK) ERK2 and the chemotaxis of arterial smooth muscle cells. Additionally, transient expression of US28 results in constitutive activation of phospholipase C and the transcription factors NF-κB and CREB. It was suggested that the US28 dependent sequestration of chemokines from the environment of infected cells may represent a viral strategy to evade the human immune response.

The present study describes the generation of the first US28 specific monoclonal antibodies. They allowed the characterization of US28 as a “late protein” which is expressed during the late phase of viral replication 24-48 hours after infection of human embryonal fibroblasts with human cytomegalovirus. By using the monoclonal antibodies posttranslational modifications of the US28 molecule were analysed. It was shown that US28 undergoes strong ligand-independent phosphorylation but was not modified by *N*-linked glycosylations or tyrosine sulfation in transiently transfected HEK293A cells. Coexpression experiments characterized the US28 receptor as a substrate for G-protein coupled receptor kinase 2. Moreover, protein kinase C and casein kinase 2 were partially involved in the regulation of constitutive US28 phosphorylation levels in HEK293A cells. Phosphoamino acid analysis revealed a predominant phosphorylation at serine residues and a minor phosphorylation at threonine residues for both the unstimulated and the RANTES-stimulated US28 receptor. Site-directed mutagenesis of C-terminal serine and threonine residues identified serine residues between positions 323 and 350 as major sites of US28 phosphorylation. Signaling studies using the US28 substitution mutants demonstrated that constitutive activation of NF-κB and RANTES-induced activation of MAPK were

independent from receptor phosphorylation. However, MAPK activation by the US28 wildtype receptor was inhibited by pertussis toxin, whereas phosphorylation deficient receptor mutants remained pertussis toxin insensitive, indicating that receptor phosphorylation is required for coupling to Gα_i-proteins. Additionally, it was shown that stimulation of US28 with RANTES induced an activation of the MAPK ERK1 and p38. Flow cytometry and immuno-cytochemistry analysis employing the US28-specific antibodies revealed that US28 accumulated in intracellular vesicles of transiently transfected HEK293A, HeLa and COS-7 cells. Only a minor portion of the receptor was detectable on the surface of US28 expressing cells. Substitution of the phosphorylation sites resulted in an enhanced cell surface expression of US28 and was responsible for a delayed and strongly reduced receptor endocytosis. Thus, constitutive US28 phosphorylation regulates endocytosis and cell surface display of the receptor. Since receptor endocytosis represents a cellular mechanism for ligand internalization constitutive US28 phosphorylation may provide the structural prerequisite for the sequestration of inflammatory chemokines from the environment of infected cells.