Chemokines are small chemotactic cytokines that are produced constitutively or secreted upon pro-inflammatory stimuli. They bind to chemokine receptors that can couple to a variety of signaling pathways in a cell type specific manner. Together chemokine receptors and their ligands constitute a vast network that orchestrates many immune functions, such as immune cell recruitment, mast cell degranulation and T cell differentiation. Proper immune function requires the strict control of chemokine and chemokine receptor function. Aberrant chemokine regulation often leads to a pathophysiological condition.
Disturbed chemokine function is also found during viral infections. In particular, herpesviruses and poxviruses are highly dependent on our immune system for their persistence, replication and dissemination. These viruses have 'stolen' the genetic components of our chemokine system and use these for their own benefit. In **chapter 2** of this thesis, we discuss the various ways in which viruses attempt to modulate the chemokine network. Unraveling the function of viral chemokine modulators is a double-edged sword that may provide novel tools for anti-inflammatory therapy or identify important virulence factors to which antiviral therapy can be targeted.
In this thesis, we have examined two chemokine receptor mimetics in more detail. We found that these viral proteins are multifaceted molecules whose actions reach beyond the chemokine network, providing interesting new possibilities for anti-inflammatory and antiviral therapies. In first part, we studied the anti-inflammatory effects of M-T7, a soluble poxviral chemokine-binding protein. In the second part, we extensively investigated the role of the chemokine receptor homolog US28 in the human cytomegalovirus life cycle.

M-T7 is a rabbit IFN-γ receptor homolog that is encoded by the myxomavirus. In addition to species-specific binding of rabbit IFN-γ, it also binds a wide variety of chemokines in a non-species specific manner [208, 295]. This characteristic was attributed to its interaction with conserved heparin-binding motifs [208]. These motifs are used for interaction with glycosaminoglycans (GAGs) which is important for the biological activity of chemokines. GAGs coupled to a protein backbone, the so-called proteoglycans, are abundantly expressed on cell surfaces. Immobilization of chemokines by proteoglycans facilitates the formation of solid gradients that is required for leukocyte recruitment. Disruption of the chemokine-GAG interaction provides interesting new opportunities for anti-inflammatory therapy [143]. Previous studies have shown that infusion of the purified protein M-T7 effectively reduces the response to

injury in rats and rabbits [21, 165, 166]. In **chapter 3**, we investigated whether M-T7 inhibits to the attraction of immune cells to an implanted biomaterial. Heterotopic expression of the M-T7 gene indeed blocked the cellular influx into foreign body implants. An interesting observation we did was that this was associated with reduced formation of blood vessels. On the molecular level, we investigated MCP-1 and VEGF164, as these molecules bound to GAGs and were known to mediate macrophage chemotaxis and blood vessel formation. We found that despite invariable mRNA expression staining for both mediators was reduced. Our results confirm the applicability of M-T7 for the inhibition of inflammation, but shows that the anti-inflammatory activity of M-T7 reaches beyond the modulation of the chemokine network.

Because M-T7 is a secreted protein, it will block immune function systemically. For local application, we require broad-spectrum decoy receptors that bind and internalize chemokines. In the next part, we have investigated the anti-inflammatory properties of a herpesviral chemokine decoy receptor US28. US28 is a seven-loop transmembrane G protein-coupled receptor that is structurally related to the human β-chemokine receptor CCR1. In the last decade several hypotheses were raised concerning the significance of US28 for HCMV infection, which are discussed in **chapter 4**.

High affinity interaction with a variety of β-chemokines and a high recycling rate suggested that US28-expressing cells act as a "chemokine sink" that disrupts chemotactic gradients. A hypothesis frequently postulated is that during HCMV infection, the scavenging of chemokines by US28 protects HCMV infected cells from immune clearance by leukocytes. During active infection, HCMV can be detected in endothelial cells. Infection results in the activation or even detachment of endothelial cells from the vascular wall. After infection, endothelial cells in the vessel wall produce a significant amount of the chemokine RANTES/CCL5 that possibly triggers the arrest of passing leukocytes that play a role in virus clearance. By expressing chemokine decoy receptors viruses attempt to counteract this rapid increase of chemokines. In **chapter 5**, we have examined the impact of chemokine internalization by endothelium-expressed US28 on the leukocyte adherence. To rule out any confounding factors caused by concomitant activation of signaling pathways, we used a signaling mute receptor US28R129A. Despite the presence of US28 on a substantial number of cells, we were not able to find a reduction of adherent cells when US28R129A

was expressed. As we believe that leukocyte arrest and extravasation are cardinal events in the antiviral response, we doubt that chemokine scavenging by US28 significantly impairs leukocyte recruitment. Therefore US28 has less value for anti-inflammatory therapy.
Unfortunately, US28 was a poor choice for application as an anti-inflammatory agent. Other researchers found that ligand binding to US28 induced migration of vascular smooth muscle cells. In addition, US28 signals in a constitutive manner and activates PLC and NF-κB. These observations raised our suspicion that US28 signaling is implicated in the pathogenesis of HCMV disease. In **chapter 6**, we focused on the significance of NF-κB activation on transactivation of the HCMV major-immediate-early promoter. This promoter is important for the transcription of immediate early genes that drive the cascade of gene expression that is necessary for full replication of the virus. The current paradigm is that extracellular factors such as an inflammatory stimulus activate the MIE promoter [133]. Using a firefly luciferase reporter assay we measured MIE promoter activity in the presence and absence of US28. We found that constitutive signaling by US28 enhances MIEP activity by multiple pathways that involve NF-κB and p38 MAPK. When we investigated the effects of US28 signaling on host gene expression, we encountered problems with long-term stability of protein expression. In **chapter 7** we attempted to generate stable cell lines, but found that US28 expression increased the number of early apoptotic cells as determined by Annexin V staining. We have strong indications that this phenomenon depends on G proteins as viability was preserved in US28R129A expressing cells. We did not investigate which pathways were involved in the US28-mediated apoptosis.
Recapitulating the preceding chapters, we consider US28 rather a target for antiviral therapy than a tool to combat chronic inflammation. Enhancement of MIEP activity suggests that US28 signaling is involved in the early phases of infection. Expression profiles of *in vitro* infected fibroblast identified US28 as an early gene [333]. However, little is known about the kinetics of US28 gene expression during clinical HCMV infection and its relationship with disease activity. In **chapter 8** we measured transcript levels of US28 and IE1 in lung transplant recipients that have an active HCMV infection. We found that US28 can be detected immediately after IE1 transcripts appear. Expression of US28 did not correlate with viral load, a clinical parameter for the course of infection. US28 transcripts became undetectable when IE1 transcript levels dropped. We found that US28 gene expression is correlated to IE gene expression, which

supports our findings in chapter 6. Our data support the hypothesis that US28 is involved in viral replication in the early stages of infection by transactivation of viral promoters.

In **chapter 9** the findings in this thesis are discussed. We have demonstrated that the viral chemokine-binding protein M-T7 not only acts in the chemokine network but probably also interferes in other inflammatory networks, such as angiogenesis. This newly discovered activity of M-T7 illustrates the importance of GAG interaction in inflammation and provides an interesting opportunity to inhibit inflammation in multiple ways. In contrast, to M-T7 we discovered that the chemokine receptor homolog US28 is highly unsuitable for this purpose. Our findings suggest that US28 may not be considered as a modulator of the chemokine system, but rather a modulator of host gene transcription machinery, which may be beneficial for virus replication.