The present thesis focuses on the manifestations of HCMV in the peripheral blood during an active infection. Special emphasis is given to the pathophysiological significance of the HCMV antigen positive leukocytes as detected with the HCMV antigenemia assay, and the possible role of these cells in the dissemination of the virus throughout the body.

Chapter 1 provides a general introduction to the subject, paying attention to the virus itself (structure, replication cycle, target cell entry), to its clinical aspects (epidemiology, symptomatology, diagnosis), and to its presence in the peripheral blood during latency and during active infection, and describes the aims of the study.

In **chapter 2** the viral antigen detected with the HCMV antigenemia assay in peripheral blood leukocytes was identified as the lower matrix protein pp65, an abundant early/late structural viral matrix protein. Surprisingly, immediate early viral antigens were present in only a subset of the leukocytes that express pp65, although it is known that the presence of such immediate early HCMV antigens is necessary for transcription of the early/late pp65 gene. This suggested that the presence of pp65 in leukocytes might not be the result of *de novo* synthesis, but might result from uptake of this abundant viral matrix protein from an unknown site in the body. In addition, it could be shown in *in vitro* experiments that pp65 was taken up by cultured human fetal lung fibroblasts from a crude virus preparation with subsequent nuclear localization, which supports this hypothesis. Furthermore, it was shown in this chapter that peripheral blood leukocytes did not express two other early/late viral antigens, which suggests that they are only abortively infected and do not support a complete viral replication cycle when circulating.

Chapter 3 describes the discovery of circulating cytomegalic inclusion cells in the leukocyte fraction of the peripheral blood of patients with an active HCMV infection. It was demonstrated that these cells contained viral antigens from all three stages of the HCMV replication cycle, including a large amount of pp65. The origin of these cells was investigated with the use of several monoclonal and polyclonal antibodies directed against cell marker and differentiation antigens. The results showed that they are detached endothelial cells, indicating that HCMV may be accompanied by widespread occult vascular damage. It was hypothesized that these cells may represent the site where leukocytes pick up their pp65.

Chapter 4 describes the results of the electronmicroscopic study of the circulating cytomegalic cells. They were shown to contain numerous mature and defective virus particles (e.g. dense bodies). These cells are therefore definitively productively infected with the virus and can be assumed to be able to disseminate HCMV throughout the body. In addition, the finding of a cluster of cytomegalic cells in the peripheral blood linked together by zonula adherens type cell junctions is further evidence for the endothelial origin of these cells and suggests that the endothelial damage may be extensive.

Chapter 5 examines the manifestations of HCMV in the tissues during an active infection. Immunohistochemical double staining was used to determine which of the cell types present in the tissues are infected with HCMV, and which stage of infection they represent. Both lung and gastrointestinal tissue sections were examined. The results showed that epithelial cells, endothelial cells, fibroblasts, and smooth muscle cells were the predominantly infected cell types *in vivo*. All of them seemed to be able to support a

complete viral replication cycle as indicated by a characteristic cytomegalic (owl's eye) appearance. A minority of infected cells were monocytes/macrophages. They were also capable of supporting a complete viral replication cycle, as demonstrated by their owl's eye appearance. In addition, polymorphonuclear cells were found containing viral immediate early antigens. These cells, however, never displayed the cytomegalic appearance belonging to a productive virus infection, nor did they express the viral early DNA-binding protein p52, and it is assumed that they are most probably only abortively infected. This confirms the findings in peripheral blood polymorphonuclear cells as described in chapter 2. The chapter ends with a schematic representation of hypothetical routes of HCMV spreading, one of which hematogeneously, during an active infection, based on these findings.

Chapter 6 describes the development of an mRNA *in situ* hybridization procedure to detect pp65 mRNA and immediate early mRNA in peripheral blood leukocytes during an active HCMV infection to investigate whether transcription of the HCMV genome occurs in these cells. For the development of the method HCMV-infected fibroblasts were used as positive control cells. **Chapter 7** describes the use of the *in situ* hybridization procedure to investigate isolated mononuclear and polymorphonuclear leukocytes from patients with an active HCMV infection for the presence of pp65 and immediate early antigens and mRNAs. The aim is to test the working hypothesis postulated in chapter 2, i.e. uptake rather than synthesis of pp65 in leukocytes. Both cell fractions were found to contain HCMV immediate early antigen and pp65, just as demonstrated in chapter 2. However, only mRNAs encoding immediate early antigen were found in these cells, whereas mRNAs encoding pp65 were not detected. In contrast, both viral antigens and mRNAs were detected in the circulating cytomegalic inclusion cells that were also present in the leukocyte fractions investigated. This demonstrates that restricted viral gene expression, i.e. transcription of immediate early genes, does occur in mononuclear and polymorphonuclear leukocytes during an active HCMV infection. However, the presence of the early structural antigen pp65, without the corresponding mRNA, strongly indicates uptake of this protein by the phagocytic leukocytes, rather than *de novo* synthesis.

In **chapter 8** several attempts are described to generate pp65 positive polymorphonuclear leukocytes *in vitro*, with the intent to elucidate the mechanism(s) that might play a role *in vivo*. Experiments in which cell-free HCMV preparations (both laboratory strain AD169 and recently isolated wild type HCMV), with or without several cell differentiation and activation agents were used, failed to induce the formation of pp65 positive leukocytes. For the successful generation of pp65 positive leukocytes the adherence to a HCMV-infected monolayer of fibroblasts appeared to be a necessary pre-requisite. This *in vitro* finding suggests that *in vivo* polymorphonuclear leukocytes may become pp65 positive via cell-cell contact with HCMV-infected cells, endothelial cells being the most likely candidate. However, polymorphonuclear cells did not consistently show pp65 expression when 'infected' this way *in vitro*, and did not express immediate early viral antigens either, indicating that additional factors are likely to be involved *in vivo*.

Chapter 9 discusses the findings described above with regard to the questions raised in the introduction. First, the pathophysiological significance of the HCMV antigen positive leukocytes circulating during an active HCMV infection is discussed. It is concluded that the majority of these cells contain the structural viral matrix protein pp65 most probably as a result of uptake. In addition, a subset of these cells contains viral

immediate early antigens and their encoding transcripts, suggesting that the viral genome is actively transcribed in these cells, and that they may play a role in the dissemination of the virus. Second, the role of the circulating late-stage infected endothelial cells in the dissemination of HCMV through the body is discussed. Third, a working model regarding the mutual relationships between these cell types hypothesized to be involved in virus dissemination is presented. In addition, suggestions for further research in this area are made.