Vascular pathophysiology of cytomegalovirus infection after kidney transplantation
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Summary
The endothelium is one of the tissues that can become infected during cytomegalovirus (CMV) infection. Because of its location and function endothelium plays a crucial role in the dissemination and pathophysiology of the virus. In the present thesis a series of studies is described concerning to the role of endothelium during CMV infection is described. In these studies, kidney transplant recipients were monitored for CMV infection and during the infection several parameters related to vascular damage were studied and related to acute rejection episodes. Furthermore some of the CMV patients underwent functional lung tests and the relationship between pulmonary function and endothelial cells in blood was investigated (Chapter 3 – 5). We also performed in vitro experiments to examine the viral dissemination and viral pathophysiology at endothelial function (Chapter 6 – 8).

Chapter 1 provides an overview of CMV infection in relation to the vasculature and the scope of the thesis is presented in Chapter 1.2. The overview starts with features specific to this virus, viral infection and clinical aspects of infection with CMV. The immunological response against CMV is explained alongside with evasion strategies of the virus. The relation of CMV with endothelium is reviewed, beginning with infection kinetics in vivo and in vitro. We discus factors of endothelial cell tropism, as well as the effects of CMV on morphology and function of EC. Finally, the significance of CMV in development of atherosclerosis and chronic rejection is described.

Chapter 2 reports on the development of a quantitative method to isolate cytomegalic endothelial cells (CEC). Peripheral blood mononuclear cells were stained for endothelial specific markers and FACS sorted onto adhesion slides. This method was compared to the conventional method of mononuclear cell isolation and cytocentrifugation, followed by a staining using CMV and endothelial specific markers. Although the recovery of endothelial cells from peripheral blood of both methods was comparable, the FACS sorting method appeared to be ten times more sensitive than the cytocentrifugation method.

In Chapter 3 we describe a study of the incidence and frequencies of endothelial cells in peripheral blood samples of kidney transplant recipients during CMV infection. Two types of endothelial cells were identified: late-stage CMV infected cytomegalic endothelial cells (CEC) and uninfected EC. The CEC were detected only in transplant recipients with moderate or high CMV antigenemia whereas uninfected EC observed in patients irrespective of CMV infection. The incidence of CEC, EC or both was associated to HCMV-related clinical symptoms. Remarkably, most patients with the highest numbers of CEC and EC in blood during CMV infection had suffered from acute rejection before CMV infection. Apparently, the sequence of acute rejection and CMV infection enhanced the extent of vascular involvement during CMV infection.

In Chapter 4 we investigated plasma levels of soluble markers during HCMV infection in kidney transplant recipients. Plasma levels of Von Willebrand Factor (VWF), sICAM-1, sVCAM-1 and sE-sel were analyzed during the course of HCMV infection and related to the presence of endothelial cells in blood and to the occurrence of acute rejection before CMV infection. In our study plasma levels of VWF and sVCAM-1 doubled during severe HCMV infection of which the plasma levels of VWF also correlated to the presence of CEC and EC.
in blood. The kinetics of changes in VWF and endothelial cells (CEC and EC) exhibited the relationship with HCMV induced vascular damage. Consistent with the findings described in Chapter 3, a combination of HCMV infection and preceding acute transplant rejection was accompanied by the highest increases of VWF and sVCAM-1 plasma levels.

In **Chapter 5.1** a study is presented of the pulmonary diffusion capacity for CO (KCOc) in kidney transplant recipients during CMV infection. By measuring parameters of elements specific for the capillary flow (Vcap) or the diffusion across the membrane between alveolus and capillary (Dm) the effect of the blood flow and for instance swelling of the interstitial tissues by fibrosis or inflammation could be assessed. According to our hypothesis, their large size would cause CEC plug into capillary vessels and which would impede the blood flow. During CMV infection this would predict a reduced Vcap and an unaffected Dm. However, we observed a decreased KCOc, composed of a decreased Vcap and a decreased Dm. We concluded that the observed reduction of the pulmonary diffusion capacity is caused by an inflammatory process and not by plugging of CEC in capillary vessels.

In **Chapter 5.2** we report the relationship between detectable CEC in blood, the severity of CMV antigenemia and the pulmonary diffusion capacity for CO. In nine patients, the pulmonary diffusion capacity was determined on the same day of blood sampling in order to study the presence of CEC and EC. In four of these patients, CEC were detected in blood during CMV infection, whereas five patients were free of CEC during infection. All HCMV patients showed a decreased KCOc with both a decreased Dm and Vcap and thus no differences were observed between patients with or without CEC. Analysis of the relation between the severity of CMV infection and the pulmonary diffusion capacity for CO revealed that patients with high HCMV displayed a larger decrease of Dm but not of KCOc or Vcap than the patients with lower levels of HCMV antigenemia.

In **Chapter 6** the role of CMV specific serum antibodies was investigated in an *in vitro* model of CMV antigenemia. This model is based on coculture of polymorphonuclear cells (PMNs) in the presence of endothelial cells infected with an endothelial-adapted strain of CMV.

In co-culture experiments between PMN and infected EC, the cell-to-cell contact between the PMN with CMV infected endothelial cells triggered the PMN to take up CMV protein pp65. Five minutes after first contact pp65 could already be detected in the nucleus of the PMN (CMV pp65 positive antigenemia). In this chapter we describe the inhibition of pp65 uptake by the PMN in the presence of patient sera. After a 2h co-culture period, inhibition of pp65 uptake by PMNs was observed using patient sera, purified IgG from the same patient as well as by anti-CMV hyperimmune globulin.

In **Chapter 7** we provide a closer look on the *in vitro* generated pp65 positive PMNs by investigating the mechanism by which PMN take up viral particles and proteins. Although phagocytosis seems the most plausible mechanism for uptake of viral particles, it did not explain how pp65 might end up in the nucleus and why it was not degraded in the lysosomal pathway. Moreover, by coating with lactoferrin or CMV specific antibodies the uptake of pp65, resulting in expression of pp65 in the nucleus, was inhibited, whereas these molecules do not affect phagocytosis. The inhibition of cytoskeleton-associated transport or fusion of multiple PMNs argues against phagocytosis as well. Therefore we propose that in addition to
phagocytosis, the fusion of viral particles with the membrane of PMNs is an additional mechanism for the uptake of pp65. Then, viral capsids (virions) or (dense bodies) proteins are released into the cytoplasm and rapidly transported to the nucleus.

**Chapter 8** describes the enhanced expression of ecto-ATP/ADPase (CD39) and ecto-5’nucleotidase (CD73) on CMV infected endothelial cells. Together with ecto-ATPase, ecto-5’ nucleotidase has an important role in regulation of platelet aggregation and activation of PMNs.

Activated platelets release ATP and ADP, which is rapidly hydrolyzed normally. This turnover prevents extensive platelet recruitment and activation of the attracted platelets. Adenosine inhibits the release of oxygen metabolites by PMNs. Our study shows that the antigen levels and enzyme activities of ecto-ATPase and ecto-5’ nucleotidase were increased on CMV infected cells and we demonstrated the enhanced enzymatic activity of ecto-5’ nucleotidase in experiments with *in vitro* cultured endothelial cells infected with CMV. Based on these observations, we suggest that the virally induced upregulation of ecto-ATPase and ecto-5’ nucleotidase serves as an evasion strategy to counteract some of the harmful effects of CMV infection on endothelial cells.

In **Chapter 9** the results described in this thesis are discussed. An evaluation is given about the meaning and significance of CEC in relation to CMV associated clinical symptoms. We discuss the putative origin of uninfected EC in blood. From the *in vitro* experiments we concluded that a major activity of CMV infected endothelial cells in viral dissemination already occurred before the appearance of CEC in blood, namely transmission of viral particles to PMNs and monocytes. Finally, further investigation is suggested on the vascular involvement during CMV infection as well as a proposal for studies in the context of endothelial pathology in the post-transplantation period.