CHAPTER 12

FINAL CONCLUSIONS

Cytomegalovirus infection is it still important in the transplant clinic? Is cytomegalovirus a vanishing or changing problem? The aim of this thesis is to elucidate the pathofysiological processes that play a role in the protean manifestations of cytomegalovirus infection after organ transplantation.

In *chapter 1* a general introduction is given. In *chapter 2* we describe the course of CMV infection frequencies in our transplant center the last ten years. We describe that modification of our immunosuppressive regimen, switching from cyclosporine standard formulation (Sandimmune, Novartis) to microemulsion formulation (Neoral, Novartis), and later adding mycophenolate mofetil (Cellcept, Roche) was accompanied by an increased incidence of secondary CMV infections. Also we noticed higher and more prolonged CMV viremia especially after the addition of mycophenolate mofetil to our immunosuppressive regimen. However, owing to better antiviral therapy and better monitoring, most CMV infections are asymptomatic nowadays. Mortality as well as morbidity associated with CMV is low. Is the increased incidence and prolonged viremia of CMV infections clinically irrelevant? Is cytomegalovirus infection still an important issue after kidney transplantation? A related question can be posed on the subject of acute rejection. Is it still important after transplantation? As a result of better immunosuppressive drugs the incidence of acute rejection is small in most of the large multicenter studies. In my opinion acute rejection and CMV infection are still important. Although the incidence of acute rejection has decreased in most centers, acute rejection is still a significant problem. As to this latter statement, the increased acceptance of non-heartbeating donors might be one of he factors explaining why the incidence of acute rejection is still considerable. The acceptance of this category of donor organs comes along with an increased incidence of delayed graft function, primary non-function, and a higher incidence of acute rejection. If one tries to decrease the incidence of acute rejection the benefit of more potent immunosuppressive therapy has to be weighed against an increased incidence of viral infections and malignancies after transplantation. The increased incidence of CMV infections, the longer duration and higher viral load may be detrimental on the long run. Also asymptomatic infections that are supposed to be 'innocent' might have influence on graft and patient survival.

In the following chapters we describe that patients with asymptomatic so-called 'innocent' CMV infection indeed have subclinical organ dysfunction. These infections are asymptomatic but we demonstrate that they do cause damage and this might have consequences on the long term.

In *chapter 3* we describe that a significant number of patients with active CMV infection after renal transplantation have increased intestinal permeability indicating intestinal epithelial damage. This finding does not provide a useful marker of CMV infection, but it gives insight into the pathofysiology of the widespread even subclinical manifestations of CMV infection after transplantation. In my opinion the epithelial damage is an early, subclinical stage in the development of CMV ileitis or gastrointestinal ulcers.

In *chapters 4, 5 and 6* we investigate the pulmonary dysfunction found in patients with CMV infection. As noticed earlier by van Son et al. (Transplantation 1987; 44:149) pulmonary diffusion is decreased in renal transplant patients with CMV infection. This was attributed to complement activation and plugging of leukocytes. Some years later Grefte et al. (The Journal of Infectious Diseases 1993; 167:270) found large cytomegalic endothelial cells (CECs; diameter 30-35 μm) in the peripheral blood of patients with CMV infection. We hypothesized that these large cells could cause the pulmonary diffusion disturbances by plugging in the capillary bed of the lungs. However in *chapter 4* we describe that the decrease in lung diffusion in renal transplant recipients with CMV infection was due to both a lower capillary volume as well as a lower membrane factor. This is an argument against just plugging of CECs, because plugging would predominantly cause a lower capillary volume. In *chapter 6* we describe that the pulmonary diffusion capacities between patients with and without CEC were similar. Also no tendency towards a diminished capillary volume was observed in patients with CECs, although numbers were small. Because the occurrence of CECs is strongly related to high CMV antigenemia levels, reflecting a higher viral load, we analysed the severity of infection, as indicated by CMV antigenemia levels, in relation to CO diffusion as well. Patients were stratified into two groups with high CMV antigenemia levels and moderate to low antigenemia levels. In the high CMV antigenemia group, the membrane factor decreased more than in the group showing low CMV antigenemia. This represents increased diffusion resistance in the patients with high antigenemia levels. An inflammatory reaction with production of cytokines, fluid extravasation and cellular infiltrate probably underlies these findings. A logical consequence of the inflammation might be that the lungs are more susceptible to other opportunistic infections. Possible long-term consequences of CMV infection on pulmonary function need to be investigated.

In *chapter 9 and 10* we address another important effect of CMV infection for the patient and his graft. We demonstrate the relation between CMV and the endothelium. In *chapter 9* we describe the detection of CECs in patients with CMV infection with high or moderate antigenemia levels. Non-infected circulating endothelial cells (ECs) were even observed in all patient categories independent of the severity of infection. Significantly more CMV-associated clinical symptoms were found in patients with CECs or ECs. Patients with rejection episodes preceding CMV infection had increased frequencies of CECs or ECs. The type of rejection was not significantly related to the detection of endothelial cells, but a tendency could be observed to higher frequencies in patients with vascular or steroid resistant interstitial rejection. We conclude that CMV infection, especially in combination with vascular or steroid resistant rejection and also the perseverance of viral load (*chapter 2*), results in extended endothelial damage, and may contribute to the development of chronic transplant failure and atherosclerosis even in asymptomatic patients. Many studies have shown both CMV infection and acute rejection to be risk factors for chronic transplant failure. With our data we demonstrate that rejection and CMV infection in the first weeks after transplantation have cumulative effects at the endothelial cell surface, which may predispose these patients toward chronic graft failure and atherosclerosis.

In *chapter 10* we describe that CMV infection in liver transplant recipients is associated with significantly lower platelet counts. The lower platelet counts in liver transplant recipients with CMV infection, compared to liver transplant recipients without CMV infection, was already observed several days before detectable CMV antigenemia. Infection of endothelial cells is an early and central phenomenon during CMV infection. Subsequent to infection of endothelial cells, CMV can spread hematogenously to different parts of the body. Circulating cytomegalic endothelial cells probably play a role in the spreading of the virus. Polymorphonuclear leukocytes take up pp65 from the infected endothelial cells. Before appearance of pp65-positive leukocytes in the blood, infection of endothelial cells has already begun and can affect thrombocytes. Apparently CMV infection occurs very early and more widespread than usually realized. In blood we only recognize the tip of the iceberg relatively late during the infection. Procoagulant properties of CMV and increased adhesion to endothelial cells may be responsible for the early difference in platelet counts.

Symptomatic CMV infection is often a flu like syndrome with fever, arthralgia, and in the blood tests leukocytopenia, thrombocytopenia and elevated liver transaminases may be demonstrated. In particular in patients with long lasting CMV infection, all organ systems can be involved. For example gastroenteritis, ulcers,

vasculitis, pneumonitis and retinitis are seen. In *chapter 8* we describe a patient with a less frequently observed disease possibly related to smoldering CMV infection: demyelinating polyneuropathy.

In this thesis I state that the endothelium plays a crucial role in the patho-fysiology of cytomegalovirus infection. First there is infection of endothelial cells by the virus. There is a change in molecules on the surface of infected endothelial cells and neighbouring endothelial cells. This change can vary from downregulation to upregulation of adhesion molecules and HLA molecules. These molecules cause adhesion of leukocytes and thrombocytes to endothelial cells. The clotting system is activated. Detachment of infected (CECs) and non-infected endothelial cells (EC) can be demonstrated in the peripheral blood. The CECs most probably play a role in the dissemination of CMV infection. In all organs clinical or subclinical dysfunction can be demonstrated. In my opinion the endothelial damage is important in the development of organ dysfunction but also in the development of chronic transplant dysfunction and atherosclerosis.

In conclusion CMV infection is a changing problem. It is our goal to further elucidate the possible effects of CMV infection on long-term graft function and graft survival in patients receiving more immunosuppression and having more and often longer lasting CMV infection. This is a challenge.

Figure 12.1 **Schematic clockwise presentation of CMV infection. At twelve a clock endothelial cells become activated and adhesion molecules are upregulated. This is caused by CMV infection of the endothelial cells with grey nuclei at three a clock. The endothelial cells become more thrombogenic and relative thrombocytopenia develops. PMN are polymorphonuclear granulocytes that take up the viral protein pp65 from the infected endothelial cells at three a clock. This results in a positive antigenemia assay. Mφ is a macrophage, NK is a natural killer cell, Tc is a cytotoxic T cell, Th is a helper T cell and P is a plasma cell. Mφ, NK, Tc, Th, P, cytokines, immunoglobulins and complement play a role in the defense against CMV. Infected endothelial cells become cytomegalic and detach. At six a clock a circulating cytomegalic endothelial is seen (CEC). SMC are smooth muscle cells that migrate and proliferate (induced by CMV) and play a role in the formation of an atherosclerotic plaque at 9 a clock. The inside of the clock symbolizes the organs where pathology due to CMV infection is found.**