# CHAPTER 1

# CYTOMEGALOVIRUS INFECTION AFTER ORGAN TRANSPLANTATION: AN INTRODUCTION

## 1.1 HUMAN CYTOMEGALOVIRUS: A HERPES VIRUS

The herpesviridae are a large group of double-stranded enveloped DNA viruses. More than 150 members of the herpes family have been identified to date. In humans eight different herpes viruses are known. The official names human herpes virus 1 till 5 are seldom used and they are more usually known by their common names: Herpes Simplex Virus type 1 and type 2 (HSV), Varicella Zoster Virus (VZV), Human Cytomegalovirus (CMV) and Epstein Bar Virus (EBV). Human herpes viruses 6 till 8 have no other names.

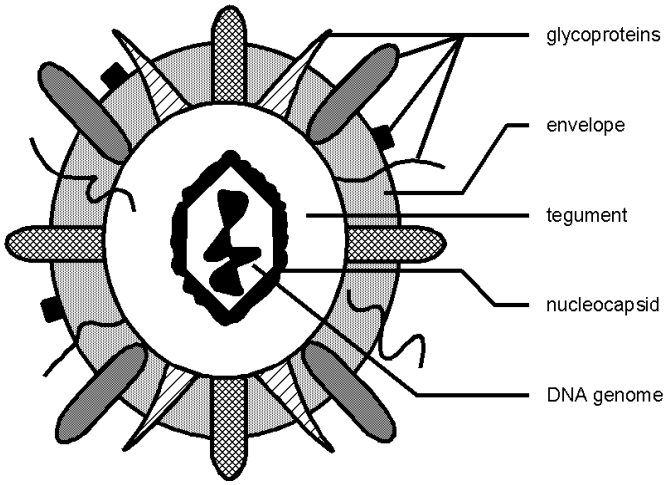
Cytomegalovirus infections were first described in the beginning of the previous century when the typical owl's eye intranuclear inclusions were found by histopathologists in tissues from fetuses stillborn following cytomegalic inclusion disease. Long time the infection was thought to be of protozoan nature. In 1956 CMV was isolated for the first time and the true nature of this infectious agent became clear. The virus was given its name by Weller from the cytopathic effect produced in cell culture [1].

## 1.2 BIOLOGY OF CYTOMEGALOVIRUS

CMV or herpes virus 5 has a typical herpes virus appearance. The central double stranded DNA genome of about 235 thousand base pairs is surrounded by an icosahedral capsid composed of 162 capsomeres. An area called the tegument surrounds the capsid and is surrounded itself by a loosely applied envelope. The tegument is constituted of about 20 proteins with pp65 (ppUL83) as major representative. The pp65 is of special interest because it is used as a diagnostic tool in the pp65 antigenemia assay. In active infection pp65 can be found in the nuclei of a small fraction of polymorphonuclear granulocytes. These granulocytes have acquired the pp65 from infected endothelial cells [2, 3]. Upon in vitro infection the pp65 protein is

transported to the nucleus of infected fibroblasts immediately after fusion of the virion with the cell membrane. The protein has kinase activity and auto-phosphorylation capacity [4].

Figure 1.1 A schematic of cytomegalovirus




The entry of CMV in target cells has been investigated in vitro in fibroblasts, endothelial cells and phagocytes [5]. First there is attachment and fusion of the virion with the cell membrane. Glycoproteins of the viral envelope [5-11] play a determining role in this process. Also HLA class I (Human Leukocyte Antigen class I) molecules are reported to have a role in viral entry [12]. The capsid moves to the nucleus and the process of transcription and translation of early and late antigens starts, following a very strict timetable [13, 14]. Due to especially the cellular immune response, infected cells will be destroyed and further replication and dissemination of the virus will be stopped. The virus comes in a stage of latency and may reactivate during a state of decreased immunity i.e. by the use of immunosuppressive drugs. Especially the potent monoclonal drug OKT3 (Orthoclone Muromonomab) and the polyclonal antithymocyte globulin (ATG) are known as potent inducers of CMV reactivation. This is probably mediated by TNF-alpha release during for example OKT3 treatment. TNF-alpha induces the transcription factor NF-κB that

causes enhancement of the major Immediate Early enhancer/promoter gen of CMV [15].

## 1.3 CYTOMEGALOVIRUS AND THE IMMUNE SYSTEM

After primary CMV infection CMV IgM antibodies are produced before IgG antibodies and persist for a few months. CMV IgG antibodies are produced after secondary and primary infections and persist lifelong [16]. This persistence is the reason that IgG antibodies are used as sign that the patient had CMV infection in the past. Donor and recipient CMV serology determine the risk of CMV infection and the type of infection after transplantation. Primary infections are infections in the positive-negative donor-recipient combinations, and secondary infections or reactivation in the neg-pos or pos-pos combinations. Whether CMV antibodiesplay an important role in the defense against CMV is still unclear. The importance of the humoral immunity is suggested by the clinical observation that without humoral immune response the patient will not clear the virus. If this is caused by the absence of humoral response or by absence of cellular response that causes also absence of humoral response by not functioning CD4 T helper cells remains unclear. Another argument for a role of antibodies in the clearance of the virus is the effectiveness of prophylactic administration of CMV immunoglobulin in seronegative recipients of kidneys from seropositive donors in preventing morbidity and mortality associated with CMV [17, 18]. The importance of the humoral immune response is also demonstrated by the finding that CMV specific antibodies reduce the generation of pp65 positive granulocytes by inhibiting uptake of pp65 by granulocytes from infected endothelial cells in vitro [3].

The cellular immune response by CD4 helper T-lymphocytes, CD8 cytotoxic T-lymphocytes and Natural Killer (NK) cells is important in the defense against Cytomegalovirus [19, 20, 21]. But Cytomegalovirus has developed several mechanisms to evade cellular immune response [22, 23]. For example the virus can prevent HLA class I loaded with viral peptides to be delivered to the cell surface. This makes the infected cell invisible to CD8 cytotoxic T-lymphocytes. Also the virus encodes a glycoprotein homologous to class I MHC antigens to prevent attack by NK cells.

CMV has been associated with increased subsequent risk for acute rejection in kidney and other solid organ transplant recipients [24]. Whether the risk is conferred by the reduction of immunosuppressive medication used to treat CMV infection or is a direct immunomodulating effect of the virus, is not known. Another possibility

is that CMV infection does not predispose to rejection but that rejection predisposes to active CMV infection.
In contrast Cytomegalovirus can enhance suppression of the immune response. CMV predisposes to subsequent opportunistic infections like Pneumocystis Carinii pneumonia (PCP) or fungal infections. Another argument for immunosuppression by CMV could be that CMV and for example HSV or VZV infections are often seen simultaneously.

## 1.4 DIAGNOSIS

CMV monitoring can be done in various ways. In our center pp65 antigenemia is detected in preparations of granulocytes from peripheral blood. These granulocytes are reacted with monoclonal antibodies against pp65 (phosphoprotein ppUL83) followed by immunoperoxidase staining. The phagocytic activity of the granulocyte has led them to ingest this protein from virus-infected cells. Maybe the protein is derived from dense bodies, which consist almost entirely of pp65 and probably are products of defective viral replication [25]. Other methods of virus detection in peripheral blood are viral DNA detection by polymerase chain reaction (PCR), viral mRNA detection by NASBA, conventional cell culture or detection of early antigen fluorescent foci in cultures.

## 1.5 HUMAN CYTOMEGALOVIRUS CLINICAL ASPECTS

Cytomegalovirus infection comprises the whole lifespan of humans. It can be acquired even before birth. The incidence of seropositivity increases with age. Almost 60-90% of the dialysis patients is seropositive, depending on age, and for example socioeconomic circumstance [26]. The virus can be acquired by intimate contact between mother and child, or from child to child in daycare centers, by sexual contact, by blood transfusion (leukocytes) and by organ transplantation. Latently infected monocytes and endothelial cells could be the origin of the virus after kidney transplantation of a seropositive kidney to a seronegative recipient [27-32]. The incubation period is between 4 and 8 weeks. In the general population most CMV infections will not be noticed because they are asymptomatic or only cause flu-like

symptoms. Also when the infection gives rise to a mononucleosis like syndrome the diagnosis of CMV probably will not be made.

During the last three decades due to the increasing possibilities of organ transplantation cytomegalovirus has become a frequent infection in transplanted patients and the diagnosis and treatment of the virus have become important issues. Nowadays incidence of CMV infection (not disease) is about 60 % of patients at risk in our center (this thesis chapter 2).

The majority of transplant patients with CMV infection diagnosed by for example a routinely performed pp65 antigenemia assay remain asymptomatic. They have CMV *infection* but no CMV *disease*. Nevertheless in these patients without clinical symptomatology subtle organ dysfunction can be demonstrated (this thesis).

If the infection is symptomatic most patients develop a flu like syndrome with fever, arthralgia, and in the blood tests leukocytopenia, thrombocytopenia and elevated liver transaminases. 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.

The incidence, course and severity of CMV infection differ in the different fields of organ transplantation. This is mainly due to the different kinds of immunosuppressive regimen. In the European Mycophenolate Mofetil (MMF) Cooperative Study the incidence of CMV disease was 36% for patients receiving a high dose of MMF (3 gram/day) compared with 8% for the patients who received either placebo or 2 gram/day MMF) [33]. We also found an increase in CMV infections after changing our immunosuppressive regimen (this thesis chapter 2).

In the long run chronic transplant dysfunction ('chronic rejection') and atherosclerosis are important threats to the transplanted patient. CMV might be important in the development of atherosclerosis as is for example demonstrated by the association of prior CMV infection and the risk of restenosis after coronary atherectomy after angioplasty [34, 35, 36]. Upregulation of adhesion molecules such as VCAM-1 might play a role in the development of vascular sclerosis [37, 38]. VCAM-1 would facilitate adhesion of leucocytes to the endothelial lining and could play a role in the process of endothelialitis and the forming of plaques.

Evidence for endothelialitis in patients comes from biopsies but also from peripheral blood samples from transplanted patients. During severe CMV infection cytomegalic endothelial cells can be demonstrated in peripheral blood [39, 40]. These cytomegalic cells have a diameter of 35-45 μm and show the characteristic owl's eye appearance. CMV proteins of all replication stages could be detected proving that these cells are late stage CMV infected cells. The large size of these CEC compared to normal endothelial cells is remarkable. The CEC could obstruct capillaries and

cause organ dysfunction by diminished perfusion of these organs (chapter 3, 4, 5 and 6). Another role of CEC in the pathogenesis of CMV infection could be the dissemination of the virus via the blood stream.

Another interesting finding is that CMV stimulates smooth muscle cells to migrate and proliferate after infection [41, 42, 43]. These smooth muscle cells play a role in the development of atheroma.

Cytomegalovirus is one of the risk factors for chronic transplant dysfunction [44, 45]. Probably this explains that CMV infection has been associated with reduced patient and graft survival rates [46, 47]. For example among 47.146 patients in the UNOS registry, kidneys from CMV positive donors demonstrated approximately 4% lower graft survival rates at 3 years after transplantation, compared with kidneys from CMV-negative donors [47]. In the study of Humar et al. [45] CMV is a risk factor for chronic allograft rejection but only in the presence of acute rejection. The combination of acute rejection and CMV infection is associated with a higher incidence of chronic rejection than acute rejection without CMV infection. Interesting is that we demonstrated increased levels of circulating endothelial cells especially in patients with CMV infection after a period of acute rejection. This illustrates that the combination of CMV infection and acute rejection gives rise to more chronic transplant dysfunction. Keeping this in mind chronic transplant dysfunction can be seen as a vascular pathology just like atherosclerosis (this thesis chapter 9 and 10).

Another hypothesis is that CMV infection may mimic or predispose to late rejection. In this hypothesis chronic transplant dysfunction is explained by immunological mechanisms. Possible mediators of CMV related rejection are for example intracellular adhesion molecule-1 (ICAM-1) and lymphocyte functioning antigen-3 (LFA-3) which are upregulated during CMV infection [48, 49]. Also the transcription and expression of interleukin-2 (IL-2) and the IL-2 receptor are upregulated by CMV gene products [50]. These products can also prevent the inhibitory effect of cyclosporine on IL-2 gene transcription [51]. Other authors found that CMV is able to stimulate alloreactive cytotoxic T-cells [52].

In both the vascular and the immunological view on chronic transplant dysfunction the endothelium plays an important role. Cytomegalovirus infecting and upregulating endothelial cells causing chronic transplant dysfunction, atherosclerosis and disturbed organ perfusion will be discussed in this thesis.

Last but not least CMV infection can be seen as a risk factor for post transplant lymfoproliferative disease [53].

## 1.6 CYTOMEGALOVIRUS THERAPY

Only using seronegative donors prevents CMV infection, but shortage of donors will not allow this strategy. Matching for CMV prevents primary CMV infections: seronegative kidneys are reserved for seronegative recipients. The point is that as a result of CMV matching, HLA matching will be worse. Further this approach will lead to an increase in waiting time. Eventually graft survival is important and it remains a question how CMV matching affects graft survival [46]. Using seronegative or leukocyte free blood products in seronegative patients who have been transplanted with a seronegative organ is important to prevent CMV infection.

Ganciclovir and foscavir are the two antivirals used in the Netherlands against CMV nowadays. Ganciclovir has to be phosphorylated by the product of the UL97 gene of CMV [54] and then the triphosphate inhibits the viral DNA polymerase (UL54). Foscavir inhibits viral replication by noncompetitively blocking the pyrophosphate binding site of viral DNA polymerase, preventing cleavage of pyrophosphate from deoxynucleoside triphosphate and elongation of the viral DNA chain. Unlike ganciclovir, foscavir does not require viral thymidine kinase for activation. As mentioned earlier antibody titers are useful in assessing the risk for CMV infection at the time of transplantation, by determination whether the donor and recipient were infected with CMV previously. This risk stratification allows selective use of antiviral therapy to prevent CMV [55, 56]. Selective prophylactic treatment of high-risk patients avoids unnecessary adverse reactions and the development of resistance [57]. In our center no prophylactic therapy is used after kidney transplantation. Preemptive therapy in patients with a positive CMV pp65 antigenemia but still without disease has been adopted in our center last years. The preemptive strategy aims at eliminating CMV infection prior to its manifestation as active disease. The advantage of preemptive therapy is to target patients at risk for CMV disease and eliminate unnecessary treatment. The disadvantage of not preventing CMV infection but treating it very early (preemptive approach) could be not preventing indirect damage of the viral infection like the subclinical organ dysfunction and chronic transplant dysfunction. Most centers consider tapering of immunosuppression as the first line and cornerstone of management of CMV infection after organ transplantation. For instance, reducing the dose of Cellcept has proven to be helpful to recover from CMV infection (this thesis chapter 2).

## 1.7 SCOPE OF THIS THESIS: CYTOMEGALOVIRUS AS A SYSTEMIC INFECTION IN THE RENAL TRANSPLANT RECIPIENT

We know from earlier studies that CMV infection after organ transplantation involves infection of endothelial cells. In this thesis the role of CMV endothelialitis in the pathophysiology of CMV infection is explored in different organ systems. The possible relation between on the one hand organ dysfunction and on the other hand viral load, circulating endothelial cells and other signs of endothelial damage was studied.

Cytomegalovirus *disease* is seen less last years due to better diagnostic and therapeutic tools. On the contrary the incidence of Cytomegalovirus *infection* diagnosed in our center by a positive CMV pp65 antigenemia assay is increasing (chapter 2). We demonstrated a longer duration and higher levels of pp65 positive granulocytes in peripheral blood (pp65 antigenemia assay) after introduction of mycophenolate mofetil (Cellcept). The higher incidence especially of the secondary infections, and the prolonged periods of higher viremia could be important in the development of chronic transplant dysfunction and atherosclerosis. The *disturbance of organ function* in clinical asymptomatic patients described in the following chapters (lung, intestines), and the unknown long-term consequences of these subclinical effects, could be the importance of the increased incidence of CMV infections. In chapter 3 we describe that the gastrointestinal barrier is not intact during cytomegalovirus infection. In chapter 4 we hypothesize that cytomegalic endothelial cells that may plug into the capillary bed, cause the pulmonary dysfunction found by van Son et al. in patients with cytomegalovirus infection. However, we found that the mechanism is more complicated than only plugging of cytomegalic endothelial cells. In chapter 8 we describe a patient with a long period of positive antigenemia and the development of a chronic inflammatory demyelinating polyneuropathy after kidney transplantation. We speculate about the pathogenesis of chronic inflammatory demyelinating polyneuropathy in kidney transplant recipients. In chapter 9 cytomegalic and non-cytomegalic circulating endothelial cells in kidney transplant recipients with CMV infection were studied. In chapter 10 we found lower thrombocyte counts in patients with CMV infection after liver transplantation and discuss the relation between CMV infection, the endothelium and thrombocytes. In chapter 12 we try to give an answer to one of the central questions in this thesis: is CMV still important in the transplant clinic? Do we have to be afraid of the smoldering asymptomatic cytomegalovirus infections? Is cytomegalovirus infection a vanishing or changing problem?

## 1.8 REFERENCES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