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Changing images of cytomegalovirus infection

Maar, Eltjo Fredericus de

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CHAPTER 7

PULMONARY INVOLVEMENT DURING CYTOMEGALOVIRUS INFECTION IN IMMUNOSUPPRESSED PATIENTS

E.F. de Maar, E.A.M. Verschuuren, M.C. Harmsen,
T.H. The, and W.J. van Son

Submitted

ABSTRACT

Although Cytomegalovirus (CMV) pulmonary involvement after solid organ transplantation is infrequently seen nowadays, CMV pneumonitis is still a potential lethal complication, especially in bone marrow transplant recipients. However, subclinical manifestation of pulmonary involvement is a frequent phenomenon. In this review we discuss pulmonary involvement of CMV infection in the immunocompromised host with the emphasis on transplant recipients. The clinical course, diagnosis, therapy, prophylaxis and pathophysiology of CMV pneumonitis are discussed.

7.1 INTRODUCTION

Cytomegalovirus (CMV) infection is a frequent complication after organ transplantation. Nowadays CMV infection occurs in more than 50% of all kidney transplant recipients, but a substantial number of these infections are asymptomatic. Asymptomatic cytomegalovirus infections can become chronic and smolder like a subsoil fire in a peat bog. In this manner CMV could play a role in the development of chronic transplant dysfunction and atherosclerosis [1, 2, 3, 4]. Symptomatic patients usually have a self-limiting CMV syndrome consisting of fever, malaise, arthralgias, leukocytopenia, thrombocytopenia and elevated transaminases. Clinical involvement of the lungs [5, 6], gastrointestinal tract [7, 8, 9], eyes, kidney, heart or nervous system is seen less frequently.

In this review we will focus on pulmonary involvement during cytomegalovirus infection in immunosuppressed patients with emphasis on transplant recipients.

7.2 DIAGNOSIS OF CMV PNEUMONITIS

Clinical manifestations of CMV pneumonitis range from mild dyspnea to severe respiratory insufficiency. Diagnosis of CMV pneumonitis is usually based on symptoms of fever, dyspnea and diffuse infiltrates on the chest X-ray in combination with CMV found in the bronchoalveolar lavage (BAL) by either a positive virus culture of the fluid, or detection of cells with CMV-positive immunostaining or cytopathogenic effects. The definitive diagnosis of CMV pneumonitis is made on transbronchial or open lung biopsy specimens showing cytomegalic cells associated with inflammation.

However, transbronchial biopsies are performed rarely because of the risk of bleeding especially in thrombocytopenic patients, and even with multiple transbronchial biopsies the diagnosis may be missed. To illustrate the rather poor sensitivity of transbronchial biopsies for diagnosing CMV pneumonitis the following case history is disconcerting [10]. A fatal CMV pneumonia is described in a renal transplant patient on triple immunosuppressive therapy (cyclosporin, mycophenolate mofetil and prednisolon). CMV-PCR from alveolar cells and lung biopsy material was repeatedly negative despite high CMV pp65 antigenemia [11]. Diagnosis was made by open lung biopsy. A severe chronic destructive, consolidating pneumonia with partial necrosis of lung tissue and thrombosis of smaller vessels was seen. In addition a widespread and intense positivity of immunostaining for CMV was detected while

there was no evidence of other pathogenic agents including fungi and mycobacteria. The thrombosis of smaller vessels found in this case is compatible with the suspected role of endothelial damage and clotting in the pathogenesis of CMV infection [12].

In different groups of immunosuppressed patients Tamm et al. [13] reported the incidence of positive CMV culture found in bronchoalveolar lavage by viral culture between 24.2-29.0% (resp. HIV, stem cell transplantation, renal transplantation and autoimmune disease). In patients who were treated with high dose chemotherapy the incidence was lower (4.4%), probably due to the relatively short duration of immunosuppression in comparison with organ transplant recipients. In contrast with the incidence of positive CMV culture in BAL, clinical CMV pneumonitis was only diagnosed in respectively 4.4, 5.8, 8.2 and 0% of the same patients. This indicates a low predictive value of positive CMV culture for CMV pneumonitis. In this study [13] CMV pneumonitis was better predicted by a positive immunostaining of BAL fluid. Especially in HIV patients the discrepancy between the high frequency of CMV in BAL culture and the low frequency of clinically overt CMV pneumonitis is evident. Uberti-Foppa et al. found in 40 AIDS patients who died within 20 days after undergoing BAL a predictive value for the diagnosis of CMV pneumonia of 61% for positive and 100% for negative virus isolation in BAL fluid [14].

The finding of CMV-specific IgG in BAL is not a good marker for the presence of CMV infection since the local humoral immune response in transplant patients with pneumonitis is not specific. The local humoral response is probably the result of polyclonal B cell activation or facilitated transport of IgG from serum to lung secretions. Local production or facilitated transport of CMV-specific IgG was found in patients with CMV pneumonitis and in patients with pneumonitis where no CMV was detected. Also Herpes Simplex Virus IgG can be found in patients with or without CMV pneumonitis [14].

Also CT-scan findings in cytomegalovirus pneumonia are not specific. In 10 transplant patients (one kidney, four lung and five bone marrow) with pathologically proven isolated pulmonary cytomegalovirus infection the most common patterns were small nodules and areas of consolidation. The consolidation was most marked in the lower lung zones [16].

In conclusion, most methods for diagnosing CMV pneumonitis have a poor positive predictive value. The false equalizing of CMV positive BAL and CMV pneumonitis confuses the literature concerning the incidence of CMV pneumonitis in immunosuppressed patients. CMV pneumonitis is a diagnosis made clinically in patients with pulmonary infiltrates and cytomegalovirus in BAL, in the absence of other opportunistic pathogens like *Pneumocystis carinii* or *Candida*.

7.3 CMV PNEUMONIA IN DIFFERENT PATIENT GROUPS

7.3.1 Renal transplantation

Following solid organ transplantation the incidence of CMV pneumonia depends on the type of allograft and the immunosuppressive regimen [13, 17, 18, 19]. A relation with the immunosuppressive regimen was recently demonstrated by Reichenberger et al [20]. They found in 71 renal transplant recipients with pulmonary symptoms an increase in CMV in BAL after a change of immunosuppressive regimen from Cyclosporin-A, azathioprine and prednisolon (C) to tacrolimus, mycophenolate mofetil and prednisolone (T) [20]. Diagnosis of CMV was made by the finding of cytopathogenic effects in the cells harvested from BAL fluid or by culture or immunostaining of the cells harvested. These patients did not receive CMV-prophylaxis. Of 91 BAL procedures in these 71 patients 27% were positive for CMV. CMV was found in 41% vs. 19% $P < 0.005$ compared T vs. C treated patients. In one third of all positive BAL procedures CMV was the only pathogen found, in the other procedures also PCP, bacteria, or other viruses were found. Nowadays in renal transplant recipients CMV pneumonia is infrequent and mortality is low due to prophylactic or preemptive treatment with ganciclovir.

7.3.2 Liver transplantation

In 13 of 141 liver transplant recipients who had interstitial pulmonary infiltrate cytomegalovirus pneumonia was diagnosed by histological evidence in lung tissue or in bronchoalveolar lavage fluid or by culture in BAL specimens. CMV pneumonia was diagnosed at median day 38 after transplantation. One-year mortality rate was higher in patients with CMV-pneumonia (84.6 vs. 17.2% $P = 0.0001$). Death occurred at a median of 17 days after the diagnosis of CMV pneumonia [21].

7.3.3 Lung transplantation

It is difficult to investigate the pulmonary effects of CMV infection in lung transplant recipients because they are intermingled with rejection and super infection in these patients. Pneumonitis is the most common presentation of CMV disease after lung transplantation and its clinical features can easily be mistaken for acute rejection. Duncan [22] showed reduced expiratory flows 6 months after the transplantation in lung transplant recipients with CMV infection. Lung transplant recipients with CMV infection developed more bacterial and fungal superinfections and had a lower survival rate (70 vs. 36% 2-year survival). Ganciclovir improved patient survival and decreased rates of superinfections.

7.3.4 Bone marrow or stem cell transplantation

Compared with solid organ transplant recipients CMV pneumonitis is frequently observed in allogenic bone marrow or stem cell transplant recipients and is associated with a high mortality [23]. CMV interstitial pneumonitis after allogenic stem cell transplantation develops 7-10 weeks after transplantation and the mortality exceeds 70% [24]. Fascinatingly, the incidence of CMV pneumonitis is higher in MHC disparate allogenic stem cell transplantation compared to autologous stem cell transplantation or HLA compatible allogenic transplantation [25]. Whereas the incidence of CMV pneumonitis in HLA compatible donors is lower the fatality rate seems to be similar [26]. These clinical observations give rise to interesting hypotheses concerning pathophysiology of CMV pneumonitis and the relation with graft versus host disease or graft immunodeficiency after stem cell transplantation that will be discussed later.

7.4 PULMONARY DIFFUSION DISTURBANCES: SUBCLINICAL CMV PNEUMONITIS?

In addition to overt pneumonia with pulmonary infiltrates, cytomegalovirus may also cause more subtle pulmonary dysfunction. In kidney transplant recipients Van Son et al. demonstrated pulmonary dysfunction measured as a decreased diffusion capacity for Carbon monoxide (KCOc) in symptomatic as well as asymptomatic kid-

ney transplant recipients with CMV infection [5]. Concomitant activation of complement during CMV infection could be demonstrated [6] and it was speculated that activation of complement could play a role. This hypothesis was based on the similarity of pulmonary dysfunction concomitant with complement activation by dialysis membranes. It was stated that complement activation and sequestration of aggregated leukocytes in lung capillary [27] could explain the dysfunction.

Grefte et al. [28] found large cytomegalic endothelial cells in the peripheral blood of kidney transplant recipients with CMV infection. As an alternative explanation for the pulmonary dysfunction we argued that these cells could plug in the lung capillaries and thus affect the diffusion capacity. But additional pulmonary diffusion studies revealed that the disturbed pulmonary diffusion during CMV infection was due to both a decrease in capillary volume as well as a decrease in membrane factor, pointing to interstitial pneumonitis and not capillary obstruction as the cause of the pulmonary diffusion [29]. So the hypothesis that CMV induced pulmonary dysfunction is caused by plugging of circulating cytomegalic endothelial cells is probably not correct.

Other authors demonstrated that ^{99m}Tc -DTPA clearances, a different method for measuring pulmonary diffusion, changed significantly during CMV infection on days 45 and 60 after renal transplantation. This also confirms decreased pulmonary diffusion capacity during CMV infection [30].

In AIDS patients it was demonstrated that a decline in TLCO couldn't be used as an early marker to predict CMV lung disease in asymptomatic persons [31].

7.5 THERAPY AND PROPHYLAXIS

The incidence of CMV pneumonitis has been diminished due to the use of successful strategies to prevent or treat severe CMV infection in the first months after organ transplantation.

Using only seronegative donors and seronegative or leukocyte free blood products in seronegative recipients would be the easiest way to CMV prevention, but shortage of donors will not allow this strategy. Other problems of matching for CMV are that HLA matching probably will be worse and waiting times will be longer for seronegative recipients. The question how graft survival is affected by CMV matching has not been answered yet [32].

Ganciclovir and foscavir are the two antivirals used against CMV. Ganciclovir has to be phosphorylated by kinases of the virus [33] and subsequently the triphos-

phate of the drug inhibits the viral DNA polymerase. 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. These drugs must be administered intravenously. The oral Ganciclovir formula only supplies low blood levels. Valganciclovir, which has a far better absorption, will be available soon for clinical practice in The Netherlands.

Antibody titers are useful in assessing the risk of CMV infection at the time of transplantation, by determination whether the donor and recipient were previously infected with CMV. This risk stratification allows for the selective use of antiviral therapy to prevent CMV [34, 35]. Selective prophylactic treatment of high-risk patients avoids unnecessary adverse reactions and the development of resistance [36]. In our kidney transplant unit no prophylactic therapy is used. Patients are monitored twice weekly with the pp65 CMV antigenemia assay. When the assay becomes positive the patients are treated with ganciclovir before they develop symptoms. This 'preemptive' strategy aims at eliminating CMV infection prior to its manifestation as active disease. The advantage of preemptive therapy is to target patients with CMV infection at risk for CMV disease and eliminate unnecessary toxic treatment. The disadvantage of preemptive therapy could be that it does not prevent the suspected pro-inflammatory effects of the viral infection. The antiviral drugs inhibit viral DNA synthesis and the forming of new virions but not the early and immediate early effects of the virus like upregulation of adhesion molecules and cytokine production, causing a state of inflammation [37]. Probably the most important treatment of CMV infection is reduction of immunosuppression. At our center we reduce especially the Cellcept, because we found that after the introduction of Cellcept patients had longer periods of CMV-viremia and higher viral loads [38].

7.6 PATHOPHYSIOLOGY OF CMV PNEUMONITIS: THE ROLE OF THE ENDOTHELIUM

CMV can productively infect endothelial cells causing a cytomegalic appearance of these cells. These infected cytomegalic endothelial cells can detach from the basal membrane and circulate in peripheral blood [28]. Most of the circulating endothelial cells in kidney transplant recipients originate from the transplanted organ [39]. During CMV pneumonitis after organ transplantation infection of alveolar epithelial

cells but also of lung endothelial cells can be found [40]. Interesting is the case report of a patient with AIDS who developed pulmonary hypertension in the months before his death. Postmortem examination showed extensive infection of endothelial cells with cytomegalic appearance and protruding into the vascular lumina. These cytomegalic cells compromised the lumen of the small vessels they lined [41] explaining the pulmonary hypertension.

The upregulation of molecules like intercellular adhesion molecule (ICAM-1), vascular adhesion molecule (VCAM-1) or Von Willebrand Factor (VWF) on the surface of endothelial cells might play a role in the pathogenesis of CMV pneumonitis [42-46]. In rat extensive vascular involvement during Rat CMV infection was found. Endothelial activation, leukocyte adhesion and activation of the clotting system were found [47]. Infection of endothelial cell layers with Herpes Simplex virus type 1 (HSV-1) or Cytomegalovirus results in an increased monocyte (MC) and polymorphonuclear (PMN) leukocyte adherence. The augmentation of MC or PMN adherence to virus infected endothelial cells is found to be sensitive to tunicamycin (hindering glycosylation), suggesting that both virus infections induce the expression of glycoproteins on the endothelial cell membrane which is responsible for the MC and PMN adhesion. This may be the first step in CMV induced endothelial damage [48, 49].

7.7 PATHOPHYSIOLOGY OF CMV PNEUMONITIS: IMMUNOPATHOLOGY OR CYTOPATHOLOGY?

The observation that cytomegalovirus related pneumonitis is frequent and severe in bone marrow transplant recipients and rare and mild in HIV infected patients might give clues for understanding the pathogenesis of CMV pneumonitis and may point to underlying immunopathological mechanisms. The first authors who suggested that immunological phenomena might play an important role in the development of CMV pneumonitis were Grundy and coworkers [50]. In 1987 they proposed that interstitial pneumonitis associated with CMV infection in transplant recipients is an immunopathologic process due to a vigorous T-cell response to a virally induced antigen expressed on cells in the lung. Others advocate the 'direct viral pathology' or 'cytopathology hypothesis'. They state that the absence of an adequate protective CD8 T-cell response causes uncontrolled CMV proliferation, cytokine storm and CMV pneumonitis [51]. Clinical data and studies in mice give conflicting results and will be discussed below.

Squire et al. [52] studied 58 episodes of pneumonitis in 68 patients with HIV infection. In nine episodes CMV was detected as the only infectious agent in BAL fluid. Only two episodes were severe and ventilatory support was required. In both cases the CD4 counts were relatively high (above 0.2×10^9 /l) and HIV infection appeared to have been acquired shortly before presentation. They conclude that a relatively well preserved immune function is needed for the development of CMV pneumonitis and used this as an argument for the immunopathology hypothesis.

This is in concert with the knowledge that AIDS-associated or idiopathic interstitial pneumonitis, which is characterized by lymphocytic infiltration of the lung tissue and pulmonary dysfunction, is likely the result of an antiviral or autoimmune response. In a murine model of retroviral associated interstitial pneumonitis CD8+ T-cell depleted mice developed interstitial pneumonitis similarly to undepleted mice. In contrast, depletion of CD4+ T-cells prevented the development of interstitial pneumonitis and inhibited inflammatory cytokine expression [53].

Other studies in mice show that CMV can replicate in the lungs of immunocompetent mice with minimal or no histological evidence of disease. Induction of pneumonitis in mice during CMV infection requires a significant change in host immunity like GVH disease [51] or Cyclophosphamide [52, 53]. Shanley et al. conclude from experiments with cyclophosphamide in CMV infected mice that CMV pneumonitis must be an immunological phenomenon. In these mice they observed large active spleens with in BAL material activated T-cells and NK-cells. Antiviral agents had no effect on the pneumonitis despite nearly total suppression of virus replication. Neutralizing pan T-cell antibodies slightly increased viral replication in the lung of these mice but almost completely inhibited the development of interstitial pneumonitis pointing out to immunopathological mechanisms in the development of pneumonitis. To summarize the studies favoring the immunopathology hypothesis so far, it can be concluded that viral replication in the lung is unrelated to the development of pathological effects and that immune response is prerequisite for the development of CMV pneumonitis.

However, other clinical and experimental data give clues that it is not the immunological reaction against CMV infected cells that leads to the development of pneumonitis, but that a deficient cellular immune response causes uncontrolled viral replication and pneumonitis (cytopathology hypothesis). There are several clinical arguments for this hypothesis. In bronchoalveolar lavage studies during episodes of CMV pneumonitis a predominance of natural killer (NK) cells and CD8 cells was found [56]. Also observations in both immunocompetent individuals and bone marrow transplant (BMT) patients support the protective role of CMV-specific CD8 T-lymphocytes [57, 58]. Waxman et al. identified 9 AIDS patients with

cytomegalovirus pneumonitis and low CD4 counts ($29,6 (\pm 22)$ cells/mm³). In the 5 patients treated with ganciclovir they found resolution of pulmonary symptoms and the patients not treated died [59].

Another argument for cytopathology is the clinical observation that in renal transplantation CMV pneumonitis is more commonly seen in primary infections than it is seen in secondary infections, suggesting that prior strain-specific immunity has some protective instead of a damaging effect. Also an argument for cytopathology is that the use of ATG or high doses of steroids has been reported to be a risk factor for CMV pneumonitis.

In a murine BMT model, adoptive transfer of syngeneic BM cells was associated with massive increases in lung CD8 cells, that resulted in the resolution rather than the exacerbation of CMV pneumonitis [58]. Obviously, the absence of protective CD8 lymphocytes, which causes uncontrolled CMV proliferation, is important for the development of this type of CMV pneumonitis.

After intranasal inoculation in immunocompetent mice, CMV can replicate in the lungs without causing pneumonitis, however in T-cell deficient nude mice CMV produced a progressive focal pneumonitis, also after subcutaneous inoculation [60]. In the lungs of these mice focal aggregates of interstitial mononuclear cells were found. Occasionally, the mononuclear cells contained viral nucleocapsids in the nucleus as well as cytoplasm. Inclusion bearing cells were occasionally found in the endothelium of the small blood vessels. Airways were consistently normal throughout the course of infection. Although late in the course of infection some mice had accumulation of acidophilic fluid in the alveoli. In these nude mice direct damage by the viral infection is the most likely mechanism in the development of CMV pneumonitis [60].

In bone marrow transplant recipients graft versus host disease (GVH) seems to play a role in the development of CMV pneumonitis. In 100 patients receiving syngeneic bone marrow from identical twins, no GVH reaction is expected to occur and no cases of CMV pneumonitis were found. However in interesting experiments in mice, Podlech et al. [58] found in MHC disparate bone marrow transplant recipients no evidence for the generation of recipient-specific alloreactive donor cytotoxic T-cells and no histological evidence for graft versus host disease. Instead they found dramatic lung destruction by viral replication, suggesting a failure in the generation of protective antiviral CD8 T-cells. They propose that the cause of pneumonitis was functional graft failure, and not graft versus host disease [58]. So the precise role of GVHD in the development of CMV pneumonitis has still to be defined.

Cytokine release during CMV infection is probably also important in the pathogenesis of CMV pneumonia. Anti-CD3 antibodies induce lethal interstitial pneumonitis with elevated levels of tumor necrosis factor alpha (TNF-alpha) and interferon gamma four weeks after intraperitoneal injection of murine cytomegalovirus in mice. Despite pneumonitis CMV was only detectable in the salivary glands and not in the lungs. These authors conclude that murine CMV pneumonitis is not mediated by virus in the lung, but probably by cytokines released from T cells, of which responsiveness has been modified by CMV infection [61]. Also the observed correlation between cytokine secretion and incidence of active CMV infection in solid organ transplant recipients points out to the importance of cytokine excretion in the pathogenesis of CMV infection [62].

So, how to deal with the conflicting results obtained by researchers of the '*immunopathology adherents*' and the '*cytopathology supporters*'? Aside from the given fact that considerable inevitable bias, such as differences in species studied (animal versus man, different CMV strains, different patient groups) is hindering the final judgment who is right, the truth might prove to be a combination of both possible explanations of this enigmatic condition. Although speculative, the delicate balance between achieving a distinct immune response which is sufficient for protection, and on the other hand does not cause overt immunopathology might be pivotal whether or not a patient will develop CMV pneumonitis.

7.8 CONCLUSION

CMV pulmonary involvement after organ transplantation is frequently seen. The clinical course ranges from subclinical pulmonary dysfunction to serious pneumonitis. The pathophysiology remains speculative. In our opinion endothelialitis caused by CMV infection, upregulation of adhesion molecules, activation of the clotting system and cytokine production are important factors in the development of CMV pneumonitis. In addition the delicate balance between achieving an immune response which is sufficient for protection, and on the other hand does not cause overt immunopathology might be pivotal in the individual patient determining whether or not an overt CMV pneumonitis will develop. Finally, pathophysiology in the individual patient will be influenced by factors such as the type of immunosuppression, the antiviral drugs used, as well as the type of transplant.

7.9 REFERENCES

1. Adam, E., J.L. Melnick, J.L. Probstfield, B.L. Petrie, J. Burek, K.R. Bailey, C.H. McCollum, M.E. DeBakey. High levels of cytomegalovirus antibody in patients requiring vascular surgery for atherosclerosis. *Lancet* 1987; 8554:291-293.
2. Hendrix, M.G., M. Daemen, C.A. Bruggeman. Cytomegalovirus nucleic acid distribution within the human vascular tree. *American Journal of Pathology* 1991; 138:563-567.
3. Grattan, M.T., C.E. Moreno-Cabral, V.A. Starnes, P.E. Oyer, E.B. Stinson, N.E. Shumway. Cytomegalovirus infection is associated with cardiac allograft rejection and atherosclerosis. *JAMA: The Journal of the American Medical Association* 1989; 261:3561-3566.
4. Pouria, S., O.I. State, W. Wong, B.M. Hendry. CMV infection is associated with transplant renal artery stenosis. *QJM: Monthly Journal of the Association of Physicians* 1998; 91:185-189.
5. Son, W.J. van, R. Peset, J.C. Duipmans, Th. W. van der Mark, T.H. The, A.M. Tegzess. Cytomegalovirus infection after renal transplantation. Pulmonary dysfunction measured by decreased diffusing capacity for carbon monoxide in patients with symptomatic and asymptomatic infection. *Transplantation* 1987; 44: 149-150.
6. Son, W.J. van, A.M. Tegzess, T.H. The, J.C. Duipmans, M.J.H. Slooff, Th. W. van der Mark, R. Peset. Pulmonary dysfunction is common during a cytomegalovirus infection after renal transplantation even in asymptomatic patients. Possible relationship with complement activation. *The American Review of Respiratory Disease* 1987; 136:580-585.
7. Goodgame, R.W. Gastrointestinal Cytomegalovirus Disease. *Annals of Internal Medicine* 1993; 119:924-935.
8. Maar, E.F. de, J.H. Kleibeuker, W. Boersma-van Ek, T.H. The, W.J. van Son. Increased intestinal permeability during cytomegalovirus infection in renal transplant recipients. *Transplant International* 1996; 9:576-580.
9. Son, W.J. van, E.J. van der Jagt, F.J. van der Woude, M.J.H. Slooff, S. Meijer, T.H. The, A.M. Tegzess, L.B. van der Slikke, A.J.M. Donker. Pneumatosis intestinalis in patients after cadaveric kidney transplantation. Possible relationship with an active cytomegalovirus infection. *Transplantation* 1984; 38:506-510.
10. Ketteler, M, L. Preuschhof, A. Mertz, M. Stöffler-Meilicke, H. Schäfer, A. Distler, G. Offermann. Fatal cytomegalovirus pneumonia after preemptive anti-

- viral therapy in a renal transplant recipient. *Clinical Nephrology* 2000; 5:418-424.
11. The, T.H., M.C. Harmsen, W. van der Bij, A.P. van den Berg, W.J. van Son. Relationship between monitoring the viral load in blood, human cytomegalovirus pathophysiology and management strategies of patients after transplantation. *Monographs in Virology* 1998; 21:262-279.
 12. Sutherland, M.R., C.M. Raynor, H. Leenknecht, J.F. Wright, E.L. Pryzdial. Coagulation initiated on herpesviruses. *Proceedings of the National Academy of Sciences of the United States of America* 1997; 94:3510-13514.
 13. Tamm, M., P. Traenkle, B. Grilli, M. Soler, C.T. Bolliger, P. Dalquen, G. Cathomas. Pulmonary cytomegalovirus infection in immunocompromised patients. *Chest* 2001; 119:838-843.
 14. Uberti-Foppa, C., F. Lillo, M.R. Terreni, A. Puglisi, M. Guffanti, N. Gianotti, A. Lazzarin. Cytomegalovirus Pneumonia in AIDS Patients. *Chest* 1998; 113: 919-923.
 15. Milburn, H.J., J.E. Grundy, R.M. du Bois, H.G. Prentice, P.D. Griffiths. Is the measurement of virus-specific antibody in the lungs of transplant recipients with cytomegalovirus pneumonitis of diagnostic or prognostic value? *Journal of Medical Virology* 1988; 26:197-206.
 16. Kang, E., E. Patz, N.L. Müller. Cytomegalovirus pneumonia in transplant patients: CT findings. *Journal of Computer Assisted Tomography* 1998; 20:295-299.
 17. Smyth, R.L., J. Sinclair, J.P. Scott, J.J. Gray, T.W. Higenbottam, T.G. Wreghitt, J. Wallwork, L.K. Borysiewicz. Infection and reactivation with cytomegalovirus strains in lung transplant recipients. *Transplantation* 1991; 52: 480-482.
 18. Ettinger, N.A., T.C. Bailey, E.P. Trulock, G.A. Storch, D. Anderson, S. Raab, E.L. Spitznagel, C. Dresler, J.D. Cooper. Cytomegalovirus infection and pneumonitis. Impact after isolated lung transplantation. *The American Review of Respiratory Disease* 1993; 147:1017-1023.
 19. Falagas, M.E., D.R. Snyderman, M.J. George, B. Werner, R. Rutzhaizer, J. Griffith, R.H. Rohrer, R. Freeman. Incidence and predictors of cytomegalovirus pneumonia in orthotopic liver transplant recipients. *Transplantation* 1996; 61:1716-1720.
 20. Reichenberger, F., M. Dickenmann, I. Binet, M. Soler, C. Bolliger, J. Steiger, F. Brunner, G. Thiel, M. Tamm. Diagnostic yield of bronchoalveolar lavage following renal transplantation. *Transplant Infectious Disease* 2001; 3:2-7.
 21. Falagas, M.E., D.R. Snyderman, M.J. George, B. Werner, R. Rutzhaizer, J. Griffith, R.H. Rohrer, R. Freeman. Incidence and predictors of cytomegalovirus

- pneumonia in orthotopic liver transplant recipients. *Transplantation* 1996; 61:1716-1720.
22. Duncan, S.R., I.L. Paradis, S.A. Yousem, S.L. Similo, W.F. Grgurich, P.A. Williams, J.H. Dauber, B.P. Griffith. Sequelae of cytomegalovirus pulmonary infections in lung allograft recipients. *The American Review of Respiratory Disease* 1992; 146:419-1425
 23. Enright, H., R. Haake, D. Weisdorf, N. Ramsay, P. McGlave, J. Kersey, W. Thomas, D. McKenzie, W. Miller. Cytomegalovirus pneumonia after bone marrow transplantation. Risk factors and response to therapy. *Transplantation* 1993; 55:1339-1346.
 24. Meyers, J.D., N. Flournoy, E. Thomas. Risk factors for cytomegalovirus infection after human bone marrow transplantation. *The Journal of Infectious Diseases* 1986; 153:478-488.
 25. Meyers, J.D. Cytomegalovirus infection following marrow transplantation: Risk, treatment, and prevention. *Birth Defects Original Article Series* 1984; 20:101-117.
 26. Ljungman, P., P. Biron, A. Bosi et al.. Cytomegalovirus interstitial pneumonia in autologous bone marrow transplant recipients. *Bone Marrow Transplantation* 1994; 13:209-212.
 27. Craddock, P.R., J. Fehr, K.L. Brigham, R.S. Kronenberg, H.S. Jacob. Complement and leukocyte-mediated pulmonary dysfunction in hemodialysis. *The New England Journal of Medicine* 1977; 296:769-774.
 28. Grefte, A., M. van der Giessen, W.J. van Son, T.H. The. Circulating cytomegalovirus (CMV)-infected endothelial cells in patients with an active CMV infection. *The Journal of Infectious Diseases* 1993; 167:270-277.
 29. Maar, E.F. de, A.M. Kas-Deelen, Th.W. van der Mark, T.H. The, A.M. Tegzess, R.J. Ploeg, W.J. van Son. Cytomegalovirus pneumonitis after kidney transplantation is not caused by plugging of cytomegalic endothelial cells only. *Transplant International* 1999; 12:56-62.
 30. Burgeon, B., P. Lang, J. Rosso, F. Zerah, C. Buisson, C. Baron, J. Fleury, L. Desforges, F. Lebargy, M. Meignan, B. Weil. Prospective study of pulmonary function for cytomegalovirus infection after renal transplantation. *Transplant International* 1994; 7:S339-342.
 31. Kvale, P.A., M.J. Rosen, P.C. Hopewell, N. Markowitz, N. Hansen, L.B. Reichman, J.M. Wallace, J. Glassroth, W. Fulkerson, L.A. Meiselman. A decline in the pulmonary diffusing capacity does not indicate opportunistic lung disease in asymptomatic persons infected with the human immunodeficiency virus.

- Pulmonary Complications of HIV Infection Study Group. *The American Review of Respiratory Disease* 1993; 148:390-395.
32. Schnitzler, M.A., R.S. Woodward, D.C. Brennan, E.L. Spitznagel, W.C. Dunagan, T.C. Bailey. The effects of cytomegalovirus serology on graft and recipient survival in cadaveric renal transplantation: implications for organ allocation. *American Journal of Kidney Diseases* 1997; 29:428-434.
 33. Sullivan, V., C.L. Talarico, S.C. Stanat, M. Davis, D.M. Coen, K.K. Biron. A protein kinase homologue controls phosphorylation of ganciclovir in human cytomegalovirus-infected cells. *Nature* 1992; 358:162-164.
 34. Brennan, D.C., K.A. Garlock, G.G. Singer, M.A. Schnitzler, B.J. Lippmann, R.S. Buller, M. Gaudreault-Keener, J.A. Lowell, S. Shenoy, T.K. Howard, G.A. Storch. Prophylactic oral ganciclovir compared with deferred therapy for control of cytomegalovirus in renal transplant recipients. *Transplantation* 1997; 64:1843-1846.
 35. Couchoud, C., M. Cucherat, M. Haugh, C. Pouteil-Noble. Cytomegalovirus prophylaxis with antiviral agents in solid organ transplantation: a meta-analysis. *Transplantation* 1998; 65:641-647.
 36. Bienvenu, B., E. Thervet, J. Bedrossian, C. Scieux, M.C. Mzeron. Development of cytomegalovirus resistance to ganciclovir after oral maintenance treatment in a renal transplant recipient. *Transplantation* 2000; 69:182-184.
 37. Craigen, J.L., J.E. Grundy. Cytomegalovirus induced up-regulation of LFA-3 (CD58) and ICAM-1 (CD54) is a direct viral effect that is not prevented by ganciclovir or foscarnet treatment. *Transplantation* 1996; 62:1102-1108.
 38. Maar, E.F. de, E.A.M. Verschuuren, J.J. Homan vd Heide, A.M. Kas-Deelen, D. Jagernath, T.H. The, R.J. Ploeg, W.J. van Son. Effects of changing immunosuppressive regimen on the incidence, duration and viral load of cytomegalovirus infection in renal transplantation: a single center report. *Transplant Infectious Disease* 2002; 4:17-24.
 39. Popa, E.R., A.M. Kas-Deelen, B.G. Hepkema, W.J. Van Son, T.H. The, M.C. Harmsen. Donor-derived circulating endothelial cells after kidney transplantation. *Transplantation* 2002; 74:1320-1327.
 40. Sinzger, C., A. Grefte, B. Plachter, A.S. Gouw, T.H. The, G. Jahn. Fibroblasts, epithelial cells, endothelial cells and smooth muscle cells are major targets of human cytomegalovirus infection in lung and gastrointestinal tissues. *The Journal of General Virology* 1995; 76:741-750.
 41. Smith, F.B., J.H. Arias, T.H. Elmquist, J.T. Mazzara. Microvascular cytomegalovirus endothelialitis of the lung: a possible cause of secondary pulmonary hypertension in a patient with AIDS. *Chest* 1998; 114:337-340.

42. Kas-Deelen, A.M., M.C. Harmsen, E.F. de Maar, W.W. Oost-Kort, J.W. Cohen Tervaert, J. van der Meer, W.J. van Son, T.H. The. Acute rejection before cytomegalovirus infection enhances von Willebrand factor and soluble VCAM-1 in blood. *Kidney International* 2000; 58:2533-2542.
43. Koskinen, P.K., K.B. Lemstrom, M.S. Nieminen, P.J. Hayry, I.T. Lautenschlager. Vascular cell adhesion molecule-1 (VCAM-1) is induced during cytomegalovirus infection in vascular structures of heart allografts. *Transplant International* 1994; 7:S363-S364.
44. Koskinen, P.K. The association of the induction of vascular cell adhesion molecule-1 with cytomegalovirus antigenemia in human heart allografts. *Transplantation* 1993; 56:1103-1108.
45. Waldman, W.J., D.A. Knight. Cytokine-mediated induction of endothelial adhesion molecule and histocompatibility leukocyte antigen expression by cytomegalovirus-activated T cells. *American Journal of Pathology* 1996; 148:105-119.
46. Sedmak, D.D., D.A. Knight, N.C. Vook, J.W. Waldman. Divergent patterns of ELAM-1, ICAM-1, and VCAM-1 expression on cytomegalovirus-infected endothelial cells *Transplantation* 1994; 58:1379-1385.
47. Persoons, M.C., F.S. Stals, M.C. van Dam Mieras, C.A. Bruggeman. Multiple organ involvement during experimental cytomegalovirus infection is associated with disseminated vascular pathology. *The Journal of Pathology* 1998; 184:103-109.
48. Span, A.H., M.C van Dam-Mieras, W. Mullers, J. Endert, A.D. Muller, C.A. Bruggeman. The effect of virus infection on the adherence of leukocytes or platelets to endothelial cells. *European Journal of Clinical Investigation* 1991; 21:331-338.
49. Vossen, R.C.R.M., M.C. van Dam-Mieras, C.A. Bruggeman. Cytomegalovirus infection and vessel wall pathology. *Intervirology* 1996; 39:213-221.
50. Grundy, J.E., J.D. Shanley, P.D. Griffiths. Is cytomegalovirus interstitial pneumonitis in transplant recipients an immunopathological condition? *Lancet* 1987; 31:996-999.
51. Barry, S.M., M.A. Johnson, G. Janossy. Cytopathology or immunopathology? The puzzle of cytomegalovirus pneumonitis revisited. *Bone Marrow Transplantation* 2000; 26:591-597.
52. Squire, S.B., M.C.I. Lipman, E.K. Bagdades, P.M. Mulvenna, J.E. Grundy, P.D. Griffiths, M.A. Johnson. Severe cytomegalovirus pneumonitis in HIV infected patients with higher than average CD4 counts. *Thorax* 1992; 47:301-304.

53. E.A. Fitzpatrick, M. Avdiushko, A.M. Kaplan, D.A. Cohen. Role of T cell subsets in the development of AIDS-associated interstitial pneumonitis in mice. *Experimental lung research* 1999; 25:671-687.
54. Shanley, J.D., C.S. Via, S.O. Sharrow, G.M. Shearer. Interstitial pneumonitis during murine cytomegalovirus infection and graft-versus-host reaction. *Transplantation* 1987; 44: 658-662.
55. Shanley, J.D., E.L. Pesanti. The relationship of viral replication to interstitial pneumonitis in murine cytomegalovirus lung infection. *The Journal of Infectious Diseases* 1985; 151:454-458.
56. Shanley, J.D. Murine models of cytomegalovirus-associated pneumonitis. *Transplantation Proceedings* 1991; 23(S3): 12-16.
57. Walter, E.A., P.D. Greenberg, M.J. Gilbert, R.J. Finch, K.S. Watanabe, E.D. Thomas, S.R. Riddell. Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. *The New England Journal of Medicine* 1995; 333:1038-1044.
58. Podlech, J., R. Holtappels, M.F. Pahl-Seibert, H.P. Steffens, M.J. Reddehase. Murine model of interstitial cytomegalovirus pneumonia in syngeneic bone marrow transplantation: persistence of protective pulmonary CD8-T-cell infiltrates after clearance of acute infection. *Journal of Virology* 2000; 74:7496-4507.
59. Waxman, A.B., S.J. Goldie, H. Brett-Smith, R.A. Matthay. Cytomegalovirus as a primary pulmonary pathogen in AIDS. *Chest* 1997; 111:128-134.
60. Shanley, J.D., R.S. Thrall, S.J. Forman. Murine cytomegalovirus replication in the lungs of athymic BALB/c nude mice. *The Journal of Infectious Diseases* 1997; 175:309-315.
61. Tanaka, K., Y. Koga, Y.Y. Lu, X.Y. Zhang, Y. Wang, G. Kimura, K. Nomoto. Murine cytomegalovirus-associated pneumonitis in the lungs free of the virus. *The Journal of Clinical Investigation* 1994; 94:1019-1025.
62. Fietze, E., S. Prösch, P. Reinke, J. Stein, W.D. Döcke, G. Staffa, S. Loening, S. Devaux, F. Emmrich, R. von Baehr, D.H. Krüger, H.D. Volk. Cytomegalovirus infection in transplant recipients: The role of tumor necrosis factor. *Transplantation* 1994; 58:675-680.