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Cytomegalovirus pneumonitis after kidney transplantation is not caused by plugging of cytomegalic endothelial cells only

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Abstract In addition to life-threatening pneumonia, cytomegalovirus (CMV) may also cause subclinical pulmonary dysfunction after kidney transplantation. To investigate the role of plugging of cytomegalic endothelial cells in the pulmonary capillary bed, we prospectively determined specific carbon monoxide diffusion capacity (KCOc) and its components: the pulmonary diffusing membrane factor (Dm) and pulmonary capillary blood volume (Vcap) before and during CMV infection in 13 kidney transplant recipients and 13 controls. During CMV infection, mean KCOc decreased significantly by 28% of the initial value (mean KCOc 79 vs 109; $P < 0.005$) due to a decrease in both Vcap and Dm. The KCOc in controls showed a significantly smaller decrease due to a slightly lower Vcap. We conclude that kidney transplant recipients with CMV infection have significant pulmonary diffusion disturbances due to a combination of lower Vcap and lower Dm. The most likely explanation for this phenomenon is a local inflammatory process due to CMV and not plugging of cytomegalic endothelial cells only.

Key words Cytomegalovirus, kidney transplantation · Pneumonitis, kidney transplantation · Kidney transplantation, pulmonary diffusion · Pulmonary diffusion, cytomegalovirus

Introduction

Cytomegalovirus (CMV) infection is the most frequent infectious complication after kidney transplantation. Although CMV infections occur in many kidney transplant recipients, a substantial number of these infections are asymptomatic. Most patients with a symptomatic CMV infection have a self-limiting CMV syndrome that consists of fever with malaise and arthralgias, leukocytopenia, thrombocytopenia, and elevated serum liver enzymes (especially transaminases). Less common is involvement of the lungs [28, 29], gastrointestinal tract [10, 20, 30], eyes, kidney, heart, or nervous system. Clinical manifestations of CMV-related pulmonary involvement range from mild dyspnea to severe respiratory insufficiency due to CMV pneumonitis.

We hypothesize that subclinical organ involvement may be common during CMV infection in kidney transplant recipients. This hypothesis is in accordance with the systemic nature of this type of viral infection. In a previous study we reported on an increased intestinal permeability in patients with CMV infection without gastrointestinal symptomatology [20]. Van Son et al. [28, 29] found pulmonary dysfunction during CMV infection after kidney transplantation in symptomatic as well as asymptomatic patients [28, 29]. Concomitant activation of complement was seen during CMV infection [27], and there was speculation about the role of complement activation in pulmonary diffusion disturbances in these pa-

tients. A similar explanation for pulmonary dysfunction was found during hemodialysis treatment, when complement activation due to the dialysis membrane causes leukocyte aggregation and subsequent plugging in the lung capillaries [8]. These findings might be attributable to a decrease in the pulmonary capillary blood volume.

Recently, we described the presence of large cytomegalic endothelial cells (diameter 30–35 μm) in the peripheral blood of patients with an active CMV infection [12]. This prompted us to study whether plugging of these large cells in the capillary bed of the lungs might play a causative role in the pulmonary dysfunction found in these patients. To study this possibility we measured the diffusion of carbon monoxide (CO) from the alveolar space to the pulmonary capillary blood. The ability of oxygen (O_2) and CO to diffuse through the alveolar-capillary membrane is similar. Yet, we chose to use CO and not O_2 since the transport of CO from alveolar gas to blood is limited by diffusion.

Briefly, after a single breath of a gas mixture consisting of O_2, helium, and CO, the CO that is taken up in the expired alveolar gas is measured. The uptake of CO is called the "transfer factor" for CO (TlCO) and represents a very sensitive method to determine pulmonary diffusion. Since the total transfer of CO is also influenced by the alveolar volume, the specific CO diffusion capacity (TlCO divided by the alveolar volume: KCOc) is used. The KCOc is used in routine clinical practice to indicate pulmonary diffusion capacity, e.g., to detect subtle changes during bleomycin chemotherapy [1].

A drop in CO diffusion may be caused by a number of factors including a decrease in pulmonary capillary blood volume (Vcap; as might be the case in plugging of cytomegalic endothelial cells into the pulmonary capillary bed), a change in the barrier that exists between the alveolar space and the blood (the membrane diffusion capacity, Dm), or a decrease in both components. By measuring TlCO at high and low O_2 concentrations, Dm and Vcap can be determined.

In this study we investigated the cause of disturbed CO diffusion by measuring KCOc and its components, Dm and Vcap. To detect any relationship between decreased CO diffusion and changes in Dm, e.g., interstitial edema, changes in Vcap that are compatible with plugging of endothelial cells or leukocytes in the capillary bed, or possible changes in both components that are compatible with pneumonitis, all pertinent recipient data were evaluated.

Materials and methods

Patients

Thirty-nine patients who underwent transplantation from April 1995 through March 1997 were included in this study. None of the patients had a history of pulmonary disease and all had a normal physical examination and chest x-ray during the study period. Patients with postoperative cardiopulmonary complications, such as myocardial ischemia or infarction, pulmonary embolism, or bronchopneumonia, were excluded from the study. Twenty-five patients were men and 14 women. Their mean age was 43 years (range 18–66 years). The median dialysis period prior to transplantation was 46 months (range 0–141 months). The study population consisted of 37 first kidney transplant recipients, 2 retransplant recipients, and 3 living related kidney transplant recipients. Fifteen patients were seropositive for CMV before transplantation. Patients were considered seropositive when IgG antibodies against CMV late antigen (CMV LA) were present [9]. No CMV prophylaxis (acyclovir, gancyclovir, or anti-CMV immunoglobulins) was given. Initial immunosuppression consisted of cyclosporin A and low-dose prednisolone. Patients with a second or third transplant (both in the control group) received monoclonal antibody induction therapy with OKT3, followed by triple therapy with azathioprine or mycophenolate mofetil, cyclosporin A, and low-dose prednisolone. All three living related kidney transplant recipients were in the control group and were treated with triple therapy. Rejection episodes were treated with 1 gram of methylprednisolone (Solu-Medrol; Upjohn, Kalamazoo, Mich., USA) intravenously on 3 consecutive days. For steroid-resistant rejection, a course of ATG (Rabbit ATG, Merieux) was administered. All patients gave informed consent before participating in the study.

Pulmonary function

Pulmonary function was determined twice in all patients between postoperative days 10 and 25. The values obtained were taken as baseline values. In one patient, only one baseline value could be taken. In 13 patients with active CMV infection, pulmonary function was measured at least twice (mean number of measurements 5; range 2–16) during the infection. In the subsequent analysis, the results of the pulmonary function with the lowest specific CO diffusion were used. In the first eight patients with active CMV infection, the lowest pulmonary diffusion values were determined on median postoperative day 56. For this reason pulmonary function assessment in the control group of patients without CMV was also performed on day 56.

Forced expiratory volume in 1 s (FEV_1) and slow inspiratory vital capacity (VC_{max}) were determined by spirometry. The transfer factor (diffusing capacity) for CO (TlCO) and its components, i.e., diffusing capacity of the alveolar-capillary membrane (Dm) and volume of blood in the pulmonary capillaries (Vcap), were determined from triplicate measurements of TlCO at high (88%) and low (19.2%) concentrations of inspired oxygen. The single breath technique of Krogh, as modified by Cotes [6], was used. Carbon monoxide was measured with an infrared spectrophotometer and helium using a thermal conductivity method (ML-Masterlab-Transfer; Jaeger, Germany). The TlCO values were corrected for hemoglobin concentrations (TlCOc), according to Cotes [6]. Corrected, specific diffusion capacity (KCOc) was calculated by dividing TlCOc by the alveolar volume. The Dm and the Vcap were derived from the equation of Roughton and Forster [25]:

$$1/TlCO = 1/Dm + 1/\theta.[Hb].Vcap$$

where θ is the reaction rate of CO with hemoglobin (Hb) at the average normal Hb concentration (9 mmol/l). [Hb] is the hemoglobin concentration as a fraction of the average normal Hb concentration. Values were expressed as percentages of those predicted, with the predicted values being taken from Cotes et al. [7] and Quanjer et al. [23].

Cytomegalovirus infection

The diagnosis of active CMV infection was made using the CMV antigenemia assay, as described by Van der Bij et al. [3, 4] and reviewed by Chou [5] and by Ljungman and Griffiths [19] during the Fourth International CMV Workshop (Paris, 1993). Briefly, peripheral blood leukocytes were isolated, cytocentrifuged, and incubated with a mixture of monoclonal antibodies directed against a 65–66 kD CMV antigen, followed by immunoperoxidase staining. The number of antigen-positive cells per 50000 leukocytes was counted in duplicate. The antigenemia assay was performed at least once weekly starting on postoperative day 12. In all patients antigenemia was followed by either seroconversion (primary infection) or a significant rise (reactivation) in CMV IgG antibodies. IgM and IgG CMV antibodies were measured quantitatively by ELISA using late-stage, CMV-infected fibroblasts as antigens [9].

Statistics

Statistical analysis was performed using Student's *t*-test for paired and unpaired samples. *P* values below 0.05 were considered significant.

Results

A total of 194 pulmonary function tests were performed on 39 patients. Eighteen patients contracted active CMV infection: ten primary infections (donor seropositive and recipient seronegative: pos-neg combination), one reactivation (neg-pos) and seven pos-pos combinations. In 13 patients with active CMV infection, pulmonary function was tested at least twice during infection (10 primary infections and 3 pos-pos combinations). The remaining five patients with CMV infection were not tested because of less frequent outpatient clinic visits and a short duration of low antigenemia. Twenty-one patients without CMV infection were studied to obtain baseline values; pulmonary function in 13 patients was also studied on day 56 (control group).

None of the 18 patients with a CMV infection had any pulmonary symptoms. Of 13 patients tested during CMV infection, 6 patients were asymptomatic. Six others had at least four of the following symptoms: fever, malaise, leukocytopenia, thrombocytopenia, and elevated liver enzymes. In one patient, only a small increase in transaminases was observed. Seven patients were treated with gancyclovir intravenously. Eight patients had rejection periods before CMV infection and were treated with methylprednisolone ($n = 4$) or with methylprednisolone and subsequent ATG ($n = 4$). The median time period between the last ATG dose and the measurement of KCOc during CMV infection was 6.5 days (3, 6, 7, and 75 days).

During CMV infection the mean KCOc decreased significantly by 28% of the initial value (mean KCOc 79 vs 109; $P < 0.005$, Fig. 1) due to a decrease in both Vcap (mean Vcap compared to baseline 79 vs 106; $P < 0.005$, Fig. 2) and Dm (mean Dm compared to baseline 67 vs 86; $P < 0.01$, Fig. 3). In controls without CMV, the mean KCOc decreased by only 9% from baseline values (100 vs 110; $P < 0.05$) due to a slightly lower Vcap (88 vs 100; $P < 0.005$) but a similar Dm (87 vs 85; P = NS). The decrease in Vcap in controls was significantly smaller than that in recipients with CMV infection (12% vs 26%; $P < 0.05$). The KCOc during CMV infection was significantly lower than that in controls on postoperative day 56 (mean KCOc 79 vs 100; $P < 0.01$, Fig. 4). There was no difference in KCOc between symptomatic and asymptomatic patients (mean KCOc 76 vs 83; P = NS). No differences were found in spirometry (mean VC_{max} 109 vs 99, and mean FEV_1 102 vs 101), weight (75 vs 77 kg, P = NS), or renal function (creatinine clearance 55 vs 48, $P = 0.12$) before and during CMV infection. No correlation was found between cyclosporin levels and KCOc, Vcap, or Dm.

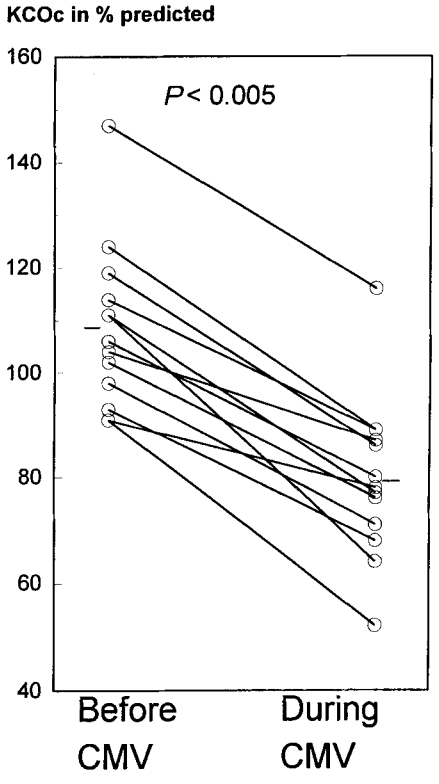

Fig. 1 Individual (o) and mean (—) values for Hb-corrected, specific CO diffusion capacity (*KCOc*) in kidney transplant recipients before and during active CMV infection

Discussion

Respiratory insufficiency due to CMV pneumonitis is a well-known complication with high mortality in kidney transplant recipients. Fortunately, it does not occur

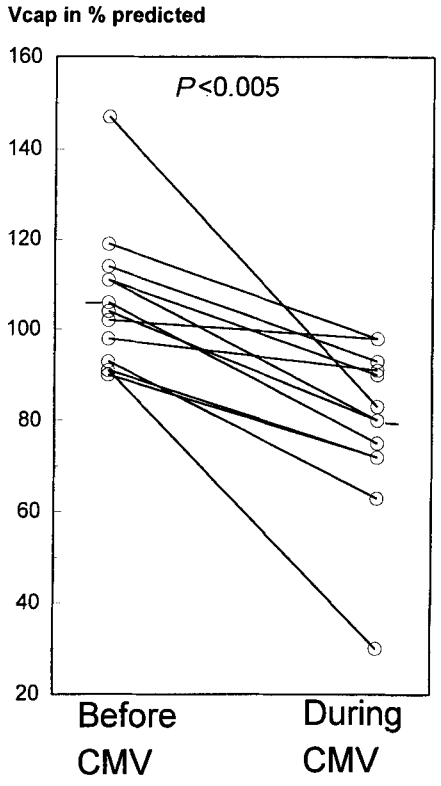

Fig. 2 Individual (o) and mean (—) values for pulmonary capillary volume (Vcap) in kidney transplant recipients before and during active CMV infection

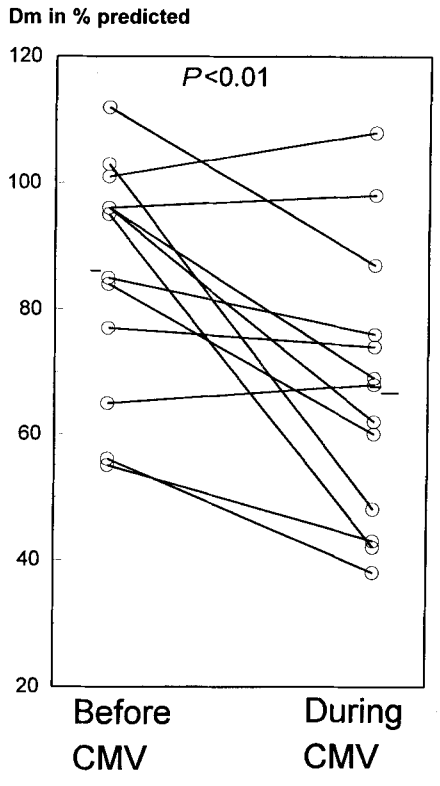

Fig. 3 Individual (o) and mean (—) values for membrane factor (Dm) in kidney transplant recipients before and during active CMV infection

very often. In this study we showed that all of our kidney transplant recipients with an active CMV infection had a subclinical pneumonitis, even in the absence of clinical symptoms. Van Son and coworkers [28, 29] demonstrated that kidney transplant recipients with CMV infection have a disturbed specific diffusion capacity for CO. In this study we found that the disturbed pulmonary diffusion during CMV infection is due to both a decrease in capillary volume (Vcap) and a decrease in membrane factor (Dm).

The decrease in diffusion cannot be explained by fluid overload or fibrosis. In the case of fluid overload, one would expect a smaller Dm but a normal Vcap [18]. Also, there were no differences in body weight or renal function at the time of CMV infection compared to controls or baseline values. The presence of fibrosis is not likely since the changes in KCOc due to CMV infection are reversible [28, 29].

It has been suggested that cyclosporin is responsible for a decrease in pulmonary diffusion. In heart transplant recipients in particular, a correlation has been found between cyclosporin levels and pulmonary diffusion. This correlation could not be related to Vcap and, as a consequence, was related to a decrease in Dm [14]. Other authors have not found a relationship between cyclosporin and pulmonary diffusion in kidney transplant recipients [22]. In our patient population, a correlation between cyclosporin levels and pulmonary diffusion capacity could not be detected. In the control group, we found a small decrease in Vcap that contradicts the normal Vcap in heart transplant recipients with cyclosporin [14].

It has been suggested that a more widespread infection of endothelial cells by CMV might play a pivotal role in the pathophysiology of CMV infection, possibly explaining the protean symptomatology of the infection [11, 12]. The virus is latently present in cells of the transplanted donor organ or of the recipient. The virus may be reactivated due to immunosuppression and/or by cytokines such as TNF-α induced by infection (septicemia), rejection, and drugs like OKT3. Cell-to-cell spread of the virus happens until, at some point during this process, endothelial cells become infected. In the next phase, mononuclear and polymorphonuclear leukocytes adhere to the infected endothelium and take up the virus and the pp65 matrix protein, which has been proven to be the viral protein detected in the CMV antigenemia test [13]. Finally, the infected endothelial cells detach and can be found in the peripheral blood as cytomegalic endothelial cells [11, 12]. Plugging of these cytomegalic endothelial cells may obstruct the pulmonary capillaries

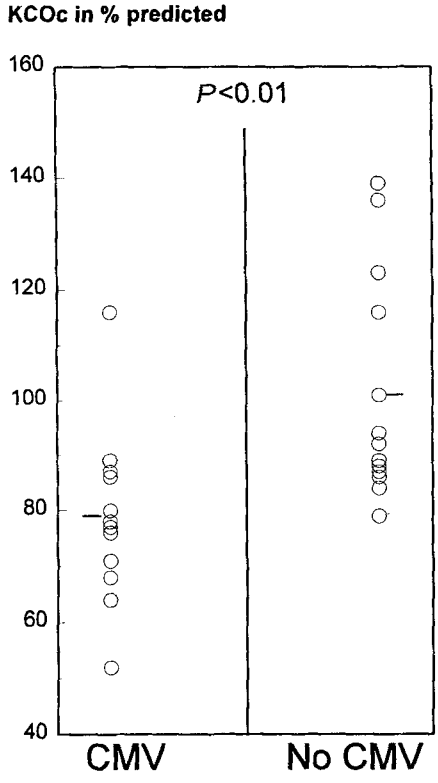

Fig. 4 Individual (o) and mean (—) values for Hb-corrected, specific CO diffusion capacity (*KCOc*) in kidney transplant recipients during CMV infection (median postoperative day 56) and in a control group without CMV infection (postoperative day 56)

since these infected endothelial cells can be as large as 35 μm and probably cannot (at least theoretically) pass through the pulmonary capillaries (diameter 5 μm). Plugging alone, however, is not an explanation for the decrease in diffusion as it should only affect Vcap. Some of the smaller cytomegalic endothelial cells most likely pass through the pulmonary capillaries.

Another possibility is complement activation. Complement activation has been demonstrated in the presence of active CMV infection [27]. In dialysis hypoxemia, complement activation causes aggregation of leukocytes, which obstructs pulmonary capillaries [8]. A similar process could take place during CMV infection. This process, however, should also only affect Vcap.

Although pulmonary involvement in CMV infection is in accordance with the systemic nature of this type of infection, the cause for the decrease in Vcap and Dm remains unknown. We speculate that a local inflammatory process due to CMV causes interstitial edema and capillary obstruction. This subclinical pneumonitis would explain both the decrease in Dm and Vcap. Mere plugging of cytomegalic endothelial cells or leukocytes does not explain the disturbed pulmonary diffusion, but the cytomegalic endothelial cells may contribute to the decrease in KCOc by, for example, spreading infection in the lungs. Cytomegalic endothelial cells contain active replicating CMV [11, 12], which makes spreading of the virus in the pulmonary capillary bed possible. This may lead to a locally CMV-induced inflammatory response that gives rise to local cytokine production. Cytokines such as IL-1, IL-6, RANTES, and TNFα have been implicated in the pathogenesis of pneumonitis (both pro-inflammatory as well as inflammatory effects of cytokines have been mentioned) in experimental pneumonia [2, 24, 31], pulmonary toxicity during bleomycin therapy [26], and CMV pneumonitis after lung transplantation [16, 17, 21]. In the latter category of patients, local cytokine production was found in material obtained by bronchoalveolar lavage (BAL). Invasive procedures like BAL are mandatory and justified in patients after lung transplantation since pulmonary symptoms may be due either to an infectious complication such as CMV pneumonitis or to rejection. Since none of our renal transplant recipients had pulmonary symptoms, invasive procedures like BAL did not seem justified. Since we did not perform BAL, we have no data concerning cytokines produced locally during CMV infection. However, locally produced cytokines during CMV infection (caused by cytomegalic endothelial cells transported to the lungs) may, indeed, have been at least partially responsible, via edema formation, for the decreased Dm found in our patients. Finally, during rejection or after ATG, cytokines are produced that may cause pneumonitis. Yet, the time between rejection and KCOc measurement (mean 42 days, range 7–81 days) and the time between ATG and KCOc measurement (median 6.5 days) makes the influence of rejection or ATG on KCOc less likely.

Subclinical pneumonitis in the transplant recipients with CMV was not followed by clinical pneumonitis. This could be explained by careful CMV monitoring in these patients, early tapering of immunosuppressive therapy during active CMV infection, and the use of antiviral medication in CMV-infected patients during polyclonal (ATG) antirejection therapy. In patients with CMV infection, pulmonary infections with opportunistic pathogens such as *Pneumocystis carinii*, fungi, or yeast are often seen. Many of these opportunistic infections are provoked by the immunosuppressive state caused by the antirejection regimen as well as by the CMV virus itself [15]. Another reason for these opportunistic infections may be that lungs are more susceptible to infections due to, even subclinical CMV pneumonitis.

In conclusion, all of our renal transplant recipients with CMV infection had a decrease in lung diffusion due to both a lower capillary volume and a lower membrane factor. A local inflammatory process due to CMV would therefore appear to be the most likely explanation for this phenomenon. We believe that this might render the lungs more susceptible to other opportunistic infections.

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