Pulmonary diffusion abnormalities in relation to cytomegalovirus antigenemia and cytomegalic endothelial cells in blood

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Abstract The pathophysiology of HCMV infection may involve many different organs including the lungs. In this study we investigated HCMV antigenemia levels and cytomegalic endothelial cells (CEC) in blood in relation to the pulmonary diffusion capacity. Patients with high HCMV antigenemia (≥ 100 pp65+ PMNs/50,000) (n = 8) showed a more extensive decrease in the membrane factor (Dm) than patients with lower levels of HCMV antigenemia (n = 7). The decline of the diffusion capacity of the alveolar capillary membrane (KCOc) and of the pulmonary capillary volume (Vcap) was the same in both groups. Four out of nine patients had CEC in the range of 0.22 CEC/ml to 30.26 CEC/ml. All the HCMV patients showed a decreased KCOc together with a decrease of Dm and Vcap but no difference was observed between patients with and without CEC. We conclude that a higher viral load is associated with a more extensive decrease in the membrane factor and therefore with more subclinical pneumonitis. No relation was observed between CEC and pulmonary dysfunction. Therefore, we postulate that CEC levels are related indirectly to subclinical pneumonitis mediated via the viral load.

Keywords CMV · Renal transplantation · Cytomegalic endothelial cells · Pulmonary diffusion

Abbreviations CEC Cytomatic endothelial cells · Dm Membrane factor · HCMV Human Cytomegalovirus · KCOc Diffusion capacity of the alveolar-capillary membrane · Vcap Pulmonary capillary volume

Introduction

Although cytomegalovirus (HCMV) infection is one of the most frequent complications after organ transplantation, the pathophysiology of HCMV disease remains speculative. Introduction of the cytomegalovirus pp65 antigenemia assay enabled early and rapid diagnosis of HCMV-viremia prior to symptoms in transplant patients [15]. After kidney transplantation, cytomegalovirus infection most often causes a flu-like syndrome with fever, arthralgia, leucocytopenia, thrombocytopenia, and elevated liver enzymes in symptomatic patients. The number of pp65 positive polymorphonuclear cells per 50,000 leukocytes is related to clinically symptomatic HCMV disease [1]. The majority of patients with positive HCMV antigenemia remains asymptomatic because of preemptive treatment with ganciclovir in case of institution of antirejection therapy. However, those still asymptomatic patients may have subclinical manifestations of HCMV. We demonstrated, for example, increased intestinal mucosal permeability for disaccharides like lactulose in kidney transplant recipients with HCMV infection [7]. Cytomegalovirus causes subclinical pulmonary dysfunction in kidney transplant recipients with and without HCMV-associated symptoms [6, 13]. Whether the disturbed pulmonary diffusion or dam-
age of the intestinal integrity precede or coincidence with clinical pneumonitis or colitis is unknown. An important pathophysiological role was suggested for cyto-
megalic endothelial cells (CEC). CEC can be detected in the peripheral blood of half of the patients with high HCMV pp65-antigenemia levels [5] and are related to high antigenemia levels [4, 9].

In a previous study [6] we found a decrease in pulmonary diffusion during HCMV infection due to a combi-
nation of a lower membrane factor (Dm) and a lower pulmonary capillary volume (Vcap). We concluded that a local inflammatory process is the most likely explanation for the decrease in pulmonary diffusion as opposed to plugging of circulating cytomegaloc endothelial cells (diameter 35–45 μm) in the pulmonary capillaries. In our opinion, endothelitis and dissemination of the virus by circulating cytomegaloc endothelial cells is important in the pathophysiology of HCMV disease. In a recent study we demonstrated that the incidence of CEC was associated with HCMV-related clinical symptoms [5]. In this report we investigated the relation between anti-
genemia levels, CEC in blood, and the decline of pulmonary diffusion in 15 kidney transplant patients.

**Patients and methods**

The study was approved by the ethics committee of our hospital. Fifteen kidney transplant patients who developed an HCMV infec-
tion were included in this study. All patients had received a cadav-
eric transplant. 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 cardiopul-
monary complications, such as myocardial ischemia or infarction, pulmonary embolism, or bronchopneumonia, were excluded from the study. Initial immunosuppression consisted of cyclosporin A and low dose prednisolone. One patient received an induction course of OKT3. All patients gave their informed consent before participating in the study.

Pulmonary function was determined in all patients at approxi-
mately 15 days after transplantation (baseline value) and at least twice during HCMV infection (median number of measurements 5, range 2–15). The transfer factor (diffusion capacity) for CO (TICO) and its components, the diffusion capacity of the alveolar-
capillary membrane (Dm) and the volume of blood in the pulmo-

nary capillaries (Vcap), were determined from triplicate measure-
ments of TICOc at high (88%) and low (19.2%) concentrations of inspired oxygen. The single breath technique of Krogh, as modified by Cotes was used [2]. Carbon monoxide was measured with an in-
frared spectrophotometer and helium using a thermal conductivity method (ML-Master-lab-transfer; Jaeger, Germany). The TICO values were corrected for hemoglobin concentrations (TICOc), ac-
cording to Cotes [2]. Corrected, specific diffusion capacity (KCOc) was calculated by dividing TICOc by the alveolar volume. Dm and Vcap were derived from the equation of Roughton and Forster [12]:

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1/TICO = 1/Dm + 1/θ[Hb]Vcap
\]

In this reaction, θ is the reaction rate of CO with hemoglobin (Hb) at the average normal Hb concentration (9 mmol/l). [Hb] is the he-
moglobin concentration as a fraction of the average normal Hb concentration. Values are expressed as percentages of those pre-
predicted; the predicted values being taken from Cotes et al. [3] and Ouanj et al. [11].

Patients were monitored for HCMV pp65-antigenemia twice a week. This test was performed according the procedure recently reviewed for standardization [15]. No HCMV prophylaxis such as ganciclovir, acyclovir or hyperimmune gammaglobulin was given. Eight patients received ganciclovir because of clinical symptoms associated with rising HCMV antigenemia values or preemptive because of anti-rejection treatment. CEC in blood was studied at approximately 15 days after transplantation (i.e. before infection) and weekly during HCMV infection. This was continued until the HCMV antigenemia was negative (n = 4) or less than 5/50,000 cells

CEC in blood were analyzed as has been described recently [5]. In brief, mononuclear cells (MNC) were isolated by density cen-
trifugation using Lymphoprep (Nycomed Pharma AS, Oslo, Nor-
way). 1 × 10^5 MNC were cytocentrifuged on a slide. The cyto-

sps were stained by indirect immunofluorescence with the following antibodies: C10/C11 directed against HCMV pp65 and E1/1/2.3 di-
rected to a 90kD cell surface antigen of endothelial cells [10]. Four cyto-

sps were analyzed if the concentration of MNC/ml blood was 1.5 × 10^5 or less, otherwise 6–8 cytosps were analyzed. The num-
ber of analyzed slides represented a detection limit of 20 CEC/ml

blood in 95% of all samples. Statistical analysis was performed using Student’s t-test for paired and unpaired samples.

**Results**

In this study, CO diffusion was determined in 15 patients (9 male / 6 female) with active HCMV infection. The median age was 42 years (range 18–63 years). Eleven patients had a primary infection (positive donor organ, negative recipient), and the remaining 4 patients had secondary infections (positive–positive combination). Nine patients had clinical symptoms such as fever, mal-
aise, leukocytopenia, thrombocytopenia and elevated liver enzymes. Nine out of the 15 patients were studied for CEC (Table 1). CEC were observed in four out of these nine patients. In two patients, CEC were observed once; in one patient twice, and in the last patient even at times during the course of HCMV antigenemia. The CEC concentrations ranged from 0.22 CEC/ml to 30.26 CEC/ml (median 1.28 CEC/ml).

The decrease in KCOc observed during HCMV infec-
tion was similar for patients with and without CEC (22.28 ± 22.0 versus 23.0 ± 20.5, P = ns). The decrease in Vcap and Dm was also similar (17.03 ± 18.9 versus 19.94 ± 18.0, P = ns and 28.7 ± 23.85 versus 17.56 ± 13.8, P = ns, respectively) (Table 1A). The CO diffusion was analyzed in relation to the severity of infection. Patients with high HCMV antigenemia (≥ 100 pp65+ PMNs/50,000) showed a more extensive decrease of Dm than patients with low or moderate HCMV levels (≥ 100 pp65+ PMNs/50,000) (29.48 ± 20.33 versus 6.69 ± 12.26, P < 0.05). These differences were not ob-
served in the KCOc and Vcap levels (26.01 ± 6.12 versus 25.0 ± 10.5, P = ns and 21.01 ± 11.89 versus 31.67 ±