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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

Abbrevations *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 cytomegalic 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 combination 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 oppossed to plugging of circulating cytomegalic endothelial cells (diameter 35–45 μm) in the pulmonary capillaries. In our opinion, endothelitis and dissemination of the virus by circulating cytomegalic 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 antigenemia 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 infection were included in this study. All patients had received a cadaveric 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 cardiopulmonary 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 approximately 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 (TlCO) and its components, the diffusion capacity of the alveolar-capillary membrane (Dm) and the volume of blood in the pulmonary capillaries (Vcap), were determined from triplicate measurements of TlCOc 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 infrared spectrophotometer and helium using a thermal conductivity method (ML-Master-lab-transfer; Jaeger, Germany). The TlCO values were corrected for hemoglobin concentrations (TlCOc), according to Cotes [2]. Corrected, specific diffusion capacity (KCOc) was calculated by dividing TlCOc by the alveolar volume. Dm and Vcap were derived from the equation of Roughton and Forster [12]:

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

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 hemoglobin concentration as a fraction of the average normal Hb concentration. Values are expressed as percentages of those predicted, the predicted values being taken from Cotes et al. [3] and Quanjer 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 ($n = 5$).

CEC in blood were analyzed as has been described recently [5]. In brief, mononuclear cells (MNC) were isolated by density centrifugation using Lymfoprep (Nycomed Pharma AS, Oslo, Norway). 1×10^5 MNC were cytocentrifuged on a slide. The cytospots were stained by indirect immunofluorescence with the following antibodies: C10/C11 directed against HCMV pp65 and E1/1 2.3 directed to a 90kD cell surface antigen of endothelial cells [10). Four cytospots were analyzed if the concentration of MNC/ml blood was 1.5×10^6 or less, otherwise 6–8 cytospots were analyzed. The number 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, malaise, 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 infection was similar for patients with and without CEC (22.28 ± 22.0 versus 23.0 ± 25.0, 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 observed in the KCOc and Vcap levels (26.01 ± 6.12 versus 25.0 ± 10.52, P = ns and 21.01 ± 11.89 versus $31.67 \pm$

Table 1 Differences in pulmonary CO diffusion before and during HCMV infection

A	+ CEC ($n = 4$)	–CEC ($n = 5$)	P
Δ KCOc (mean ± SD)	22.28 ± 22.0	23.0 ± 25.0	N.S.
Δ Vcap (mean ± SD)	17.03 ± 18.9	19.94 ± 18.0	N.S.
Δ Dm (mean ± SD)	28.7 ± 23.85	17.56 ± 13.8	N.S.
B	HCMV-Ag ≥ 100* ($n = 8$)	HCMV-Ag ≤ 100* ($n = 7$)	
Δ KCOc (mean ± SD)	26.01 ± 6.12	25.0 ± 10.52	N.S.
Δ Vcap (mean ± SD)	21.01 ± 11.89	31.67 ± 21.25	N.S.
Δ Dm (mean ± SD)	29.48 ± 20.33	6.69 ± 12.26	$P < 0.05$

21.25, P = ns, respectively) (Table 1B). Patients with and without HCMV-associated clinical symptoms did not differ in decreases of KCOc, Vcap and Dm (KCOc: 25.57 ± 9.91 versus 25.0 ± 5.36, P = ns; Vcap: 28.34 ± 22.08 versus 22.45 ± 4.40, P = ns; Dm 17.99 ± 21.4 versus 20.12 ± 20.27, P = ns).

In Figure 1, the course of KCOc, Vcap and Dm in a kidney transplant patient with HCMV infection is illustrated. CEC were studied weekly and appeared at four days after the maximal HCMV antigenemia value. At that time, the Dm and KCOc were decreased (32% and 8%) while the Vcap showed an increase of 22%.

Discussion

During HCMV infection, patients had a decreased pulmonary diffusion capacity that affected both Vcap and Dm. The balance between disturbances of the individual components was influenced by the severity of the infection, but we did not observe a specific influence of CEC in blood on either the Vcap or the Dm. To clarify the contribution of CEC in blood, nine patients were studied for the occurrence of CEC. No evident differences in pulmonary diffusion capacity were observed between patients with and without CEC. Although the numbers were small, no tendency towards a diminished Vcap was observed. The numbers of CEC in patients varied from 0.22 CEC/ml–30.26 CEC/ml, equivalent to 1,100 to 151,300 cytomegalic cells per 5 l of blood at that moment. Apparently, these numbers are too low to cause a measurable obstruction of the blood flow in the lungs, in addition to the decrease already observed in all HCMV patients. Alternatively, the CEC might either be disrupted in the capillaries or be deformed and circulate normally, like genuine blood cells.

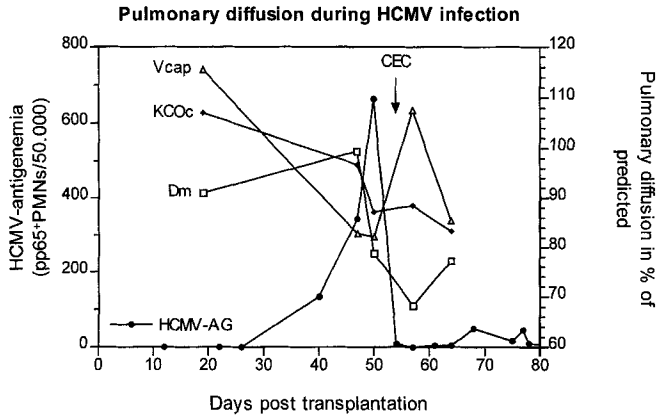

Fig. 1 Typical course of HCMV antigenemia (*left Y-axis*) and KCOc, Vcap and Dm (*right Y-axis*) in a kidney transplant recipient with HCMV infection. The *arrow* indicates the occurrence of CEC

Because CEC are strongly related to high HCMV antigenemia levels, we analyzed the severity of infection, as indicated by HCMV antigenemia levels, in relation to CO diffusion as well. Patients were divided into groups with high HCMV antigenemia levels (≥ 100 pp65$^+$ PMNs/50.000) and such with moderate to low antigenemia levels (< 100 pp65$^+$ PMNs/50.000). In the high HCMV antigenemia group, the Dm decreased more than in the group with low HCMV antigenemia, representing a longer diffusion resistance from the alveolus to the capillaries. An inflammatory reaction with production of cytokines, fluid extravasation and cellular infiltrate could underlie these findings. The infiltrating cells can be composed of T cells, monocytes and macrophages [14]. Monocytes and macrophages are capable of producing nitric oxide (NO). NO has, beside immunomodulatory properties, strong vasoregulatory effects [8], which may partially compensate the decrease in Vcap.

In conclusion, we found a significant decrease in Dm during more severe HCMV infection. This indicates that the severity of subclinical pneumonitis is related to maximal antigenemia levels. We have not proven a relation between CEC and the decrease in Dm and Vcap. In the post, high viral loads expressed by high maximum antigenemia levels were related to CEC levels. We think that CEC levels are indirectly related to more extensive decreases in pulmonary diffusion, but because of low numbers of patients this could not be demonstrated.

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References

1. Berg AP van den, Bij W van der, Son WJ van, Anema J, Giessen M van der, Schirm J, Tegzess AM, The TH (1989) Cytomegalovirus-antigenemia as a useful marker of symptomatic cytomegalovirus disease after renal transplantation: a report of 130 consecutive patients. Transplantation 48: 991–995
2. Cotes JE (1979) Lung function. 4th edn. Blackwell, Oxford
3. Cotes JE, Chinn DJ, Quanjer PhH, Roca J, Yernault JC (1993) Standarization of the measurement of transfer factor (diffusion capacity). Eur Resp J [Suppl] 16: 41–52
4. Grefte A, Giessen M van der, Son WJ van, The TH (1993) Circulating cytomegalovirus (CMV)-infected endothelial cells in patients with an active CMV infection. J Infect Dis 167: 270–277
5. Kas-Deelen AM, Maar EJ de, Harmsen MC, Driessen C, Son WJ van, The TH (2000) Uninfected and cytomegalic endothelial cells in blood during cytomegalovirus infection: effect of acute rejection. J Infect Dis 181: 721–724
6. Maar EF de, Kas-Deelen AM, Mark TW van der, The TH, Tegzess AM, Ploeg RJ, Son WJ van (1999) Cytomegalovirus pneumonitis after kidney transplantation is not caused by plugging of cytomegalic endothelial cells only. Transpl Int 12: 56–62
7. Maar EF de, Kleibeuker JH, Boersma-van Ek W, The TH, Son WJ van (1996) Increased intestinal permeability during cytomegalovirus infection in renal transplant recipients. Transpl Int 9: 576–580
8. Meyer KC, Love RB, Zimmerman JJ (1998) The therapeutic potential of nitric oxide in lung transplantation. Chest 113: 1360–1371
9. Percivalle E, Revello MG, Vago L, Gerna G (1993) Circulating endothelial giant cells permissive for human cytomegalovirus (CMV) are detected in disseminated CMV infections with organ involvement. J Clin Invest 92: 663–670
10. Pober JS, GimbroneJr MA, Lapiere LA, Mendrick DL, Fiers W, Rothlein R, Springer TA (1986) Overlapping patterns of activation of human endothelial cells by interleukin 1, tumor necrosis factor, and immune interferon. J Immunol 137: 1893–1896
11. Quanjer PhH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC (1993) Lung volumes and forced ventilatory flows. Eur Resp J [Suppl] 16: 15–40
12. Roughton FJW,Forster RE (1957) Relative importance of diffusion and chemical reaction rates in determining the exchange of gases in the human lung with special reference to the true diffusing capacity of pulmonary membrane and volume of blood in the lung capillaries. J Appl Physiol 11: 290–302
13. Son WJ van, Bij W van der, The TH, Peset R, Mark TW van der, Tegzess AM (1989) Evidence for pulmonary dysfunction in all patients with symptomatic and asymptomatic cytomegalovirus (CMV) infection after renal transplantation. Transplant Proc 21: 2065–2068
14. Steinmüller C, Steinhoff G, Bauer D, You XM, Denzin H, Franke-Ullmann G, Hausen B, Bruggemann C, Wagner TOF, Lohmann-Matthes ML, Emmendörffer A (1997) Analysis of leukocyte activation during acute rejection of pulmonary allografts in noninfected and cytomegalovirus- infected rats. J Leukocyte Biol 61: 40–49
15. The TH, Harmsen MC, Bij W van der, Berg AP van den, Son WJ van (1998) Relationship between monitoring the viral load in blood, human cytomegalovirus pathophysiology and management strategies of patients after transplantation. Monogr Virol 21: 262–279