Chemokines and soluble adhesion molecules in renal transplant recipients with cytomegalovirus infection

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SUMMARY

Cytomegalovirus (CMV) infection is associated with leucocyte infiltration in various organs, which supports a role for chemokines and adhesion molecules in the pathogenesis of CMV infection. In a prospectively conducted study of renal transplant recipients, 10 patients with CMV disease, five patients with asymptomatic CMV infection and 10 patients who did not have any CMV infection were included. During CMV infection, and in particular during CMV disease, plasma levels of the chemokines IL-8, macrophage inflammatory protein-1α (MIP-1α) and monocyte chemotactic protein-1 (MCP-1) and the soluble adhesion molecules vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and L-selectin increased and were positively correlated with the degree of CMV pp65 antigenaemia. Furthermore, a decrease in plasma levels of these chemokines and adhesion molecules was observed following ganciclovir therapy in the patients with CMV disease. This could suggest a role for these molecules in the pathogenesis of CMV infection.

Keywords   CMV infection   chemokines   adhesion molecules   renal transplant recipients

INTRODUCTION

Cytomegalovirus (CMV) infection is often associated with organ involvement, and the infiltration of leucocytes in these areas is associated with production of chemokines and up-regulation of adhesion molecules on endothelial cells and leucocytes [1]. Chemokines are cytokines which, in addition to activating leucocytes, have specific chemo-attractive properties [2]. Up-regulated adhesion molecules on leucocytes and endothelial cells mediate the recognition, adherence and extravasation of leucocyte subsets to sites of inflammation [3]. Indeed, *in vitro* infection of various cells with CMV has demonstrated an effect on the production of various chemokines [4,5], and on the expression of several adhesion molecules on the membrane surface [6,7].

IL-8, macrophage inflammatory protein-1α (MIP-1α) and monocyte chemotactic protein-1 (MCP-1) are important chemokines in the attraction and activation of granulocytes, T cells and monocytes, all of which have been implicated in the spread or control of CMV. Furthermore, three adhesion molecules of particular interest in the leucocyte–endothelial interaction are L-selectin, expressed on most resting leucocytes in peripheral blood; vascular cell adhesion molecule-1 (VCAM-1), expressed by activated endothelial cells and macrophages; and intercellular

adhesion molecule-1 (ICAM-1), expressed by both activated leucocytes and endothelial cells. In renal transplant recipients CMV infection is common and is a major cause of morbidity [8]. Based on the possible important role of chemokines and adhesion molecules in the immune response during CMV infection, we hypothesized that these molecules could contribute to the development of infection and clinical manifestations of CMV-related disease in renal transplant recipients. Plasma concentrations of the above listed molecules were investigated in a prospectively conducted study of renal transplant recipients with and without CMV infection.

PATIENTS AND METHODS

Patients
Patients and blood sampling have previously been described [9]. Briefly, blood samples were prospectively collected and monitored for pp65 antigenaemia in 25 consecutively recruited renal transplant patients after transferral from the surgical to the medical ward. At time of transferral all patients had undetectable pp65 antigen levels in circulating neutrophils and no signs of any clinical complications. They were included for further analysis based on the following criteria: Group A (CMV disease) was composed of all patients ($n = 10$) with CMV disease, defined by the presence of pp65 antigen in circulating neutrophils combined with fever $> 38°C$ and/or evidence of organ dysfunction of no other cause [10]. These patients were treated with ganciclovir

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(5 mg/kg bid) for 14 days; Group B (asymptomatic CMV infection) consisted of all patients (*n* = 5) with asymptomatic infection, as defined by the presence of pp65 antigenaemia without any clinical signs of infection; Group C (no CMV infection) had neither detectable pp65 antigenaemia nor clinical manifestations suggesting CMV disease during follow up (*n* = 10).

At time of blood sampling all patients received immuno-suppressive therapy with prednisolone, azathioprine and cyclosporin. No patients received prophylactic or pre-emptive anti-CMV treatment. For characteristics and time relations between the inclusion point and other events see Table 1. Controls consisted of 10 healthy volunteer blood donors. Informed consent for blood sampling was obtained from all patients and blood donors.

Selection of blood samples and detection of pp65 antigen
The first blood samples were taken on the morning immediately after transfer from the surgical to the medical ward, a median of 26 days after transplantation (baseline; Table 1). During follow up, blood samples were taken every 2 weeks for further analysis. If CMV antigenaemia occurred, blood samples were taken weekly. For more extensive analysis, two samples were selected in addition to samples taken at baseline. In Group A, samples were taken immediately prior to ganciclovir therapy (disease) and after cessation of therapy in a period with no clinical suggestion of CMV disease or undetectable pp65 antigenaemia (follow up). In Group B, further samples were taken during peak pp65 (peak antigenaemia) and later in a phase when pp65 was declining or not detectable (follow up). In Group C, samples were selected that had a time span between baseline, disease/peak antigenaemia, and follow up comparable to that observed in Groups A and B (Table 1). Plasma was obtained from peripheral venous blood and CMV pp65 antigen detection was performed as previously described [9]. The results are given as numbers of CMV pp65 antigen-positive cells per 100 000 leucocytes.

Enzyme immunoassays
Enzyme immunoassays (EIAs) (all from R&D Systems, Minneapolis, MN) were used to measure plasma levels of IL-8 (detection limit 2 pg/ml), MIP-1α (detection limit 6 pg/ml), MCP-1 (detection limit 5 pg/ml), soluble (s) L-selectin (detection limit 0·03 ng/ml), sVCAM-1 (detection limit 2 ng/ml) and sICAM-1 (detection limit 0·35 ng/ml).

Statistical analysis
When comparing more than two groups of individuals the Kruskall–Wallis test was used. If a significant difference was found, the Mann–Whitney *U*-test was performed. When comparing paired data Wilcoxon's matched pairs test was used. Coefficients of correlation were calculated by the Spearman rank test. Data are given as medians and ranges if not otherwise stated. *P* values are two-sided and considered significant when < 0.05. All statistical calculations were performed using the STATISTICA (Statsoft, Tulsa, OK) software package.

RESULTS

At inclusion, pp65 was not detectable in any patient. At disease/peak antigenaemia, the median levels were 233 (range 22–700) and 71 (range 7–150), and at follow up the median levels were 20 (range 0–330) and 65 (range 0–148), in Group A and B, respectively. In three patients treatment with ganciclovir and methylprednisolone was initiated simultaneously, and was continued for 4, 6 and 7 days, respectively. However, kidney biopsies did not demonstrate histological alterations suggestive of graft rejection.

Plasma levels of IL-8, MIP-1α and MCP-1
As previously reported [11], plasma levels of IL-8 and MCP-1 in Group A were significantly elevated, while levels of MIP-1α were significantly reduced at inclusion compared with controls. During follow up, in Group A levels of IL-8, MIP-1α and MCP-1

Table 1. Characteristics of 25 renal transplant recipients at baseline of the study

	Group A (*n* = 10)	Group B (*n* = 5)	Group C (*n* = 10)
Sex (female/male)	3/7	2/3	1/9
Age (years)	40·5 (19–68)	40 (24–61)	49 (23–60)
CMV-IgG pre-Tx (neg/pos)	8/2	0/5	4/6
Total leucocyte count (10^9/l)	4·7 (3·1–9·6)	6·1 (4·3–8·2)	8·0 (4·1–10·8)
Creatinine (μmol/l)	171 (96–196)*	134 (99–169)	125 (101–176)
Previous rejection therapy	5 (4 with ATG)	0	2
Rejection after inclusion	1	3	2
Days from end of rejection therapy to pp65 antigenaemia	13 (10–36)		
Days from transplantation to baseline	28 (16–59)	23 (20–40)	24 (17–51)
Days from transplantation to pp65 antigenaemia	51 (33–67)	70 (32–90)	
Days from baseline to pp65 antigenaemia	22 (4–32)	32 (10–55)	
Days from baseline to ganciclovir therapy	29 (11–36)		
Days from baseline to disease/peak antigenaemia	25 (6–32)	30 (12–45)	25 (1–32)
Days from disease/peak antigenaemia to follow up	20·5 (14–33)	12 (7–18)	27 (14–50)

Group A, patients with cytomegalovirus (CMV) disease; Group B, patients with asymptomatic CMV infection; Group C, patients without CMV infection; ATG, anti-thymocyte globulin.
*$P < 0.05$ *versus* Group C.
Data are given as medians and ranges where appropriate.

Table 2. Plasma levels of IL-8, macrophage inflammatory protein-1α (MIP-1α) and monocyte chemotactic protein-1 (MCP-1) in 25 renal transplant recipients at different time intervals and in 10 controls (data are given as medians and ranges)

	Baseline	Disease/peak antigenaemia	Follow up
IL-8 (pg/ml):			
Controls	0 (0–0)		
Group A	3·4 (0–13)[1,2,3]	16·1 (0–36·8)*	0 (0–14·1)†
Group B	0 (0–0)	0 (0–6·7)	3·2 (0–6·4)
Group C	0 (0–21·5)	0 (0–0)	0 (0–0)
MIP-1α (pg/ml):			
Controls	16·4 (11·9–21·4)		
Group A	10·7 (5·4–23·9)[1,2]	25·2 (7·6–34·3)*	12·1 (0–18·4)†
Group B	19·8 (16·5–34)	28 (14·8–31·1)	25·6 (15·3–28·8)
Group C	17·2 (14·6–24·7)	18·2 (17·1–25)	19·5 (14·3–27)
MCP-1 (pg/ml):			
Controls	92 (19–151)		
Group A	366 (220–778)[1]	771 (430–1976)**	474 (138–696)†
Group B	424 (272–564)[1]	604 (450–960)	737 (492–988)
Group C	330 (210–736)[1]	485 (270–710)*	419 (151–558)

Group A, patients with cytomegalovirus (CMV) disease; Group B, patients with asymptomatic CMV infection; Group C, patients without CMV infection.
[1] $P < 0.05$ *versus* controls; [2] $P < 0.05$ *versus* Group C; [3] $P < 0.05$ *versus* group B.
*$P < 0.05$ *versus* time 0; **$P < 0.005$ *versus* time 0.
†$P < 0.05$ *versus* time 1; †$P < 0.05$ *versus* time 1 in the same group.

increased from baseline to disease, though not significantly for MIP-1α levels (Table 2). The levels decreased significantly for all three chemokines from time of disease to follow up. In Group B there was a slight and not significant rise in these chemokines from baseline to peak antigenaemia, while no changes were observed from peak antigenaemia to follow up (Table 2). In Group C, MCP-1, but neither IL-8 nor MIP-1α levels, increased from baseline to follow up (Table 2).

Plasma levels of VCAM-1, ICAM-1 and L-selectin
Plasma levels of VCAM-1 was increased in all patients compared with controls at inclusion. The levels increased further in Groups A and B from baseline to disease/peak antigenaemia and tended to decrease at follow up in Group A (Table 3). No changes were observed in Group C in the same period. sICAM-1 and sL-selectin levels tended to be lower in all patient groups compared with controls at inclusion, and both molecules increased in Groups A and B from baseline to disease/peak antigenaemia, though not significantly in Group B. In Group A, sICAM-1 levels decreased significantly from disease to follow up. In Group C, sICAM-1 also increased from baseline to follow up, while no difference was observed in sL-selectin levels.

Correlations to pp65 antigenaemia
Including data from all patients ($n = 25$), IL-8 and MCP-1 levels correlated with pp65 antigen levels at time of disease/peak antigenaemia ($r = 0.63$, $P < 0.001$ and $r = 0.44$, $P < 0.05$, respectively), and for IL-8 levels also at follow up ($r = 0.57$, $P < 0.005$). Plasma levels of sVCAM-1, sICAM-1 and sL-selectin correlated positively with pp65 antigenaemia at time of disease/peak antigenaemia ($r = 0.51$, $P < 0.005$; $r = 0.40$, $P < 0.05$; $r = 0.51$, $P < 0.01$, respectively), and for sVCAM-1

such a correlation was also found at follow up ($r = 0.61$, $P = 0.02$).

DISCUSSION

The immune response to CMV replication is probably of the utmost importance both for the defence against CMV infection and for clinical manifestations. Increased levels of chemokines and adhesion molecules have previously been reported in several infectious and inflammatory diseases [12–14], and our findings suggest that these molecules also appear to play an essential role in the response to CMV. Although not specific for CMV infection, the increase in plasma levels of both chemokines and adhesion molecules during CMV infection, and particularly during CMV-related disease, which significantly correlated with the degree of CMV replication, could suggest a role for these molecules in the pathogenesis of CMV infection and disease.

Previous reports on chemokines during human CMV infection include elevated levels of MCP-1 in the cerebrospinal fluid in HIV patients with CMV encephalitis [15] and high concentrations of RANTES in bronchoalveolar lavage fluids from patients with CMV pneumonitis [16]. Furthermore, serum IL-8 has been reported elevated in bone marrow transplant recipients with CMV infection [17]. In the present prospectively conducted study on renal transplant recipients, plasma levels of IL-8 increased during infection and particularly during disease. In addition to its chemotactic and activating effects on granulocytes, IL-8 also recruits a subset of T cells and natural killer (NK) cells, and although only moderately chemotactic on monocytes, IL-8 has recently been reported to activate these cells under flow conditions [18]. From *in vitro* studies we know that IL-8 may increase CMV replication and enhance viral dissemination [4,19,20]. In view of the pleiotropic actions of this

Table 3. Plasma levels of vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and L-selectin in 25 renal transplant recipients at different time intervals and in 10 controls (data are given as medians and ranges)

	Baseline	Disease/peak antigenaemia	Follow up
sVCAM-1 (pg/ml):			
Controls	353 (228–589)		
Group A	910 (413–1929)[1]	1580 (794–2055)*	963 (566–1579)
Group B	861 (246–1086)[1]	1338 (682–2224)	1651 (938–2174)
Group C	722 (223–2228)[1]	677 (249–1693)	678 (220–1098)
sICAM-1 (pg/ml):			
Controls	215 (159–315)		
Group A	166 (62–230)[1]	272 (146–446)*	204 (48–310)†
Group B	207 (88–239)	270 (202–324)	273 (257–320)
Group C	187 (83–237)	221 (131–276)*	170 (43–252)
sL-selectin (pg/ml):			
Controls	768 (169–1524)		
Group A	412 (233–1101)	712 (359–1620)*	555 (330–977)
Group B	343 (250–683)	518 (327–668)	498 (449–562)
Group C	284 (128–682)[1]	341 (103–655)	382 (92–569)

Group A, patients with cytomegalovirus (CMV) disease; Group B, patients with asymptomatic CMV infection; Group C, patients without CMV infection.

[1]$P < 0.01$ *versus* controls.

*$P < 0.05$ *versus* time 0.

†$P < 0.05$ *versus* time 1.

chemokine, its role during CMV infection is uncertain. However, our finding that the increase of IL-8 is most pronounced during CMV disease might suggest a role for IL-8 in the establishment of symptoms.

The role of MIP-1α and MCP-1 is also difficult to ascertain. Increased synthesis of MIP-1α may contribute to suppression of haematopoiesis [21], which is often seen during CMV disease. MCP-1 may enhance viral replication through activation of monocytes. On the other hand, MIP-1α recruits potentially protective NK cells to sites of CMV infection in mice [22] and both MIP-1α and MCP-1 activate lymphocytes and may therefore contribute to the antiviral defence. A functional chemokine receptor encoded by CMV was recently demonstrated to sequester soluble chemokines, leading to a reduction of the extracellular concentration of CC-chemokines *in vitro* [23]. While this may also happen *in vivo*, such effects are not obvious in our study, since the levels of MIP-1α and MCP-1 increased during ongoing infection.

In both heart and liver transplant recipients an up-regulation of adhesion molecules has been observed on the endothelium in the allograft during CMV infection, and is probably of central importance in the development of inflammatory lesions [24,25]. In renal transplant recipients elevated levels of sICAM-1 and sVCAM-1 have been reported during CMV infection [26,27]. We confirmed these findings and add to this an increase in plasma levels of L-selectin. This could reflect an increased activation of leucocytes and contributes to the notion that adhesion molecules are involved in the CMV immune response.

One interesting observation in the present study was the decrease in levels of chemokines and sICAM-1 after the initiation of ganciclovir. This is not in accordance with previously published *in vitro* studies, where ganciclovir treatment was reported to increase rather than reduce IL-8 production and ICAM-1 expression [6,28]. Ganciclovir fails to inhibit the production of

CMV immediate early antigens, which are reported to stimulate the production of ICAM-1 and IL-8. It has been reported that such therapy, in spite of effective inhibition of replication [29], does not necessarily improve disease manifestations [28]. In our patients, 14 days of ganciclovir treatment was associated with a reduction of plasma levels of these molecules and resolution of disease. The reason for the discrepancy between our observations and those referred to above is not at present clear.

The fact that some patients, in spite of ongoing viral replication, do not develop clinical disease is intriguing. CMV disease has been associated with high CMV load, particularly in HIV co-infected patients [30], and indeed our patients with disease had higher CMV load compared with asymptomatic patients. However, the difference in clinical manifestations may also indicate qualitative and quantitative differences in the anti-CMV immune responses in these patient groups. Certain components of the immune response such as total lymphocyte numbers in renal transplant recipients are lower [9,31] and serum levels of cytokines such as IL-6 in bone marrow recipients are higher [17] in patients with symptomatic disease compared with patients with asymptomatic disease. We find that particularly elevated plasma levels of IL-8 are associated with CMV disease, though larger studies are needed to confirm this. These alterations of the immune system might not necessarily be induced through viral replication [32] and could contribute to pathogenesis.

In conclusion, during CMV infection, and in particular during CMV disease, a considerable increase is seen in plasma concentrations of both chemokines and soluble adhesion molecules, probably reflecting central roles for these molecules in the immunopathogenesis of CMV infection. While some of the biological effects of these multifunctional molecules may be protective, others may contribute to disease manifestations. Further studies may clarify the importance of the various molecules in human CMV disease.

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