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Cytomegalovirus-related disease and risk of acute rejection in renal transplant recipients: a cohort study with case-control analyses

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Abstract The relationship between a cytomegalovirus (CMV) infection and the acute rejection of a renal transplant is not well established. The aim of the study was to document whether the clinical presentation of a CMV infection as a diffuse inflammatory disease or as a clinically asymtomatic illness is a risk factor of acute renal transplant rejection. One hundred and ninety-two consecutive renal transplant recipients were included in a historical cohort study for exposed – non exposed analyses. CMV infection after transplantation was the exposure factor. Before transplantation, 113 patients had antibodies against CMV and 79 were seronegative. The patients were divided into three groups: Group 1 consisted of 64 patients who had neither clinical signs of CMV disease nor CMV serological changes after transplantation, Group 2 consisted of 77 seropositive patients with asymptomatic viremia, and Group 3 consisted of 51 seropositive patients with clinical signs of diffuse inflammation that included fever, neutropenia, and various visceral involvements (CMV disease). Groups 2 and 3, the seropositive patients, were paired with Group 1 patients. Acute rejection was considered as CMV-induced when it occurred within one month following viremia, during the first year after transplantation. Transplant patients with CMV disease, had a significant likelihood of developing acute rejection after CMV infection or reactivation ($P < 0.01$). The odds ratio for developing rejection was 5.98, 95% confidence interval: 1.21–29.40. Such a link was not documented for recipients with asymptomatic CMV infection. In conclusion, CMV disease, but not asymptomatic viremia, is a risk factor of acute renal transplant rejection. On epidemiological grounds, these results support the hypothesis that factors controlling both the viral replication and the diffuse inflammatory process are implicated in acute graft rejection.

Keywords Renal transplantation · Acute rejection · Cytomegalovirus infection · Cytomegalovirus disease · Risk factors of rejection

Introduction

Cytomegalovirus (CMV) infection is no longer a life-threatening complication of renal transplantation. The use of efficient antiviral drugs such as ganciclovir has dramatically improved the prognosis of the disease, leading to recommendations for their use as prophylactic agents [1, 2, 4, 13]. However, there is evidence that CMV has an immunostimulatory effect increasing responsiveness to alloantigen, and the concept of CMV infection causing an acute allograft rejection which, in turn, may lead to graft loss, has been suggested by sever-

al authors [10, 18, 20, 27]. Whether antiviral prophylaxis has been initiated in a renal transplant recipient or not, the relationship between CMV infection, as defined by its appearance in blood, and the occurrence of acute graft rejection is still a subject of controversy.

The seroprevalence of CMV infection in renal transplant recipients is about 80% in western countries [3]. Among infected patients, one quarter to one third will develop a clinically patent disease after transplantation [6, 15], particularly in seropositive patients receiving an infected transplant [10]. On clinical grounds, the presentation of CMV infection as either a CMV disease or a clinically asymptomatic infection may not bear the same risk of acute rejection. The hypothesis we considered was: does the diffuse inflammatory reaction that characterizes CMV-induced disease promote acute rejection? This hypothesis, the immunological background of which is not clearly understood, lacks epidemiological support.

In search of clinical relevance for such a hypothesis, we carried out a retrospective study comparing CMV-exposed patients to non-exposed controls, to document a possible relationship between the clinical presentation of CMV infection and the subsequent occurrence of acute graft rejection in renal transplant recipients treated with the same immunosuppressive protocol.

Materials and methods

Patients

One hundred and ninety-two consecutive recipients of a cadaver renal transplant from January 1st 1989 to December 31st 1995, were admitted to the study. The study was started in 1989, when ganciclovir was made available in our institution for every patient requiring antiviral therapy at that time, allowing the selection of a homogeneous group. The prophylactic immunosuppressive regimen was the same throughout the study: a quadruple sequential therapy with corticosteroids and azathioprine started at day 0, antilymphocyte globulins (ALG) during the first 10–15 days, in order to keep blood CD_2 lymphocytes undetectable, then cyclosporine (8 mg/kg per day), when ALG were resumed. At 6 months post-transplantation, corticosteroids were stopped when possible. The mean age of the recipients was 49.0 ± 10.9 years, 175 patients had a first tranplantation, and 17 a second one. The main characteristics of the patients are shown in Table 1. Data were collected during the first year of follow up, the period when CMV infection is likely to occur.

CMV infection

CMV infection was the exposure factor. Its definition was the first detection of positive viremia. According to the clinical status of the patient, CMV in blood and urine specimens was detected in culture by inoculation into human embryonic fibroblasts (MRC5). Cultures were maintained for at least 4 weeks. In order to avoid the delayed diagnosis due to slow cytopathic effect, an earlier diagnosis was made by detection of CMV immediate early antigen using the immunoperoxidase technique with monoclonal antibody (Clone E13, Argene-Biosoft). More recently, monitoring for pp65 antigenemia in circulating polymorphonuclear leucocytes was used to confirm the diagnosis of CMV infection [23]. During the 6 months following transplantation, the presence of CMV in blood and urine was systematically checked every week for seropositive patients, and every 2 weeks for seronegative patients. From month 7 to 12, the search for CMV infection was made on a monthly basis. In case of seroconversion during the first semester, the search was made every 2 weeks.

Three groups of patients were considered: group 1 consisted of 64 patients with no detectable viremia throughout the study; group 2 consisted of 77 patients with an asymptomatic CMV viremia, and group 3 of 51 patients with CMV disease. CMV disease was defined as positive viremia associated with one or more of the following clinical symptoms: fever with leukopenia, lung disease, liver disorders, gastrointestinal disorders, encephalitis, retinitis, and even death. All of the patients with CMV disease received ganciclovir. When used, the protocol for CMV prophylaxis included either aciclovir or specific anti-CMV immunoglobulins.

Acute graft rejection

The diagnosis of acute graft rejection was made on histological criteria. A renal biopsy was performed for the diagnosis of any significant increase of baseline plasma creatinine that was clinically suspected to be due to acute rejection, before the administration of antirejection corticosteroid bolus. The Banff classification [24] was used for reviewing all the biopsies included in the study. Plasma creatinine was measured on a weekly basis during the first 6 months after transplantation and every 2 weeks therafter. We considered that acute rejection could be the consequence of CMV infection when it occurred at least 2 days, and at the most 1 month, after the infection.

Analytical method

Exposed- non-exposed studies were made in order to test the possible role of CMV infection as a risk factor of acute graft rejection episode. Patients in Group 2 (asymptomatic CMV) and patients in Group 3 (CMV disease) were paired with Group 1 control patients who received a renal transplant during the same period and remained free of CMV infection or reinfection thereafter. Since the prevalence of CMV-positive patients in our waiting list for transplantation is about 60%, we found only 64 renal transplant recipients for inclusion in the control group. They were paired with CMV-positive patients, allowing the analysis of a group of 64 pairs in the setting of asymptomatic CMV infection and a group of 51 pairs in the setting of CMV disease.

Statistics

Results are expressed as mean ± standard deviation (SD) or percentage. Patient- and transplant-survivals were calculated according to the non-parametric Kaplan-Meier method. For this analysis, the end-point of follow-up was June 30, 1998, corresponding to a survey lasting from 2.5–9.5 years. The Log-rank test was used for survival comparison between groups of patients. Pairs of exposed-non-exposed patients were compared for both patients with asymptomatic CMV infection and patients with CMV disease using the MacNemar χ^2 test. When required, quantitative variables were cut into qualitative variables. Potential confounding factors

Table 1 Clinical characteristics of transplant recipients at baseline

Variable	All patients	Non-exposed controls	Exposed patients	
			asymptomatic infection	CMV disease
	(n = 192)	(n = 64)	(n = 77)	(n = 51)
Recipient's characteristics				
Age (years)	42.0 ± 0.9	40.2 ± 12.9	43.0 ± 11.9	42.6 ± 12.8
Sex (male)	119 (62.0%)	42 (65.6%)	46 (72.7%)	31 (60.8%)
Pretransplant CMV status				
Seropositive	113 (58%)	19 (30%)	63 (82%)	31 (61%)
Seronegative	79 (42%)	45(70%)	14 (18%)	20 (39%)
Renal disease				
Primary glomerular disease	61 (31.8%)	23	22	16
Chronic interstitial nephritis	29 (15.1%)	8	13	8
Polykystic renal disease	21 (10.9%)	11	8	2
Diabetes	13 (6.8%)	4	5	4
Other diseases	40 (20.8%)	18	29	21
Duration of hemodialysis (years)	3.8 ± 9.7	3.3 ± 10.5	4.5 ± 11.1	3.2 ± 4.2

Table 2 Summary of CMV serological status of donors and recipients (D refers to donor and R to recipient with (+) or without (–) CMV antibodies)

Variable	All patients	Non-exposed	Exposed	
			asymptomatic infection	CMV-disease
	(n = 192)	(n = 64)	(n = 77)	(n = 51)
CMV seropositive donor	94 (49%)	17 (27%)	42 (55%)	35 (69%)
CMV seronegative recipient	113 (58%)	19 (30%)	63 (82%)	31 (61%)
D^- / R^-	42 (22%)	35 (55%)	5 (6%)	2 (4%)
D^- / R^+	56 (29%)	12 (19%)	30 (40%)	14 (27%)
D^+ / R^+	57 (30%)	7 (11%)	33 (43%)	17 (33%)
D^+ / R^-	37 (19%)	10 (16%)	9 (12%)	18 (35%)

were tested by univariate analysis using Student's t test for quantitative variables and Pearson χ^2 test, or Fisher exact test for qualitative variables, when appropriate. The Cornfield method was used to calculate 95% confidence interval (CI) of the odds ratio. Variables were selected at a 0.20 threshold for inclusion in the multivariate analysis. Multivariate analysis was performed with logistic regression. Calculations were performed using DBASE IV and BMDP 386 software.

Results

The clinical characteristics of the patients, documented at baseline, are shown in Table 1. The mean age was 42.0 ± 0.9 years, and some two thirds of the patients were male. The various primary renal diseases were similarly distributed in the groups of recipients.

Forty-nine percent of the donors were seropositive (Table 2) . A similar proportion of seropositive recipients (58%) was observed. In this setting, there was no CMV serological matching between donors and recipients, since a seronegative transplant was allocated to 22% and 29% of seronegative and seropositive recipients, respectively, by the random distribution. All the seronegative patients with a seropositive transplant received anti-CMV prophylaxis during the first 3 months post-transplantation.

Table 3 summarizes the main characteristics of the two paired groups of patients. In patients with asymptomatic CMV infection, the transplant was CMV seropositive in 36 patients (56%), whereas the recipient was CMV seropositive prior to transplantation in 51 (80%) cases. In non-exposed patients, these proportions were significantly lower: 17 (27%) and 19 (30%), respectively. In the group of patients developing CMV disease, 35 (69%) exposed recipients received a seropositive transplant and 31 (61%) were CMV seropositive prior to transplantation. The only significantly different variable between groups was the anti-HLA-DR mismatch in the asymptomatic CMV infection group. None of the other variables included in the study was significantly different between exposed and non-exposed patients.

Table 4 shows patient follow up. An episode of acute rejection was observed in the asymptomatic infection

Table 3 Main characteristics of the two groups of exposed patients who had asymptomatic CMV infection (group 2) or CMV disease (group 3). Values of non exposed controls (group 1) are also indicated

Variable	Asymptomatic CMV infection			CMV disease		
	exposed ($n = 64$)	non exposed ($n = 64$)	P	exposed ($n = 51$)	non exposed ($n = 51$)	P
Donor						
Age (years)	32	33	NS	37	33	NS
Male	48	48	NS	37	35	NS
CMV seropositive	36	17	< 0.001	35	14	< 0.001
Cold ischemia (min)	1497	1363	NS	1413	1359	NS
Recipient						
Age (years)	41	40	NS	44	41	NS
Male	38	41	NS	32	32	NS
CMV seropositive prior to transplantation	51	19	< 0.001	31	16	< 0.003
First transplantation	57	60	NS	47	48	NS
Prior anti-HLA immunisation	15	13	NS	11	10	NS
Anti HLA-A mismatch	0.82	1.01	NS	0.92	1.03	NS
Anti HLA-B mismatch	1.07	1.23	NS	1.33	1.21	NS
Anti HLA-DR mismatch	1.07	0.98	< 0.02	0.86	0.94	NS
CMV prophylaxis	13	9	NS	16	8	NS

Table 4 Clinical events occurring in the follow up of both groups of exposed and non exposed patients

Variable	Asymptomatic CMV infection			CMV disease		
	exposed ($n = 64$)	non exposed ($n = 64$)	P	exposed ($n = 51$)	non exposed ($n = 51$)	P
Follow-up						
Delayed graft function (n)	13	9	NS	9	7	NS
Acute rejection episode before CMV infection (n)	19	13	NS	11	14	NS
Acute rejection after CMV infection (n)	4	3	NS	10	2	< 0.01
Return to dialysis (n)	11	7	NS	4	7	NS
Plasma creatinine at 1 year (μmol/l)	135 ± 5	140 ± 4	NS	139 ± 5	140 ± 4	NS
Death (n)	3	5	NS	3	4	NS

group in 4 exposed and in 3 non-exposed patients (NS). By contrast, a significant difference ($P < 0.01$) was observed in the CMV disease group, between exposed ($n = 10$) and non-exposed ($n = 2$) patients.

The relative odds ratio for developing acute transplant rejection after CMV disease was 5.98 (95% CI: 1.21–29.40; $P < 0.01$). In contrast, no significant risk was calculated for patients with asymptomatic CMV infection. However, in the latter, the risk of acute rejection was significantly higher after a second renal transplantation (OR: 10.40, 95% IC: 1.91–56.30 $P < 0.001$) (Table 5). No significant difference was observed between the other variables that might induce risk of acute rejection such as: sex, delayed graft function, HLA mismatch and rejection episode prior to CMV infection or reactivation.

At the end of the first year of follow up, CMV infection, as well as related acute rejection episode, were neither predictive of a patient's death nor of his return to dialysis. The patients were also evaluated in terms of survival after the first year of follow up. Figures 1 and 2 show the survival rate of patients and transplants, respectively: no significant difference was found between the subgroups of patients.

Discussion

The role of CMV in fostering acute transplant rejection is still a subject of controversy. The present epidemiological study, based on an exposed- non-exposed methodology, documents the significant temporal relation-

Table 5 Risk of acute graft rejection after either asymptomatic CMV infection or CMV disease (multivariate analysis)

Variable	OR	95% IC	*P*
Asymptomatic CMV infection (exposed/non exposed)			
Asymptomatic CMV infection	1.09	0.21–5.59	NS
Second vs first renal transplantation	10.40	1.91–56.30	< 0.001
CMV disease (exposed/non exposed)			
CMV disease	5.98	1.21–29.40	< 0.01

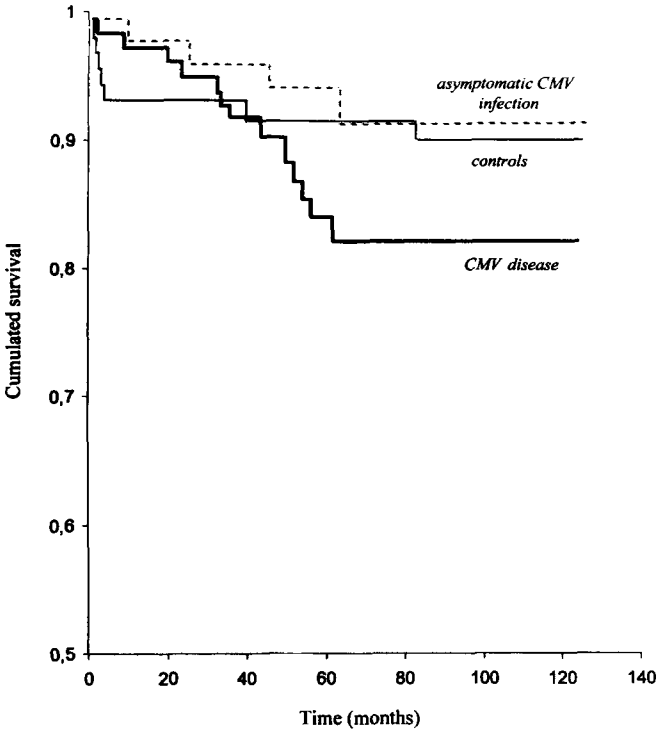

Fig. 1 Graft survival in renal transplant recipients with asymptomatic CMV infection, CMV disease, and controls who remained free of viremia during the first year post-transplantation. No significant difference was observed between groups

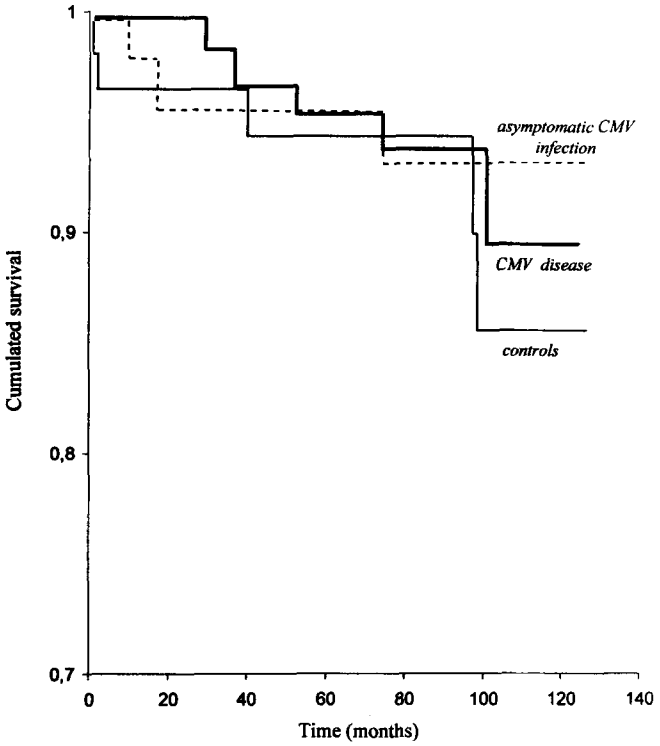

Fig. 2 Patient survival in renal transplant recipients with asymptomatic CMV infection, CMV disease, and controls who remained free of viremia during the first year post-transplantation. No significant difference was observed between groups

ship between the onset of CMV disease and the occurrence of acute transplant rejection. The risk of acute rejection was documented for the clinical presentation of CMV infection as CMV disease only, and not for the asymptomatic infection. A significant odds ratio of 5.98 ($P < 0.01$), was calculated for this condition. We did not document any difference between primary infection, reactivation or reinfection with CMV. Since a time lag of a few days is sometimes required to ascertain the presence of CMV in the specimen [25], some episodes of acute rejection requiring aggressive immunosuppressive treatment and followed by CMV disease would have been misdiagnosed and considered as a consequence of strong immunosuppression, and not the opposite. Consequently, the sensitivity of this study would have been increased using more recent markers of CMV infection, in all included patients, such as monitoring for pp65 antigenemia in circulating polymorphonuclear leucocytes [23].

In a study dealing with the same subject and compromising 242 consecutive renal transplant patients, Pouteil-Noble et al. [19] presented 157 patients with CMV infection, among whom 107 were considered as clinically symptomatic, corresponding in our study to the transplant recipients with CMV disease. Fifty of their patients had asymptomatic CMV infection. Comparing the whole group of CMV-positive patients with their 85 CMV-negative recipients, they calculated an odds ratio of 8.25 for developing acute rejection. However, they did not comment on the respective risk of rejection associated with either asymptomatic infection or CMV disease.

The immunological mechanisms that predispose a patient to CMV infection and to CMV disease are poorly understood and may differ between bone marrow and

solid organ transplant recipients [14, 15]. Cellular immunity is strongly implicated in the control of CMV replication. Cytotoxic T lymphocytes, CMV-specific CD4 lymphocytes, and NK cells play a major role in the defense against CMV infection [26]. The role of various cytokines, chemokines, and adhesion molecules is also debated in the development of viral infection [5, 8, 12]. The recent study of Nordoy et al [16] documented major findings in the field of renal transplantation and proposed the establishment of an immunological "risk profile"16 for later development of CMV disease. They have shown that low MIP-1α and elevated IL-8 levels are strongly associated with later development of CMV disease. They confirmed that these chemokines are involved in the viral pathophysiology, but moreover, they may also predict later CMV disease. Contrasting other studies [7], they found conflicting results between IL-10 and TNF-α levels with later development of CMV disease [17]. The complexity of the pathogenesis of CMV disease is also outlined by the fact that effective CMV infection therapy with currently used antiviral agents exacerbate rather than prevent adhesion molecule upregulation, and such treatment could accentuate rather than prevent inflammatory responses in vivo [11]. The link between CMV infection and acute rejection is also debated. Its immunological basis refers to numerous factors [4, 9]. Direct renal colonization by CMV induces a cytopathic effect in glomerular and tubular epithelial cells as well as in capillary endothelial cells [18]. Upregulation of adhesion molecules facilitates the inflammatory process and the infiltration of allograft parenchyma by lymphocytes, polymorphonuclears and macrophages. The ensuing inflammatory response induces interstitial nephritis and even a distinct glomerulopathy [3, 22]. Acute CMV infection with concurrent alloantigen stimulation may activate cytotoxic T cells which can trigger acute rejection. It is postulated that CMV infection could increase major histocompatibility complex antigens on the surface of graft cells through mediators such as interferons. Nevertheless, indirect evidence identifies CMV as a risk factor for graft rejection, which is correlated to poor long-term graft survival. The recent study of Lowance et al [13] has clearly demonstrated that prophylaxis with valacyclovir significantly reduced the incidence of CMV disease in seronegative recipients receiving a seropositive transplant. In those patients, the incidence of acute graft rejection was reduced by 50%.

The present investigation was not designed to study the risk factors of mortality in renal tranplant recipients with CMV infection. Nevertheless, CMV infection did, whatever its intensity not result in an increased mortality when compared with CMV-negative patients. In renal transplantation, CMV infection is not the risk factor of mortality it was before the development of active antiviral drugs and the widespread use of prophylactic protocols in transplantation centers. A recent meta-analysis supports this assumption, at least in sero-negative renal transplant recipients [6]. Considered at risk of CMV infection, 21% of our patients received CMV prophylaxis with either gammaglobulins or acyclovir. Unfortunately the study was not designed to document the pertinence of our prescription since both prophylactic and preemptive therapy may led to the emergence of late CMV disease in transplant recipients after cessation of therapy [11]. The role of CMV disease in the development of chronic rejection [21] was not investigated, nor was the role of human herpesvirus 6 which may induce viral disease in association with CMV [20].

In conclusion, CMV disease associated with a diffuse inflammatory condition was found to be a definite risk factor of acute rejection. The result of the present epidemiological investigation argues for systematic prophylaxis of the viral infection at least in sero-negative recipients of a CMV-positive transplant.

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