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Risk factors for cytomegalovirus infection and disease in renal transplant recipients: HLA-DR7 and triple therapy

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Abstract In a prospective study, an analysis of risk factors for the development of cytomegalovirus (CMV) infection and disease was performed on 77 renal allograft recipients. Twenty-five out of the 77 recipients (32 %) had a CMV infection. Twenty-two of the recipients received triple immunosuppressive therapy (cyclosporin A, prednisolone, and azathioprine) while the remaining 55 received standard therapy (cyclosporin A and prednisolone). In 23 recipients (30 %) acute rejection was diagnosed and the first positive parameter of infection occurred 22 days after rejection therapy. Infection occurred in 10 out of 18 HLA-DR7-positive recipients (56 %) and in 15 out of 59 HLA-DR7-negative recipients (25 %; $P < 0.02$). In multiple regression analysis, HLA-DR7 was found to be a significant predictor of CMV infection ($P < 0.005$). CMV disease was diagnosed in only 9 out of 25 recipients with an acute infection. Six recipients (67 %) with CMV disease received triple therapy for maintenance immunosuppression; this was significantly correlated to CMV disease ($P < 0.05$) as compared to three recipients (33 %) with CMV disease maintained with standard therapy. Our data suggest that HLA-DR7-positive recipients are more susceptible to CMV infection and that CMV disease is associated with triple immunosuppressive therapy.

Key words CMV, kidney transplantation, HLA-DR7 · CMV, kidney transplantation, triple therapy · Kidney transplantation, CMV, HLA-DR7 · HLA-DR7, CMV, kidney transplantation · Triple therapy, kidney transplantation, CMV

Introduction

In renal transplant recipients, cytomegalovirus (CMV) disease is influenced by many factors, such as pretransplant CMV serostatus of donor and recipient, immunosuppressive drug regimen, and graft rejection therapy. In CMV-seronegative recipients of a seropositive renal allograft in particular, the incidence of CMV infection and disease is high [10].

The type of maintenance immunosuppressive therapy seems to play an important role in the development of CMV disease after transplantation [13]. In patients treated with monoclonal or polyclonal lymphocyte antibody preparations for rejection, CMV disease is diagnosed more frequently than in patients without antilymphocyte globulin therapy [10].

The association between the occurrence of viral infections and virus-associated disease and the presence of certain HLA-DR antigens in the host has been described [1, 3, 17, 20].

In the present study, several possible risk factors for CMV infection and CMV disease are studied in a prospective study of kidney allograft recipients. The risk factors analyzed included pretransplant CMV serostatus

of donor and recipient, type of maintenance immunosuppression, occurrence of acute graft rejection, and recipient HLA-DR type.

Patients and methods

Patients and specimens

Eighty-two consecutive renal allograft recipients transplanted in our hospital between May 1990 and July 1992 were enrolled in this study. Five patients were excluded because of transplantectomy within the 1 week after transplantation. Seventy-seven recipients were then prospectively monitored for CMV infection. Blood specimens for serology, heparin blood, and urine specimens for virus culture were collected weekly during the hospitalization period. Thereafter, the collection of serum samples was continued monthly for up to 1 year after transplantation.

Serological methods

Latex agglutination test

The presence of CMV antibodies was determined using the sensitive latex agglutination test (CMV Scan, Becton Dickinson Microbiology Systems, Cockeysville, Maryland, USA) according to the instructions of the manufacturer. All serum samples obtained from pretransplant CMV recipients were checked for the presence of CMV antibodies using this test.

Complement fixation test

The complement fixation test was performed as described elsewhere [12].

Enzyme-linked immunosorbent assays (ELISA)

The antibody capture ELISA Vironostika anti-CMV IgM Micro Elisa System (Organon Teknika, Boxtel, The Netherlands) was used for the detection of CMV IgM antibodies and was performed according to the instructions of the manufacturer. For this assay the serum samples were diluted 1 : 100. Optical densities were measured at 492 nm and compared to the value of a reference serum supplied by the manufacturer. To exclude a false-positive IgM reaction due to the presence of the rheumatoid factor (RF), all sera were checked using the Rapi Tex RF latex agglutination test for RF detection (Behring, Marburg, Germany). Interfering IgG was removed in all RF-positive sera with a protein A preparation (Behring, Marburg, Germany) to avoid false-positive reactions associated with the presence of RF.

Virus isolation and identification

Leukocytes were separated from 10 ml of heparinized whole blood using 6% dextran (Pharmacia Biotechnology, Sweden) and suspended, after lysis of the remaining erythrocytes, in Eagle's minimal essential medium (Gibco, Paisly, UK) with 2% newborn calf serum.

For the determination of CMV viremia and viruria, four human embryonal fibroblast (HEF) monolayers grown in shell vials were inoculated and centrifuged for 30 min at 1500 rpm at room temperature. In parallel, conventional isolation and early identification were carried out using the HEF cultures.

Early identification of CMV isolates was accomplished with an immunofluorescent assay 24 and 48 h after inoculation of the specimen with the monoclonal antibody E13 (Biosoft, Paris, France) to the CMV immediate early nuclear antigen [7]. Monolayers containing one or more cells with the characteristic bright nuclear staining were considered positive.

For conventional isolation of CMV, the inoculated HEF monolayers were checked twice a week for appearance of cytopathic effect (CPE). The cultures were again subcultured blindly after 3 weeks and maintained for another 3 weeks. Each CMV-induced CPE was confirmed by immunofluorescent staining of the cells using the monoclonal antibody E13.

Definition of CMV infection and CMV disease

CMV infection was defined as one or more of the following: detection of the virus in blood and/or urine, seroconversion of CMV antibodies, and or a fourfold increase in CMV antibody titer [12].

Criteria for CMV disease were as described previously [13], namely, illness with two of the following otherwise unexplainable symptoms: fever (> 38 °C, measured axillary) for at least 3 consecutive days, leukocytopenia ($< 3 \times 10^9$ / l), thrombocytopenia ($< 100 \times 10^9$ / l), liver abnormalities (> 2.5 times the normal upper limit), gastrointestinal, lung, or central nervous system involvement. This syndrome had to be confirmed by concomitant positive CMV culture or serology. Organ involvement had to be confirmed by culture or biopsy from the diseased organ.

Immunosuppression therapy

Maintenance immunosuppression consisted of cyclosporin A (CyA), the doses adjusted to reach a trough level of 15–20 ng/ml in whole blood by high-pressure liquid chromatography (HPLC) in the first 3 months after grafting and 10–15 ng/ml thereafter, and prednisolone (P; starting dose 10 mg/day, tapered to 0 mg after 3 months for recipients with an uneventful course post-transplantation, i.e., not highly immunized (< 80% allo-T-cell antibodies), and to 5 mg/day for all other recipients (highly immunized, with acute rejection or with retransplants). Azathioprine (Aza) was included for recipients who were highly immunized or who were retransplanted because the former transplantation was lost due to rejection (triple therapy).

Acute rejection

Allograft rejection was assessed clinically and confirmed histologically by needle-core biopsy. Rejection treatment was started on the 1st day that acute allograft rejection was clinically suspected.

Rejection treatment conisted of a 10-day course of rabbit antilymphocyte globulin (RATG, RIVM, Bilthoven, The Netherlands). Subsequent rejections were treated with methylprednisolone, 0.5–1.0 g, on 3 alternating days. In recipients who were severely cardiovascularly compromised or who previously received RATG, the rejection episode was treated with methylprednisolone.

HLA typing

HLA class was typed by means of the two-color fluorescence test [18]. A panel of 90 selected antisera was used to type for 18 DR specificities (DR1–16, DR52, and DR53) as well as 9 DQ specifi-

Table 1 Risk factors for CMV infection after transplantation

The difference between * and the total of ** is $P < 0.05$

Group		CMV infection $n = 25$ (%)	No CMV infection $n = 52$ (%)	Total number $n = 77$
1	CMV serostatus donor/recipient:			
	Pos/pos	7 (26)*	20 (74)	27
	Pos/neg	9 (50)**	9 (50)	18
	Neg/pos	7 (30)*	16 (70)	23
	Neg/neg	2 (22)*	7 (78)	9
2	Immunosuppression:			
	CyA + P	16 (29)	39 (71)	55
	Aza + CyA + P	9 (41)	13 (59)	22
3	Acute rejection:			
	Positive	10 (43)	13 (57)	23
	Negative	15 (28)	39 (72)	54
	Treatment for rejection:			
	RATG	3 (50)	3 (50)	6
	Solumendrol	7 (41)	10 (59)	17
	None	15 (28)	39 (72)	54
4	HLA-DR 7:			
	Positive	10 (56)*	8 (44)	18
	Negative	15 (25)**	44 (75)	59

cities (DQ1–9). All donors were typed on spleen cells; typing of recipients was carried out on peripheral lymphocytes.

Statistical analysis

Data analysis was performed using both the general linear regression model and nonparametric tests (the χ^2 goodness-of-fit test for crosstabulations). A logit analysis was also performed for the prediction of both CMV infection and CMV disease. Differences with a P value less than 0.05 were considered to be statistically significant. All data analysis was done using SPSS-pc programs.

Results

Occurrence of CMV infection

In 25 out of 77 recipients (33 %), active CMV infection was diagnosed. An analysis of risk factors for CMV infection in transplant recipients is summarized in Table 1.

The risk factors were divided into four groups. In group 1 the effect of the presence or absence of CMV antibodies in the donor and recipient on the occurrence of CMV infection is given. CMV infection was significant more frequently diagnosed in seronegative recipients receiving an organ from a seropositive donor than in the other combinations.

In group 2 the influence of the immunosuppressive therapy on the occurrence of CMV infection is shown. The type of immunosuppressive therapy did not have a statistically significant effect on the incidence of CMV infections in our patient population.

The effect of the occurrence of acute rejection on the incidence of CMV infection is given in group 3. No significant difference was found in the occurrence of CMV infection between the group of patients with acute rejection and the group of patients without acute rejection. In addition, the effect of the type of antirejection therapy on the incidence of CMV infection was evaluated in the group of patients with acute rejection. We could not find any differences in incidence of CMV infection between the two treatment groups.

CMV infection was detected in ten patients with signs of acute rejection. The first positive parameter of CMV infection occurred at a mean of 35 days (range 18–63 days) after transplantation while the 1st day of antirejection therapy came at a mean of 13 days (range 4–36 days) post-transplantation. In nine of these patients the diagnosis of infection was made several days after the start of rejection treatment.

As the tendency to be infected with CMV may be linked with the HLA type of the donor and recipient, the correlation between CMV infection and HLA type was analyzed (group 4). A correlation between the development of CMV infection and the presence of HLA-DR7 in the recipient (HLA class II antigens were matched in such a way that almost all HLA-DR7-positive recipients received an HLA-DR7-positive allograft) was in fact, found. Infection occurred in 10 out of 18 (56 %) HLA-DR7-positive recipients and in 15 our of 59 (25 %) HLA-DR7-negative recipients ($\chi^2 = 5.71$; $P < 0.02$). None of the other HLA types were correlated with the occurrence of CMV infection.

In multiple regression analysis, the dependent variable CMV infection remained significantly related to HLA-DR7 (beta = 0.35; $P = 0.003$) and to the combination of seropositivity of donor and seronegativity of recipient (beta = 0.33; $P = 0.05$). Variance explained by both factors was 0.15. Logit analysis confirmed the results of regression analysis. Interaction effects of both factors did not reach statistical significance. No sig-

Table 2 Risk factors for CMV disease after transplantation in recipients with CMV infection

The difference between * and ** is $P < 0.05$

Group		CMV disease $n = 9$ (%)	No CMV disease $n = 16$ (%)	Total number $n = 25$
1	CMV serostatus donor/recipient:			
	Pos/pos	2 (29)	5 (71)	7
	Pos/neg	2 (22)	7 (78)	9
	Neg/pos	3 (43)	4 (57)	7
	Neg/neg	2 (100)	0 (0)	2
2	Immunosuppression:			
	CyA + P	3 (19)*	13 (81)	16
	Aza + CyA + P	6 (67)**	3 (23)	9
3	Acute rejection:			
	Positive	5 (50)*	5 (50)	10
	Negative	4 (27)**	11 (73)	15
	Treatment for rejection:			
	RATG	1 (33)	2 (67)	3
	Solumedrol	4 (57)	3 (43)	7
	None	4 (27)	11 (73)	15
4	HLA-DR7:			
	Positive	4 (40)	6 (60)	10
	Negative	5 (33)	10 (67)	15

nificant correlation was found between HLA-DR7 and rejection treatment or between HLA-DR7 and preoperative CMV serostatus of donor and recipient.

Occurrence of CMV disease

The analysis of risk factors for CMV disease in recipients with CMV infection is summarized in Table 2. CMV disease was diagnosed in 9 out of 25 recipients (36 %) with an infection.

In recipients with CMV infection, no correlation was observed between the serostatus of donor and recipient (group 1) and the development of CMV disease. An analysis of the type of immunosuppressive therapy given (group 2) showed that of 25 recipients with CMV infection, 9 (36 %) were on triple therapy (CyA + P + Aza), and 6 of these (67 %) developed CMV disease. Of 16 patients (64 %) treated with CyA + P, 3 developed CMV disease (19 %). The difference between standard and triple therapy was statistically significant ($\chi^2 = 5.74$, $df = 1$; $P < 0.05$).

As for the effect of acute rejection and therapy (group 3), it was shown that CMV disease developed in five out of ten (50 %) of the patients who needed rejection treatment versus 4 out of 15 (27 %) recipients who did not need rejection treatment. The type of rejection treatment was not significantly related to the development of CMV disease.

No correlation was found between the presence of HLA-DR7 or any other class II antigen and the development of CMV disease.

In multiple regression analysis with the dependent variable CMV disease, the only significant factor found was triple therapy versus CyA + P (beta = 0.45; $P = 0.003$). The occurrence of acute rejection reached borderline significance ($P = 0.05$). A logit analysis confirmed the results of the regression analysis for the 25 recipients with CMV infection.

Discussion

It is well known that CMV infections occur frequent after renal transplantation. In our study 33 % of the recipients of a kidney graft were infected. The incidence of CMV infection in our patient population is low compared to that of other centres [10, 13]. This might be due to the low dose of steroids we used and to the lesser need for rejection treatment.

Our results show that infection occurred more frequently in seronegative recipients of seropositive donor organs than in the other donor/recipient combinations. From this observation we clonclude that the transplanted organ seems to be an important source of infection.

Transmission of CMV from donor to recipient did not occur in all cases. In our study, only 50 % (9/18) of the seronegative recipients of a seropositive kidney were infected. This observation is in agreement with the finding of Chou [5]. Although the exact mechanism is not known, it suggests that in some donors the virus persists in a state in which it cannot be reactivated.

An observation worth noting was that infection was detected in two out of nine seronegative recipients of a seronegative organ. Although infection in this group was not expected, it has also been reported by others [14, 16]. A possible explanation for this observation could be that latent virus is present in seronegative individuals. Previous work in our laboratory and in other laboratories has shown that the CMV genome is detectable in leuko-

cytes and organs of seronegative patients, suggesting that in some seronegative donors the virus is present [2, 11, 19]. In the group treated with standard therapy (CyA + P), CMV infections were found in 16 out of 55 patients (29%), while in the triple therapy group CMV infections occurred in 9 out of 22 patients (41%). Although the difference in incidence between the two groups was not statistically significant, it supports the observation that CyA therapy is less often associated with infections than is the combination of CyA and Aza therapy [6, 13].

A difference that reached borderline significance was found between the occurrence of acute rejection and the development of CMV disease. The notion that CMV infection can cause an acute allograft rejection which, in turn, may lead to graft loss, has been suggested by several authors [9, 15, 21]. However, in our study, we could not confirm this observation. Moreover, in our patient group, the first positive parameter of CMV infection was found at a mean of 22 days after the start of rejection therapy. The time delay between the start of rejection treatment and CMV infection makes a correlation between CMV infection and the total immunosuppressive load more likely.

In the literature there is some evidence of a correlation between the occurrence of viral infections and the major histocompatibility complex (MHC). Studies in animals have shown a genetic susceptibility to CMV infection, that is controlled by the MHC [4, 8]. The immune response to CMV infection may be linked to HLA-DR genes, as has been demonstrated in recipients of renal transplants [1]. With herpes simplex virus, another member of the herpes virus family, an association between HLA-DR antigens and the cellular immune response has been found [20]. Although the mechanism of enhanced susceptibility to an infection is not clear, there is some evidence that the immune response is influenced by the HLA type of the host, which could lead to enhanced infections and/or infection-related disease. This last phenomenon was found by Blancho et al. [3] in their retrospective study. An association between HLA-DR7 and the development of CMV disease was found. We found a correlation between HLA-DR7 and CMV infection, but if a patient was infected with CMV, we found no correlation between CMV disease and HLA-DR7.

Our prospective study contradicts the study of Roenhorst et al. [17], who found that recipients positive for HLA-DRw6 but not for DR7 had an increased incidence of active CMV infection. As for the development of CMV disease, the only difference we were able to detect with regard to triple therapy reached borderline significance. Using Aza + CyA + P, more CMV disease was found, which is in agreement with results obtained in other center studies [10]. This finding suggests the possibility of a causal relationship between the intensity of immunosuppression and CMV disease, rather than an induction of graft rejection by CMV infection.

In conclusion, this study demonstrated an association between HLA-DR7 and the occurrence of CMV infection. CMV disease was shown to be correlated with the total immunosuppressive load, such as triple therapy (Aza + CyA + P) and acute rejection treatment.

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