

Is Combined Pretransplantation Seropositivity of Kidney Transplant Recipients for Cytomegalovirus Antigens (pp150 and pp28) a Predictor for Protection against Infection?

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Key Words

Kidney transplantation · Cytomegalovirus infection · Antibody responses · Peptide antigens

Abstract

Objective: This study was aimed at detecting antibodies to the antigens which may contribute to protection against cytomegalovirus (CMV) infection after organ transplantation. **Materials and Methods:** A total of 203 kidney transplant patients were enrolled in the study. Based on CMV antigenemia assay, 23 patients were antigen-positive and of the remaining 180 antigen-negative patients, 46 were selected as controls matched for age, gender and source of kidney. The 69 kidney recipients (KR) had CMV antibody due to previous infection and were followed up for a period of 6 months after transplantation for the development of active CMV infections by the antigenemia assay. Antibody responses to five CMV-related peptide antigens (pp65, gB, pp150, pp28 and pp38) were investigated by enzyme immunoassay and their presence was correlated with the results of the CMV antigenemia assay. **Results:** Of the five CMV-related peptide antigens, only gB antigen showed response to the antibody in 10/23 (43.5%) antigen-positive patients and 9/46 antigen-negative patients and the difference was statistically significant ($p = 0.048$). On the other hand, there was no significant difference in antibody responses between the antigen-positive and antigen-negative KR to the other four CMV peptide antigens ($p > 0.05$). However, among the antigen-positive KR there was only 1 patient who had antibodies to both pp150 and pp28 antigen, while among the antigen-negative KR, 22 of 46 (47.8%) had the antibodies ($p < 0.001$). **Conclusion:** The findings suggest that the combined presence of antibodies against the pp150 and pp28 antigens may indicate a lower risk of CMV reactivation after kidney transplantation.

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Introduction

A common complication following organ transplantation is infection with cytomegalovirus (CMV) [1, 2], a ubiquitous member of the β -herpesvirus group. CMV infection occurs in the majority of humans, mainly during the first two decades of life. Although the main host defense against CMV is cell-mediated immunity, several

Table 1. Age, source of kidney and gender distribution in antigen-positive and -negative kidney transplant recipients

Age	Source of kidney				Gender		
	cadaver pos/neg	LR pos/neg	LUR pos/neg	total pos/neg	female pos/neg	male pos/neg	total pos/neg
<20 years	0/0	0/0	2/4	2/4	1/2	1/2	2/4
21–40 years	3/6	0/0	0/0	3/6	3/6	0/0	3/6
41–60 years	3/6	0/0	10/20	13/26	7/14	6/12	13/26
>60 years	0/0	1/2	4/8	5/10	2/4	3/6	5/10
Total	6/12	1/2	16/32	23/46	13/26	10/20	23/46

Pos = Positive; neg = negative; LR = life related; LUR = life unrelated.

studies suggest that humoral immunity and particularly the level of neutralizing virus-specific antibodies may also modify the disease caused by this virus [3, 4]. The deleterious effects of CMV in transplant recipients result from direct viral invasion of various organ systems [5, 6]. Currently the treatment of CMV disease in solid-organ transplant recipients consists of the administration of intravenous ganciclovir for 3 weeks; however, CMV disease still occurs after the completion of oral antiviral prophylaxis [7, 8]. Despite the advent of antiviral chemotherapy, CMV infections remain a significant cause of morbidity in organ transplant patients, including kidney recipients (KR).

Controlling an active CMV infection requires a joint action of both cell- and antibody-mediated immunity [9–17] and this is well maintained in healthy individuals. However, in transplant patients the immune responsiveness is hampered by the immunosuppressive therapy. Despite this fact, immunity still plays a role in determining the outcome of CMV infection in KR.

The combination of proteins that should be included in the antigen mixture for investigation of protective cell-mediated and humoral immunity is not fully defined. However, a number of CMV proteins may be good candidates [18–21]. The tegument protein pp150 (UL32) is recognized by most antigen-positive individuals during activated viral infections [22, 23]. On the other hand, the major tegument protein pp65 (UL83) is recognized early during infection and can stimulate T cell responses [24, 25]. Other CMV proteins that evoke cellular responses include the tegument proteins pp28 (UL99) and major tegument protein (UL48), viral glycoprotein gB (UL55), pp38 (UL80a), peptide 981–1003 (UL86), IE2 (UL122), IE1 (UL123) and pp71 (UL82) [26–29].

Immunological factors that confer protection against disease development are not well characterized. Identifying those antigens that provoke protective humoral immunity against CMV infection and/or disease may help to provide markers for categorizing patients into high- and low-risk groups for developing CMV-related complications. In this respect we investigated the antibody responses of KR to the peptide antigens gB, pp150, pp65, pp28 and pp38.

Subjects and Methods

Study Population

A total of 203 KR patients were enrolled in the study. Based on CMV antigenemia as described below, 23 KR were antigen-positive and of the remaining 180 antigen-negative KR, 46 were selected as controls matched for age, gender and source of kidney as shown in table 1.

The 69 KR (42 male and 27 female) were monitored over a period of 6 months from the day of transplantation. The average age for men and women was 39 years with a range from 16 to 72. All recipients and donors were CMV IgG-positive prior to transplantation. KR were assigned to receive induction antithymocyte globulin and maintenance immunosuppression (prednisolone, cyclosporine, and mycophenolate mofetil). Prophylactic intravenous ganciclovir was administered for 2 weeks at a dose of 5 mg/kg/day (adjusted to kidney function) starting from the day of surgery. CMV disease was treated with intravenous ganciclovir 5 mg/kg twice daily for 21 days. Patients with systemic manifestations continued ganciclovir therapy for 3 months. Blood samples from the patients were collected as follows: one sample before transplantation, 4 samples during the first month, 4 samples during the second and third months and finally 3 samples 4–6 months after transplantation.

CMV Antigenemia Assay

The antigenemia assay was carried out as described earlier [30]. Blood samples were separated by dextran sedimentation method and pp65 antigen was detected according to the procedure recommended by the manufacturer (INCSTAR Corp., Salt Lake City, Utah, USA). The number of cells containing the CMV-specific pp65 antigen was counted under a light microscope ($\times 400$) and samples containing ≥ 5 cells/50,000 with the pp65 antigen were considered positive for current CMV replication.

CMV Antigens for Enzyme Immunoassay (EIA)

Five peptide antigens (Thermobaid, Ulm, Germany) derived from the most reactive epitope domains of gB, pp150, pp28, pp65 and pp38 were tested as described by Greijer et al. [31]. These five peptides were as follows: CMV-envelope component (gB), matrix proteins (pp65, pp28, pp150) and fusion protein (pp38). The amino acid position of the five CMV peptides is shown in table 2.

EIA for the Detection of Peptide-Specific IgG Antibody

EIA was performed as described by Greijer et al. [31]. Briefly, micro-EIA plates were coated with 1 $\mu\text{g/ml}$ of the selected peptides in 50 mM sodium bicarbonate buffer (pH 9.6) for 16 h at 4°C. Free binding sites were saturated by incubation with 3% bovine serum albumin in phosphate-buffered saline (PBS). After 1 h of incubation at 37°C, wells were washed 4 times with PBS containing 0.05% Tween 20 (PBST). Sera were diluted 1:100 in PBST with 20% normal goat serum containing 0.5% Triton X-100, and the mixture was incubated for 1 h at 37°C. After washing, horseradish peroxidase-conjugated antibody to human IgG (The Binding Site, Birmingham, UK) diluted 1,000 times with PBST containing 1% bovine serum albumin was added for 1 h. Wells were washed

four times with PBST and the bound horseradish peroxidase label was detected with 3,3',5,5'-tetramethylbenzidine as substrate for 30 min in the dark, after which the color reaction was stopped by the addition of 1 M H₂SO₄. Absorbance was measured at 450 nm by an EIA reader. The test was considered positive when the mean optical density values were more than twice the mean of the antigen control values.

Statistical Analysis

All statistical analysis was carried out using the SPSS software package (SPSS Inc. 2002, version 11.5.1, Chicago, Ill., USA). Proportions were analyzed by Fisher's exact probability test (2×2 contingency table), the p values are two-sided.

Table 2. Description of CMV peptide antigens used in this study

CMV peptide antigens	Amino acid positions
gB (UL55)	792-809 60-81
pp28 (UL99)	15-45 130-160
pp150 (UL32)	595-614 615-636 595-636 1011-1048
pp65 (UL83)	297-510
pp38 (UL80a)	117-373

Table 3. EIA reactivity of serum samples to the five CMV-related antigens

Antigens	Antigen-negative group (n = 46)		Antigen-positive group (n = 23)		p value ²
	positive ¹	average of OD values	positive ¹	average of OD values	
pp65	14 (30.4)	0.192	5 (21.7)	0.056	0.572
gB	9 (19.5)	0.139	10 (43.5)	0.085	0.048
pp150	28 (60.9)	0.383	9 (39.1)	0.174	0.125
pp28	26 (56.5)	0.282	9 (39.1)	0.149	0.208
pp38	8 (17.4)	0.103	2 (8.7)	0.069	0.477
pp150/28	22 (47.8)	NA	1 (4.3)	NA	<0.001
pp150 and 65	11 (23.9)	NA	1 (4.3)	NA	0.050
pp150 and 38	7 (15.2)	NA	0	NA	0.086
pp65 and 28	12 (26.1)	NA	2 (8.7)	NA	0.119
pp28 and 38	9 (19.5)	NA	1 (4.3)	NA	0.148
pp65 and gB	0	NA	1 (4.3)	NA	0.333
pp150 and gB	7 (15.2)	NA	5 (21.7)	NA	0.517
pp38 and gB	1	NA	0	NA	0.999
pp28 and gB	4 (8.7)	NA	3 (13.0)	NA	0.679
pp65 and 38	3 (6.5)	NA	1 (4.3)	NA	1.000

NA = Not applicable. ¹ Number and percentage (in parentheses). ² Fisher's exact test.

Results

The results of the 69 patients are summarized in table 3. Antibodies to the gB antigen were present in 10 of 23 (43.5%) and in 9 of 46 (19.6%) antigen-positive and -negative recipients, respectively, and this difference was statistically significant ($p = 0.048$). Regarding the other CMV-related peptide antigens pp65, pp150, pp28 and pp38 there was no statistically significant difference between the two groups as calculated by Fisher's exact probability test.

When combining the presence of antibodies to CMV-related antigens we found that 22 (48%) of the antigen-negative KR had antibodies to both the pp150 and the pp28 antigens while among the antigen-positive kidney transplant recipients, only 1 patient reacted with these two antigens. The difference between the two groups was highly significant ($p < 0.001$). The other combinations that resulted in no significant difference include pp65 and 150, pp65 and 28, pp150 and 28, pp28 and 38, pp65 and 38, gB and pp150, gB and 28, gB and 38 and pp150 and 38 antigens are also shown in table 3.

CMV infection/reactivation occurring after transplantation triggered the production of antibodies to the CMV-peptide antigens. A number of those KR who were negative just prior to the detection of CMV infection became positive for one or more CMV-peptide antigens after CMV infection. This 'seroconversion' to the five antigens is shown in table 4.

Discussion

Humoral antibody responses of KR to major structural and nonstructural CMV-specific antigens seem to be correlated with the outcome of CMV infection/disease. Indeed a strong correlation between the presence of antibodies to the two peptide antigens (pp150/pp28) and the development of an active CMV infection/reactivation suggests that CMV-antigen-specific humoral immune responses before transplantation may be used as markers regarding the outcome of CMV infection after kidney transplantation.

It is well established that CMV-specific antibodies cannot prevent virus dissemination in an immune-compromised host [32]. However, various studies have provided evidence that antiviral antibodies have a modulatory effect on the infection and the subsequent clinical course of CMV disease [33, 34]. In the murine CMV system, neutralizing antibodies effectively limit viral spread

Table 4. Antibodies of KR in relation to the results of the antigenemia assay

CMV peptide antigens	Antigen-positive group (n = 23)	
	antibodies positive prior to CMV infection	antibodies positive after CMV infection
pp65	4 (17.4)	5 (21.7)
gB	5 (21.7)	10 (43.5)
pp150	6 (26.1)	9 (39.1)
pp28	4 (17.4)	9 (39.1)
pp38	1 (4.3)	2 (8.7)

Number and percentage (in parentheses) are given.

during reactivation, an effect that is dependent on antibody titer [32, 35]. A similar situation seems to exist in bone marrow transplant patients; high CMV glycoprotein-specific antibody titers were associated with the absence of PCR positivity, indicating an effective suppression of viral dissemination [36]. Thus, in situations in which transplant recipients maintained or developed high CMV glycoprotein-specific humoral immune responses, viral replication could not be detected and clinical symptoms related to CMV infection were not seen. These results are in agreement with the findings published in relation to liver transplantation [37].

Conclusion

Data of the study suggest that the combined presence of antibodies against pp150 and pp28 antigens may indicate a lower risk of CMV infections/reactivation after kidney transplantation.

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