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# Cellular Immune Response to Cytomegalovirus Infection After Renal Transplantation

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A prospective study of 15 patients who received renal transplants defined the effect of renal transplantation on the cellular immune response to cytomegalovirus infection. Of 15 patients, 14 developed cytomegalovirus infection, usually in the first 2 months after transplantation, and all infections were accompanied by a normal humoral immune response. After the initiation of immunosuppressive therapy and transplantation, there was a general depression of lymphocyte transformation, as reflected in the response to phytohemagglutinin, accompanied by a specific defect in cellular immunity, as indicated by lymphocyte transformation to cytomegalovirus antigen. Eleven patients had cellular immunity to cytomegalovirus before transplantation, and all of these became negative in the first month after transplantation. In subsequent months, only 6 of the 14 study patients with cytomegalovirus infection developed specific cellular immune responses to cytomegalovirus. This occurred most often in patients who had severe febrile illnesses in association with infection. The specific cellular immune response which developed in the posttransplant period did not persist in three of the patients. This study demonstrates the dissociation of the humoral and cellular immune response to cytomegalovirus infection in renal transplant patients and indicates the importance of the loss of cellular immunity in the appearance of infection. Previously infected patients lost their cell-mediated immunity and had reactivation infections despite the presence of serum antibody.

Cytomegalovirus (CMV) infection is a common problem after renal transplantation (12). These patients receive immunosuppressive therapy at the time of transplantation, and most of them are maintained on therapy indefinitely. After they develop CMV infection, they continue to excrete the virus for months. Therefore, a defect in cell-mediated immunity, similar to that described in relation to congenital CMV infection, may be present (4, 7). The current study was undertaken to define the specific humoral and cellular immune response to CMV infection in a group of patients receiving renal allografts. Immune status was determined before the initiation of immunosuppressive therapy and transplantation and for at least 6 months after transplantation.

## MATERIALS AND METHODS

**Study population.** Fifteen patients on chronic hemodialysis and awaiting renal transplantation were studied prospectively. The ages ranged from 22 to 53 years, with a mean of 37 years, and included eight females and seven males. Specimens for viral culture and measurement of immune status were obtained before transplant and at monthly intervals after transplantation for at least 6 months. A flow sheet was maintained for each patient, which included clinical and laboratory data during hospitalization and outpatient visits. Whenever patients were hospitalized for possible infection, additional cultures were obtained to determine the cause of the illness.

Twelve of the patients received renal allografts from cadaver donors, and three received them from living related donors. All patients received prednisone and azathioprine. This therapy was started on the day of transplantation in most patients, but those receiving allografts from living related donors began therapy 1 to 2 days before surgery. The patients were continued on immunosuppressive therapy throughout the study period with the one exception of a patient who received a kidney from an identical twin. She was given prednisone and azathioprine only during the first posttransplant month. Thirteen of the patients also received antilymphocyte globulin. The exceptions were two of the three who had living, related donors.

**Antibody tests.** Serum for antibody assays was frozen at −70°C until tested. All serum specimens from an individual patient were tested simultaneously for antibody. Complement fixation (CF) antibody was measured in a microtiter system using two units of

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antigen and two exact units of complement derived from pooled guinea pig sera (11). The antigen used in the test was obtained by freeze-thawing of cultures of the AD 169 strain of CMV. Twofold dilutions of serum were tested, beginning at a 1:4 dilution. Immunofluorescent (IF) antibody was measured by an indirect technique with slides containing human fibroblasts infected with the AD 169 strain and fluorescein-conjugated goat anti-human globulin (2). Twofold dilutions of sera, beginning at a 1:8 dilution, were incubated on the slides for 30 min at room temperature before the addition of the conjugate. Brilliant green counterstain was added to the slides before examination with a fluorescent microscope.

**Lymphocyte transformation.** Lymphocyte transformation to phytohemagglutinin (PHA) was measured as previously described (5). Specific cellular immunity was defined by lymphocyte transformation to CMV-infected cells by a modification of the technique of Thurman et al. (14). Confluent monolayers of human embryonic lung fibroblast (WI-38) cell cultures were inoculated with the AD 169 strain of CMV and incubated at 35°C for 4 days. Infected and noninfected cells were harvested by trypsinization of monolayers. Cells were washed with minimum essential medium and treated for 30 min with 25 μg of mitomycin C (ICN Pharmaceuticals, Cleveland, Ohio) to block DNA synthesis. Cells were washed three times with minimum essential medium, counted, and diluted to a concentration of $25 \times 10^4$ cells per ml.

Peripheral blood mononuclear cells were obtained using a Ficoll-Isopaque gradient (3). These cells were washed three times in minimum essential medium, counted, and diluted to a concentration of $25 \times 10^5$ lymphocytes per ml in minimum essential medium with 2 mM L-glutamine and 10 μg of streptomycin per ml. A 0.1-ml amount of the lymphocyte suspension was mixed with 0.08 ml of CMV-infected cells (or noninfected cells for controls) and 0.02 ml of heat-inactivated autologous serum in glass culture tubes (6 by 50 mm). Simultaneous cultures were done with 20% fetal calf serum instead of autologous serum. All cultures were performed in triplicate. Cultures were incubated at 37°C in a 5% $CO_2$ humidified atmosphere for 72 h. At 4 h before harvesting, 1 μCi of [*methyl*-$^3$H]thymidine (specific activity, 6 Ci/mmol) was added to each tube. Harvesting was performed by methods previously described (5). DNA synthesis was measured in a Beckman beta-scintillation counter, and mean disintegrations per minute for each triplicate set of cultures were determined. A specific cell-mediated immune response to CMV was defined as a stimulation index (disintegrations per minute in CMV-stimulated cultures/disintegrations per minute in unstimulated cultures) greater than 3.

## RESULTS

**Humoral immunity.** Of the 15 patients, 14 developed CMV infection after transplantation as indicated by a fourfold rise in antibody titer in all 14 patients and also by isolation of CMV in 10 of them. The CF antibody test was less sensitive than the IF test in detecting antibody before transplantation (Table 1). Only 6 of 15 (40%) patients had CF antibody before transplantation, in contrast to 11 of 15 (73%) patients who had IF antibody. Both the CF and IF tests were equally as sensitive in detecting rising antibody titers. Twelve of 15 (80%) patients had a fourfold increase in CF antibody after transplantation. The same number of patients had an increase in IF antibody. The IF antibody rose slightly earlier than the CF antibody. Only one patient had a fourfold increase in CF antibody by the end of posttransplant month 1, nine had a fourfold increase by month 2, and two had a fourfold increase by month 3. In contrast, the rise in IF antibody occurred by the end of posttransplant month 1 in four patients and by posttransplant month 2 in eight. Although both the CF and IF tests used the same strain of CMV for antigen, three patients who developed IF antibody never developed CF antibody. Two patients had persistently elevated titers of IF antibody, but showed a fourfold increase in CF antibody. By the end of posttransplant month 2, all study patients had IF antibody titers ≥1:64.

**Cellular immunity.** The specific cellular immune response to CMV before transplantation correlated with the presence of IF antibody (Table 2). Of 15 patients, 11 had lymphocyte trans-

TABLE 1. *Humoral immune response to CMV infection in 15 renal transplant patients studied prospectively*

| Type of antibody | Antibody status | | | | | |
|---|---|---|---|---|---|---|
| | Pretransplant | | | Posttransplant | | |
| | Absent[a] | Present | Positive (%) | No change | Fourfold increase | Responders (%) |
| CF | 9 | 6 | 40 | 3 | 12 | 80 |
| IF | 4 | 11 | 73 | 3 | 12 | 80 |

[a] Absent = titer <1:4 for CF and <1:8 for IF antibody.

TABLE 2. *Cellular immune response to CMV infection in 15 renal transplant patients studied prospectively*

| Antibody status pretransplant | No. studied | No. with lymphocyte response to CMV antigen[a] | | |
|---|---|---|---|---|
| | | Pretransplant | Posttransplant | |
| | | | Mo 1 | Subsequent mo |
| Absent[b] | 4 | 1 | 0 | 1 |
| Present | 11 | 10 | 0 | 5 |

[a] Lymphocyte response defined as a stimulation index (disintegrations per minute in CMV-stimulated cultures/disintegrations per minute in unstimulated cultures) >3.

[b] Absent = IF antibody titer <1:8.

formation to CMV, including 10 of 11 with IF antibody and only 1 of 4 without antibody ($P < 0.05$, Fisher exact test). In month 1 after transplantation, all patients lacked a specific cellular immune response to CMV (Fig. 1). In subsequent months, 6 of the 15 patients developed cellular immunity, including 5 who had had a specific response pretransplant and 1 who had not. Responses in autologous and fetal calf serum were similar. Lymphocyte transformation to PHA was normal before transplantation and decreased in posttransplant month 1 and subsequent months (Fig. 1). The mean stimulation index and standard deviation for PHA was 175.1 ± 82.2 pretransplant, and 18.4 ± 19.3 at posttransplant 1 month.

The patients who developed cellular immunity to CMV in the posttransplant period were compared with those who did not. Four of the six who developed responses had severe disease associated with their CMV infection, defined as a prolonged febrile illness of at least 2 weeks duration and fevers which exceeded 101°F (ca. 38.3°C). Only one of nine nonresponders had severe disease ($P < 0.05$, Fisher exact test). The two groups also differed in that only one of the six responders developed a dual infection with herpes simplex virus, in contrast to six of nine nonresponders. No significant differences could be demonstrated in regard to the type of donor kidney, degree of histocompatibility matching, immunosuppressive therapy, rejection episodes, or excretion of CMV. Three of the six patients who developed a specific cellular immune response lost the response within 3 months.

Only 1 of the 15 study patients was taken off all immunosuppressive therapy. This was a 24-year-old woman who received a kidney from an identical twin. Before transplantation, she had both humoral and cellular immunity to CMV. She received prednisone and azathioprine during posttransplant month 1. During this time, she

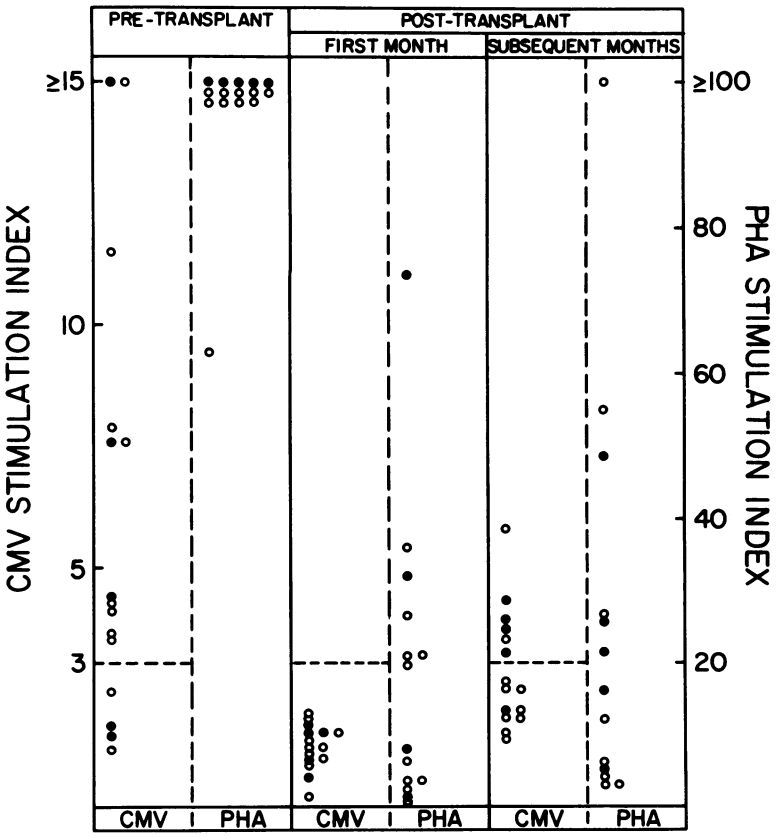


FIG. 1. *Lymphocyte transformation in autologous serum to CMV antigen and PHA in renal allograft recipients before and after transplantation. The highest CMV response after transplantation is given in the column for subsequent months, together with the simultaneous PHA response. Three patients did not have a PHA response measured at the same time that the CMV responses were measured. Patients with severe CMV infections (●) and those with subclinical or mild infection (○) are indicated.*

lost her specific cellular immune response to CMV, but had a fourfold increase in antibody and began to excrete CMV in the urine. She was not symptomatic with the infection. Her immunosuppressive therapy was discontinued in posttransplant month 2, but she continued to excrete CMV in the urine over the next 6 months and did not regain her cellular immune response.

## DISCUSSION

The humoral immune response to CMV infection was normal in renal transplant patients, as has been observed by others (9). By the end of posttransplant month 2, all patients had an IF antibody titer ≥1:64. Differences are apparent between the CF and IF antibody responses. IF antibody was a more sensitive indicator of previous infection than CF antibody, but both assays were effective in demonstrating rising antibody titers (Table 1). The CF antigen used in the present study was prepared by freeze-thawing of infected cells. Betts et al. have recently shown that the sensitivity of the CF antibody test in renal transplant patients can be increased by using an antigen prepared by glycine extraction (2). The correlation between humoral immunity defined by the IF antibody test and specific cellular immunity was demonstrated (Table 2). One patient who did not have detectable IF antibody at a 1:8 dilution of serum had a cellular immune response to CMV. This suggests that he had been infected with CMV previously, but his IF antibody titer had decreased to undetectable levels.

The specific cellular immune response to CMV disappeared in renal transplant patients after the initiation of immunosuppressive therapy and transplantation (Table 2). This was associated with a general depression of cellular immunity as reflected by impaired lymphocyte transformation to PHA. Although 14 patients had evidence of CMV infection after transplantation, only 6 of them developed a cellular immune response to CMV in the posttransplant period. This occurred most often in those with severe clinical disease, but the response was transient in three of the patients. Interestingly, one patient who was taken off immunosuppressive therapy after she had experienced a reactivation infection did not develop a specific cellular immune response and continued to excrete CMV. Using a lymphocytotoxicity assay to measure cellular immunity to CMV, Rola-Pleszczynski et al. also demonstrated depressed cellular immunity in two normal adults with persistent CMV viruria (7). They did not study their patients before infection. Results similar to those described for renal transplant patients in the present study have also been reported for cardiac transplant patients (6). Pollard et al. noted a loss of specific cell-mediated immunity to CMV after cardiac transplantation, but responses returned to normal in 60% of the patients who survived for more than 3 years.

This prospective study of CMV infection in renal transplant patients clearly demonstrates the dissociation of humoral and cellular immunity in relation to the development of infection. Cellular immunity, if present before transplantation, disappeared with immunosuppressive therapy and was followed by reactivation of infection accompanied by a normal humoral immune response. Understanding the defect raises questions as to how CMV infections in renal transplant patients should be prevented or treated. Immunization, transfer factor, and antiviral chemotherapy have been suggested as possible approaches. Immunization to provide humoral immunity may not be a useful approach because these patients have a normal antibody response without clearing of the infection. Recently, the use of transfer factor has been used (8, 13). Preliminary studies in infants with congenital CMV showed only a transient increase in negative cultures after treatment, and treatment of one patient with CMV retinitis was followed by improvement for at least 5 months. The need for continuing immunosuppressive therapy in renal transplant patients may limit this approach. Even when specific cellular immunity developed after infection in the present study, it was transient in several patients. The same problems apply to antiviral chemotherapy, which can suppress CMV infection, but only temporarily (10). Perhaps treatment during acute disease produced by CMV would be useful, even though chronic infection persists. Because of these problems in developing a method to treat CMV infection, an attempt should be made whenever possible to prevent CMV infection in uninfected recipients of renal allografts by selecting uninfected donors (1).

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### ADDENDUM

Since the preparation of this manuscript, Rytel et al. (Cell. Immunol. 37:31-40, 1978) reported on cellular immunity to CMV in renal allograft recipients ≥6 months after transplantation. Their results agree in part with the present study in that only one third of the patients had cell-mediated immunity to CMV in the late posttransplant period.

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