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Cytomegalovirus Infection and Immunity in Renal Allograft Recipients: Assessment of the Competence of Humoral Immunity

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Cytomegalovirus (CMV) infections are prevalent in renal allograft recipients. The purpose of this ongoing study is to attempt to elucidate the mechanism(s) responsible for the enhanced susceptibility to CMV infections on the part of transplant patients and for their apparent inability to eradicate the infection once it starts. The present report assesses the competence of humoral immunity to CMV in renal allograft recipients. The total study population was comprised of 41 renal allograft recipients (10 followed prospectively) and 38 age-matched control subjects. The overall CMV infection rate in renal allograft recipients was 90.2%, and in 11 cohort control subjects it was 45.5%. Active infection was present in 61.0% of transplant patients (24.0% of these had CMV disease) and in 18.2% of the cohorts. These differences are significant. CMV complement fixation and neutralization antibody prevalence was similar in 10 patients with renal failure undergoing hemodialysis before transplantation and in 23 control subjects. There was similarly no difference in antibody response between allograft recipients in whom the infection was primary (eight subjects) or secondary (17 subjects). We conclude that despite immunosuppressant therapy (with azathioprine and corticosteroids), humoral immunity to CMV is not impaired in transplant patients with either a primary or secondary infection.

Renal allograft recipients are known to have a high prevalence of infections with cytomegalovirus (CMV) (20). Once an active CMV infection occurs, it persists indefinitely until the patient dies or immunosuppression is reduced. The purpose of this ongoing study is to investigate the possible mechanism(s) responsible for the enhanced susceptibility to CMV infections on the part of renal allograft recipients and for their inability to eradicate these infections once they become established. Information of this kind would be useful from both the theoretical and the practical standpoints. For example, it would define the requirements and could suggest the means for effective passive or active immunoprophylaxis or therapy. We have recently reported that the in vitro interferon response is suppressed in lymphocytes from renal allograft recipients (16). The present report analyzes the prevalence of CMV infection and disease in transplant patients and assesses the competence of their humoral antibody response. Investigation of cell-mediated immune response in renal allograft recipients is in progress in our laboratory and will be the subject of a separate report.

MATERIALS AND METHODS

Subjects and study design. Forty-one renal allograft recipients were entered into the study over a period of 4 years (1970 to 1974). Thirty-three were males and eight were females. The mean age was 37.4 years and the range was 15 to 66 years. Ten patients were studied prospectively (i.e., before transplantation) to determine the possible influence of the state of chronic renal disease with azotemia and of hemodialysis on the prevalence of CMV infection. The other 31 patients were studied in a retrospective-prospective manner. Because of attrition due to mortality (11 patients) or departure from the area, as well as the staggered addition of subjects studied prospectively, not all of the patients were followed over the entire 4 year period of the study. The range of participation was from 6 weeks (patient died with CMV pneumonia) to 4 years, with a median of 1.5 years. Viral isolation was attempted and antibody determinations were performed repeatedly on the study subjects, generally every few weeks immediately after transplantation and then every 6 to 12 months.

All of the transplant patients were receiving azathioprine in the dose range of 125 to 175 mg/day and corticosteroids such as methylprednisolone, 8 to 32 mg/day, throughout the period of observation. The dose of immunosuppressants would generally be in-

creased during rejection episodes, and the patient would receive, in addition, local X irradiation of the transplanted kidney.

The nonimmunosuppressed control population was comprised of three categories of subjects. (i) Renal unit personnel comprised 11 subjects, including both doctors and nurses who were followed concurrently with the renal allograft recipients with serial CMV serological studies and were given one initial screening of body fluids (see above) for viral isolation. Five were males and six were females; mean age was 34.7 years (range, 26 to 51 years). (ii) Hospital personnel comprised 23 individuals who were randomly selected for a point prevalence study of CMV infection as determined by complement fixation (CF) and neutralization (NT) antibody titers. There were 5 males and 18 females; mean age was 31.8 years (range, 26 to 56 years). (iii) Patients with CMV diseases comprised four patients with "post-perfusion" mononucleosis. They were all males; mean age was 45.3 years (range, 24 to 69 years).

Viral isolation and serology. These studies were performed as reported in detail previously (15). They were based on standard methods (3).

Definition of infection and disease. CMV infection was considered to be present in a given individual if the following criteria were met: CMV isolation from any body fluid; CF antibody titer of ≥4 and NT antibody titer of ≥8. The infection was considered to be "active, intercurrent" if during the period of observation CMV was isolated from a body fluid and/or there was at least a fourfold change in either CF or NT antibody titer (or both). If the initial CF antibody titer was <4 and the NT titer was <8, these intercurrent infections were felt to be primary; otherwise they were considered secondary (5). The latter undoubtedly included both reactivated (endogenous) as well as recurrent (exogenous) infections, but since the protective role of preexistent antibodies against infection is unknown (6), it is impossible to establish this distinction. If the patient had a persistently elevated antibody titer, i.e., CF ≥4 and NT ≥8, unaccompanied by isolation of the virus, the infection was considered to be "inactive, preexistent." Patients with titers lower than this were considered to be noninfected unless CMV was isolated from a body fluid or tissue obtained at biopsy or autopsy.

No attempt was made to detect specific immunoglobulin M antibodies by the immunofluorescent antibody test (20). These antibodies are thought by some to be indicative of a primary active CMV infection (9). However, as pointed out by Jordan et al., specific immunoglobulin M response may occur after a second exposure to certain viral antigens in patients and experimental animals (7). Thus, this information would not materially contribute to the classification of infections as primary or secondary. The definition of "activity" in the present study was based on other acceptable criteria, such as viral isolation or antibody rises in serial specimens.

Infections were considered to be clinically significant (i.e., CMV disease) if the patient had a clear-cut syndrome previously shown to be characteristic of or at least reported to be associated with infection with this virus (1, 20).

RESULTS

CMV infection rate in renal allograft recipients and control subjects. The CMV infection rate in renal allograft recipients is contrasted in Table 1 with the rate in nonimmunosuppressed control cohorts—renal unit personnel. The overall infection rate in transplant patients was 90.2% (37/41), with 61.0% (25/41) having an active, intercurrent infection. In 18 of these 25 patients (72.0%), CMV was isolated from a body fluid (urine in 17 of 25 sampled, buffy coat in 3 of 12, saliva in 4 of 13, and subretinal fluid in 1 of 1) or from tissues obtained at biopsy or autopsy (lung in two). Sixteen of 21 serially bled patients had at least a fourfold CF or NT antibody rise. Six of these 25 patients (24.0%) were considered to have CMV disease: two cases of retinitis, three of interstitial pneumonia, and one of disseminated disease, the prominant feature of which was ulcerative lesions of the gastrointestinal tract. All of these syndromes are well-recognized clinical entities associated with CMV infection (1, 20). In eight of these 25 patients (32.0%), the infection was considered to be primary; four of these were in the prospectively studied group. Interestingly enough, four patients remained entirely free of infection (CF titer <4, NT<8) for from 1 to 2.5 years of their follow-up.

Only 45.0% (5/11) of the cohort control subjects (renal unit personnel) had evidence of infection. In two subjects, the infection could be classified as active, intercurrent on the basis of a greater than fourfold rise in both the CF and NT antibody titers. These were probably secondary infections, since both subjects had detectable antibody titers initially (NT = 1:8, al-

TABLE 1. *CMV infection rates in renal allograft recipients and control subjects during a 4-year follow-up*

Category	No.	% of category
Renal allograft recipients	41	100.0
Infected group	37	90.2[a]
Active, intercurrent infection[b]	25	61.0[a]
Inactive, previous infection[c]	12	29.2
Noninfected group[d]	4	9.8
Control subjects—renal unit personnel	11	100.0
Infected group	5	45.5[a]
Active, intercurrent infection[b]	2	18.2[a]
Inactive, previous infection[c]	3	27.3
Noninfected group[d]	6	54.5

[a] Statistically significant differences between corresponding groups in the two categories (Fisher's exact test: P = 0.006 and 0.004, respectively).

[b] Viral isolation (18 patients) and/or ≥fourfold change in CF or NT antibody titer (16 patients).

[c] CF antibody titer ≥4, NT antibody titer ≥8; no change in titer and no viral isolation.

[d] CF antibody titer <4, NT antibody titer <8; no change in titer and no viral isolation.

though CF titers were <1:4). They remained asymptomatic, however, and no attempts at viral isolation (other than at the inception of the study) were made.

The differences between the two groups in the total infection rate and in the active, intercurrent infection rate are statistically significant (Fisher's exact test: $P = 0.006$ and 0.004, respectively; this test is suited for analysis of differences between "small numbers" [18]).

CMV infection prevalence in patients on hemodialysis and in control subjects. It has been postulated that patients with chronic renal failure and azotemia are unusually susceptible to infections (11). Hemodialysis per se has also been implicated as a risk factor (4). To ascertain whether or not the high CMV infection rates in renal allograft recipients may be at least in part due to increased frequency of such infections in the transplant candidates, a point prevalence study was done. CMV infection prevalence as determined by CF and NT antibody survey was virtually identical in chronic renal disease patients on hemodialysis (40.0% [4/10]) and control subjects (randomly selected hospital personnel) (39.1% [9/23]) (Table 2).

Effect of age and gender on CMV infection rates. Age of the population is an important variable that may influence the CMV infection rate. Prevalence increases with advancing years. The mean age of hemodialysis patients was 10 years higher than that of hospital personnel controls (i.e., 42.7 versus 31.8 years). According to most reports in the literature, however, the CMV infection prevalence is not different in subjects with this age differential (10, 12). Other categories of patients and control subjects in this study were matched for age more closely (e.g., allograft recipients [37.4] versus renal unit cohorts [34.7]).

Sex may also be a factor influencing CMV infection rates. Luby and Shasby have recently reported a higher prevalence of CMV CF antibodies in females than in age-matched males (10). There was a predominance of males (nine to one) in our hemodialysis group and a predominance of females in the hospital employee controls (18 to 5), and therefore the CMV prevalence rate in this control group could be spuriously high. However, no sex-related difference in CMV antibody prevalence could be demonstrated in the present study in the total group of normal control subjects (i.e., renal unit personnel plus other hospital personnel). CMV infection prevalence was 40.0% in the males (10 subjects) and 41.7% in the females (24 subjects). Admittedly, the group analyzed in the present study was smaller (34 subjects) than that of Luby and Shasby (195 subjects). Other investigators studying a Danish population (9) also could not confirm the findings of these authors, and the gender-influenced differences in CMV infection rates cited by Luby and Shasby from other studies were less striking than in their own (10). Perhaps an explanation may lie in the different racial makeup of the populations studied. Luby and Shasby's group was exclusively nonwhite, whereas the one in the present study (as well as in the one from Denmark) was Caucasian.

TABLE 2. *CMV infection point prevalence in patients with chronic renal disease on hemodialysis and control subjects*

Category	No.	% of category
Patients on hemodialysis	10	100.0
Infected group[a]	4	40.0
Noninfected group[b]	6	60.0
Control subjects—hospital personnel	23	100.0
Infected group[a]	9	39.1
Noninfected group[b]	14	60.9

[a] CF antibody titer ≥4, NT antibody titer ≥8; there were no viral isolations from subjects in either category.

[b] CF antibody titer <4, NT antibody titer <8.

Antibody response in allograft recipients and control subjects. The competence of humoral immunity to CMV was assessed by comparing the CF and NT antibody response in allograft recipients with active intercurrent infection to the response in a corresponding group of control subjects. There were 25 transplant patients and six controls. Four of the latter were patients with post-perfusion CMV mononucleosis and two were renal unit employees who were asymptomatic but had serological evidence of intercurrent infection. Results are summarized in Table 3. The difference between the two groups in NT and CF antibody titers, both in terms of median titers and geometric mean titers (GMTs), is not statistically significant. (Two-sample t test was used for assessing differences in GMTs, and the Mann-Whitney rank sum test was used for median titers [18].)

Comparison of antibody response in allograft recipients with primary versus secondary infection. As indicated under Materials and Methods, primary infection was defined as an infection occurring in individuals with an initial CF antibody titer of <4 and NT antibody titer of <8. It is important to point out that according to some authors, absence of CF antibodies does not totally exclude previous CMV infection (14, 20), and there are reports of patients with absent CF antibodies who nonethe-

TABLE 3. *Comparison of NT and CF antibody response in renal allograft recipients and control subjects with evidence of active, intercurrent infection*[a]

Category	No.	NT antibody[b]		CF antibody[b]	
		Median	GMT	Median	GMT
Renal allograft recipients	24[c]	64[d]	57.0[e]	32[d]	33.0[e]
Control subjects .	6[f]	48[d]	50.8[e]	64[d]	64.0[e]

[a] Established by viral isolation and/or ≥fourfold change in antibody titer.

[b] Highest titer observed.

[c] One subject not included in the analysis because of undetectable antibody level.

[d] No statistically significant difference between median titers in the two categories (two-sample *t* test).

[e] No statistically significant difference between GMTs in the two categories (Mann-Whitney rank sum test).

[f] Four subjects with CMV mononucleosis, two renal unit personnel with asymptomatic intercurrent infection.

less had elevated NT antibody titers (19). In this study, however, we have determined NT as well as CF antibody titers, and the patient was considered to be initially uninfected only when both were undetectable at the lowest dilution used.

There was no significant difference in patients with primary versus secondary active, intercurrent CMV infection in either CF or NT antibody median titers and GMTs (Table 4). Antibody response and viral studies in a typical patient (B.B.) who had been studied prospectively for 3 years and who developed a primary CMV infection are summarized in Table 5. It will be seen that he developed and maintained high titers of both CF and NT antibodies. It is also important to point out the lag of antibody response by at least 2 weeks after infection (viruria) was established.

DISCUSSION

The main purpose of this ongoing study is to elucidate the mechanisms responsible for the known high prevalence of active CMV infections in renal allograft recipients (5, 14, 20). However, in pursuing this goal, we had first to define the scope of the problem in our study population. Renal allograft recipients were found to have a rate of active CMV infection of 61.0% compared with 18.2% in cohort controls. Significant, life-threatening CMV disease was present in 24.0% of transplant patients with active infection. The relatively low degree of contagiousness of CMV for normal hosts is suggested by the fact that over the 4-year period of the study, only two intercurrent infections occurred in the 11 cohort control subjects (18.2%), renal unit personnel involved in the care of both the renal allograft recipients and transplant candidates undergoing hemodialysis.

No exhaustive attempt was made in this study to identify all the possible risk factors involved, since the main thrust was on the immunological mechanisms of host resistance. However, certain risk factors were assessed. For example, it had been reported that patients with chronic renal failure (transplant candidates) with a prolonged state of azotemia have an impairment of both humoral and cell-mediated immunity (21) and of interferon production (17). In the present study, the prevalence of CMV CF and NT antibodies in transplant candidates on hemodialysis was found to be comparable to that in a control group of hospital employees. This confirms other reports indicating that CMV infection is no more common in uremic patients than in control subjects (8, 19). Apparently, the state of immunosuppression in uremia is not great enough to predispose to CMV infection, although uremic patients seem to be more susceptible to other infections (11).

Craighead reported that patients with a primary CMV infection had a lower ratio of NT antibody seroconversion than patients experiencing a secondary infection (5). Data reported herein do not support this finding. Only one of the eight patients with a primary infection who developed a rapidly progressive CMV pneumonia leading to death in a few weeks failed to mount an antibody response. This could have been due to the reported lag in CMV antibody response relative to recovery of the virus (12,

TABLE 4. *Comparison of NT and CF antibody response in renal allograft recipients with primary or secondary infection*[a]

Type of infection	No.	NT antibody[b]		CF antibody[b]	
		Median	GMT	Median	GMT
Primary	7[c]	32[d]	64.0[e]	32[d]	35.3[e]
Secondary	17	64[d]	54.1[e]	32[d]	31.8[e]

[a] Primary infection defined as active, intercurrent infection in individual with initial CF antibody titer <4 and NT antibody titer <8. Secondary infection comprises both reactivation and reinfection.

[b] Highest titer observed.

[c] One subject not included in the analysis because of undetectable antibody level.

[d] No statistically significant difference between median titers in the two categories (two-sample *t* test).

[e] No statistically significant difference between GMTs in the two categories (Mann-Whitney rank sum test).

TABLE 5. *Viral studies in a renal allograft recipient[a] with a primary CMV infection*

Date	Time post-surgery (mo)	Virus isolation			Serology	
		Urine	Saliva	Buffy coat	NT	CF
12/29/70	−1 day	−	−	−	<1:8	<1:4
2/1/71	1	+	−	−	<1:8	<1:4
2/18/71	1.5	+	−	+	1:16	1:16
3/22/71	3.75	+	ND[b]	ND	ND	ND
9/13/71	9.5	+	−	−	1:256	1:32
12/4/72	23	ND	ND	ND	1:32	1:32
2/5/73	25	$10^{3.0c}$	ND	ND	ND	ND
1/5/74	35	+	ND	ND	1:64	1:128

[a] Date of transplantation: 30 December 1970.
[b] ND, Not done.
[c] Mean tissue culture infective dose per 0.2 ml.

20; Table 5) rather than to the patient's humoral anergy.

There are several other reports in the literature suggesting that the CMV antibody response in renal allograft recipients appears to be unaffected by immunosuppressive therapy (2, 6, 13). This is the first study, however, in which the question of the competence of humoral immunity has been looked at in great detail by comparing both NT and CF antibody titers in matched groups of renal allograft recipients and nonimmunosuppressed subjects experiencing active, intercurrent primary or secondary CMV infections.

The results of the present study indicate that it is not the impairment of humoral immunity that is responsible for the observed high prevalence of active CMV infections in transplant patients. Studies assessing the status of cell-mediated immunity to CMV in transplant patients and control subjects are in progress in our laboratory at the present time and will be the subject of a subsequent report.

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LITERATURE CITED

1. **Aaberg, T. M., T. J. Cesarz, and M. W. Rytel.** 1972. Correlation of virology and clinical course of cytomegalovirus retinitis. Am. J. Ophthalmol. 74:407–415.
2. **Andersen, H. K., and E. S. Spencer.** 1969. Cytomegalovirus infection among renal allograft recipients. Acta Med. Scand. 186:7–19.
3. **Benyesh-Melnick, M.** 1969. Cytomegaloviruses, p. 701–732. *In* E. H. Lennette and N. J. Schmidt (ed.), Diagnostic procedures for viral and rickettsial infections. American Public Health Association, Inc., New York.
4. **Coulson, A. S., Z. T. Lucas, M. Loudy, and R. Cohn.** 1974. An epidemic of cytomegalovirus disease in a renal transplant population. West. J. Med. 120:1–7.
5. **Craighead, J. E.** 1969. Immunologic response to cytomegalovirus infection in renal allograft recipients. Am. J. Epidemiol. 90:506–513.
6. **Craighead, J. E., J. B. Hanshaw, and C. B. Carpenter.** 1967. Cytomegalovirus infection after renal allotransplantation. Am. Med. Assoc. 201:725–728.
7. **Jordan, M. C., W. E. Rousseau, J. A. Stewart, G. R. Noble, and T. D. Y. Chin.** 1973. Spontaneous cytomegalovirus mononucleosis. Clinical and laboratory observations in nine cases. Ann. Intern. Med. 79:153–160.
8. **Kantor, G. L., and B. L. Johnson, Jr.** 1969. Incidence of cytomegalovirus infection in uremic patients, p. 347–349. Antimicrobial. Agents Chemother. 1968.
9. **Lang, D. J., and J. B. Hanshaw.** 1969. Cytomegalovirus infection and the postperfusion syndrome. N. Engl. J. Med. 280:1145–1149.
10. **Luby, J. P., and D. M. Shasby.** 1972. A sex difference in the prevalence of antibodies to cytomegaloviruses. J. Am. Med. Assoc. 222:1290–1291.
11. **Montgomerie, J. Z., G. M. Kalmanson, and L. B. Guze.** 1968. Renal failure and infection. Medicine 47:1–32.
12. **Plummer, G.** 1973. Cytomegaloviruses of man and animals. Prog. Med. Virol. 15:92–125.
13. **Prince, A. M., W. Szmuness, S. J. Millian, and D. S. David.** 1971. A serologic study of cytomegalovirus infections associated with blood transfusions. N. Engl. J. Med. 284:1125–1131.
14. **Rifkind, D., N. Goodman, and R. B. Hill, Jr.** 1967. The clinical significance of cytomegalovirus infection in renal transplant recipients. Ann. Intern. Med. 66:1116–1128.
15. **Rytel, M. W., T. M. Aaberg, T. H. Dee, and L. H. Heim.** 1975. Therapy of cytomegalovirus retinitis with transfer factor. Cell Immunol. 19:8–21.
16. **Rytel, M. W., and J. Balay.** 1973. Impaired production of interferon in lymphocytes from immunosuppressed patients. J. Infect. Dis. 127:445–449.
17. **Sanders, C. V., Jr., J. P. Luby, J. P. Sanford, and A. R. Hull.** 1971. Suppression of interferon response in lymphocytes from patients with uremia. J. Lab. Clin. Med. 77:768–776.
18. **Siegel, S.** 1956. Nonparametric statistics for the behavioral sciences. McGraw-Hill Book Co., Inc., New York.
19. **Spencer, E. S.** 1974. Cytomegalovirus antibody in uremic patients prior to renal transplantation. Scand. J. Infect. Dis. 6:1–4.
20. **Weller, T. H.** 1971. The cytomegaloviruses: ubiquitous agents with protean clinical manifestations. N. Engl. J. Med. 285:203–214, 267–274.
21. **Wilson, W. E. C., C. H. Kirkpatrick, and D. W. Talmage.** 1965. Suppression of immunologic responsiveness in uremia. Ann. Intern. Med. 62:1–14.