

## The Relationship of Immunosuppression to Cytomegalovirus Infection<sup>1</sup>

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It has been pointed out that immunosuppression with cytotoxic, nonsteroidal drugs is associated with the development of cytomegalovirus (CMV) infection after renal transplantation, and that without such immunosuppression infection may not occur (3). What is not known is whether immunosuppression alone, without other factors peculiar to the transplant recipient, can cause CMV infection. One approach to this question is to study patients receiving such therapy for reasons other than to prevent allograft rejection. Patients with rheumatologic disorders were chosen for this purpose because there is no known association between these diseases and CMV.

This investigation may conveniently be divided into two parts. The first was a prevalence survey conducted among patients attending a rheumatology outpatient clinic during a 7-month period. Blood and urine samples were obtained on a single occasion from essentially all clinic attendees. Isolation of CMV was attempted from buffy coat and urine specimens, and complement-fixing (CF) antibody was measured on each serum.

Table 1 is a summary of the serologic results among 131 rheumatology clinic patients. The geometric mean titer and the fraction of rheumatology patients with a CF titer of  $\geq 1:4$  are compared to a control group of 211 unselected blood donors. As would be expected in an adult population, both the geometric mean titer and the fraction who were seropositive increased with age up to 55 years. Above 55, CF titers appeared to level off or even decrease slightly. As shown in Table 1, the geometric mean titers of females are greater than those of males, both in rheumatology patients and in blood donors. This relationship between sex and titer is statistically significant in both of these two populations.

Therefore, when the influence of other factors on CMV serology was examined, appropriate statistical methods to adjust for age and sex differences were used. In each instance the effect of a particular factor was examined with respect both to the observed proportion of relevant persons who had antibody and to the magnitude of the titer among those who were seropositive. In order to adjust for the effects of varying age and sex distributions when testing for significant differences between population subgroups in the observed proportion of individuals with antibody, Mantel-Haenszel summary chi-square statistics (4) were employed. Analysis of variance techniques were used to identify those variables which are significantly related to the magnitude of titer among seropositive individuals. By analysis of

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TABLE 1  
Cytomegalovirus CF Antibody Titers of Rheumatology Patients and Blood Donors

Sex	Rheumatology patients			Blood donors			Total		
	Number	1/GMT	Titer $\geq 1:4$ (%)	Number	1/GMT	Titer $\geq 1:4$ (%)	Number	1/GMT	Titer $\geq 1:4$ (%)
Male	41	7.5	51.2	109	6.4	50.5	150	6.7	50.7
Female	90	15.8	64.4	102	8.4	55.9	192	11.3	59.9
Total	131	12.5	60.3	211	7.3	53.1	342	9.0	55.8

variance, the effect of any factor was examined after simultaneous adjustment for every other factor.

As shown in Table 1, both the proportion of rheumatology clinic patients who are seropositive and their geometric mean titer appear to be higher than blood-donor controls. However, when adjusted for age and sex, there is no significant difference between clinic patients and blood donors with respect to either the proportion with antibody or the magnitude of the titer. The only significant source of variation within the combined population is attributable to the effects of sex ( $P = 0.002$ ), indicating that the apparent difference between patients and donors is explained by different distribution of the sexes, females predominating in the clinic population.

The antibody titers of clinic patients according to diagnostic categories are shown in Table 2. It appears that a greater proportion of patients with progressive systemic sclerosis are seropositive, and that those with antibody tend to have a higher titer. Likewise, it seems that a smaller fraction of systemic lupus erythematosus patients are seropositive. However, in an analysis adjusted for age and sex, there is no significant difference between any diagnostic group and blood-donor controls with respect to the proportion having titer. Among seropositive rheumatology patients, there is no significant relationship between diagnosis and magnitude of titer after adjusting for age.

The effect of the therapy employed on the prevalence and magnitude of CF titers is presented in Table 3. The proportion of patients receiving corticosteroids with titer is lower than the rest of the clinic population. Despite having a similar proportion who are seropositive, the titers of seropositive patients receiving cytotoxic immunosuppressive drugs appear to be higher than the titers of the remaining patients. These observations are borne out by appropriate analyses. The proportion of patients on

TABLE 2  
Cytomegalovirus CF Antibody Titers in a Rheumatology Clinic Population According to Diagnosis

Diagnosis	1/Titer							Total	1/GMT	Titer $\geq 1:8$ (%)
	<4	8	16	32	64	128	$\geq 256$			
Progressive systemic sclerosis	10	1	3	9	9	7	1	40	23.1	75.0
Rheumatoid arthritis	12	0	3	7	1	1	0	24	8.0	50.0
Systemic lupus erythematosus	7	0	2	0	0	1	1	11	6.6	34.4
Other	23	3	8	9	8	3	2	56	11.0	58.9
Total	52	4	16	25	18	12	4	131	12.5	60.3

TABLE 3  
Cytomegalovirus CF Antibody in a Rheumatology Clinic Population According to Therapy

Therapy	1/Titer							Total	1/GMT	Titer ≥ 1:8 (%)
	<4	8	16	32	64	128	≥ 256			
Immunosuppression <sup>a</sup>	5	0	0	1	0	5	3	14	30.5	64.3
Corticosteroid	18	1	4	3	4	0	1	31	6.5	41.9
Neither or none	29	3	12	21	14	7	0	86	13.6	66.3
Total	52	4	16	25	18	12	4	131	12.5	60.3

<sup>a</sup>Azathioprine or cyclophosphamide.

corticosteroids who are seropositive is significantly lower regardless of whether comparison is made with blood-donor controls or with the remainder of the rheumatology clinic population ( $P < 0.025$ ). Examination of only those patients who are seropositive in an analysis of variance adjusted for age, sex, and diagnostic effects reveals a significant effect of treatment on titer magnitude ( $P = 0.02$ ). This is due to the higher titers of immunosuppressed patients ( $P = 0.04$ ); the titers of seropositive corticosteroid-treated patients are not significantly different from those of clinic patients on neither form of therapy.

The results of isolation attempts from buffy coat and urine specimens of the rheumatology clinic patients are shown in Table 4. CMV was not isolated from any of the 126 available buffy coat specimens. No isolations were made from the urines of 123 patients not receiving azathioprine or cyclophosphamide therapy, including 37 who were on corticosteroids. Three of 15 (20%) patients receiving cytotoxic immunosuppressive agents had cytomegalovirus recovered from their urine. This difference in the prevalence of viremia between patients receiving and not receiving immunosuppressants is highly significant (Fisher's exact test,  $P = 0.001$ ). All three patients with viremia had high CF antibody titers. In one of the three viremic patients, it was possible to correlate the presence of CMV in the urine with initiation and cessation of immunosuppressive medication. Cyclophosphamide was discontinued in this patient after the first positive urine was obtained, and a second specimen 3 months later was negative for virus. At that time, the drug was reinstated, and cytomegalovirus was isolated from the urine 7 months later. Therapy was once again stopped and urine, nearly 10 months thereafter, was again free of virus.

To determine when immunosuppressed patients become infected and whether infection is of a primary or reactivation type, a prospective study was undertaken of patients with a rheumatologic condition who were scheduled to begin a course of cyclophosphamide, azathioprine or, in one case, chlorambucil. Specimens for isola-

TABLE 4  
Frequency of Cytomegalovirus Isolation from the Buffy Coat and Urine of Rheumatology Clinic Patients

Therapy	Buffy coat		Urine		Total patients
	Positive	Tested	Positive	Tested	
Immunosuppression <sup>a</sup>	0	14	3	15	15
Corticosteroid	0	31	0	37	37
Other or none	0	81	0	86	89
Total	0	126	3	138	141

<sup>a</sup>Azathioprine or cyclophosphamide.

TABLE 5  
Cytomegalovirus Infection in Rheumatology Patients Following Immunosuppression

Prior status	Number	Greater than fourfold titer increase	Viruria	Total infected
Seropositive	9	3	5	5 (55.6%)
Seronegative	5	1	0	1 (20.0%)
Total	14	4	5	6 (42.9%)

tion attempts and CF antibody determinations were obtained before, and at intervals following, the initiation of immunosuppressive therapy. Table 5 summarizes the results to date in the 14 patients studied. CMV was not isolated from buffy coat specimens in any case. Six of the 14 patients (42.9%) had evidence of CMV infection which in each case was first observed between 6 and 12 weeks following the initiation of the immunosuppressive drug. In five patients, infection was demonstrated by isolation of CMV from the urine, preceded or accompanied in three by a fourfold or greater rise in antibody titer. All five patients who yielded virus in their urine had a titer of 1:8 or greater prior to initiation of immunosuppressive medication. One patient who was seronegative prior to therapy manifested CMV infection by seroconversion which was not accompanied by viruria. Thus, infection developed in one of five (20.0%) seronegative and five of nine (55.6%) seropositive patients. This relationship, although suggestive, is not statistically significant, perhaps because of the small sample sizes.

These studies reaffirm the increasing prevalence of CMV CF antibody with age among adults and confirm a previous observation (6) that seropositive females have significantly higher titers than seropositive males. It is reassuring that rheumatology clinic patients are not significantly different from controls in respect both to the proportion who are seropositive and to the magnitude of titer, after adjustment for all other relevant factors. Furthermore, there is no suggestion that serologic or virologic evidence of CMV is related to the specific rheumatologic diagnosis. Since the underlying disease does not in itself promote CMV infection, patients with rheumatologic conditions are an appropriate group in which to study the effects of therapy on infection.

The proportion of patients treated with corticosteroids who are seropositive is significantly lower than that of other patients or controls, but the distribution of the titers of seropositive patients on corticosteroids is not significantly different. There is no evidence from this investigation, previous human studies, or results with animals that corticosteroids in any way protect against infection. The explanation of the observed lower proportion of corticosteroid-treated patients who are seropositive may rest with the effect of these agents on antibody production. Recently, methylprednisolone administration was found to reduce the levels of circulating immunoglobulins in normal men (1). It is possible that the maintenance of CMV antibody after primary infection requires constant B lymphocyte stimulation by antigen during the clinically latent infection, a process interfered with by corticosteroid administration.

In contrast to corticosteroid therapy, cytotoxic immunosuppressive drugs increase the magnitude of titers in seropositive patients but do not significantly affect the pro-

portion with antibody. In the prospective investigation five of the six patients who showed evidence of CMV infection after initiation of immunosuppressive therapy had preexisting antibody titer, indicating that such therapy acts to reactivate clinically latent, endogenous infection. The serologic and isolation results in the prevalence survey then result from sampling a partially seronegative, and presumably partially uninfected, population at varying intervals following initiation of the immunosuppressive drug.

The mechanism by which cytotoxic agents promote reactivation of CMV is not elucidated by the present investigation. These drugs are employed in the allograft recipient and in rheumatology patients to suppress immunologic responsiveness, and reduction in immune defense may allow virus activation. Alternatively, potentiation of CMV could be a result of direct effects of cytotoxic agents on the cells harboring the virus. This possibility is enhanced by the recent report (5) that pretreatment with the DNA inhibitor, 5-iodo-2'-deoxyuridine, permits normally nonpermissive cells to support the growth of CMV. We are studying these two alternative mechanisms in mice.

Assuming that immunosuppressive therapy is the sole determinant for the development of CMV infection in the rheumatology patient group, their infection rate can be applied to renal transplant recipients to determine the risk in the latter ascribable to cytotoxic drugs. As described elsewhere in this volume (2), 21 of our 32 renal allograft recipients developed CMV infection. If the rate of infection in immunosuppressed rheumatology patients (42.9%) applies to the transplant recipients, 65% of infections in the latter could be explained by the drugs. Moreover, eight of ten (80%) seropositive transplant recipients became infected compared with 56% of seropositive rheumatology patients begun on azathioprine, cyclophosphamide, or chlorambucil. By the same reasoning, approximately 70% of cytomegalovirus infections in seropositive kidney recipients may be due solely to the immunosuppressants employed. Immunosuppressive therapy appears to be the predominant risk factor in seropositive renal transplant recipients. The remainder of CMV infections in transplant patients, particularly seronegative recipients, must be explained by other factors (2).

## SUMMARY

The prevalence of complement-fixing antibody to cytomegalovirus (CMV) among adult rheumatology clinic patients and blood donors was significantly related to age and sex. After adjustment for age and sex differences, CMV antibody among patients was influenced by the therapy employed, but not diagnosis. The proportion of patients receiving corticosteroids who were seropositive was significantly lower than other patients. Despite having a similar proportion who had antibody, the titers of seropositive patients receiving cytotoxic immunosuppressive drugs were significantly higher. CMV was isolated from the urine of 20% of rheumatology patients on cytotoxic immunosuppressive drugs but not from those on other forms of therapy. In a prospective investigation, 6 of 14 patients begun on cytotoxic immunosuppressants developed evidence of CMV infection. Five of the six had preexisting CMV antibody, indicating that immunosuppression acts primarily by reactivating endogenous infection. It is estimated that about two-thirds of the CMV infections in renal allograft recipients may be explained by the immunosuppressive therapy employed.

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