INFECTION AND IMMUNITY, Sept. 1983, p. 1380–1382
0019-9567/83/091380-03$02.00/0

Vol. 41, No. 3

T Lymphocyte Subpopulations in Congenital Cytomegalovirus Infection

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Received 10 December 1982/Accepted 25 May 1983

T lymphocyte subpopulations in the peripheral blood of children with congenital cytomegalovirus infections and in controls were enumerated with monoclonal antibodies. Infants of less than 1 year of age with symptomatic infections showed significant increases in the proportion of suppressor cells and decreases in the ratio of helper to suppressor cells, whereas T lymphocyte populations in older symptomatic patients and asymptomatic infants and children did not differ from those in controls.

Changes in proportions of T lymphocyte (T3) subpopulations, resulting in a dramatically decreased ratio of helper T cells to suppressor T cells, have been described in patients with mononucleosis due to cytomegalovirus (CMV) and Epstein-Barr virus (4, 6, 12, 16). Although the meaning of these observations in relation to pathogenesis is not yet clear, they are of interest because of evidence from a number of sources that at least some CMV infections impair measurable host immune responses and resistance to other pathogens (2, 4, 5, 7, 9, 10). To gain additional perspective on the relationship between CMV infection and T3 subpopulations, we studied peripheral blood from congenitally infected infants and controls, using monoclonal antibodies to enumerate T3, helper T cells, and suppressor T cells.

Forty-eight children with congenital CMV infections (proven by isolation of virus from the urine of newborns) and 27 controls were studied. The patients included 17 children with symptomatic congenital infection (S-CMV) (i.e., clinical abnormalities were present in the newborn period) and 31 children who were asymptomatic (A-CMV). The congenitally infected children ranged in age from 1 month to 10 years at the time of study. Controls included infants, children, and adults.

Mononuclear cells were separated from peripheral blood by centrifugation on Ficoll-Hypaque (3). Lymphocyte subpopulations were enumerated as previously described (15) with indirect immunofluorescence with monoclonal antibodies (Ortho Pharmaceuticals, Raritan, N.J.) to T3 (OKT3), to helper T cells (OKT4), and to suppressor T cells (OKT8) (12–14). Briefly, 0.5×10^6 to 1×10^6 peripheral blood mononuclear cells (PBMC) were pelleted at $200 \times g$ in a test tube (6 by 50 mm) and incubated for 5 min with 5 μl of normal human serum. The cells were then incubated for 20 min on ice with 10 μl of a 1:5 dilution of monoclonal antibody. After a wash with phosphate-buffered saline containing 0.1% sodium azide, cells were again pelleted for a 20-min incubation with fluorescein-conjugated goat anti-mouse immunoglobulin (Meloy, Springfield, Va.). After being washed, 10^5 cells were cytocentrifuged onto glass slides, air dried, fixed in 95% ethanol–5% glacial acetic acid, and examined with a Leitz Dialux 20 fluorescent microscope. A total of 400 cells per slide were counted, and results were expressed as the percentage of PBMC positive with each antibody. Total leukocyte and differential counts on peripheral blood were not obtained simultaneously for all patients, so results were not expressed as concentration in peripheral blood. Results in infected and control groups were compared by the Wilcoxon rank-sum test.

The children in the S-CMV group who were less than 1 year of age (median age, 6 months) had a significantly higher percentage of suppressor T lymphocyte (T8) PBMC (mean ± standard deviation, 29 + 8.0) as compared with controls (median age, 4 months) (18 ± 3.7; $P < 0.01$ [Fig. 1]. In addition, the ratio of helper T lymphocytes (T4) to T8 was lower in infants in the S-CMV group (1.4 ± 0.60) than in the control group (2.3 ± 0.67; $P < 0.01$). The percentage of T8+ PBMC and the T4/T8 ratio were similar in the A-CMV and control groups for both infants and older individuals. Elevation of the percentage of T8+ PBMC to more than 30% was noted among 6 infants (≤1 year of age), including 4 of 10 in the S-CMV group, 2 of 20 in the A-CMV group, and 0 of 12 in the control group. Only one child over 1 year of age (16 months) in the S-CMV group

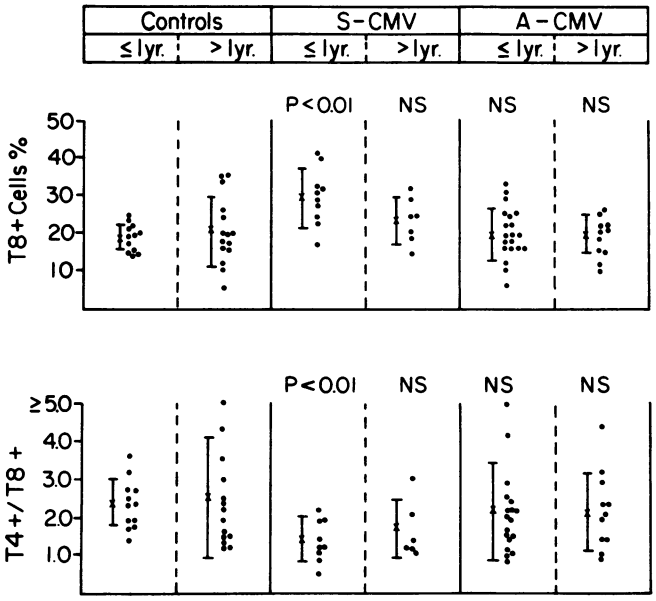

FIG. 1. Comparison of percentage of T8 and T4/T8 ratio in children in the S-CMV group, in the A-CMV group, and controls. The mean ± 1 standard deviation are indicated by brackets. The *P* values are for comparison with controls by the Wilcoxon rank-sum test.

had >30% T8+ PBMC. T4/T8 ratios of <1.0 were found in four patients, including two infants in the S-CMV group, one infant in the A-CMV group, and one older, asymptomatically infected child. Only one of the four patients with a T4/T8 ratio of <1.0 had more than 30% T8+ PBMC. The percentages of T4+ PBMC found in these children were respectively 20, 22, 21, and 32, indicating that a reduced number of helper cells contributed more to the low T4/T8 ratio than did an increase in suppressor cells. The patients in the S-CMV group had abnormal physical or laboratory findings at birth that have been previously described (11). There was no correlation between the percentage of T8+ PBMC or the T4/T8 ratio and the severity of the newborn disease or the presence of sequelae such as microcephaly, psychomotor retardation, or sensorineural hearing loss. Two of the six S-CMV patients with abnormalities in percentages of T8+ PBMC or T4/T8 ratio had hepatosplenomegaly with a normal serum serum glutamic oxalacetic transaminase, and the other four patients were free of any clinical evidence of reticuloendothelial involvement. In children in the S-CMV group under 1 year of age, leukocyte and differential counts revealed total lymphocyte counts from 4.6×10^3 to 10.3×10^3 per mm^3, with a mean of 7.2×10^3 per mm^3, all within normal limits for infants.

Neither the S-CMV nor A-CMV groups differed significantly in percentage of T3+ or T4+ PBMC from controls during the first year of life or later (Table 1). Although children in the A-CMV group who were less than 1 year of age had a lower percentage of both T3+ and T4+ mononuclear cells than did controls, differences were not statistically significant. Within each group as well, results with OKT3 and OKT4 antibodies were similar for infants and older individuals.

Congenital CMV infection results in persistent viral excretion during infancy and early childhood, even in patients with no clinical evidence of infection (1). The majority of these children will shed CMV in urine for more than 4 years. Approximately 5% of infants with congenital CMV infections are symptomatic at birth; clinical findings often include jaundice, hepatosplenomegaly, petechiae, increased serum transaminases, elevated conjugated bilirubin, thrombocytopenia, and microcephaly. Hematological and hepatic abnormalities usually clear by 6 months of age, but irreversible damage to the central nervous system occurs in over 90% of patients who are symptomatic at birth, resulting in mental retardation, hearing loss, visual impairment, or neurological abnormalities. Approximately 5 to 10% of patients who are initially asymptomatic will also have late sequelae affecting the central nervous system (1, 11). Patients with congenital CMV infections consistently produce antibody to CMV, but they usually lack the lymphocyte blastogenic response to CMV that is so readily detectable in normal seropositive adults (8, 17, 18). Neither generalized defects in cellular immunity nor inability to

TABLE 1. T3 and T4 in S-CMV and A-CMV children compared with controls

Age (yr) and group	No.	% PBMC positive (mean ± SD)	
		T3	T4
≤1			
Control	12	59.8 ± 10.9	40.8 ± 8.6
S-CMV	10	58.0 ± 10.7	37.1 ± 13.7
A-CMV	20	51.3 ± 10.8	33.9 ± 7.1
>1			
Control	15	59.7 ± 11.6	38.0 ± 9.7
S-CMV	7	57.8 ± 9.5	35.6 ± 9.1
A-CMV	11	54.0 ± 11.0	36.8 ± 11.5

produce antibody to other antigens have been recognized. In summary, congenital CMV infection results in a chronic infection in virological, immunological, and clinical terms.

Although we found an increased proportion of T8+ and lower T4/T8 ratios in some infants in the S-CMV group, our results are not very striking when compared with findings reported in patients with CMV mononucleosis. Carney et al. found consistent increases in the percentage of T8 cells in the peripheral blood of patients with CMV mononucleosis (up to ≥80% lymphocytes [4]). When compared with patients recovering from CMV mononucleosis, our study population is perhaps more noteworthy by its lack of abnormality in distribution of T3 between helper and suppressor subpopulations. The proportions of T3-, T4-, and T8-positive mononuclear cells in infants of less than 1 year of age with asymptomatic congenital infection and in older patients from both categories were not different from those of controls. However, our patients acquired CMV during fetal life; even those of less than 1 year of age at the time of this study were likely to be many months beyond acquisition of CMV. Serial studies of congenitally infected patients during the first weeks of postnatal life might reveal more abnormalities. The results reported here indicate that the persistent viral shedding and impaired specific lymphocyte blastogenic response that are characteristic of congenital CMV infection are not accompanied by the same type of disturbance in distribution of peripheral blood lymphocytes between helper and suppressor subpopulations that accompanies CMV mononucleosis.

This work was supported by Public Health Service Program Project grants HD10699 and 5-MOI RR32 from the National Institutes of Health.

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