Longitudinal Studies of Cytomegalovirus-Specific Cell-Mediated Immunity in Congenitally Infected Infants

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Characteristics of specific cell-mediated immunity in infants with congenital cytomegalovirus (CMV) infection were studied by in vitro lymphocyte transformation test, using the whole blood culture technique. Subjects were 6 symptomatic congenitally infected infants, 11 asymptomatic congenitally infected infants, 3 postnatally infected infants, and 5 CMV-free infants. Among them, three symptomatics and seven asymptomatics were studied temporally, together with three postnatally infected infants and five controls. In the congenitally infected group, 18 of 47 (38%) specimens proved to be negative in terms of stimulation index values. Studies of temporal changes in CMV lymphocyte transformation tests revealed that in the postnatally infected group, the response turned positive in accordance with the appearance of CMV viruria and remained positive afterwards. In the congenitally infected group, the delay in development of positive reaction followed initial negativity, a phenomenon conspicuously observed in the symptomatic subjects. No significant difference was seen in phytohemagglutinin-induced lymphocyte transformation tests among congenital, postnatal, and control groups.

The role of specific cell-mediated immunity in human cytomegalovirus (CMV) infection has been suggested in a number of experimental studies (1, 5, 7, 12–15). There have appeared lately several reports implying the disorder in specific cell-mediated immune response in infants with congenital CMV infection (2, 8, 10, 11). This phenomenon seems to include important problems. If the disorder is explored in comparison with appropriate age-matched subjects with postnatal CMV infection, it could provide us with valuable information suggesting the CMV infection to be prenatal, or a kind of parameter indicating introduction of appropriate immunotherapy. From this point of view, longitudinal study of virus-specific cell-mediated immunity by the use of lymphocyte transformation (LTF) tests was carried out in infants with congenital CMV infection in comparison with those with postnatal infection and CMV-free infants.

MATERIALS AND METHODS

Study population. Sera and peripheral blood lymphocytes were obtained from 17 congenitally CMV-infected infants, of whom 11 were asymptomatics having positive urine culture in the first week of life. Six were symptomatics with viruria and typical stigmata of congenital CMV infection (i.e., petechiae, hepatomegaly, etc.). Three postnatally CMV-infected infants with hepatitis were confirmed by negative CMV culture in the first week of life. Five were CMV-free infants born to seronegative mothers; they remained negative in CMV cultures and CMV antibody throughout the period of observation. Written consents were obtained from all mothers of the subjects after full explanation of the plan.

Serovirological study. Sera were tested for CMV antibody by complement fixation test, using an initial serum dilution of 1:4. CMV strain AD-169 grown in human embryonic fibroblasts was used for antigen production. The antigen was prepared by extraction of cell-associated antigen with the glycine-buffer method (3). CMV isolation was performed by inoculation of fresh urine samples into human embryonic fibroblast cultures.

LTF test. (i) Antigen preparation. An antigen for LTF test was prepared by the same method as that used for the preparation of complement fixation antigen. A control antigen was prepared from uninfected cells treated in exactly the same way. Antigens were inactivated by UV irradiation with a 10-W germicidal lamp at a distance of 10 cm for 60 min. The highest response, obtained from stimulation by antigen of three kinds of dilution (1:2, 1:8, and 1:32), was applied to experimental data.

(ii) Mitogen preparation. Lymphocyte responses to mitogen stimulation were tested by using phytohemagglutinin (PHA) at a concentration of 100 μg/ml.

(iii) Lymphocyte culture and harvest. Lymphocytes and either CMV antigen diluted from 1:2 to 1:32 or PHA were incubated together by using the whole blood culture technique (4, 6). Blood, which was collected in preservative-free heparin (100 U/ml), was diluted with 15 volumes of RPMI 1640 culture medium. The final lymphocyte concentration was adjusted...
to $2 \times 10^5$ cells per ml. Triplicate cultures (0.5 ml per tube) were set up and contained 0.05 ml of antigen per tube. The cultures with CMV antigen were maintained at 37°C in air with 5% CO$_2$ for 6, 7, and 8 days. At 24 h before harvest, 10 $\mu$Ci of $[^3H]$thymidine per ml was added to each culture. The cultures with PHA were harvested after incubation for 3 days. The cells were harvested and processed for scintillation counting as previously described (4, 6). The arithmetic mean and standard deviation of triplicate cultures were calculated. Virus-induced lymphocyte stimulation, expressed as stimulation index (SI), was calculated as follows: SI = (mean counts per minute in stimulated cultures)/ (mean counts per minute in unstimulated cultures). Statistical significance was determined by Student’s $t$ test.

RESULTS

Distribution of CMV-LTF responses. The CMV-specific LTF test was performed in CMV excretors and nonexcretors into which subjects were grouped (Fig. 1). In postnatally infected subjects, SI values were all greater than 3. Only two of the nonexcretors had SI values of $\geq 3.0$. These two subjects were also seronegative. Congenitally infected subjects showed SI values of $<3.0$ soon after birth, followed by significant responses in later months. This phenomenon was conspicuous in the symptomatic infants.

Temporal changes in CMV-LTF responses in individuals. Five CMV-free infants showed SI values lower than 3, except for one positive episode in each case of K.H. and T.M. with concomitant negative serological findings. Three postnatally infected infants, all of whom showed negative CMV viruria in the first week of life, developed positive CMV-LTF responses when they had CMV viruria at several months of age (Fig. 2). In the congenitally infected group, longitudinal assessment could be done in 10 patients. Five of them changed to positive (SI $\geq 3.0$), two remained positive throughout, and two returned to positive from transient negative responses (Fig. 3). Only one subject remained negative. The delay in conversion to positive LTF response was more remarkable in the symptomatic infants.

PHA-LTF responses. No significant differences in PHA-LTF responses were observed among three groups: the mean SI ($\pm$1 standard deviation) of the congenitally infected group was 202.7 ($\pm$133.1); for the postnatally infected group it was 195.0 ($\pm$96.2); and for the CMV-free group it was 195.6 ($\pm$120.4).

FIG. 1. Distribution of SI values of LTF tests by CMV antigen in congenitally or perinatally CMV-infected infants and CMV-free infants. Symbols: $\Delta$, CMV excretor (congenital, symptomatic); $\star$, CMV excretor (congenital, asymptomatic); $\bullet$, CMV excretor (postnatal); $\bigcirc$, nonexcretor.

FIG. 2. Temporal changes of CMV-specific lymphoproliferative activity in infants with postnatal CMV infection and in controls.
FIG. 3. Temporal changes of CMV-specific lymphoproliferative activity in infants with congenital CMV infection.

DISCUSSION

The results of the present study showed that CMV-specific cellular immune defect in congenital CMV infection appeared to be not a permanent but rather a transient phenomenon and that specific lymphocyte reactivity seemed to be more profoundly depressed in the symptomatic infants.

Establishment of the cutoff point in the LTF test was done in the following manner. CMV-free subjects who remained negative in viral serology always showed SI values below 3.0, except that two subjects presented values higher than 3.0 once each. These two exceptions could be interpreted as false positive, due to an unknown cause or to fortuitous products of non-specific mitogenic activity of CMV crude antigen. In contrast, the postnatally infected group permanently maintained SI values over 3.0. On the basis of the results, we made 3.0 a cutoff point instead of 2.0, which was applied in our previous study. Nonspecific cell-mediated immunity in infants with congenital CMV infection appeared to be normal, based on PHA-LTF tests. We obtained results suggesting no substantial difference in response to PHA among three groups of congenitally infected infants, postnatally infected infants, and CMV-free infants. In the studies of Gehrz et al. (2) and Starr et al. (11), nonspecific cell-mediated immune responses were also said to be generally intact in children with congenital CMV infection. However, nonspecific cell-mediated immunity in congenital CMV infection needs further study, because PHA is said to be the least sensitive of mitogens for testing nonspecific mitogen responsiveness (9).

Recently, Ten Napel and The (13, 14) presented interesting data of acute CMV infection and the host immune response in adults. They studied cellular immunity in CMV infection in 18 patients in the acute and convalescent stages and suggested that CMV infection caused a long-lasting cellular immunosuppression.
Starr et al. (11) had already suggested that impairment in CMV-specific cell-mediated immunity was transient in congenital infection, since some older children responded in the assays.

Our findings confirmed the already suggested impairment in CMV-specific cell-mediated immunity in congenital CMV infection from longitudinal study in individuals by LTF tests. In addition, symptomatic infants seem to be more profoundly depressed in CMV-specific cell-mediated immunity and may need more time to gain lymphocyte reactivity.

**LITERATURE CITED**


