Viral transmission among eight infants attending a day care center who showed acquired immunosuppression accompanied by CMV infection

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ABSTRACT

Background: We encountered sporadic cases of human cytomegalovirus (CMV) infection diagnosed by titration of IgM and of complement fixation (CF) antibodies during a period of about 18 months in a day care center. Frequent episodes of infection, fever, pneumonia and diarrhea were found among those children.

Objectives: This study was undertaken to determine whether the CMV infection in 8 out of 32 children was acquired inside or outside the day care center, and also to tentatively establish a correlation between CMV infection and episodes.

Study design: CMV DNA in urine and saliva were examined by polymerase chain reaction (PCR) analysis with one pair of primers for the immediate early region. The sequencings of the CMV genome were examined in CMV PCR-positive cases. The laboratory data of the cases which had episodes of frequent infections were studied.

Results: The 24 CMV PCR negative children showed no episodes of frequent infections. Four cases out of 8 with a high titer of antibodies against CMV were positive on CMV PCR analysis of saliva. The sequencings of the CMV genome were identical in 3 cases. Of 8 cases with CMV infection, the immunological findings showed a decrease of bacterial sterilizing power and of the IgG2 antibodies to pneumococcal capsular polysaccharides in one case. We supposed that CMV infection was acquired inside the day care center and that the viral infection might cause immunological abnormalities in the hosts, decreasing their bacterial sterilizing power.

Conclusions: This is the first report of a group showing sporadic episodes of fever, pneumonia and diarrhea accompanied with CMV infection. We are concerned that CMV infection may induce acquired immunological susceptibility to infection more frequently than has been suspected.
INTRODUCTION

Cytomegalovirus (CMV) infections are frequent in immunosuppressed hosts, particularly in patients who have had organ transplantsations and in patients who have human immunodeficiency virus (HIV) infections. Infections in immunologically normal hosts are frequent too, but are usually without symptoms.

There have been a few reports indicating that CMV infections influenced the immune mechanism—a decrease in the CD4 level and an increase in the CD8 level[1, 2].

In the United States, a high prevalence rate of CMV extraction among children in day care centers was reported. Pass et al. in Birmingham, Alabama, reported that the CMV extraction rate among children in day care centers was 51%. Adler reported that a significantly high prevalence (25%) of viruria occurred and CMV was frequently transmitted among children attending day care centers[3].

In this report, we deal mainly with sporadic cases of CMV infections, diagnosed by the antibody detection method, in a day care center during about 18 months. They had episodes of frequent infection, fever, pneumonia and diarrhea. This study was undertaken to determine, by the polymerase chain reaction (PCR) method, whether the CMV infections were acquired inside or outside the day care center.

MATERIALS AND METHODS

Subjects and samples

CMV extraction was studied in urine and saliva samples collected from 32 children attending the day care center. There were 8 cases with CMV infection during the 18-month period. (Table 1). They had episodes of frequent infection, fever, pneumonia and diarrhea. In most cases, laboratory examinations showed liver dysfunction—an increase in aspartate aminotransferase (AST) and in alanine aminotransferase (ALT). Virological examinations revealed high concentrations of specific IgM or CF antibodies against CMV. Examinations for other hepatitis markers—for example hepatitis A (HAV), hepatitis B (HBV), hepatitis C (HCV) and Epstein-Barr virus (EBV) were all negative. All cases showed only moderate changes in laboratory data, with no systemic symptoms of CMV infections, for example, cytomegalic inclusion disease. And in all cases, frequent infections took a long time to recover.

PCR methods

Oligonucleotide primers, derived from the immediate early (IE) gene sequence, 101(5’-
PCR assay
Of 64 samples—32 saliva samples and 32 urine samples), 4 (12.5%) of saliva and 1 (3.1%) of urine were CMV-positive. In all positive cases, laboratory data showed liver dysfunction, and there were frequent infection episodes.

Sequence analysis
In the 5 PCR-positive samples, the sequence data for 171 bases in IE gene (bp 1225-1396) were compared with standard strains. Three samples gave conclusive sequence results (Fig. 1). In the 3 samples, the sequence data for 171 bases were identical.

Immunological findings
In 8 cases with CMV infection, there were many episodes of frequent infection, fever, pneumonia and diarrhea. In case 3, the immunological data showed a decrease in bacterial sterilizing power (56.8%) and in IgG2 antibodies to pneumococcal capsular polysaccharides by ELISA (0.8 μg/ml). The level in IgG2 antibodies was decreased, so regularly high titer γ-globulin for CMV was administered intravenously. Recently, the episodes of frequent infection decreased.

RESULTS

CCAAGCGGCTCTTGATAACCAAGCC-3') and 102 (5'-CAGCACCATCCTCTTCTCTTCTG-3') amplified the 434-bp fragment (IE bp 1112-1546) (Genemed Biotechnologies, Inc, South San Francisco, CA 94080, USA). PCR was done with 1.5U of Taq polymerase (Perkin-Elmer, Norwalk, CT, USA) during 32 cycles of denaturation (94°C for 1min), annealing (55°C for 1min), and extension (72°C for 1min). The reaction mixture was analyzed after electrophoresis on 3.0% agarose gels. The nucleotide sequences were determined, only for PCR positive samples, by using direct sequencing with M13 primer. The sequence data for 171 bases in IE gene (bp 1225-1396) was compared with standard strains, for example - AD 169, Towne, UL122.

Immunological studies
Bacterial sterilizing power, as a marker of H2O2 production, was measured by a flow cytometer (FACS). The IgG subclass was measured by the method reported by Hayashibara and IgG2 antibodies to pneumococcal capsular polysaccharides was measured by enzyme linked-immunosorbent assay (ELISA) reported by Ishizaka et al. FACS was carried out to determine the percentages of CD4 and CD8 lymphocytes.

Fig. 1 Nucleotide sequence of the 171 nucleotides amplified between the primers
DISCUSSION

Cases with CMV infection had episodes of frequent infection, fever, pneumonia and diarrhea. Four (12.5%) saliva samples and 1 (3.1%) urine sample were CMV-positive. In all positive cases, laboratory data showed liver dysfunction with frequent infection episodes. In 3 cases, the sequence data for 171 bases in the IE gene (bp 1225-1396) were identical. The 3 cases showed frequent infections for 2 or 3 months after entering the day care center. They did not show frequent infections at the same time unseasonably. So it is unlikely that other viruses, for example the RS virus or bacterial infections, cause frequent infections in children. We suspect that the CMV infection was acquired inside the day care center by the 8 children with liver dysfunction and that there was viral transmission among the children attending the day care center. It has been reported that CMV was shed at a high rate by day care center children because of viral transmissions among the children\(^5\),\(^8\). CMV-positive children may shed CMV in their urine and saliva for a long time\(^9\),\(^10\). On the basis of our sequence data, we suspected that the prevailing viral strain might be identical. Therefore, the CMV PCR-positive children in the day care center in question were separated from CMV PCR-negative children, and disposable diapers were used instead of cloth diapers. Toys of children positive for CMV in saliva were also separated. As a result, the frequency of CMV infections decreased. Saliva is easy to collect as samples for PCR, so it facilitates sequencing in other regions, for example, in the glycoprotein (gB) region.

As for immunological studies of CMV infections, Watanabe et al. reported that CMV mononucleosis reduced H\(_2\)O\(_2\) production of neutrophils in an immunocompetent adult\(^11\) while Carney et al. reported that lymphocyte subpopulations in immunocompetent patients with CMV infection may show a decrease in OKT4-positive lymphocytes and an increase in OKT8-positive lymphocytes\(^5\). However, immunity to CMV infections is complicated and is not well understood. Our data showed a decrease of bacterial sterilizing power in CMV PCR-positive children and, in one case, a marked decrease of IgG2 antibodies to pneumococcal capsular polysaccharides. Now we strongly suspect that CMV infections influenced the immune mechanism in the infected hosts, causing frequent episodes of fever, pneumonia and diarrhea.

This is the first report of a group of sporadic cases with frequent infections and liver dysfunction induced by CMV. We are concerned that CMV infection induces acquired immunological susceptibility to infection more frequently than has been suspected.

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易感染性を示した乳児院における8人の
サイトメガロウイルス感染の流行

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要旨：乳児院において、サイトメガロウイルス感染を示し、発熱、肺炎、下痢といった症状を繰り返す
易感染性を示した8人の乳児を経験した。8人のうち4人は唾液にてCMV PCRは陽性であり、内3例は塩基配列の一致を認めた。サイトメガロウイルス感染は免疫学的影響を及ぼすこと、乳児院におけるサイトメガロウイルスは同一のウイルスである可能性が考えられた。