Breast Milk Is Not a Significant Source for Early Epstein-Barr Virus or Human Herpesvirus 6 Infection in Infants: a Seroepidemiologic Study in 2 Endemic Areas of Human T-Cell Lymphotropic Virus Type I in Japan

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Abstract: In order to evaluate the possibility of Epstein-Barr virus (EBV) and human herpesvirus 6 (HHV-6) transmission via breast milk, a total of 331 serum specimens collected from bottle-fed and breast-fed children and their mothers, in 2 endemic areas of human T-cell lymphotropic virus type I (HTLV-I) in Japan, were assayed for antibodies to EBV and HHV-6. The seroprevalences of EBV and HHV-6 were over 95% both in the mothers of bottle-fed children and in those of breast-fed children. The seroprevalence of EBV at 12–23 months of age was 54.5% (36/66) and 55.8% (24/43) in breast-fed children and bottle-fed children, respectively. The seroprevalence of HHV-6 at 12–23 months of age was 90.9% (60/66) and 93.0% (40/43) in breast-fed children and bottle-fed children, respectively. No difference was observed between the seroprevalences of EBV and HHV-6 in breast-fed and bottle-fed children at 12–23 months of age. Our seroepidemiologic data indicate that breast milk is not a significant source of early EBV or HHV-6 infection in infancy.

Key words: Breast milk, Epstein-Barr virus, Human herpesvirus 6, Serology

Herpesviruses are common pathogens in immunocompromised hosts. Seroepidemiologic studies have revealed the early acquisition of three herpesviruses: cytomegalovirus (CMV) (11, 19, 24), Epstein-Barr virus (EBV) (2, 8) and human herpesvirus 6 (HHV-6) (23, 29, 30). Breast milk has been determined to be a major route by which infants become infected with CMV in western countries (7, 14, 26) and Japan (9, 22). However, the significance of breast milk as a source of early infection of EBV and HHV-6 has not been fully investigated. In order to clarify this point, we performed a seroepidemiologic study of both viruses using serum specimens collected from bottle-fed and breast-fed children and their mothers in 2 endemic areas of human T-cell lymphotropic virus type I (HTLV-I) in Japan.

Materials and Methods

Subjects. Kyushu Island is one of the endemic areas of HTLV-I in Japan. Kagoshima City and Karatsu City are located on the southern coast and northern coast of Kyushu Island, respectively. Since mother-to-child transmission of HTLV-I via breast milk was confirmed in Japan (1, 15), Kagoshima City Hospital and Karatsu Red Cross Hospital have been undertaking a screening program of pregnant women for HTLV-I antibody as a preventive act. In this program, most of the seropositive mothers chose complete bottle feeding for the prevention of milk-borne transmission of HTLV-I. In our previous studies (9, 22), 118 serum specimens obtained at both

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Abbreviations: CMV, cytomegalovirus; EBV, Epstein-Barr virus; FITC, Fluorescein-isothiocyanate; HHV-6, human herpesvirus 6; HTLV-I, human T-cell lymphotropic virus type I; VCA, viral capsid antigen.
hospitals from bottle-fed children aged from 6 to 23 months, and whose mothers were seropositive for HTLV-I, were assayed for the CMV antibody. Among them, 108 serum specimens (81 from Kagoshima, 27 from Karatsu) were available for HHV-6 and EBV antibody assays (bottle-fed group). The remaining 10 serum specimens were not assayed since the sample volume was not sufficient. One hundred and fifteen serum specimens obtained from breast-fed children aged from 6 to 23 months at both hospitals (9, 22) and were assayed as a control (breast-fed group; 80 from Kagoshima and 35 from Karatsu). We also evaluated 61 serum specimens obtained from mothers of the bottle-fed group and 47 serum specimens obtained from mothers of the breast-fed group.

**EBV antibody assay.** The EBV viral capsid antigen (VCA) IgG antibody was assayed by the indirect immunofluorescence method (20). Briefly, the P3HR-1 cell line was maintained at 33°C in RPMI 1640 plus 10% fetal calf serum for a week. The smears containing approximately 10-15% VCA-positive cells were used as the antigen. Fluorescein-isothiocyanate (FITC)-labeled anti-human IgG rabbit serum (Dakopatts, Glostrup, Denmark) was used as the second antibody. A positive fluorescence at 1:5 serum dilution was considered as seropositive for EBV VCA.

**HHV-6 antibody assay.** The HHV-6 IgG antibody was assayed by the indirect immunofluorescence method, as described elsewhere (29). Briefly, HPB-ALL cells co-cultured for 7 days with cord blood lymphocytes infected with the HHV-6 HST strain were used as the antigen. FITC-labeled anti-human IgG goat serum (Jackson ImmunoResearch Laboratories, Avondale, Pa., U.S.A.) was used as the second antibody. A positive fluorescence at 1:10 serum dilution was considered as seropositive for HHV-6.

**Results**

The seroprevalences of EBV VCA for mothers and children of the breast-fed and bottle-fed groups are shown in Table 1. No significant difference between the seroprevalences of the bottle-fed and breast-fed groups was observed for the mothers or children at 12–23 months of age, when maternal antibody has already disappeared, in either hospital (P > 0.05, chi-square test or Fisher’s exact test).

The seroprevalences of HHV-6 for mothers and children of the breast-fed and bottle-fed groups are pre-
sent in Table 2. Similar to the seroprevalence of EBV-VCA, no significant difference between the seroprevalences of the bottle-fed and breast-fed groups was observed for the mothers or children at 12–23 months of age in either hospital (P>0.05, Fisher’s exact test).

Discussion

Our present data for children in both hospitals were compiled and compared to data from our previous studies on CMV (9, 22) in Fig. 1.

Our studies on CMV showed approximately a 40% difference in the seroprevalences of CMV between the breast-fed and bottle-fed infants at 12–23 months of age, suggesting that about 40% of children are infected with CMV via breast milk. In contrast, the seroprevalences of EBV at 12–23 months of age were 54.5% (36/66) and 55.8% (24/43) in the breast-fed and bottle-fed children, respectively, showing no difference between them. Similarly, no difference was observed between the seroprevalences of HHV-6 at 12–23 months of age in the breast-fed (90.9%, 60/66) and bottle-fed children (93.0%, 40/43). These data indicate that breast milk is not a significant source of early EBV or HHV-6 infection in infancy.

Seroepidemiologic studies have revealed the early acquisition of EBV in childhood (2, 8). Saliva has been believed to be the main source of EBV infection, based on data showing frequent virus shedding in the saliva of asymptomatic seropositive carriers (3, 10, 27). Recently, Junkar et al (17) detected EBV genome in cells shed into the breast milk of almost half of all healthy women using a dot-blot hybridization technique, and proposed the possibility of EBV transmission via breast milk. We conducted the seroepidemiologic study reported here to evaluate this possibility. However, we failed to demonstrate a significant difference between the seroprevalences of EBV in breast-fed and bottle-fed children, and could not confirm the possibility. Saliva still seems to be the significant source of EBV infection in infants.

The route of transmission of HHV-6 to infants has not yet been fully clarified. Age-specific seroprevalences of HHV-6 reported in Japan have demonstrated an abrupt increase of seroprevalence after 5 months of age, indicating that the primary infection of HHV-6 occurs immediately after the disappearance of maternal antibody in most infants (23, 29, 30). Saliva and/or breast milk could be a significant source of this early HHV-6 acquisition. The isolation, or detection by polymerase chain reaction, of HHV-6 from saliva has been reported with high frequency (4, 12, 13, 16, 21). However, several investigators observed a low detection rate (<7%) of HHV-6 DNA from the saliva of healthy donors (5, 18, 25). On the other hand, two negative data on the possibility of HHV-6 infection via breast milk have been reported. Takahashi et al (28) reported the acquisition of HHV-6 infection by 9 months of age in 11 of 12 bottle-fed infants. Dune et al (6) failed to demonstrate the HHV-6 genome in breast milk. We evaluated the possibility of milk-borne transmission of HHV-6 using serum specimens obtained from larger scale populations. Our data show no difference in seroprevalences between bottle-fed and breast-fed infants, and suggest that milk-borne transmission is not a significant factor of early HHV-6 acquisition as documented by previous studies. Further studies are needed to elucidate the significant source of early HHV-6 infection in infants.

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References


