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The distribution of antibodies to HHV-6 compared with other herpesviruses in young children

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SUMMARY

Sera from 141 infants aged 0–12 months were examined for IgG antibodies to HHV-6, HSV, CMV, VZV and EBV and for HHV-6 specific IgM. Following the decline in maternal antibody, antibody to HHV-6 was found to rise by 5–6 months and approached the level found in adults by 11–12 months. In contrast the antibody rates for the other herpesviruses were much slower to rise, especially in the case of CMV and EBV. HHV-6 IgM antibodies were detected mainly in age groups showing a rapid rise in antibody to HHV-6. HHV-6-IgM was not detected in 235 cord blood samples. The data suggest that HHV-6 infection is acquired horizontally, at a very early age in Western Australia.

INTRODUCTION

While performing lymphocyte culture on specimens from patients with lymphoproliferative disorders the unexpected discovery was recently made of the existence of a novel human herpesvirus [1].

At first the virus was thought to be B-lymphotrophic and was thus designated human B-lymphotrophic virus (HBLV). Later it became apparent that replication also occurred in a variety of haemopoietic cell lines and for taxonomic purposes the new virus was named human herpesvirus 6 (HHV-6) [2]. The first isolations of HHV-6 were made from patients with AIDS and various lymphoproliferative diseases [1] but later, isolations were made from the lymphocytes of normal adults [3]. Isolations were also made from saliva samples collected from normal adults [4, 5].

Initial reports cited a low distribution of antibody to HHV-6 in the general population [1, 6] but it soon became apparent that antibody to HHV-6 had, in fact, a very wide distribution in adults [7]. In Western Australia (WA), antibodies to HHV-6 were found to be very prevalent in adults and young children [8]. This study confirms and extends these findings and compares the distribution of antibodies to HHV-6 with that of antibodies to the other herpesviruses in children up to 12 months of age.

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MATERIALS AND METHODS

Production of HHV-6 infected cell substrates

HHV-6 (WA-1), a locally isolated virus strain [8], was first propagated in phytohaemagglutinin-stimulated human lymphocytes from healthy adults. Material from this culture was inoculated into J Jhan cells [6] using centrifugal enhancement [9]. These cells were cultured in RPM1 1640 medium (CSL, Melbourne, Australia) with 10% foetal calf serum (Flow Laboratories, North Ryde, NSW, Australia), 0·2% sodium bicarbonate, 3 mM hydrochloric acid, penicillin (100 u/ml), streptomycin (100 μg/ml), neomycin (35 μg/ml) and extra glutamine (2 mM). A 50% medium change was made every 2–3 days.
The J Jhan cells were monitored by indirect immunofluorescence antibody technique (IFAT) and harvested when about 50% of cells were infected. The cultures were washed in phosphate buffered saline pH 7·3 (PBS), air-dried on teflon-coated glass slides, fixed in acetone for 10 min at room temperature and then stored at −85 °C until required.

Immunofluorescence assay for HHV-6 antibodies

Sera were diluted 1:10 in a sonicated preparation of J Jhan cells in PBS (10^7 cells/ml) and incubated at room temperature for 30 min to absorb anticellular antibodies. Cell debris was removed by centrifugation. The sera were then tested for IgG antibodies by IFAT on HHV-6 infected, and uninfected control slides, which had been exposed to 1:5 normal rabbit serum for 1 h to block Fc receptors which may be increased in cells infected with some herpesviruses [10]. Incubation periods of 1 h at 37 °C were used for reaction with sera and antihuman IgG FITC conjugate (Tago, Burlingame, USA) which was diluted in 0·001% Evan's Blue counterstain. A 10 min wash cycle in PBS followed each incubation. For detection of IgM antibody, sera were absorbed with J Jahn cells as above but were also treated with suspensions of heat-killed streptococcus AR1 which absorbs all subclasses of IgG thus reducing competition with IgM for antigenic sites [11], and with human immunoglobulin coupled to sepharose gel to remove rheumatoid factor [11]. The IFAT for the IgM antibody was similar to that for IgG except that the serum incubation time was extended to 3 h and antihuman IgM FITC conjugate (Tago) was used. Positive control sera were included with each batch of IgG and IgM IFAT. Only sera yielding definite fluorescence in a similar percentage of cells to control sera were scored as positive. Specimens showing very weak or equivocal fluorescence were recorded as negative.

Other serological assays

IgG antibody to Epstein-Barr virus (EBV) was assayed by IFAT as previously described [11] using sera diluted 1:10. IgG antibodies to cytomegalovirus (CMV), varicella-zoster virus (VZV) and herpes simplex virus (HSV) were tested by enzyme immunoassay (EIA) using methodology, controls and cut-off values according to the manufacturer's instructions (Behringwerke, Marburg, West Germany) using sera diluted 1:40.

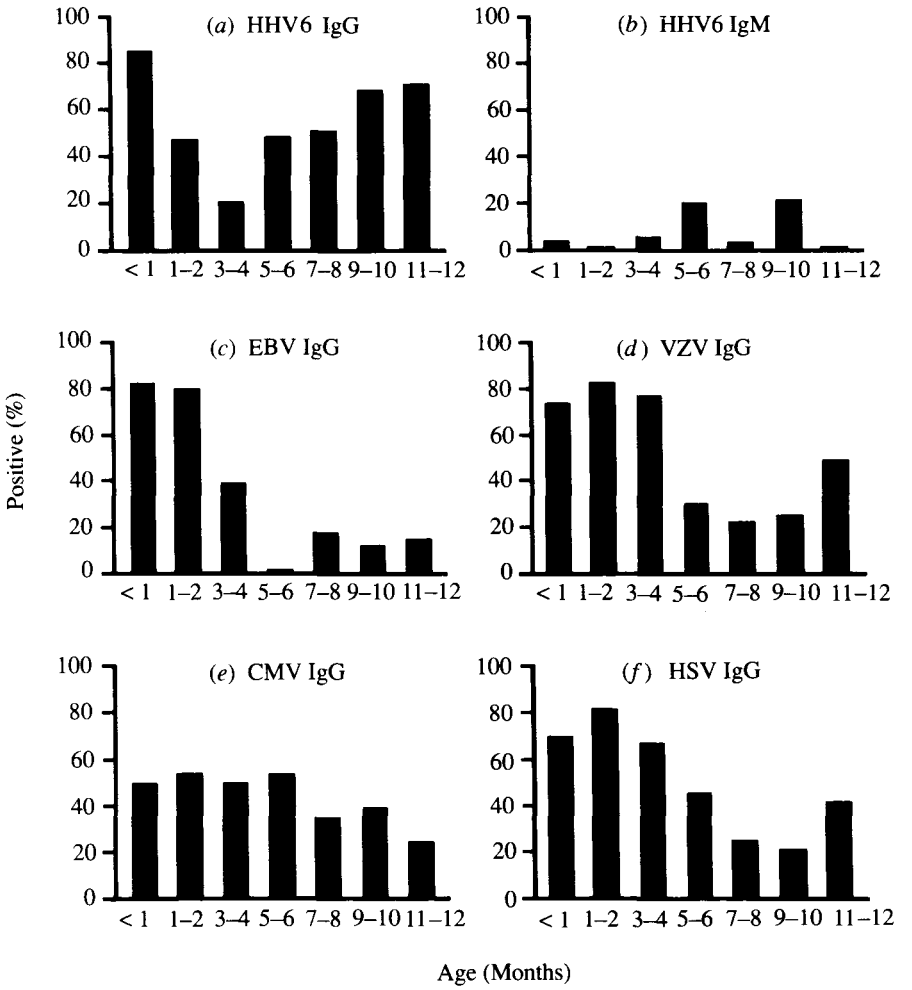

Fig. 1. Antibody distributions in sera from 141 infants aged 0–12 months, showing the percentages of sera which were positive at a dilution of 1/10 by IFAT (HHV-6 IgG and IgM, EBV IgG) or 1/40 by EIA (VZV, CMV and HSV IgG). Specimens showing very weak or equivocal results were recorded as negative.

Serum specimens

Sera from 141 infants aged 0 to 12 months were selected from specimens received for virus investigation at the State Health Virus Laboratory, WA, and from sera collected for other tests at Princess Margaret Hospital for Children, WA. Cord serum specimens were received from King Edward Memorial Hospital, WA.

RESULTS

The distribution of IgG antibodies to HHV-6, EBV, VZV, CMV and HSV in young children of various age groups is shown in Fig. 1 (*a*, *c–f*.) The positive rates for all of the herpesviruses were high in the early months of life but show differing rates of decrease and differing minimum positive rates. HSV and VZV positive

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rates were lower by 5–10 months but showed an upward trend by 11–12 months; this rise had not occurred by 12 months for CMV and EBV.

In contrast, the rise in the HHV-6 positive rate was evident in children as young as 5–6 months and by 11–12 months the positive rate was approaching the level found in the adult population [8].

Fig. 1(*b*) shows the prevalence rate of HHV-6 specific IgM antibodies in 139 infants. Of these, 10 were positive and although numbers were small, the highest prevalence is clearly associated with the age groups showing rapid acquisition of HHV-6 antibody. The presence of IgM antibody to HHV-6 in the serum of an infant aged less than 1 month suggested the possibility of congenital infection. To investigate this, 235 cord blood specimens were examined for HHV-6 IgM. No positive results were detected.

DISCUSSION

Although early reports of HHV-6 seroepidemiology indicated rather low infection rates in adults [1, 6] later reports have contradicted these findings [7, 8]. These discrepancies may have been associated with technical variables in the IFAT methods used in the various studies. In Western Australia we have found very high rates of infection in adults [8]. The data in this report confirm and extend our previous findings of early infection amongst children. This has also been noted by other workers [12]. We have also reported evidence of frequent shedding of HHV-6 in saliva of normal adults [4, 5]. This would suggest a possible vehicle for the rapid and early transmission of HHV-6 to young children.

The transmission pattern of HHV-6 in the childhood population we have studied contrasts sharply with patterns for the other human herpesviruses. While infection with HHV-6 occurs rapidly after decline of maternal antibody at 3–4 months, VZV and HSV rates are only beginning to rise at 12 months of age. Infection with CMV and EBV is even slower to occur, although a plateau effect evident in the case of CMV may represent some background congenital infection. It is doubtful if congenital infection with HHV-6 would produce the type of antibody distribution shown in Fig. 1(*a*). The appearance of HHV-6 specific IgM antibody in some children after the general decline of maternal antibody and the lack of IgM antibodies in 235 cord blood specimens also weigh against the likelihood of congenital infection being responsible for the high antibody rates noted in children.

Other studies have shown that the childhood disease of exanthem subitum is accompanied by seroconversion to HHV-6 in some cases [13] and virus has been isolated from peripheral blood lymphocytes in the acute phase of illness. Of the patients with HHV-6 specific IgM in this study only two had a rash, which in both cases was maculo-papular. The clinical histories of the other children showed no consistent pattern likely to be associated with HHV-6 which suggests that infection with this virus is often inapparent.

It has also been suggested that HHV-6 may be associated with an infectious mononucleosis-like syndrome in adults [14]. However, if the high rate of HHV-6 infection in children found in this study proves to be generally true, primary infection in adults leading to this condition is likely to be fairly uncommon.

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