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Production of IgM antibody to HHV6 in reactivation and primary infection

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SUMMARY

The cross-reaction of HHV6 antibody with that to the other herpesviruses was studied in 96 blood donors whose sera were tested for IgG antibody to human herpesvirus type 6 (HHV6), cytomegalovirus (CMV), Epstein–Barr virus (EBV), varicella zostervirus (VZV) and herpes simplex virus (HSV). No correlation was found between IgG antibody to HHV6 and that to any of the other herpesviruses in these individuals. Antibodies to HHV6 and CMV were measured in patients undergoing documented serological responses to HHV6. Eleven cases of primary HHV6 infection associated with roseola infantum in babies, 1 of whom suffered from gastroenteritis as well as pyrexia and rash, are reported. Three cases of HHV6 reactivation, 1 in a 3-year-old child and 2 in adults, 1 of whom simultaneously underwent a primary CMV infection are also reported. Our results suggest that indirect immunofluorescence is a specific way of measuring HHV6 antibody, that HHV6 IgG and IgM can be detected in the absence of antibody to CMV and that HHV6 IgM is present both in primary HHV6 infections and in reactivations.

INTRODUCTION

During attempts to isolate pathogenic retroviruses from West African patients in late 1986 a lymphotropic herpesvirus was isolated in our laboratory [1]. Two other research groups also described the isolation of similar viruses from patients with AIDS or other lymphoproliferative disorders [2, 3]. These viruses have double-stranded DNA with a genome of approximately 110 kb pairs [2]. Endonuclease restriction mapping of the genome and serological studies suggested that these isolates comprised a newly recognized human herpesvirus which has now been designated tentatively as human herpesvirus type 6 (HHV6).

A number of clinical manifestations have been suggested for primary infection with and reactivation of HHV6 in both children and adults. Yamanishi and colleagues [4] reported isolation of HHV6 and concomitant seroconversion for anti-HHV6 IgG in young children with roseola infantum (exanthem subitum). In

adults HHV6 infection has been associated, provisionally, with many clinical symptoms including enlarged lymph nodes [5] and a mononucleosis-like illness [6]. There has been discussion whether adult patients showing HHV6 seroconversion do so in relation to HHV6 infection alone or whether a CMV infection elicits a response to anti-HHV6 as well as anti-CMV. There have been two reports of concurrent CMV and HHV6 seroconversion in adults [6, 7]. Others have shown the presence of IgM antibody to both CMV and HHV6 in renal and cardiac transplant patients [8].

Initial serological studies using an indirect immunofluorescence (IF) test to detect antibody to our strain (AJ) of HHV6 showed that 52% of healthy adults were seropositive for anti-HHV6 IgG and that infection with the virus occurred very early in life [9]. To assess any serological cross-reaction between HHV6 and other human herpesviruses we examined sera from 96 blood donors for evidence of an association between anti-HHV6 and antibody to herpes simplex virus (HSV), varicella-zoster virus (VZV), CMV and Epstein–Barr virus (EBV). We have developed an assay for IgM antibody to HHV6 and have used this together with measurement of anti-HHV6 IgG to study the antibody responses in 11 children with clinical roseola infantum and 2 adults and 1 child identified retrospectively to have significant fluctuations in HHV6 antibody levels.

METHODS

HHV6 IgG was measured by IF. Briefly, JJhan cells (a T lymphocyte cell line) infected with the AJ strain of HHV6 were placed on multispot slides, air-dried and fixed with acetone. Serum which had previously been absorbed with uninfected JJhan cells was applied at a dilution of 1/50. The slides were incubated at room temperature for 40 min before washing in PBS and applying fluorescein-conjugated anti-human IgG (Dako) at a dilution of 1/100. The slides were left at room temperature for a further 20 min, washed again, mounted in PBS/glycerol and examined. Dilutions of serum and conjugate were made in PBS.

Indirect immunofluorescence was also used to detect IgM antibody to HHV6. The protocol used was similar to that for IgG except that prior to screening for IgM the sera were treated with an anti-IgG compound (Behring) or passed down an ion-exchange chromatography column (Isolab) to remove competing IgG. The sera were tested at a dilution of 1/24 and the incubation time was increased to 3 h at room temperature. After washing, fluorescein-conjugated anti-human IgM (Dako) at 1/30 was applied to the slides for 1 h at room temperature. The slides were then rewashed, mounted and examined as before. In some cases Evans blue (1/1000 in the diluted conjugate) was used to counterstain the non-fluorescing cells.

Sera positive by immunofluorescence were scored +, ++, or +++ depending on the intensity of the fluorescent staining as previously described [9]. On evaluating the test it was found that the titre by end-point dilution closely correlated with the score given at a fixed dilution. Where reactivation of the virus was thought to have taken place, sera were titrated in doubling dilution to an end point so that slight differences in IgG titre could be more easily shown. Anti-HSV and anti-VZV were measured using the complement fixation test (CFT). Antibody

to CMV was measured either by CFT or by using one of the commercial solid-phase assays (Pharmacia enzyme immunoassay kits for IgM and IgG and Mercia Diagnostics CMV ELA-IgM kit). P3HR1 cells were used in a standard indirect immunofluorescent test for anti-EBV IgG. Sera to be tested for EBV antibody were screened at a dilution of 1/200, positive sera were scored +, ++, +++ or ++++ depending on the intensity of the fluorescent staining.

Blood samples

Blood donors. Serum samples were available from 96 random blood donors attending the North London Blood Transfusion Centre.

Patients; children. Patients 1–9 were infants aged between 2 and 22 months who were examined, and in some cases admitted to hospital (patients 1–3 and 5), because of a recent onset of high fever and rash consistent with a diagnosis of roseola infantum. The clinical symptoms of 'classical' roseola infantum have been reviewed by Stammers [10]. The onset is marked by abrupt fever (the temperature may rise to 39–41 °C) in a child who is often listless and irritable. Enlargement of the posterior cervical lymph nodes is common as is mild pharyngeal inflammation. The temperature usually lasts 3–5 days and the appearance of the rash coincides with subsidence of the fever. The rash consists of small pink discrete macules which fade on pressure. The exanthem appears first on the neck and trunk but may spread to involve other parts of the body and typically lasts 1–2 days.

Patient 10 (a 9-month-old child) was described as having a pyrexia of unknown origin. The pattern of fever was similar to that found in roseola infantum but no rash was reported. It is known, however that in some cases the roseola rash may only be visible for a few hours.

Patient 11 had all the symptoms of roseola infantum but also suffered from moderately severe gastroenteritis. This 5-month-old child was hospitalized 2 days after the onset of a head cold when she began to have diarrhoea and moderate vomiting. At this time a rash also began to appear over her cheeks and upper trunk. The child had a temperature of 38·8 °C.

Patient 12 was a 3-year-old child who was hospitalized with a mononucleosis-like illness and tender lymphadenopathy. On admission to hospital he was pale with moderate anterior cervical lymphadenopathy and hepatosplenomegaly; his blood film showed an iron-deficiency anaemia and many glandular-fever-like lymphocytes. A serum sample taken at this time was monospot positive and a presumptive diagnosis of EBV infection was made. Four weeks prior to admission the child had had a throat infection with multiple mouth ulcers which did not respond to penicillin. After this treatment he had a poor appetite and loose pale stools which had become watery by 2 days prior to his admission. Over the next month his diarrhoea persisted and he failed to gain weight although no microbiological cause was found. Four months after his illness began he started to improve and put on weight and, in spite of occasional episodes of diarrhoea, he remained healthy. Serum samples taken during his long illness were tested for anti-HHV6 IgM and IgG as well as antibody to CMV and EBV.

Control serum samples were available from seven cases of childhood pyrexia and rash associated with diagnosed enteroviral or rubella infections.

Patients: adults. Patient 13 was a male aged 50 years who had tender

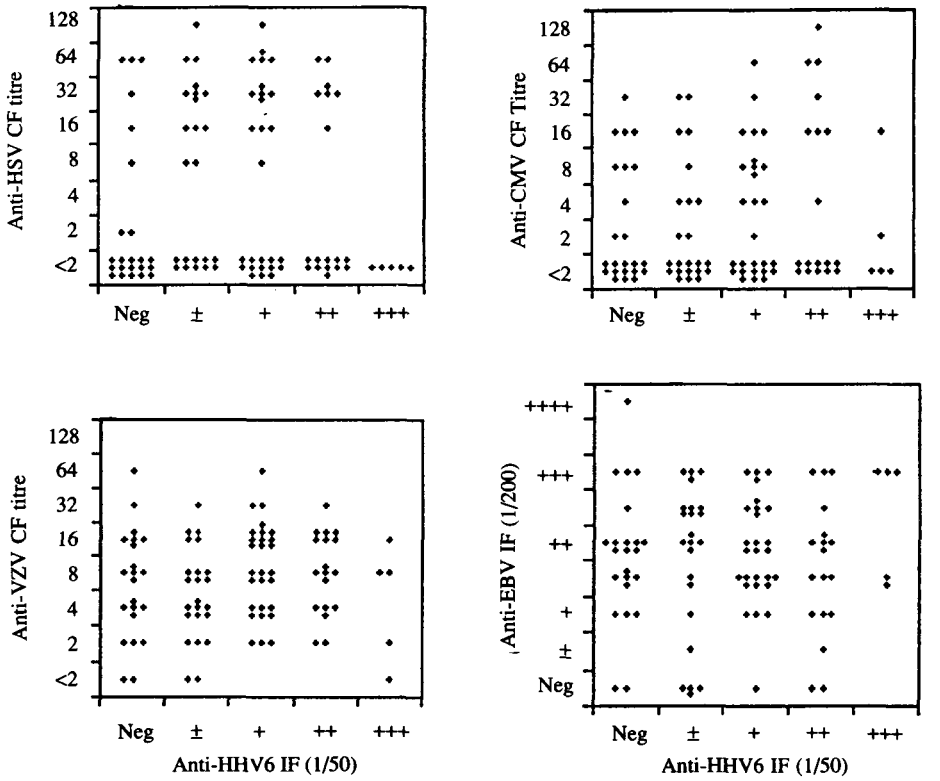

Fig. 1. Correlation graphs comparing antibody levels to HHV6 and each of the other herpesviruses in 96 blood donors. Antibody to CMV, HSV and VZV was measured by the complement fixation test (CF). Anti-HHV6 and EBV IgG was measured by indirect immunofluorescence.

lymphadenopathy, atypical lymphocytosis and persistent pyrexia (39 °C). Blood samples were taken regularly (from 11–167 days after onset) during the investigation of his persisting febrile illness.

Patient 14 was a healthy adult who was found, by random screening, to have a very high anti-HHV6 IgG titre. She had had mild symptoms of neck stiffness and fever (38 °C) 3 months prior to the HHV6 test. Sera were available from this individual from samples taken 8 months prior to this episode and from days 1, 42, 71, 92 and 127 after onset of illness.

RESULTS

Blood donors. The level of antibody to HHV6 and to each of the other herpesviruses was compared in sera from 96 blood donors (Figure). There was no correlation between the presence or level of HHV6 antibody and presence or level of antibody to any of the other herpesviruses in these individuals.

Patients: children. Serum samples from the infants with roseola infantum (patients 1–11, Table 1) showed seroconversion for anti-HHV6 IgG and all had detectable levels of anti-HHV6 IgM in one or more of the samples tested (except patient 4 who had equivocal levels of IgM in the first sample). Where there was

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Table 1. *Antibody to HHV6 and CMV in 11 children with roseola infantum*

Patient	Day serum taken*	Anti-HHV6 IgG	Anti-HHV6 IgM	Anti-CMV IgM
1	−2	−	−	−
	3	±	+	−
	44	+++	±	−
2	−1	±	−	−
	6	+/++	++	−
3	2	±	+	−
	14	+	++	−
4	1	±	±	−
	13	++	−	−
5	5	++	+	−
	22	++	+	−
6	1	−	+	−
	29	+/++	+	−
7	1	±	−	−
	30	++	+	N.D.
8	1	−	−	N.D.
	17	+	+	−
9	1	−	−	N.D.
	43	+/++	++	N.D.
10	6†	−	+	N.D.
	76	++	++	N.D.
11	−2	−	−	N.D.
	12	+++	+	N.D.

* Day 1, day of onset of pyrexia and rash. † No rash was reported in this case.
N.D., not done.

sufficient serum to test for CMV IgM those samples from patients 1–11 were negative. Fifteen serum samples from seven cases of childhood pyrexia and rash associated with enteroviral or rubella infection were also tested for HHV6 IgM and CMV IgM with negative results (data not shown). The average age of the infants in this group was similar to that of the cases of roseola infantum described.

Routine serology and microbial culture on samples from patient 11 gave no other diagnostic results, although rotavirus antigens were detected by ELISA. The patient seroconverted for HHV6 IgM and IgG, consistent with the diagnosis of roseola infantum.

The first sample from patient 12 was taken at the time of admission to hospital, 4 weeks after the child's illness began, and was positive for both HHV6 IgM and IgG (Table 2). It was positive for CMV IgM and EBV VCA IgM but negative for CMV and EBV VCA IgG. The second sample was taken at about the time the child began to improve and this was again positive for HHV6 IgM and IgG. The patient was now EBV IgM negative but EBV VCA positive, consistent with having had a primary EBV infection. The third sample which was taken 6 months after the illness began was positive for HHV6 and EBV VCA IgG but was negative for IgM. The second and third samples from patient 12 were CMV IgG negative.

Patients: adults. A serum sample taken from patient 13 on day 11 of his illness contained anti-HHV6 IgG. IgM antibody to HHV6 was first detected on day 18 and there was an eightfold drop in HHV6 IgG between days 25 and 167. CMV IgM and IgG was not detected in any sample.

Table 2. *Antibody to HHV6 and CMV in three cases of HHV6 reactivation*

Patient	Day serum taken*	Anti-HHV6 IgG titre	Anti-HHV6 IgM	Anti-CMV IgM
12	28	512	+	+
	167	256	+	N.D.
	237	256	−	−
13	11	1024	−	−
	18	512	+/++	−
	19	512	++	−
	24	2048	+/++	−
	25	2048	+	−
	167	256	N.D.	−
14	−213	128	−	−
	1	128	+	−
	42	8192	++	+
	71	8192	+++	+
	92	4096	+	±
	127	4096	±	±

* Day 1, day of onset of illness.

A sample taken from patient 14, 213 days before the onset of illness showed a low level of anti-HHV6 IgG. On the first day of illness low level anti-HHV6 IgM was present and by the time of the second sample after onset (day 42) there had been a 64-fold increase in anti-HHV6 IgG together with a more marked anti-HHV6 IgM response. IgM antibody to CMV was detected and seroconversion for CMV IgG antibody were consistent with a primary CMV infection. Coxsackie A9 was isolated from a throat swab taken on day 1 of the patient's illness.

DISCUSSION

The prevalence and titre of antibodies to HSV, VZV, CMV, EBV, and HHV6 in the sera from blood donors gave no indication of cross-reactivity between HHV6 antibody and antibody to any of the other four human herpesviruses. Though minor cross-reactions might be detected by techniques such as Western blotting and radio-immune precipitation of antigen (RIPA), with the methods used here there was no evidence to suggest that such cross-reactions might lead to diagnostic difficulties.

Primary infections with HHV6 were shown to occur in the first year of life. It is likely that many of these infections would have occurred in the presence of waning levels of transplacental antibody which might have attenuated the infants' antibody response. In spite of this we were able to demonstrate easily the presence of IgM antibody to HHV6 in these infants. The IgM response appeared at about the same time as IgG antibody in infants undergoing seroconversion for HHV6 and we consider the presence of anti-HHV6 IgM to be a good marker of primary HHV6 infection in the investigation of cases of pyrexia and skin rash in infants. In our group of 11 patients only 1 infant with roseola infantum (patient 4) failed to develop convincing levels of HHV6 IgM antibody. Yamanishi and colleagues [4] have described anti-HHV6 seroconversion with virus isolation in children with roseola but, unlike ourselves and others [11] did not describe the detection of IgM anti-HHV6.

It is possible that the rotavirus gastroenteritis suffered by patient 11 was a more severe form than is seen normally due to coincident primary HHV6 infection. In at least one child a dual infection was documented and showed the possibility of a synergistic effect. Patient 12 suffered a primary EBV infection and possibly a primary HHV6 infection. The presence of HHV6-specific IgM in 2 of the 3 samples tested indicates a productive HHV6 infection during the same time span. The first sample from this patient was taken 4 weeks after the first clinical symptoms and so it was not possible to distinguish between a primary HHV6 infection or reactivation in this case. The patient did not have the 'classical' symptoms of pyrexia and rash and was older than most children who suffer from roseola infantum. CMV IgM was detected in the first sample from patient 12 but, because no CMV IgG was detected in any of the samples, this IgM response was likely to have been non-specific.

The diagnostic value of anti-HHV6 IgM among adult patients is less clear. The question of cross-reactivity between CMV and HHV6 in primary infections remains partly unanswered. Kirchesch and colleagues [6] have described concomitant rises of IgM antibody to HHV6 and CMV accompanying an episode of fever and rash in an adult undergoing a chronic glandular-fever like illness. Reports both of CMV reactivation and HHV6 seroconversion [7] and of HHV6 IgM recrudescence in a patient with primary EBV infection [6] indicate that the presence of IgM anti-HHV6 should be interpreted cautiously. In the two adults in this series (patients 13 and 14) we found clear evidence of the production of IgM anti-HHV6 in individuals already seropositive for IgG. The causes of HHV6 reactivation remained unidentified but patient 14 had both a Coxsackie A9 and a primary CMV infection and it is possible that one of these might have been a trigger for reactivation.

Demonstration of a rise in IgG titre and the appearance of IgM must be interpreted with caution when seeking an aetiological association for HHV6 infection in patients with illness. Because of the possibility of reactivating the virus HHV6 the isolation of HHV6 or the localization of its genome in diseased tissue should also be assessed with care since either could be the result of reactivation of latent virus and not primary infection. Though this caveat is most relevant to disease in adults where reactivation may be the most likely explanation of the presence of HHV6 IgM, older infants like patient 12 may also show serological responses compatible with reactivation. Nevertheless, in infants, detection of HHV6 IgM is likely to be a reliable marker of acute primary HHV6 infection. In view of the pattern of illness seen in patient 11, in whom severe rotavirus gastroenteritis was present together with roseola infantum, we feel that it may be valuable to broaden the diagnostic use of tests for HHV6 IgM in infants to examine the spectrum of illness associated with primary HHV6 infection.

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