ORIGINAL ARTICLES

Roseola infantum and other syndromes associated with acute HHV6 infection

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Abstract

Eight cases of acute human herpesvirus type 6 (HHV6) infection in infants were diagnosed serologically by the demonstration of IgM anti-HHV6 (8/8) and a significant change in total anti-HHV6 antibody titre (6/8). Four infants were sufficiently ill to require admission to hospital and further investigations: one with encephalitis and three with gross hepatosplenomegaly, two of whom had evidence of simultaneous infection with another herpesvirus. The remaining four infants had an illness compatible with roseola infantum, although this diagnosis had not been made clinically. Sera from two of those infants with rash had been sent for analysis to exclude rubella because the infants' mothers were pregnant. The other two had received antibiotics when febrile, and the subsequent appearance of the roseola rash had raised the possibility of antibiotic allergy.

The data suggest that there are clinical syndromes in addition to roseola infantum associated with the presence of IgM anti-HHV6, in which serological screening for evidence of acute HHV6 infection may be useful.

Human herpesvirus type 6 (HHV6) is a recently identified human lymphotropic virus of diverse cell tropism in vitro.[1–3] Serological surveys have shown a high prevalence of antibodies to HHV6, and have suggested that the majority of HHV6 infections occur in the first year of life.[4–6] Descriptions of clinical illness in infants associated with seroconversion to HHV6 are limited to the syndrome of roseola infantum (also known as exanthem subitum), two infants with a rash and lymphadenopathy and two infants with a hepatitic illness, one fatal.[6–9] There are additional reports of acute HHV6 infection in older children and adults associated with hepatitic and infectious mononucleosis like illnesses.[10 11]

In order to identify possible clinical consequences of acute HHV6 infections we have devised a strategy for screening sera for the presence of IgM anti-HHV6.[10] This has been applied to sera from infants presenting with a variety of illnesses, resulting in the identification of eight acute HHV6 infections, none of which had been diagnosed clinically as roseola infantum. The clinical features of those eight infants described here illustrate that HHV6 serology may be useful in the investigation of infants with encephalitis or hepatosplenomegaly, in the investigation of a rash in an infant whose mother is pregnant, and to exclude either the need to prescribe antibiotics in a febrile child, or the diagnosis of antibiotic allergy.

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Methods

SELECTION OF SERA

During the course of this study (December 1988 to February 1990) all sera sent to the virology laboratory, Westmead Hospital, from infants up to the age of 3 years were tested for evidence of recent HHV6 infection. In addition, sera from infants presenting with hepatosplenomegaly were kindly referred to us from the Royal Alexandra Hospital for Children, Sydney, the Royal Children's Hospital, Melbourne, and the Royal Brisbane Hospital, Brisbane. The infants fell into the following diagnostic categories (number of sera): neonates with jaundice, congenital abnormalities, or those labelled for screening for toxoplasma, other viruses, rubella, cytomegalovirus, and herpes virus (n=31); children with fever (n=6); rash (n=21); encephalitis (n=6); hepatomegaly and/or splenomegaly (n=10); glandular fever like illness (including tonsillitis, lymphadenopathy (n=17); clinical details not given (n=7); and miscellaneous (father carrier of hepatitis B virus, nephrotic syndrome, ?idiopathic thrombocytopenia, ?mumps, ?dengue (n=5).

SEROLOGY

Sera were screened by indirect immunofluorescence for the presence of IgM and IgG anti-HHV6 antibodies as previously described, using acetone fixed HHV6 infected J Jhan cells (a T cell line) as substrate.[10] Briefly, sera were screened at dilutions of 1/100 for IgG and 1/20 for IgM in phosphate buffered saline containing 1% casein. Screen positive sera were retested for IgM after a 1/10 dilution in an anti-IgG reagent (Gullsorb, Gull Laboratories) for absorption of IgG and removal of rheumatoid factor. Fluorescein conjugated second antibodies used were sheep antihuman immunoglobulin (Wellcome), and $F(ab')_2$ fragment goat antihuman IgM (Kallestad). No non-specific fluorescence was seen when sera were tested using acetone fixed uninfected J Jhan cells as a negative control.

Commercially available enzyme immunoassay kits were used for the detection of IgG and IgM anticytomegalovirus (Pharmacia), and IgG and IgM antirubella antibodies (Behring). IgM anti-Epstein-Barr virus was assayed using an in house enzyme immunoassay,[12] and IgG anti-Epstein-Barr virus by indirect immunofluorescence using Epstein-Barr virus infected

Acute HHV6 infection in infants: serological data

Case No (sex)	Age (months)	Illness	Date of serum sample (days)*	HHV6		Cytomegalovirus		Epstein-Barr virus			Other negative serology
				IgG†	IgM	IgG	IgM	IgG	IgM	Epstein-Barr nuclear antigen	
1 (F)	13	Encephalitis	0	1024	Positive		Negative				Herpes simplex virus,‡ mumps, mycoplasma, measles, enterovirus, varicella
			10	1024	Positive	Positive	Negative		Negative		
			24	512	Negative						
			185	256	Negative						
2 (M)	10	Hepatosplenomegaly	0	16	Negative	Negative	Negative				
			16	128	Positive	Negative	Negative		Negative		
			42	128	Negative						
3 (M)	6	Hepatosplenomegaly	0	8	Positive	Negative	Negative	2560	Negative§	Negative	
			14	2048	Positive	Negative	Negative	2560	Positive	Negative	
4 (M)	16	Hepatosplenomegaly	0	512	Positive	Equivocal	Positive	Positive	Negative		
			13	512	Positive						
5 (M)	12	Roseola infantum	0	8	Negative						Measles, adenovirus, enterovirus, rubella
			15	256	Positive	Negative	Negative		Negative		
6 (F)	8	Roseola infantum	0	8	Positive	Positive	Negative	Negative	Negative		Measles, rubella
			11	256	Positive	Positive	Negative	Negative	Negative		
7 (M)	19	Roseola infantum	0	<4	Positive	Negative	Negative	Negative	Negative		Measles
			27	64	Negative						
8 (F)	8	Roseola infantum	0	64	Positive						Measles, rubella
			7	128	Positive						

*Day 0 taken as the date of the first serum sample.
†Titre as determined by immunofluorescence.
‡Herpes simplex virus, complement fixation titre less than 4 in all samples.
§Epstein-Barr virus IgM negative by enzyme immunoassay but positive by fluorescence.

marmoset lymphoblastoid cells as substrate. Anti-Epstein-Barr nuclear antigen antibodies (anti-EBNA) were determined by an anti-complementary immunofluorescent technique.[13] All other antibody titres were assayed by a standard complement fixation test.

Results

Sera from eight patients were found to contain IgM anti-HHV6. The serological results are summarised in the table.

CASE REPORTS

Case 1

A 13 month old girl presented with right sided focal seizures. She had been unwell for one week, initially with rhinorrhoea and cough, and in the two days before admission became increasingly lethargic and began vomiting. Four right sided seizures with variable involvement of face, arm, and leg, each lasting less than 10 minutes, occurred on the morning of admission.

On examination she was drowsy and miserable, with a temperature of 37·9°C. A blotchy erythematous rash was present over her upper trunk. Her eyes tended to deviate to the left and there were signs of mild right sided weakness. Results of investigations were haemoglobin concentration 13 g/l, white cell count $15{\cdot}1\times10^9$/l with 75% granulocytes and platelets 236×10^9/l. Serum electrolytes, blood glucose, calcium, magnesium, and phosphate concentrations were all normal. In the cerebrospinal fluid there was one polymorph, 14 mononuclear cells and 85 red blood cells $\times10^9$/l. Cerebrospinal fluid protein was 160 mg/l and glucose 5·5 mmol/l. Cerebral computed tomography showed loss of grey white differentiation in the left frontotemporal regions with loss of the sulcral pattern indicating mass effect. Electroencephalography showed pronounced slowing from the left hemisphere with high voltage 1/2–3 hertz activity appearing at times in a semiperiodic fashion.

She was treated with phenobarbitone (loading dose of 10 mg/kg followed by 5 mg/kg/24 hours) and acyclovir (10 mg/kg every eight hours for 10 days). No further seizures occurred and by 24 hours after admission her neurological state had returned to normal. She continued to spike temperatures up to 38·5°C over the next few days but otherwise completed her course of acyclovir without further problems. When last reviewed at age 19 months there were no definite neurological signs. She had been walking for three months and her parents stated she had a vocabulary of 50 words. Repeat computed tomography showed atrophic changes in the left frontotemporal region.

Case 2

This 10 month old boy had been admitted to hospital with a febrile fit and pneumonia, and then discharged. One week later he presented with a history of two days of fever followed by the appearance of a dense fine rubelliform rash over the limbs and trunk (but not face), 7 cm hepatomegaly and 4 cm splenomegaly, and shotty diffuse lymphadenopathy, none of which had been present during his hospital stay. A full blood count showed a haemoglobin concentration of 10·9 g/l, a leucocyte count of $12{\cdot}6\times10^9$/l with a lymphocytosis of 55% and 26% atypical mononuclear cells. Liver function tests were normal. The rash lasted for 24 hours only. The cause of his hepatosplenomegaly remained unclear. His physical signs resolved slowly over the following three months, eventually disappearing completely.

Case 3

This 6 month old boy presented with fever and cervical lymphadenopathy 48 hours after triple antigen immunisation. Twenty four to 48 hours later he developed a fine rubelliform rash over the face and trunk, cervical lymphadenopathy, and mild splenomegaly. At this stage a full blood count showed a microcytic normochromic

anaemia and neutropenia, with atypical mononuclear cells. His rash disappeared after 48 hours. Two days later he was admitted to hospital with hepatosplenomegaly, anaemia, neutropenia, and thrombocytopenia. Several investigations were performed to ascertain the cause of the child's illness, including bone marrow aspiration, but no diagnosis was reached. The physical signs and the abnormal haematology resolved spontaneously over a period of several months.

Case 4
This 16 month old boy presented with fever, respiratory distress, hepatosplenomegaly, and cervical lymphadenopathy. He was also noted to have a few petechial lesions over one cubital fossa, of 24 hours duration. A diagnosis of bronchopneumonia was made. A chest radiograph showed patchy consolidation throughout both lung fields which resolved within a week. Respiratory syncytial virus was isolated from a nasopharyngeal aspirate. Investigation of his hepatosplenomegaly included a full blood count (haemoglobin concentration 10·8 g/l, white cell count of $19{\cdot}2\times10^9$/l with 51% lymphocytes and 16% atypical mononuclear cells). Liver function tests were also performed and his aspartate aminotransferase activity was 80 U/l (normal range 15–55); γ-glutamyltransferase and bilirubin were normal. A diagnosis of cytomegalovirus infection was made on the basis of a positive IgM anticytomegalovirus assay and isolation of cytomegalovirus from a urine sample taken one week after presentation. He was well on review a month later, and no further mention has been made of hepatosplenomegaly on subsequent clinical examinations.

Case 5
This 12 month old boy was admitted to hospital with a two week history of rhinorrhoea and a dry cough, and 48 hours of fever (40°C). There was no lymphadenopathy, hepatomegaly, or splenomegaly noted. The fever settled spontaneously after a further 24 hours, and a rash noted 24 hours later. As his mother was six weeks pregnant, acute and convalescent serum samples were sent to the laboratory for rubella, measles, adenovirus, and enterovirus serology.

Case 6
This 8 month old girl presented with a mild fever and a rubelliform rash confined to the trunk, described as flat pink macules 3·5 mm in diameter, without crusting or excoriation. Mild cervical lymphadenopathy was noted, but no hepatomegaly or splenomegaly. The rash resolved after four days. Acute and convalescent sera were sent to the laboratory for rubella and measles serology as the child's mother was pregnant.

Case 7
This 19 month old boy presented with a four day history of fever (>38°C), cough, and sore throat. Mild cervical lymphadenopathy was noted. A provisional diagnosis of streptococcal pharyngitis was made, and penicillin prescribed. Forty eight hours later his fever was resolving, but a rubelliform rash was noted, ascribed to either measles virus infection, or to penicillin allergy. The rash lasted three to four days, by which time all other symptoms and signs had resolved.

Case 8
This 8 month old girl presented with an upper respiratory tract infection, treated with ampicillin and subsequently erythromycin. A morbilliform rash then developed, lasting four days. A convalescent serum was sent to the laboratory for measles serology.

SEROLOGY
IgM anti-HHV6 was demonstrated in at least one serum sample from each infant. In five of the infants it was also possible to demonstrate a rise in IgG anti-HHV6 titre in paired sera, while in two of the remaining three infants (cases 1 and 4), the titre in the acute serum was already raised. IgM anti-Epstein-Barr virus was detected only in sera from case 3, and IgM anticytomegalovirus only in sera from case 4. Additional negative serology from individual patients is given in the table.

Discussion
HHV6 has recently been identified as the causative agent of roseola infantum.[7] This disease is characterised by an abrupt onset of fever, which lasts three to five days before subsiding, when a maculopapular rash appears on the neck and trunk. The rash may be fleeting, or may not be detected at all.[14] Roseola is generally considered to be a benign disease, but it was suggested as long ago as 1949 that atypical, more severe forms of the disease may occur.[15]

While serological diagnosis of herpesvirus infections may not be able to distinguish between primary and reactivated infections with such viruses, it is most likely that the presence of IgM anti-HHV6 in sera from infants in the first two years of life is indicative of primary infection. Thus we believe that the eight infants described in this paper had indeed suffered recent HHV6 infections.

Four of the infants we report had severe illnesses requiring admission to hospital, temporally related to the presence of IgM anti-HHV6 (4/4) and rising titres of IgG anti-HHV6 (2/4) in their sera. Case 1 was suspected of having herpes simplex encephalitis, and treated accordingly with acyclovir. However, her illness was unusually mild for this disease, and she failed to develop any antibodies to herpes simplex virus in a serum sample taken seven months after her illness. In addition, she had serological evidence of a recent HHV6 infection, as well as a rash consistent with roseola infantum, and it is likely that her encephalitic illness was related to this. Convulsions are well recognised

as a common occurrence in roseola infantum, and there are several reports of transient (stupor, coma, hemiparesis, bulging fontanelles) and permanent (hemiparesis) neurological sequelae of the disease.[16–20] The authors of those reports raised the possibility of a 'specific encephalitis due to the roseola virus', although in the absence of an identifiable causative agent none were able to substantiate the hypothesis. Prospective diagnosis of similar cases, both serologically and by virus isolation, should finally allow proof of those astute clinical observations.

The predominant feature of the illness in cases 2–4 was hepatosplenomegaly. This has not, to our knowledge, been reported previously in either roseola infantum or in acute HHV6 infection, although there are two reports of hepatitis in infants, one fulminant and fatal, due to HHV6 infection.[8 9] All three infants had a fleeting rash, noted to appear after defervescence in cases 2 and 3. The diagnosis of roseola infantum due to acute HHV6 infection seems to be clearcut in case 2. The simultaneous appearance of IgM anti-Epstein-Barr virus or anticytomegalovirus in cases 3 and 4, however, make their diagnoses more problematic. The presence of dual antibody rises to HHV6 and cytomegalovirus has been noted previously,[21] and we have shown that such dual rises are not due to cross reactive antibodies.[22] Dual primary infections, primary infection with one virus plus reactivation of the second virus, or dual reactivations are all theoretical possibilities. The age of case 3 (6 months), the very low concentration of IgG anti-HHV6 in his acute serum, and the absence of anti-EBNA in both his sera make simultaneous primary infection with HHV6 and Epstein-Barr virus the most likely explanation of his serological findings. It is not possible to distinguish between the different scenarios in case 4 on the evidence available. Primary Epstein-Barr virus infection in childhood is usually accompanied by asymptomatic seroconversion, and postnatal primary cytomegalovirus infection is rarely a severe illness. It is tempting to speculate that the combined effects of infection with either of these viruses plus HHV6 are the cause of the considerable morbidity suffered by these two patients. There have been other reports of unusually severe illnesses due to double virus infections recently.[23 24]

The remaining four infants presented with illnesses entirely compatible with roseola infantum, although none was diagnosed clinically as such. The primary indication for serological testing in cases 5 and 6 was maternal pregnancy. A rapid diagnosis of acute HHV6 infection, by the demonstration of IgM anti-HHV6 in an acute serum sample, provides considerable reassurance in cases such as these. Cases 7 and 8 illustrate a further practical advantage in encouraging the accurate diagnosis of roseola, namely the avoidance of inappropriate prescribing of antibiotics in a febrile child.[14] Furthermore, if the diagnosis is roseola, the child may be wrongly labelled as allergic to the drug.

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