SHORT COMMUNICATIONS

Isolation and Identification of Human Herpesvirus 7 from An Infant with Exanthem Subitum

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Summary

Exanthem subitum (ES) is a common childhood exanthematous disease. In a recent study of ES due to human herpesvirus 6 (HHV 6), we isolated human herpesvirus 7 (HHV 7) from the peripheral blood mononuclear cells (PBMC) of a seven month-old infant with typical symptoms of ES. The identity of the virus was confirmed by indirect immunofluorescence using HHV 7 specific monoclonal antibody and by amplification of the HHV 7 specific genomic sequences using the polymerase chain reaction (PCR). Paired serum samples from the infant showed serological conversion to the isolated virus. The clinical manifestations of ES in this infant appeared to be milder than the classical ES due to HHV 6.

Key Words: Exanthem subitum, human herpesvirus 7.

Introduction

A recent seroepidemiological study of human herpesvirus 6 (HHV 6) conducted by Chua *et al.*[1] revealed that it is an ubiquitous virus in Malaysia and primary infection occurs mainly during childhood. In the study, it was found that 83.7% of 600 randomly collected serum samples were positive for HHV 6 antibodies and that 80% of children between 2 to 5 years of age were tested seropositive. There was no significant difference between sexes or among the 3 major ethnic groups.

HHV 6 is the most common causative agent of exanthem subitum (ES) in childhood. However, Frenkel *et al*[2] in 1990 isolated a novel human herpesvirus from CD4+ lymphocytes of a healthy adult and this virus, known as human herpesvirus 7 (HHV 7) has been demonstrated to cause ES by Tanaka *et al*[3] and subsequently confirmed by Ueda *et al*[4], Hidaka *et al*[5] and Torigoe *et al*[6]. Seroepidemiological studies indicated that primary infection of HHV 7 occurs mainly in childhood but the age of infection appears to be later than that of HHV 6 (Wyatt *et al*[7]). However, HHV 7 infection as the cause of ES has not been reported outside of Japan.

In the course of studying an association of HHV 6 with exanthem subitum by Chua *et al*[8], HHV 7 was isolated from an infant with typical ES and the clinical and laboratory findings of this case are reported in this paper.

Material and Methods

Clinical history

A.H, a 7 month-old male infant was seen on 8 December 1996 with a history of sudden onset of fever, which started the previous evening. He was otherwise active, feeding normally and having normal bowel movement. His birth and neonatal history were

essentially normal. His mental and physical development was appropriate for his age and had completed the required schedule of immunization.

Physical examination revealed a small built infant with length and weight corresponding to 25th percentile of Boston Growth Chart. He was active, cheerful with normal anterior fontanelle. Axillary temperature of 38.5°C was noted. Systemic examination revealed no other abnormal finding except for the presence of 1.5 mm erythematous fleshy papules, one on each side of the junction of the base of uvula with the palatoglossal fold.

A provisional diagnosis of exanthem subitum was made and 2 ml of venous blood was collected from the infant for laboratory investigation after prior consent was taken from the parents. He was prescribed regular 6-hourly dose of syrup paracetamol and advised tepid sponging should the axillary temperature exceed 38.5°C.

On follow-up, he developed mild maculopapular rash on day 4 with subsidence of fever and the rash faded within a day. 2 ml of convalescent blood was collected for further laboratory investigation.

Virus isolation

The venous blood was separated into plasma and packed cells by centrifugation at 800 X g for 10 minutes. The plasma was kept for serological study. The packed cells were resuspended in 5 to 6 ml of phosphate buffered saline (PBS) and peripheral blood mononuclear cells (PBMC) were harvested by centrifuging in Ficoll-paque density gradient at 600 X g for 15 minutes. 2 x 10^6 PBMC were removed for detection of HHV 7 by nested PCR and the remaining cells were cultured in 25 cm^2 cell culture flasks containing 5 ml of RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), recombinant human interleukin-2 (IL-2, 10 units/ml); (Genzyme Diagnostics, USA) and phytohemagglutinin (PHA, 10 µg/ml); (Sigma Chemical Co. USA). The flasks were incubated at 37°C in 5% carbon dioxide and after 24 to 48 hours incubation, the culture medium was changed to RPMI 1640 containing 10% FCS only. The PBMC were examined daily for any cytopathic change. After 6 days of incubation, 0.2 ml of the PBMC culture was harvested and washed twice with PBS before final resuspension in 50 µl of PBS. 10 µl of the resuspended PBMC was spread over each well of a Teflon coated slide, dried over a warm plate and fixed in cold acetone for 10 minutes. The fixed cells were stained for HHV 7 specific antigen by indirect immunofluorescence using monoclonal antibody, KR-4 specific for HHV 7, and followed by a second antibody (goat anti-mouse) conjugated with fluorescein isothiocyanate before being viewed under 40 X dry objective using a BH2-RFCA Olympus UV microscope. Monoclonal antibodies OHV-1 and P150 (Chemicon, USA) specific for HHV 6 were used as controls.

Concurrently, 1.5 ml of the PBMC culture was co-cultured with 5 ml (1 to 3 X 10^5 cells per ml) of activated human cord blood mononuclear cells (HCBMC) and another 5 ml of activated HCBMC was cultured as a virus negative control. After 2 to 3 days of co-cultivation, the mononuclear cells were observed for cytopathic effect and harvested for subsequent viewing via electron microscopy.

Electron Microscopy

After 2 days of incubation, infected HCBMC were centrifuged at 800 X g for 10 minutes. The cell pellet was fixed in 4% glutaraldehyde for 8 to 12 hours. The fixed cells were then transferred into cacodylate buffer with osmiun tetroxide for 2 hours and kept in cacodylate buffer overnight. The following day, the cells were washed with double distilled water and stained with uranyl acetate for 10 minutes after which the cells were re-washed with double distilled water. The cells were then dehydrated with graded concentration of alcohol from 35% to 100%, conditioned with propylene oxide followed by propylene oxide/epon mixture, and finally embedded in epon at 60°C for 12 hours. The embedded cells were sectioned at 60-70 nm thickness, stained with uranyl acetate followed by lead acetate, and viewed with a Philip CM-12 transmission electron microscope.

Detection of HHV 7 Genomic Sequences in Patient's PBMC

The presence of HHV 7 genomic sequences was examined using nested PCR. Infected PBMC were lysed in K-buffer (50 mM KCL, 10 mM Tris-HCL [pH 8.3], 3 mM $MgCl_2$, 0.45% Nonidet P-40, 0.45% Tween 20) containing proteinase K (100 µg/ml) at a concentration

of 2 x 10^6 cells per 200 µl. The cell suspension in a microfuge tube was incubated in a water bath at 60°C overnight and for 10 minutes at 98°C to inactivate the proteinase K. Amplification was performed by adding 10 µl of each sample to 40 µl of reaction mixture [50 mM KCl, 10 mM Tris-HCl (pH 8.3), 3 mM $MgCl_2$, 200 µM each dNTP and 1.5 units of Taq polymerase (Promega)]. Specific oligonucleotide primers were used at 0.3 µM each. The outer primer pair (5'-AGTTCCAGCACTGCAATCG-3' and 5'-CACAAAAGCGTCGCTATCAA-3') and inner primer pair (5'-CGCATACACCAACCCTACTG-3' and 5'-GACTCATTATGGGGATCGAC-3')[9] were used to amplify the specific 264 base-pairs region of the reference strain of HHV 7 (KHR).

Thirty amplification cycles consisting of denaturation at 94°C for 1 minute, annealing at 60°C for 1 minute and extension at 72°C for 1 minute was performed. An additional extension step of 10 minutes at 72°C was added at the end of the 30th cycle. A second amplification step was performed using 2 µl of the PCR product as template under similar conditions as the first PCR step using the inner primers. The resulting amplified products were electrophoresed in 1% agarose gel containing ethidium bromide and viewed with a UV transilluminator at 302 nm.

Serology

The acute and convalescent antibody titres of the infant were assayed by indirect immunofluorescence according to the method described by Chua *et al.*[1] However, activated human cord blood mononuclear cells, separately infected with HHV 7 (KHR strain) and HHV 6 (HST strain), were used as antigens. Commercial ELISA kit was used to detect the presence of measles and rubella specific IgM (Radim, Italy).

Results

The haematological findings of the acute blood specimen showed that the infant had mild anemia and moderate neutropenia (Table I) with no abnormal lymphocyte detected. A small proportion of the infant's acute PBMC underwent "ballooning"-type cytopathic effect after 6 days of culture (Figure 1) and these cells

Table I
Haematological profile of the infant with exanthem subitum due to HHV 7

Blood sample	Acute	Convalescent
Haemoglobin (g/L)	101	109
Total white blood cells (10^9 / L)	4.27	8.13
Neutrophils (%)	24.2	50.1
Lymphocytes (%)	49.4	35.5
Monocytes (%)	19.6	12.3
Eosinophils (%)	2.8	1.1
Basophils (%)	4.0	1.0
Platelets count (10^9 / L)	210	233

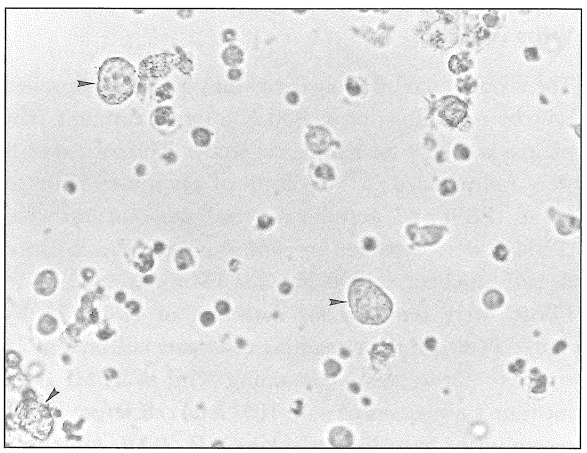

Fig. 1: Patient's peripheral blood mononuclear cells showing "ballooning"-type cytopathic changes after 6 days of culture (arrows). These cells stained positive with monoclonal antibody KR-4 (specific for HHV7) but negative with monoclonal antibodies OHV-1 and P150 (specific for HHV 6)

Table II
Serological profile of the infant with exanthem subitum against HHV 6 and HHV 7 by indirect immunofluorescence

	Acute plasma	Convalescent plasma
HHV 7 specific IgM	< 1 : 10	1 : 20
HHV 7 specific IgG	< 1 : 10	1 : 320
HHV 6 specific IgM	< 1 : 10	< 1 : 10
HHV 6 specific IgG	< 1 : 10	< 1 : 10

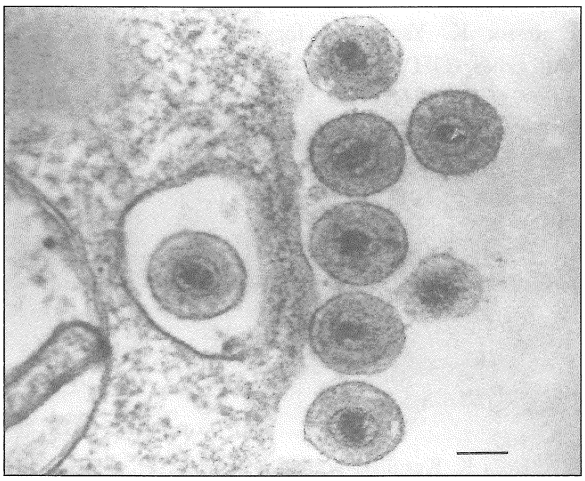

Fig. 2: Transmission electron micrograph of activated HCBMC infected by HHV 7 isolated from the patient. Bar = 100 nm

gave positive immunofluorescence staining with monoclonal antibody KR-4 specific for HHV 7 but negative with monoclonal antibodies specific for HHV 6.

Electron microscopic findings (Figure 2) showed that the virus isolated from the infant was morphologically indistinguishable from other members of the herpesvirus family. Nested-PCR shows that HHV 7 specific genomic sequence was detected in the PBMC of both acute and convalescent blood samples (Figure 3) although the acute sample of PBMC (lane 2, Figure 3) appears to have higher copies of viral genome compared

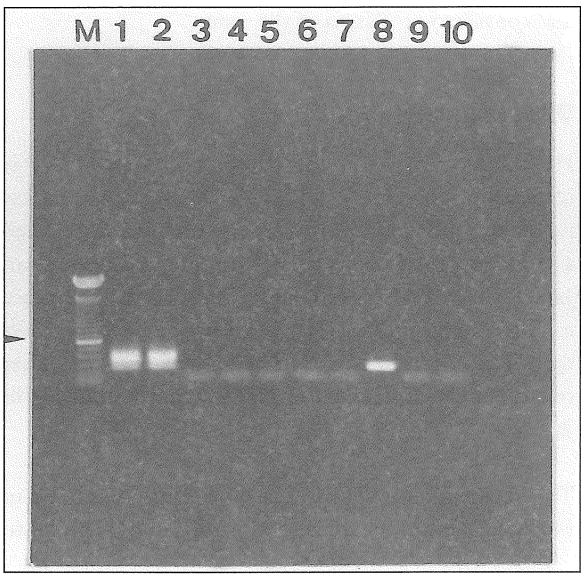

Fig. 3: Ethidium bromide stained agarose gel showing the nested-PCR products of acute and convalescent PBMC of the patient

The amplified nested-PCR products of the acute PBMC of the infant is shown in lane 2, and the convalescent PBMC is shown in lane 8. Lane 1: HHV 7 (KHR strain) infected human cord blood mononuclear cells were used as the reference HHV 7. Lane 3: MRC-5 cells infected with HHV 1. Lane 4: MRC-5 infected with HHV 3. Lane 5: MRC-5 cells infected with HHV 5. Lane 6: HCBMC infected with HHV 6, HST strain. Lane 7: HSB-2 cells infected with HHV 6, GS strain. Milli-Q water (lanes 9 and 10) was included as negative control. 100 base-pairs ladder marker (lane M) was used to indicate the molecular size of the amplified products. Arrow head indicates the position of 600 base-pairs.

to that of convalescent PBMC (lane8, Figure 3) as indicated by a brighter band which is equivalent to the reference control (lane 1).

Paired serum samples from the infant showed seroconversion to HHV 7 for both specific IgG and IgM but were negative for HHV 6 (Table II). Measles and rubella specific IgM were not detected in both the acute and convalescent plasma of the infant.

Discussion

HHV 7 was first isolated in 1990 from the lymphocytes of healthy individual. Its role in human diseases was unknown until four years later when it was implicated as another causative agent of ES. Several reports from Japan showed that, although HHV 6 is the main causative agent of ES, HHV 7 probably accounts for less than 10% of ES cases.[5,6] There have been no other reports of the association of HHV 7 and ES outside of Japan.

This is the first case of ES caused by HHV 7 reported in Malaysia. Clinically, the disease is mild and is similar to that caused by HHV 6. The laboratory evidence includes not only virus isolation and seroconversion, but also the detection of HHV 7 specific DNA by nested PCR. The detection of specific HHV 7 in peripheral blood mononuclear cells in the convalescent blood sample may imply that HHV 7, like other human herpesviruses, can remain latent in the body following primary infection.

The isolation of HHV 7 in this infant confirms the Japanese findings that the virus can cause ES as a result of a primary infection. However, the importance of this virus as a cause of ES requires further study.

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