Jpn. J. Infect. Dis., 54, 47-54, 2001

Review

Human Herpesviruses 6 and 7: Effects on Hematopoiesis and Mode of Transmission

Masao Yamada*

Department of Virology, Okayama University Graduate School of Medicine and Dentistry, Shikata 2-5-1, Okayama 700-8558, Japan

(Received February 5, 2001. Accepted March 22, 2001)

CONTENTS:

SUMMARY: Human herpesvirus 6 (HHV-6) and human herpesvirus 7 (HHV-7) were recently discovered, and are known as etiologic agents of exanthem subitum (roseola). HHV-6 and HHV-7 are T-lymphotropic, and have been classified as betaherpesviruses. In monitoring of herpesviruses after hematopoietic stem cell transplantation, each herpesvirus had a unique temporal profile of detection. HHV-6 DNA was detected most frequently at 3 weeks, whereas cytomegalovirus and Epstein-Barr virus DNA were detected later. HHV-7 DNA was not detected throughout the observation period. In in vitro hematopoietic colony assays, HHV-6 suppressed all three lineages of hematopoiesis, i.e., erythroid, granulocyte/macrophage, and megakaryocyte, whereas HHV-7 did not have any suppressive effect. Molecular epidemiological analysis revealed that HHV-7 was transmitted horizontally from grandparents to parents to children through close contact within a household. Either parent could transmit HHV-7 to the children. Follow-up studies of the amount of viral DNA in saliva samples revealed that the amount of HHV-7 DNA was rather constant for each individual, and that "high producers" and "low producers" could be distinguished. Transferred antibodies against HHV-7 tended to be higher and remain longer after birth than those of HHV-6, and these findings are consistent with the clinical observation that HHV-6 infection occurs earlier than HHV-7 infection.

Introduction

Human herpesvirus 6 (HHV-6) was first isolated from peripheral blood lymphocytes of patients with lymphoproliferative disorders in 1986 (1), and has been divided into two variants, HHV-6A and HHV-6B (2,3). Yamanishi et al.(4) clearly demonstrated an etiological relationship between primary HHV-6 infection and exanthem subitum. Human herpesvirus 7 (HHV-7) was first isolated from the peripheral blood lymphocytes (5) and saliva of healthy adults (6). HHV-6 and HHV-7 are predominantly T-lymphotropic (7-10), exhibit considerable genomic sequence homology, and are classified as members of the *Roseolovirus* genus of the *Betaherpesvirinae* subfamily (11-13). In this short review, the general characteristics of these two viruses are comparatively discussed, then two topics related to our recent studies on pathogenicity in the hematopoietic system and mode of transmission are introduced.

*Corresponding author: Tel: +81-86-235-7163, Fax: +81-86-235-7169, E-mail: masao@md.okayama-u.ac.jp

General characteristics of HHV-6 and HHV-7

The virions of HHV-6 and HHV-7 consist of four ultrastructural components that are common to all herpesviruses; a centrally-located core containing the viral genome, surrounded by three concentric structures–the capsid, the tegument, and the envelope that is studded with virus-encoded glycoproteins. The virions of HHV-6 and HHV-7 are 170-200 nm in diameter and have a distinct layer of tegument (14,15) (Fig. 1). Maturation of these viruses appears to be processed by a pathway that includes 1) capsid formation in the nucleus, 2) envelopment of the capsid at inner nuclear membranes, 3) de-envelopment into the cytoplasm, 4) re-envelopment of the resulting cytoplasmic capsid at a cytoplasmic membrane (budding into vacuoles), 5) transport to the plasma membrane, and 6) release by reverse phagocytosis (14,15).

The HHV-6 genome is approximately 160 kbp in length, and includes approximately 100 genes (16-18). The HHV-7

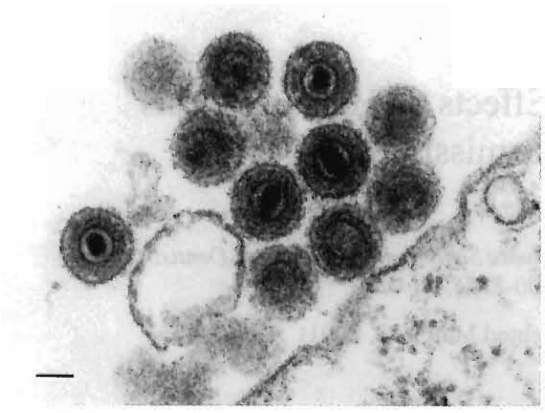

Fig. 1. HHV-7 virions on the surface of an infected cord blood mononuclear cell. Bar: 100 nm.

genome, with a length of approximately 145 kbp, is more compact than the HHV-6 genome (19). About 40 genes of HHV-6 and HHV-7 encode structural proteins or proteins that are involved in the lytic-cycles of genome replication, and are homologs of genes that are shared by all members of the family *Herpesviridae*. These are referred to as "herpesvirus core genes". Three members of the *Betaherpesvirinae* subfamily, cytomegalovirus (CMV), HHV-6, and HHV-7, are similar in the arrangement of the herpesvirus core genes, which are located in the central part of the genomes. A handful of genes are specific to the *Roseolovirus* genus. Nearly every HHV-7 gene has a counterpart in HHV-6. However, one gene (the U94 gene), a homolog of the parvovirus rep gene, is present in both HHV-6A and HHV-6B but is not present in HHV-7 (20,21).

Clinically, HHV-6B and HHV-7 are the common etiologic agents of exanthem subitum (roseola), whereas diseases caused by HHV-6A are less apparent (4, 22). While diseases caused by primary infections of either HHV-6 or HHV-7 in childhood usually resolve spontaneously, several cases of complications have been reported (23-26). HHV-6 and HHV-7 are thought to establish latent, life-long infection like other members of the herpesviruses. It has been reported that HHV-6 may contribute to life-threatening diseases in immunosuppressed conditions such as organ transplant and AIDS (27-30). A variety of diseases, such as chronic fatigue syndrome, histiocytic necrotizing lymphadenitis, mononucleosis-like syndrome, and multiple sclerosis have been reported to be associated with HHV-6 (31-35). No reliable link between reactivation of HHV-7 and a disease has been demonstrated thus far (36,37).

HHV-6: as a causative agent of myelosuppression after stem cell transplantation

1. Monitoring of herpesviruses after allogeneic peripheral blood stem cell transplantation and bone marrow transplantation

Recently, more attention has been focused on the activity of herpesviruses after bone marrow transplantation and other types of hematopoietic stem cell transplantation (38-40). CMV, HHV-6, and Epstein-Barr virus (EBV) were associated with failure of marrow engraftment and other types of complications such as pneumonitis, encephalitis, and post-transplantation lymphoproliferative disorder (PTLD).

We recently monitored the activity of four herpesviruses after allogeneic peripheral blood stem cell transplantation (allo-PBSCT) and bone marrow transplantation (allo-BMT) (41). Viral genomes in peripheral blood leukocytes of the patients were detected by PCR. Each herpesvirus had a unique temporal profile of detection. HHV-6 DNA was detected most frequently at 3 weeks after transplantation, whereas CMV and EBV DNAs were detected later, at 2-3 months after transplantation (Fig. 2). Detection rates of HHV-6 DNA at 3 and 4

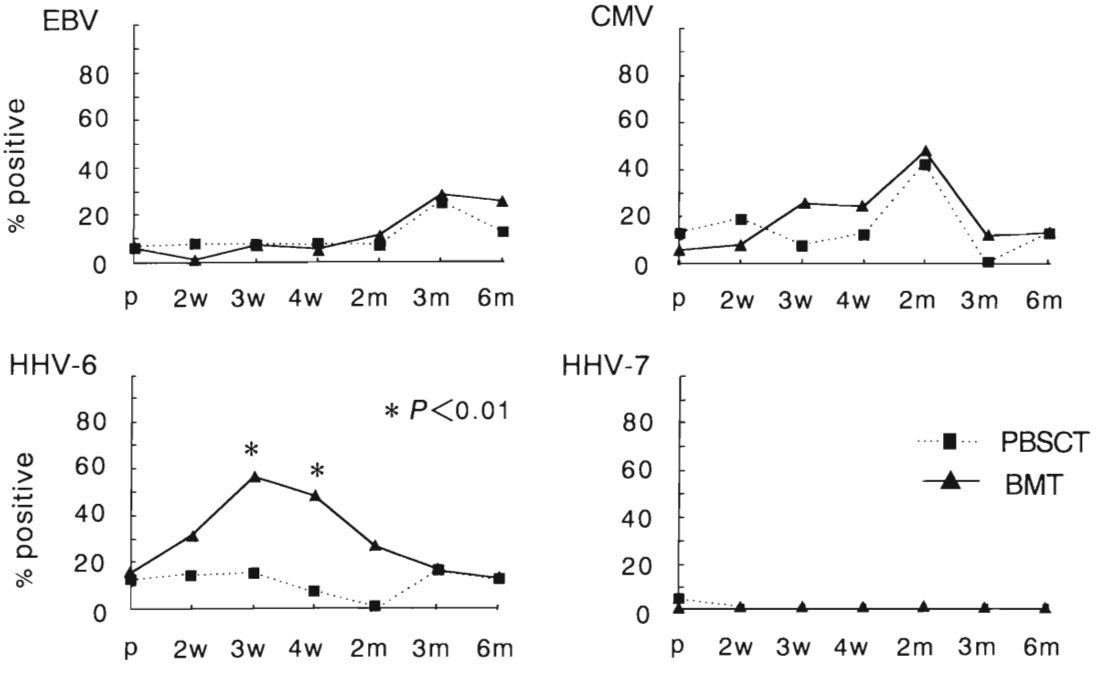

Fig. 2. Detection rates of EBV, CMV, HHV-6, and HHV-7 DNA in peripheral blood leukocytes of patients after allo-PBSCT (closed squares) and allo-BMT (closed triangles). Blood samples were collected 1-2 weeks before transplantation (p) and 2, 3, and 4 weeks (2w, 3w, and 4w), and 2, 3, and 6 months (2m, 3m, and 6m) after transplantation. (ref. 41)

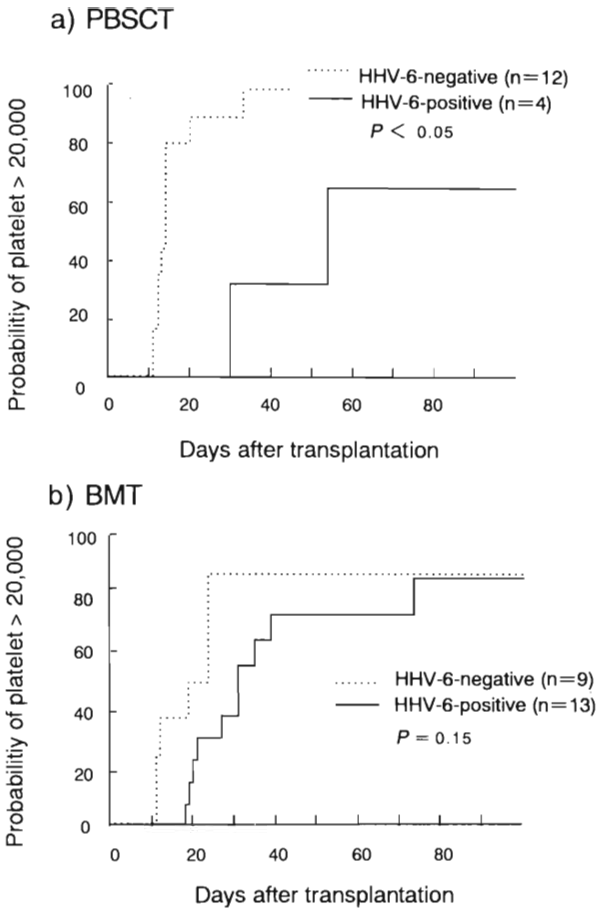

Fig. 3. Time course of platelet engraftment after a) allo-PBSCT and b) allo-BMT. (ref. 41)

weeks after allo-BMT were significantly higher than those after allo-PBSCT. However, the detection rates of the other three herpesviruses were not significantly different between allo-BMT and allo-PBSCT throughout the observation period.

Furthermore, delayed platelet engraftment was associated with detection of HHV-6 DNA at the first 4 weeks after allo-PBSCT. Figure 3 shows the kinetics of platelet engraftment of patients who were positive for HHV-6 at the first 4 weeks and of those who were negative for HHV-6 after allo-PBSCT or allo-BMT. Platelet engraftment after allo-PBSCT was significantly slower in HHV-6 positive patients than in HHV-6 negative patients.

The amounts of HHV-6 DNA detected within 4 weeks after transplantation were also compared between patients with delayed and those with normal platelet engraftment. Patients with delayed platelet engraftment exhibited a greater amount of HHV-6 DNA than did patients with normal engraftment. None of the other herpesviruses were associated with the delay of platelet engraftment in terms of detection rates or amount of viral DNA in the first 4 weeks after transplantation. These results suggest an advantage in immunological reconstruction after allo-PBSCT as compared with allo-BMT; i.e., rapid recovery of CD4+ T cells after allo-PBSCT might be more prominent in the early stage, when HHV-6 is most likely to reactivate. Thus, allo-PBSCT is more beneficial than allo-BMT in terms of prevention of complications caused by HHV-6 reactivation.

2. Suppressive effects of HHV-6 on in vitro hematopoietic colony formation

These clinical observations urged us to evaluate the inhibitory effects of HHV-6 and a related betaherpesvirus, HHV-7, on hematopoietic progenitors in vitro. We employed colony assays in a semisolid matrix supplemented with various hematopoietic cytokines. Using T-cell depleted cord blood mononuclear cells as a source (42), we established hematopoietic colony assays for three lineages, including 1) CFU-GM (colony-forming unit granulocyte/macrophage) colonies for granulocyte/macrophage lineage, 2) BFU-E (burst-forming unit erythroid) colonies for erythroid lineage, and 3) CFU-Meg (colony-forming unit megakaryocyte) colonies for megakaryocyte lineage. CFU-GM and BFU-E colonies can be assayed together in the presence of a cocktail of cytokines, stem cell factor (SCF), GM-CSF, IL-3, and erythropoietin, whereas CFU-Meg colonies require a special cytokine, thrombopoietin (TPO).

Effects of HHV-6 and HHV-7 on CFU-GM and BFU-E were examined first (43). T-cell depleted cord blood mononuclear cells were infected with one of these viruses and subjected to the hematopoietic colony assay in a semisolid matrix. Ten to 12 days after plating, CFU-GM and BFU-E colonies were counted. HHV-6B, a more prevalent variant of HHV-6, suppressed both CFU-GM and BFU-E colonies in a multiplicity of infection (MOI)-dependent manner, and the other less prevalent variant, HHV-6A, also had a suppressive effect. However, HHV-7 did not have any suppressive effect on BFU-E or CFU-GM. Compared with the mock-infected culture, the size of both types of colonies were also reduced in HHV-6 infected culture. When single colonies of each type were picked up from the infected culture and separately examined, HHV-6 was detected by PCR in most of the colonies of CFU-GM and BFU-E.

Compared to erythroid and granulocyte/macrophage colonies, megakaryocyte colonies had been very difficult to establish until the recent discovery of the genuine megakaryocyte colony stimulation factor, TPO (44,45). We took advantage of the availability of recombinant human TPO to establish culture conditions for megakaryocyte colonies (46). Two types of TPO-induced colonies, CFU-Meg and non-CFU-Meg colonies, were generated. Identification of CFU-Meg colonies was based on their characteristic morphology, i.e., translucent cytoplasm and a highly refractile cell membrane, and was confirmed by immunohistochemistry to detect the CD41 antigen. Optimization of culture conditions for TPO-inducible colonies was attempted in the presence or absence of fetal calf serum and SCF.

Effects of two variants of HHV-6 and HHV-7 on TPO-inducible colonies were examined under serum-containing conditions. T cell-depleted cord blood mononuclear cells were infected with either HHV-6 or HHV-7, and were subjected to TPO-inducible colony assay. As shown in Fig. 4, HHV-6B inhibited CFU-Meg colony formation in a MOI-dependent manner and HHV-6A had a similar inhibitory effect. On the contrary, HHV-7 had no effect. To confirm the suppressive effect of HHV-6 on CFU-Meg, experiments were repeated under several conditions. Under serum-free conditions, either in the absence or presence of SCF, HHV-6 suppressed CFU-Meg colony formation in a MOI-dependent manner. Heat inactivation and ultra-filtration of the virus sample completely abolished the suppressive effect of HHV-6, suggesting that the suppression was due to the presence of the infectious virus.

Combined with the results on the erythroid and granulocyte/

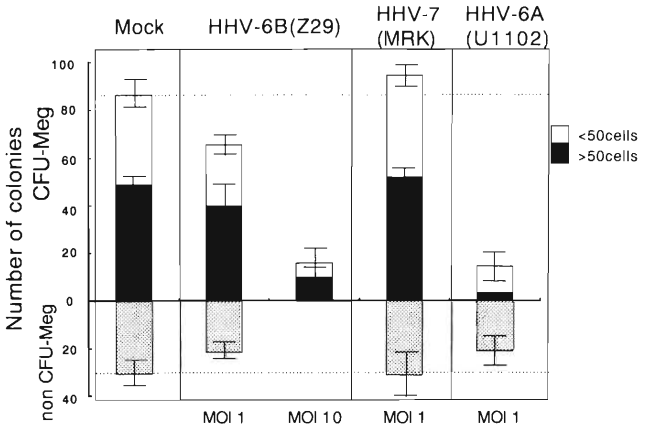

Fig. 4. Effects of HHV-6 and HHV-7 on TPO-inducible colonies. Number of CFU-Meg colonies (filled bars, colonies consisting of 50 or more cells; open bars, colonies consisting of fewer than 50 cells) and non-CFU-Meg colonies (shaded bars) after infection with HHV-6 and HHV-7. (ref. 46)

macrophage colonies, HHV-6 suppressed all three lineages of hematopoietic colony formation in vitro (Fig. 5). Thus, we focused on an earlier stage of hematopoiesis, i.e., a lineage-uncommitted progenitor step, and examined the interaction between HHV-6 and CD34+ cells, which are generally thought to be the major source of hematopoietic progenitor cells (47). In order to examine whether CD34+ cells are susceptible to HHV-6, we purified CD34+ cells with magnetic beads and infected them with HHV-6. Three days after infection, we reselected CD34+ cells and obtained more than 99% purity. The HHV-6 genome was detected in the highly-purified CD34+ cells by in situ hybridization.

Mode of transmission of HHV-6 and HHV-7

1. Molecular epidemiology of HHV-7

Serologic studies showed that the prevalences of HHV-6 and HHV-7 infections are very high throughout the world and that almost all people are exposed first to HHV-6 and second to HHV-7 in their childhood (22,48). By adulthood, most individuals are seroimmune even if no specific febrile illness was ever recognized. Thus, like most human herpesviruses, HHV-6 and HHV-7 are ubiquitous in the human population and likely to persist in a latent state after primary infection. Several investigators have reported that HHV-7 is easily isolated from the saliva of individuals who had antibodies to HHV-7 (6,49,50). Because the virus is an inhabitant in saliva of a majority of healthy adults, horizontal transmission from parent to child is highly likely, but this mode of transmission of HHV-7 has yet to be firmly documented. Therefore, we conducted a series of studies to obtain evidence of intrafamilial transmission of HHV-7 (51).

We set out to isolate HHV-7 from the saliva samples of all members of six families, including four families with three generations living in the same household. As a result, HHV-7 was isolated from saliva samples of 43 of 47 participants (91.5%). Every isolate was subjected to DNA restriction analysis. The results are summarized in Fig. 6. In family #1, the restriction patterns of the mother and three of the four children were similar, and the patterns of the paternal grandmother and the father were similar. In family #2, the patterns of the maternal grandmother, the mother, and three children were similar, and the patterns of the paternal grandmother and the father were similar. In family #3, the patterns of the maternal grandmother and the mother were similar, and the patterns of the paternal grandfather and the father were related; the pattern of the child did not match the pattern of any other family member. In family #4, which included three generations, two pairs of related patterns were observed among siblings, but neither of these patterns matched any of those of the senior members of the family. In family #5, the pattern of the father as well as those of five of six children were similar, and those of the mother and the other child were similar. In family #6, the father and a child had related patterns, and the patterns of the mother and another child

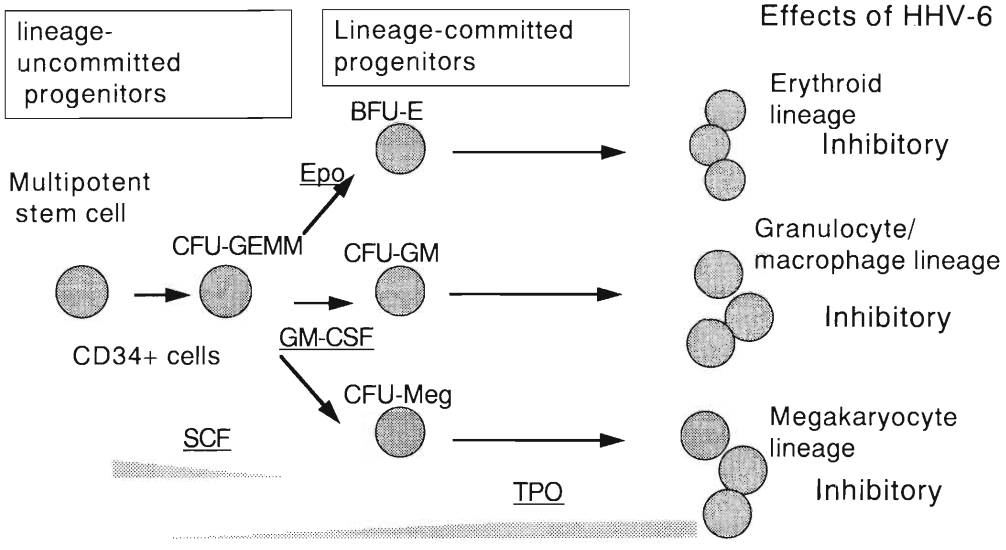

Fig. 5. Hematopoietic differentiation and the suppressive effects of HHV-6 on three lineages. Responsible hematopoietic cytokines for each lineage are underlined. Epo, erythropoietin; GM-CSF, granulocyte/macrophage-colony stimulation factor; SCF, stem cell factor; TPO, thrombopoietin.

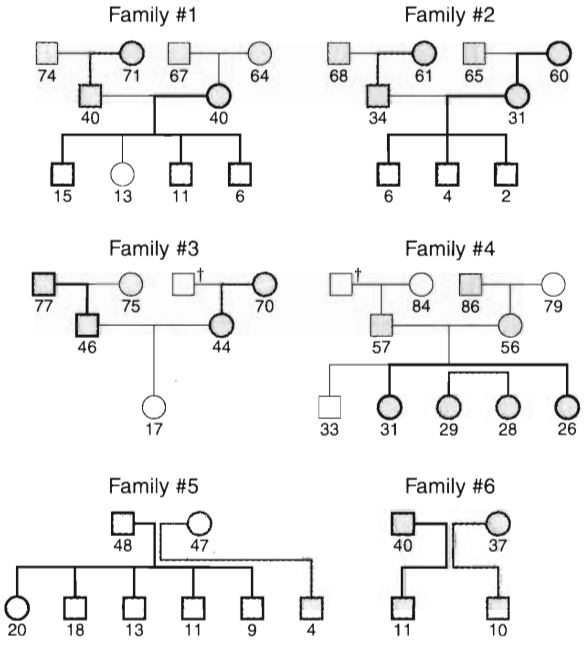

Fig. 6. Pedigrees of six families examined by restriction analysis of HHV-7 DNA. Male and female are indicated as boxes and circles, respectively. Persons from whom HHV-7 was isolated are shaded. A person who was already deceased is marked (†). Members having similar HHV-7 DNA restriction patterns are connected with bold lines. Numbers under the boxes and circles indicate ages. (ref. 51)

were related.

The similarities in restriction profiles of family members were summarized. In total, similar patterns with the mother were found in 48% of offspring of the families, and similar patterns with the father were found in 28% of offspring. History of breast feeding was also obtained from each mother in the study. But there was no apparent correlation between breast feeding and maternal transmission within a family; e.g., in family #3 with a history of breast feeding, HHV-7 transmission occurred from a father to his children. The results clearly showed that HHV-7 was transmitted horizontally from generation to generation in families living in the same household. The data also showed that fathers as well as mothers transmitted the infection.

2. Kinetics of the virus production in saliva of healthy adults

HHV-7, as well as HHV-6, is thought to be transmitted during early infancy through saliva. However, the kinetics of virus shedding in saliva of healthy adults, from whom children are assumed to acquire the viruses, is mostly unknown. HHV-7 is more readily isolated from saliva of healthy adults than is HHV-6. The next question we posed was how many copies of the HHV-6 and HHV-7 genomes were secreted in the saliva of healthy adults.

The number of copies of HHV-6 and HHV-7 genomes in saliva samples was determined by the quantitative PCR procedure (52). The genomes of HHV-6 and HHV-7 were respectively detected in 41.3 % and 89.7 % of the saliva samples from 29 healthy adults. The average DNA copy number of the HHV-6 genome in the positive samples (4.8×10^3 copies/ml) was much lower than that of the HHV-7 genome (1.4×10^4 copies/ml). Only 4 out of 29 individuals exhibited more HHV-6 DNA than HHV-7 DNA.

The virus DNA load of sequential samples from six healthy adults was monitored for 3 months, and the relationship between the number of copies of the two genomes is shown in Fig. 7. The amount of HHV-7 DNA was rather constant for each individual. Some individuals (d and f) were "high-producers", and others (b and e) were "low producers" of

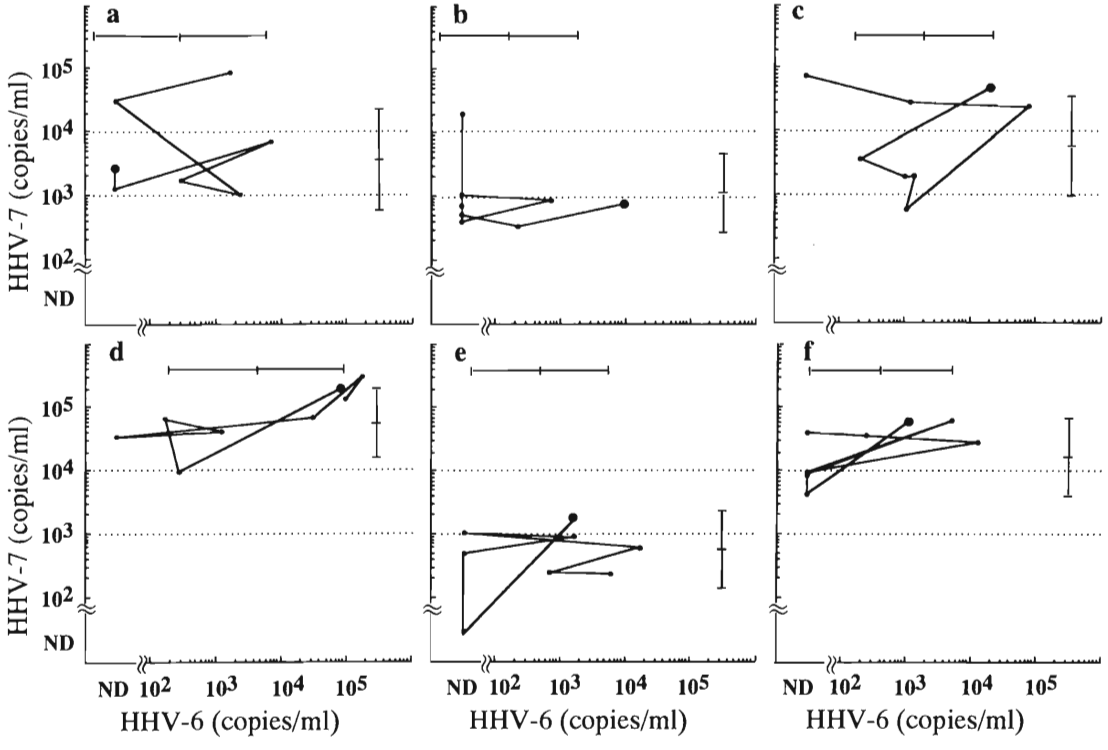

Fig. 7. Number of copies of the HHV-7 genome plotted in relation to the number of copies of HHV-6 (logarithmic scales) in saliva samples of six individuals (a-f) during 3 months of follow-up period. For each subject, the first point is shown with a large circle and the points are then connected in chronological order. (ref. 52)

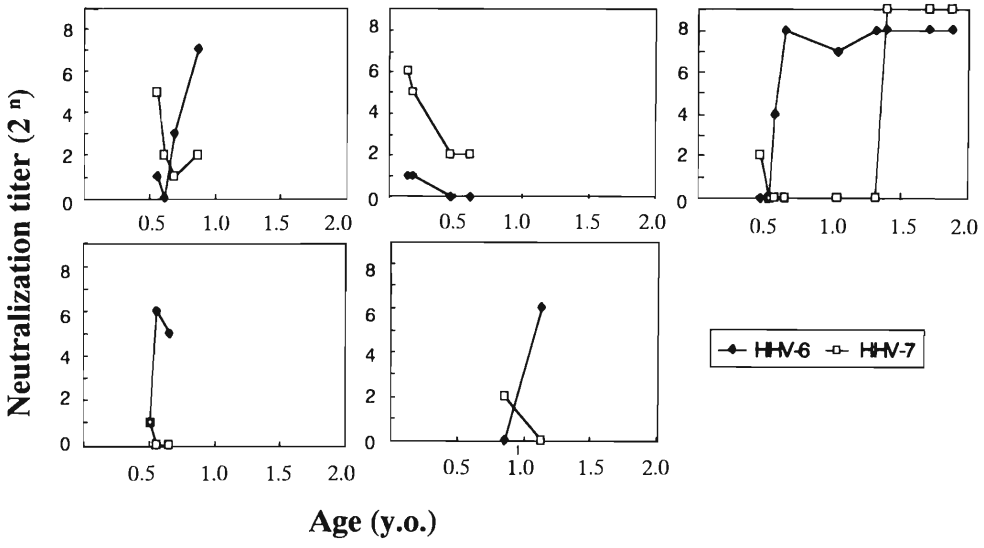

Fig. 8. Changes of neutralizing antibody titers against HHV-6 and HHV-7 in five infants.

HHV-7 DNA. In contrast, the amount of HHV-6 DNA varied drastically over time in each individual, from below detection limit to more than 1.0×10^5 copies/ml. In subjects a and c, the amounts of both HHV-6 and HHV-7 DNA were extremely variable.

We also examined the relationship between the copy number of the genome and the presence of infectious viruses. Although HHV-6 was never isolated from the saliva, HHV-7 was isolated from the saliva several times during the follow-up period. The amount of HHV-7 DNA tended to be higher at the times when the virus was isolated than at the times when the virus was not isolated. However, failure to isolate HHV-6 from the saliva samples cannot be attributed to the scarcity of the amounts of HHV-6 DNA, because at several time points the amounts of HHV-6 DNA were almost comparable to the maximum amount of HHV-7 DNA. One possibility is that HHV-6 might be present in saliva in a noninfectious form, binding to some inhibitory factors.

3. Seroepidemiology of HHV-6 and HHV-7 based on neutralizing assay

The enigma why HHV-7 infection usually follows HHV-6 infection by a few years during early childhood has not yet been resolved. We have made an effort to solve some of the problem from differences in maternal transferred antibodies against these viruses.

We developed a dot-blot neutralizing assay for HHV-6 and HHV-7, and monitored neutralizing antibody titers against these viruses in sera from healthy adults (53). In most individuals, neutralizing antibody titers against HHV-7 were much higher than those against HHV-6. Elevated neutralizing titers against HHV-7 might be attributed to continuous booster effects by persistent HHV-7 production in saliva. Furthermore, we monitored neutralizing antibody titers against these viruses in children with a documented history of exanthem subitum. Transferred antibody titers against these viruses from mothers were readily demonstrated by the neutralization test, because no cross-reaction between HHV-6 and HHV-7 was observed with use of this method. Transferred antibody titers against HHV-7 were higher and tended to remain longer after birth than those of HHV-6 (Fig. 8), and these findings are in accord with the clinical observation that HHV-6 infection usually occurs earlier than HHV-7 infection.

Conclusion

While diseases caused by HHV-6 and HHV-7 in children usually resolve spontaneously, life-threatening cases related to HHV-6 in childhood and immunocompromised hosts in cases of stem cell and solid organ transplantation have been reported. These conditions warrant the development of effective antiviral agents against HHV-6 (54-56).

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