Human Herpesvirus 6 Infection and Kawasaki Disease

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Eighteen of a total of 22 serum specimens (81.8%) from patients with Kawasaki disease were positive for immunoglobulin G or M antibodies to human herpesvirus 6, whereas 10 of 16 age- and sex-matched healthy controls (62.5%) were seropositive. Additionally, increased geometric mean antibody titers of immunoglobulin G were shown in these patients. These results suggest that the status of human herpesvirus 6 infection may be a reflection of the immunologic alterations that are associated with Kawasaki disease.

Kawasaki disease (KD) is an acute febrile vasculitis of infancy and early childhood that is recognized in patients by prolonged fever, cervical lymphadenopathy, conjunctival injection, rash, and other characteristic features (5). Well-documented epidemics superimposed upon an endemic background and seasonal peaks in winter and spring suggest an infectious etiologic basis. Additionally, KD rarely occurs in the neonatal period and beyond 5 years of age. These observations indicate that an unknown common agent(s), which likely infects the patients’ mothers asymptptomatically, appears to cause KD. We recently observed that a primary infection with adenovirus was highly associated with the development of KD in children who were seronegative for Epstein-Barr virus, without any significant differences between patients and controls in serologic results for other herpesviruses, including herpes simplex virus types 1 and 2, varicella-zoster virus, and cytomegalovirus (M. Okano, G. M. Thiele, Y. Sakiyama, S. Matsumoto, and D. T. Purtilo, submitted for publication). These data suggested that some interaction(s) of adenovirus and a delayed or suppressed Epstein-Barr virus infection were important for the development of the disease. The causative agent(s) and precise pathogenetic mechanisms for KD, however, remain unclear.

Human herpesvirus 6 (HHV-6), a novel lymphotropic virus, was isolated recently from cultured primary lymphocytes from patients with various lymphoproliferative disorders (6). Also, this virus appears to be associated with exanthem subitum (roseola infantum), a common disease in infants and young children that is characterized by high fever and rash (7). This disease is rare in the neonatal period, so maternal antibodies protect the infant from a primary infection for several months to 1 year.

The purpose of this study was to evaluate HHV-6 infection in patients with KD. Twenty-two serum specimens from Japanese patients with KD were obtained in the acute phase (within 7 days after the onset of illness) and stored at −70°C until use. The 22 patients included 13 males and 9 females from 8 months to 5 years of age. Control sera were also obtained from 16 age- and sex-matched healthy children. These sera were tested for immunoglobulin G (IgG) and IgM antibodies to HHV-6 by immunofluorescence as previously described (1, 3). The presence of IgM antibodies indicates that a primary or reactivated (secondary) infection has recently occurred in these subjects (3). Briefly, HHV-6 was propagated in HS-2 cells (an immature T-cell line from the American Type Culture Collection, Bethesda, Md.) Maximal cytopathic effect (large, refractile, balloononed cells) was typically observed starting 7 days after infection. Infected and uninfected (as a negative antigen control) cells were pelleted and washed with phosphate-buffered saline. Subsequently, these cells were smeared on Teflon-coated slides (Cell Line Associates, Inc., Newfield, N.J.), air dried, and fixed for 5 min at −20°C with acetone. The fixed-cell smears were overlaid with serial dilutions of test sera for 40 min (3 h for the IgM test) at 37°C, washed three times with phosphate-buffered saline, and incubated with fluorescein isothiocyanate-conjugated goat anti-human IgG (gamma-chain specific; Jackson ImmunoResearch Laboratories, Avondale, Pa.) or anti-human IgM (mu-chain specific) for 40 min. The slides were washed with phosphate-buffered saline, air dried, and mounted with 50% phosphate-buffered saline-glycerol. Immunofluorescence was viewed with a Nikon Labophot immunofluorescence microscope (Lincoln Microscope Co., Lincoln, Nebr.). Antibody titers were expressed as the reciprocals of the serum dilutions. Sera positive for IgM antibodies to HHV-6 were tested for false-positive results caused by the presence of rheumatoid factor with RHEUMATEX (Wampole Laboratories, Div. Carter-Wallace, Inc., Cranbury, N.J.) (4).

Eighteen of 22 patients with KD (81.8%) were seropositive for HHV-6, whereas 10 of 16 age- and sex-matched healthy controls (62.5%) were seropositive. One of 18 seropositive patients (5.6%) was positive only for IgM antibodies (IgG and IgM antibody titers: <1:10 and 1:40, respectively), indicating the occurrence of a primary infection. Seven of 18 seropositive patients (38.9%) were positive for both IgG and IgM antibodies. The geometric mean antibody titers (GMTs) were 1:117.9 for IgG (range, 1:20 to 1:2,560) and 1:24.3 for IgM (range, 1:20 to 1:40), suggesting that a recent primary or reactivated HHV-6 infection had occurred in these patients. Ten of 18 seropositive patients (55.6%) had IgG antibodies alone, with a GMT of 1:158.5 (range, 1:20 to 1:1,280) (the IgM GMT was <1:10). Four of 22 patients (18.2%) were seronegative for HHV-6. In contrast, 2 of 10 seropositive healthy controls (20.0%) had only IgM antibodies (IgG and IgM GMTs: <1:10 and 1:20.0 [1:10 and 1:40], respectively).

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with no obvious symptoms. Six of 10 seropositive controls (60.0%) had both IgG and IgM antibodies, with GMTs of 1:50.1 for IgG (range, 1:20 to 1:160) and 1:36.3 for IgM (range, 1:20 to 1:80). Two of 10 seropositive controls (20.0%) had IgG antibodies alone, with a GMT of 1:28.2 (1:20 and 1:40) (the IgM GMT was <1:10). Six of 16 healthy controls (37.5%) were negative for HHV-6. Additionally, all sera positive for IgM antibodies were negative for rheumatoid factor in this study. Furthermore, no serological cross-reactivity of IgM antibodies for HHV-6 and the other herpesviruses was found (Okano et al., submitted). Increased IgG antibody responses were demonstrated in patients with KD with or without IgM antibodies, as compared with IgG antibody responses in healthy controls, although this difference was not significant (a comparison of results for patients and controls by Student’s t test for IgG antibodies showed that P was equal to 0.148 in cases positive for IgM antibodies and that P was equal to 0.07 in those negative for IgM antibodies).

These results suggest that HHV-6 infection is not the direct cause of KD, especially since 4 of 22 patients (18.2%) had no evidence of infection. However, increased IgG antibody titers were demonstrated in patients with KD who were seropositive for HHV-6. Patients with KD have circulating activated B cells and helper T4 cells in the acute phase (2, 5). The increased titers to HHV-6 could have arisen from the activation of lymphocytes and/or HHV-6 replication caused by infection by the putative infectious agent of KD. More studies, however, are necessary to confirm this possibility. Nevertheless, the relationship between the antibody status of HHV-6 and the severity or stage of the disease should be considered as the subject of further investigation.

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