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HETEROPHILE HANGANUTZIU-DEICHER ANTIBODIES IN SERA OF PATIENTS WITH KAWASAKI DISEASES

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SUMMARY The heterophile antibody levels in sera from patients with Kawasaki disease (49 sera from 39 cases) were measured by sheep erythrocytes (SRBC) agglutination and radioimmunoassay in microplates coated with Hanganutziu-Deicher (H-D) antigen-active glycosphingolipid, equine hematoside. The antibody levels were low in the first week of illness, increased rapidly in the 2nd week, and thereafter gradually decreased. The SRBC agglutination titers and H-D antibody titers of sera from patients with Kawasaki disease from week 2 to 8 of illness were significantly higher than those of healthy children (44 sera) and normal cord blood (13 sera).

INTRODUCTION

Kawasaki disease (acute febrile mucocutaneous lymph node syndrome, MCLS) was first described by Kawasaki in 1967 (Kawasaki, 1967), and since then about 20,000 cases have been reported in Japan (Kawasaki et al., 1974: Yanagawa et al., 1979). A few cases of Kawasaki disease have also been reported from North America, Australia, Korea and elsewhere (Melish et al., 1976; Morens et al., 1980).

The cause and pathogenesis of Kawasaki disease are unknown. Some characteristics of illness, including fever, eruption, leukocytosis (increased neutrophils), abnormal acute phase reactants, cervical lymphadenitis, and usually a self-limited course of one or two weeks, suggest an infectious origin (Mortimer, 1976). However, microbiological and serological tests have failed to identify the agent responsible for the disease. There are preliminary reports of recovery of Rickettsia-like bodies in culture (Hamashima et al., 1973) and of high levels of EB virus (EBV) antibodies in sera from some patients with Kawasaki disease. Today, it is generally considered that Kawasaki disease is due to a hypersensitive reaction against some

antigenic stimulation such as an infectious agent, because of the high incidence of the disease in patients with allergic diseases and the high levels of immunoglobulin E in sera from patients with the disease (Kusakawa and Heiser, 1976).

When the levels of several kinds of antibody in sera from patients with Kawasaki disease were measured, high levels of sheep erythrocyte (SRBC) agglutinin were observed in some cases of the disease. Therefore, the time course of change in heterophile antibody level in sera from patients with the disease was tested by SRBC agglutination and radioimmunoassay, and compared with the levels in healthy children and cord blood.

PATIENTS AND METHODS

1. *Patients*

Forty-nine sera from 39 patients with Kawasaki disease admitted to Kinki University Hospital between May 1975 and January 1981 were tested for heterophile antibodies. Thirteen sera from cord blood and 44 sera from healthy children (" control "), which were preimmune sera before measles vaccine, were also examined. The sera from healthy children were kindly supplied by Dr. M. Takahashi of this Institute.

2. *Hemagglutination*

Agglutination titers were assayed using a " V "-microplate. Serial twofold dilutions of serum (25 μl) were mixed with 0.5% SRBC suspension (25 μl), kept at room temperature for 2 h, and then incubated overnight at 4 C. After further incubation at 37 C for 1 h, the end point of complete agglutination was read. Titers are shown as reciprocals of the final dilution for complete agglutination. Phosphate-buffered saline, pH 7.2, (PBS) was used for dilution.

3. *Radioimmunoassay*

H-D antigen-active glycosphingolipid hematoside was purified from equine erythrocyte stroma (Higashi et al., 1977). Antibody against human immunoglobulin G (IgG) was purified from rabbit anti-human IgG (Miles Laboratories, Inc.) by affinity chromatography on human IgG-coupled CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals). The purified antibody was labeled with [^{125}I] (New England Nuclear) by the chloramin-T method (McConahey and Dixon, 1980) (7×10^5 c.p.m./μg IgG). New polyvinylchloride " U "-microplates (Dynateck 220–24) were coated with 50 μl/well of equine hematoside (10 μg/ml in distilled water containing 0.1% sodium taurodeoxycholic acid) and dried at 37 C overnight. Then antigen-coated and uncoated plates were treated with PBS for 3 h at room temperature. Volumes of 50 μl of serum diluted 10-fold with PBS containing 0.05% Tween 20 (PBS-T) were plated in coated and uncoated plates, which were then incubated for 1 h at 37 C and washed five times with PBS-T. Then 50 μl of ^{125}I-labeled antihuman IgG (0.2 μg IgG/well) was added, and after incubation for 1 h, the radioactivity in each well was measured with a gamma counter. The titer of H-D antibody was calculated by the following formula:

$$\text{H-D antibody titer} = \frac{\text{c.p.m. in antigen-coated well with serum minus that with PBS-T}}{\text{c.p.m. in uncoated well with serum minus that with PBS-T}}$$

RESULTS

1. *H-D type heterophile antibody response in Kawasaki disease*

The heterophile antibody levels in sera from patients with Kawasaki disease were tested by measuring agglutination of SRBC which possess H-D, Paul-Bunnell and Forssman antigens, and compared with those in several types of control sera (Fig. 1). The mean SRBC agglutination titers ($\pm$S.D.) of sera from patients with Kawasaki disease were $2^{3.2}\pm2^{1.1}$ in the 1st week of illness, $2^{5.1}\pm2^{1.4}$ in the 2nd to 4th week, $2^{5.0}\pm2^{1.5}$ in the 5th to 8th week, and $2^{3.4}\pm2^{0.9}$ after week 9. The mean titers of patients with Kawasaki disease from week 2 to 8 of illness were significantly higher than those of " controls " ($2^{3.1}\pm2^{1.1}$) ($p<0.01$). None of the 13 sera from cord blood agglutinated SRBC ($<2^2$).

It was found by Davidsohn's differential absorption test (Davidsohn, 1938) that these high levels of SRBC agglutinins in sera from

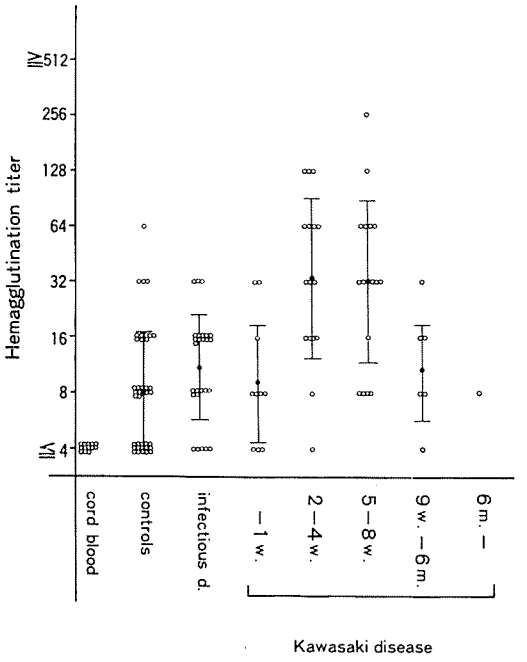

FIGURE 1. The distribution of sheep-RBC agglutination titers in sera of patients with Kawasaki disease and other sera. Closed circles and horizontal bars show geometrical mean titers±standard deviations.

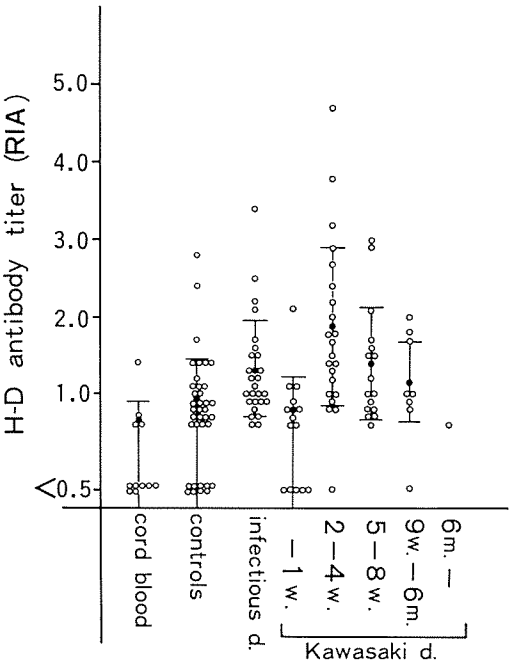

FIGURE 2. Distribution of H-D antibody titers in sera of patients with Kawasaki disease and other sera. Closed circles and horizontal bars show average titers±S.D.'s.

patients with Kawasaki disease were due to H-D-type heterophile antibody. Therfore, H-D antibody titers were examined by radioimmunoassay in microplates coated with H-D antigen-active glycosphingolipid, equine hematoside (Fig. 2). The mean titers of H-D antibody were 0.81±0.42 in the 1st week, 1.88±1.04 in the 2nd to 4th week, 1.04±0.73 in the 5th to 8th week, and 1.16±0.52 from week 9. The values in sera of " controls " and cord blood were 0.95±0.51, and 0.63±0.27, respectively. The percentage of incidence H-D antibody (H-D antibody titers≧1.0) was 9.1% in sera from cord blood and 35.1% in " controls ". On the other hand, the incidence in Kawasaki disease was 21.4% in the 1st week, 81.8% in the 2nd to 4th week, 62.5% in the 5th to 8th week, and 55.6% from week 9. The mean titers of patients with Kawasaki disease within one week after the onset of illness were similar to those of " controls ", but in the 2nd to 4th week of illness they were significantly higher than those of " controls " or cord blood ($p<0.01$). The percentage incidence of H-D antibodies from week 2 to 4 was also higher in sera of patients with Kawasaki disease than in " controls " ($p<0.01$).

2. *Time course of change in heterophile antibody titers in sera from patients with Kawasaki disease*

A transient H-D antibody response was observed in the average titers when these were measured weekly. To detect this response in individual patients, we examined the time course of change in the heterophile antibody titers in sera from individual patients with Kawasaki disease by SRBC agglutination (Fig. 3) and radioimmunoassay (Fig. 4). These

assays both showed that in almost all patients the titers were low in the 1st week of illness, and increased rapidly in the 2nd week. After this the high titers persisted for about 4 weeks, and then gradually decreased, the titers in after week 9 being similar to those in the 1st week of illness.

Kawasaki disease was divided into three clinical stages: an acute stage, a convalescent stage and a stage after recovery. The acute stage, in which the patients had various symptoms such as fever and eruption lasted 1–2 weeks in most cases. The convalescent stage, in which the patients were not febrile but had abnormal acute phase reactants, such as a positive CRP and elevated erythrocyte sedimentation rate, was usually in the period from 2 to 4 weeks after the onset of illness. The stage in which acute phase reactants had diminished was named the stage after recovery. The titers of the H-D antibody in these three stages are plotted Fig. 5. In most patients with Kawasaki disease, the H-D antibody levels were low in the acute stage, increased in the convalescent stage, and decreased again in the stage after recovery.

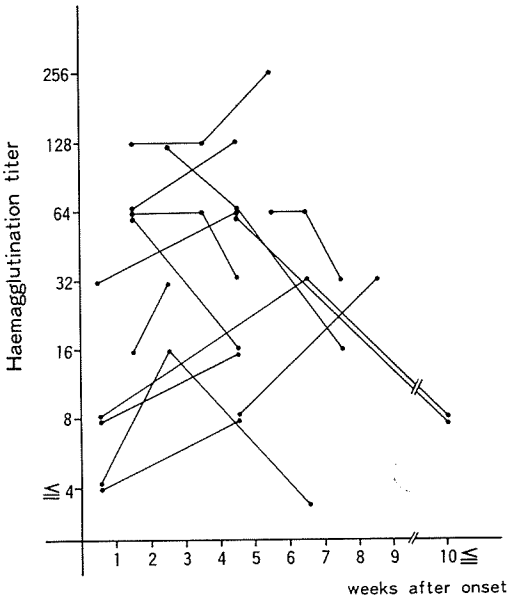

FIGURE 3. Time course of change in sheep-RBC agglutination titers in individual cases of Kawasaki disease.

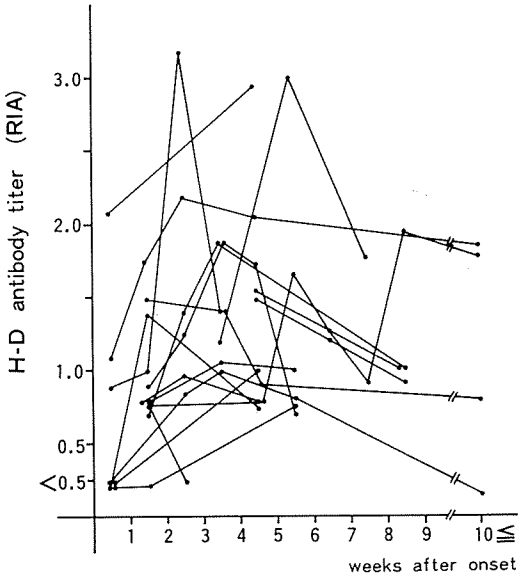

FIGURE 4. Time course of change in H-D antibody titers in individual cases of Kawasaki disease.

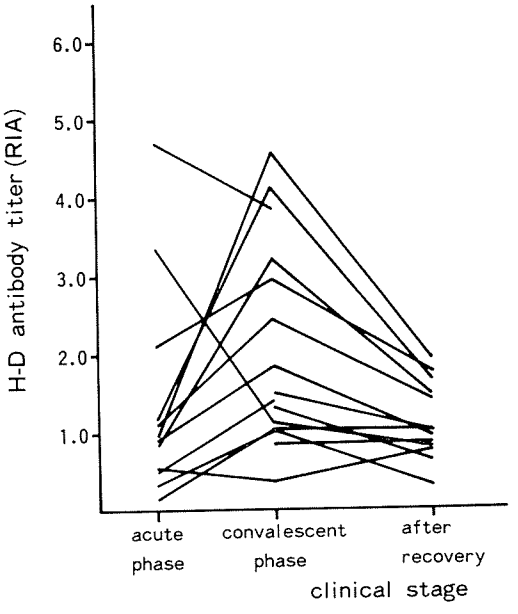

FIGURE 5. Time course of change in H-D antibody titers in clinical stages of Kawasaki disease.

Thus, the antibody response detected by radioimmunoassay with equine hematoside as H-D antigen was similar to that detected by SRBC agglutination. Therefore, most of the heterophile antibodies observed in sera of patients with Kawasaki disease seemed to be H-D-type heterophile antibodies.

DISCUSSION

The SRBC agglutinins and H-D antibodies in sera of patients with Kawasaki disease were tested and compared with those in the sera of healthy donors and of cord blood. In sera from cord blood, no agglutinins for SRBC were detected, and the H-D antibody detected by radioimmunoassay was low, although in several cases its titer was 0.5. On the other hand, the antibody levels in sera from patients with Kawasaki disease in the 1st week of illness were low and similar to those of " controls ". Then the antibody level increased rapidly in the 2nd week, and thereafter gradually decreased to the level in the 1st week. The mean titers of SRBC agglutination and H-D antibody of sera from patients with Kawasaki disease from week 2 to 4 of illness were significantly higher than those of " controls ".

H-D Antibody, a kind of heterophile antibody, was first detected in sera of patients after a therapeutic injection of horse antitoxin (Hanganutziu, 1924; Deicher, 1926), and therefore H-D antibody has been named " serum sickness antibody ". Antibody with the same specificity was detected in sera of patients with various diseases who had not received an injection of animal serum (Kasukawa et al., 1976). Recently, Naiki et al. using gel precipitation and hemagglutination inhibition, identified H-D antigen active glycosphingolipids that reacted with human H-D antibody (Naiki and Higashi, 1980).

H-D antigen is present in the cells and tissues of many mammals, including horses, cows, sheep, pigs, goats, dogs and monkeys, but not in healthy humans or chickens. However, it has also been demonstrated in sera and extracts of malignant tissues of patients with various types of cancer (Nishimaki et al., 1979). Recently, we demonstrated H-D antigen on the cell surface of EBV-transformed lymphoblastoid cell lines (Yonemura et al., in preparation).

The transient response of H-D antibody observed here indicated that Kawasaki disease is due to an infectious agent. Several kinds of viruses, such as EBV, Rickettsia, β-streptococcus, Staphylococcus aureus and Candida albicans, have been considered as causative agents of Kawasaki disease (Melish, 1981). The antibody titer against EBV-viral capsid antigen (VCA) in sera from patients with Kawasaki disease has been measured. The positive ratio of EBV-VCA antibodies ($\geq 1:10$) was much lower than that of age-matched controls, although some cases had high EBV-VCA antibody titers. However, we established an EBV nuclear antigen (EBNA) positive lymphoblastoid cell line from an EBV-seronegative patient with Kawasaki disease. Therefore, it is now considered that patients with Kawasaki disease have an immunodeficiency related to EBV. H-D antigen may be expressed as a result of EBV-infection as well as EBV-transformation. To clarify whether the significant levels of H-D antibody found in Kawasaki disease are due to EBV or other unknown agents, we are now testing for the presence of H-D antigen-positive cells in the blood and skin of patients with Kawasaki disease.

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