A SIGNIFICANT AGE SHIFT OF THE HUMAN PARVOVIRUS B19 ANTIBODY PREVALENCE AMONG YOUNG ADULTS IN JAPAN OBSERVED IN A DECADE

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SUMMARY: A seroepidemiological study on human parvovirus (B19) in Japan was undertaken with serum samples randomly collected from healthy Japanese populations (416 in 1973, 675 in 1984 and 508 in 1987/88). All samples were tested for anti-B19 IgG antibody by the indirect antigen-capture ELISA. The antibody prevalence for ages 0-9 years old in 1984 was significantly higher (16%) than that in 1973 (2%), whereas those for ages 20-29 years and 30-39 years were significantly lower in 1984 (20% and 56%) than in 1973 (67% and 80%) (p<0.005). After the erythema infectiosum (EI) outbreak in 1986/87, the antibody prevalences for ages 5-9, 10-14 and 15-19 years were 40-85% in Fukuoka, 0-10% in Gunma, and 21-41% in Chiba reflecting each EI incidence in these three prefectures, whereas those for ages 20-29 years remained low (<20%). These data indicate that B19 virus was transmitted mainly among children and no significant incidence of B19 virus infection in adults has occurred since 1973, resulting in a notable shifting of B19 susceptibility toward older ages including child-bearing females.

INTRODUCTION

In Japan, local epidemics of erythema infectiosum (EI) were first reported in the end of 1930's, followed by large epidemics with about ten-year cycles after
1960's. After the EI outbreak in 1981, the National Epidemiological Surveillance of Infectious Diseases Program (NESIDP) started in July (1). Then a large outbreak began in the spring of 1986 and spread throughout the country, involving mainly the west part of Japan from the autumn of 1986 to early summer of 1987. It was calculated from the NESIDP data that approximately 3% of the population under 15 years of age were involved in this outbreak. In this paper, we present the results of the nationwide time-comparative sero-epidemiological study of B19 virus infection in recent years.

MATERIALS AND METHODS

**Sera:** Four hundred and sixteen serum samples collected in 1973 and 675 serum samples in 1984 were obtained from the National Serum Reference Bank, NIH of Japan. The samples were randomly collected in 13 prefectures covering from northern to western parts of Japan. The same serum collections were previously used for the study of age-specific antibody prevalences for hepatitis A virus (2) and varicella-zoster virus (3). The samples for 1984 were divided into one-year age groups from 0 to 9 years, then 5-year age groups from 10-14, 15-19 and so on up to 59 and a group of 60 years of age or older with 25-35 samples in each group. The samples for 1973 were divided into 5-year age groups from 0-4, 5-9 and so on up to 59 and a group of 60 years old of age or older with 31-36 samples in each group.

A hundred and eighty serum samples from Fukuoka Prefecture and the same number of samples from Gunma Prefecture, all collected in the post-epidemic season of 1987, were divided into three age-group categories; 5-year age groups 0-4, 5-9, 10-14 and 15-19 years old, 10-year age groups 20-29, 30-39, 40-49 and 50-59 years old, and a group of 60 years of age or older. Each group contained 20 samples. A total of 148 post-epidemic serum samples from children aged 0-16 years living in Chiba Prefecture collected in 1987 to 1988 were obtained from Public Health Laboratory of Chiba Prefecture.

All serum samples were collected from healthy individuals and maintained at −70 C until use.

**ELISA:** B19 antigen was prepared from B19 antigen-positive plasma of a blood donor screened by counter-immunoelectrophoresis (CIE) (4). The plasma was clarified by centrifugation at 12,000 rpm for 30 min at 4 C and was re-centrifuged at 100,000 × g for 16 hr. Pellet was resuspended in 0.4 ml of a Tris-EDTA (50 mM Tris, 5 mM EDTA, pH 8.7) solution containing 1% NP40, 0.2% bovine serum albumin (BSA), and 0.02% NaN3 and stored at 4 C. Antigen negative control was prepared in a similar way from B19 antigen-negative sera.
collected from healthy individuals. The antigen dilution of 1:4,000 was used as a minimum essential concentration, which was calculated to be approximately equivalent to $10^7$ virus particles per 50 μl/well. The indirect antigen-capture ELISA for varicella-zoster virus previously described (3) was modified for anti-B19 IgG antibody ELISA. A 96-well plate (Nunc-Immuno Plate, Maxisorp, InterMed, Denmark) was coated with a 1:6,500 dilution of mouse monoclonal anti-B19 antibody in phosphate buffered saline (PBS) (50 μl/well) for two days at 4 C. The plate was washed twice with PBS containing 0.05% Tween 20 (PBS-T). Next, PBS-T containing 0.2% BSA (PBS-T-A) was added and the plate was incubated overnight at 4 C (250 μl/well). After washing three times, an antigen, either B19 antigen or control diluted in PBS-T-A, was dispensed into wells (50 μl/well, overnight at 4 C). After washing four times, two B19 antigen and two control wells received 50 μl each of a serum sample. Each plate contained 20 test serum samples diluted 1:250 with PBS-T-A containing 1% normal goat serum (PBS-T-A-G) and four laboratory reference sera (positive control serum, which was chosen from CIE-positive serum samples, diluted 1:1,000, 1:4,000 and 1:16,000 and negative control serum diluted 1:250) which were used for calibration of delta optical density (DOD) of each sample. After 60-min incubation at 37 C, the plate was washed five times with PBS-T. A 1:10,000 dilution of goat F(ab’)2 anti-human IgG antibody conjugated to horseradish peroxidase (HRPO) (Tago Inc., CA) in PBS-T-A-G was added to all wells (50 μl/well, 60 min at 37 C). After washing five times, the HRPO was allowed to react with the substrate in a solution (0.1 M disodium phosphate, 0.05 M citric acid, pH 5, containing 4 mg orthophenylenediamine and 2 μl of 30% H2O2 per 10 ml of buffer, 100 μl/well) for 30 min at room temperature. The reaction was stopped with 100 μl/well of 2 M H2SO4. The plates were read immediately on a spectrophotometer (Titertech, Multiscan Plus) at 492 nm (A492). Results were expressed as DOD determined for each specimen. DOD values indicate the differences between the mean A492 of two B19 antigen wells and that of two control wells.

RESULTS

ELISA results with 675 serum samples in 1984 by the first series of assays were plotted as DOD against number of cases (Fig. 1). There was a distinct difference between presumed negative group and positive one. The mean and standard deviation (SD) of the negative values were calculated at 0.0104 ± 0.0162 from the DOD values of 409 samples of presumed negative group. All DOD values of the samples in the negative group were less than the mean + 3SD (0.059). Out of 1,599 serum samples, 45 samples including border line and low
Fig. 1. Anti-B19 ELISA results with 675 serum samples in 1984. The arrow indicates the cutoff DOD value (0.070).

Fig. 2. B19 antibody prevalence by age in Japan in 1984. Percentages of anti-B19 IgG antibody positives are plotted against each age group.
positive samples, whose DOD values were 0.048-0.155, were retested at the same time to confirm the results. Of these, 31 samples showed also DOD values greater than 0.070, and they were considered to be positive including six samples which showed again low values less than 0.121. The remaining 14 samples were judged as negative.

Age-specific prevalences of anti-B19 IgG antibody in the three different years (1973, 1984 and 1987/88) were examined and the results of each respective age group were analyzed by Chi-square test (with Yates correction, if necessary).

Firstly, the 1984-collection was thoroughly investigated (Fig. 2). The mean prevalence rate of the age 0 group (18%) was higher than that of age 1 (8%), the former probably representing true incidence plus maternally acquired antibody, since four of five antibody-positive infants were under 3 months of age and one was 7 months old. At ages 1 through 4, there was no increase in incidence: the mean prevalence rate was 8%. But it increased at age 5 and kept an almost the same level (14-22%) at ages 6 through 9, 10-14, 15-19 and 20-24. Then, it slightly increased (25%) at ages 25-29, followed by a gradual increase up to 97% at ages 55-59.
Fig. 4. B19 antibody prevalence in 1987/88. Post-epidemic serum samples were collected from three prefectures. The prevalence in Fukuoka Prefecture (the large-epidemic area ○) was higher than that in Gunma Prefecture (the small-epidemic area ●) and the difference was statistically significant in the respective age groups 5-9, 10-14, 15-19 and 30-39 (Chi-square test). The prevalence among children in Chiba Prefecture (the moderate-epidemic area ▲) showed an intermediate level of those in Fukuoka and Gunma Prefectures. The dotted line indicates the mean level of the prevalence in Japan in 1984.

Secondly, the 1973-collection was compared retrospectively with the 1984 result. In younger age groups, there was a low prevalence of antibody: only one of 68 cases under 10 years of age was positive and the prevalence rate for age group 10-19 was 16%. The antibody prevalence remarkably increased to 67% at 20-29 years of age and retained at a high level (78-91%) in the older age groups. When the data were combined with those of the 10-year age groups (Fig. 3), significant differences in the prevalence rates between 1973 and 1984 were observed in the three age groups: 0-9 years (p<0.005), 20-29 years (p<0.001) and 30-39 years (p<0.005).

Lastly, the 1987/88-collection was examined to compare the antibody prevalence in different parts of Japan after the 1986/87 EI epidemic (Fig. 4). The prevalence in Fukuoka Prefecture (the large-epidemic area) was greater than that in Gunma Prefecture (the small-epidemic area) and the difference was
particularly significant in young age groups 5-9 (p < 0.005), 10-14 (p < 0.001), 15-19 (p < 0.025) and 30-39 (p < 0.05). However, the mean prevalences for older age groups were similar in these two prefectures. The antibody-positive rates of age groups 20-29 and older in Fukuoka Prefecture in 1987/88 were equivalent to the mean level in 1984. The prevalence among children in Chiba Prefecture (the moderate-epidemic area) showed an intermediate level of those in Fukuoka and Gunma Prefectures.

**DISCUSSION**

Human parvovirus (B19) was first demonstrated in the serum from healthy blood donors in 1975 (5) and it has been recognized as an etiologic agent of EI or fifth disease since 1983 (6). Anti-B19 antibody prevalences have been reported in Fukuoka of Japan (7), U.S.A. (8), West Germany (9) and England and Wales (10). In those studies, the techniques used were CIE, antibody-capture radioimmunoassay or antibody-capture ELISA. We applied the indirect antibody-capture ELISA system to anti-B19 IgG antibody assay. In the experiment of the ELISA standardization (Fig. 1), a narrow distribution of negative values and a broad distribution of positive values were observed. To ascertain the positive range, we repeated the test for border line samples and judged as positive when the DOD values were always higher than 0.070.

Out of six samples showing low positive DOD values less than 0.121, five were obtained from older persons (32, 35, 44, 49 and 63 years old). We supposed that those people were exposed to B19 infection a long time ago, perhaps in childhood, and had escaped from reinfection which is thought to be the chance necessary to boost their antibody level. Although we did not compare ELISA with CIE using the same serum collection, we observed a significantly higher prevalence of ELISA-antibody for older age groups in Fukuoka Prefecture in 1984 than previously reported B19 antibody prevalence measured by CIE in the same area and same year (7): for ages older than 30, 58-90% by ELISA and 32-55% by CIE. But the antibody prevalence rates in the age groups under 30 were almost the same in ELISA and CIE assays. We supposed that the antibody present in samples from older individuals showing low positive DOD values by ELISA could not be detected by CIE assay.
The study on the age-specific prevalences of anti-B19 antibody in the representative serum collection in Japan demonstrated that the antibody-positive rate was as low as 20% or lower in young age groups but it remarkably increased in middle ages and reached a high level (>80%) in older age groups (Fig. 3).

When the 1973- and the 1984-data were compared, the antibody prevalence in the 20-39-year olds was significantly lower in 1984 than in 1973, with average 10-year prevalence shift. Almost all (99%) of the 0-9-year-olds in 1973 were seronegative. In 1984, only 16% of the 0-9-year-olds who were born after 1973 and 20% of the 10-19-year-olds were seropositive. These findings indicate the followings: first, no large epidemics of B19 infection occurred during the decade before 1973; second, a clinically observed local EI epidemic in 1981 affected a small proportion of young population, and; third, a large number of those in their twenties including child-bearing females remained susceptible to B19 in 1984 as a result of notable shifting of B19 susceptibility toward older age groups. With the same serum collection in 1973 and 1984, the more prominent age shift was observed also in seroepidemiology of hepatitis A virus (2), whereas no significant change was observed in varicella-zoster virus prevalence (3) reflecting the epidemiological feature of these diseases in Japan (1).

Then, we examined the level of anti-B19 antibody prevalence acquired after a large EI outbreak in 1986/87 with the serum samples collected in three representative prefectures. In two prefectures, Fukuoka and Chiba, the antibody prevalences for the two age groups of 5-9 and 10-14 in 1987/88 were higher than those in 1984 as well as in 1973, reflecting an outbreak of EI in Japan in 1986/87. The levels of those in 1987/88 were low in Gunma, high in Fukuoka and middle in Chiba, correlating with the size of each EI incidence clinically observed in the three prefectures: the numbers of EI cases per reporting clinic under NESIDP in 1986/87 were 46.07 in Gunma, 83.08 in Chiba, and 97.88 in Fukuoka (1). This means that B19 is transmitted mainly among children in epidemics of EI.

Unfortunately, some pregnant women acquired B19 infection and developed EI resulting in fetal death of hydrops fetalis in this outbreak (11). However, there were no corresponding changes in young adult seroepidemiology from 1984 to 1987 to explain the increase in reported cases of perinatal B19 infection: age-specific prevalences in age groups 20-29 and 30-39 in 1987 rather decreased, compared to those in 1984. There remained a large number of susceptibles in these child-bearing-age groups. The high prevalences in adults were reported in the previous cross-sectional studies (8,9) and it was supposed that frequent asymptomatic infections with B19 occurred in adults as well as in children (12).
But, our time-comparative data suggest that asymptomatic B19 infection in adults was not so frequent as in children, if any. High prevalence in older people may suggest the pre-existence of B19 outbreaks in their childhood.

Our IgG ELISA with the monoclonal antibody was found to be very convenient and useful for seroepidemiology of B19 virus infection and can be used for antibody screening of women who are at increased risk of B19 infection from children at their places of work or at home to provide recommendations for prevention in pregnancy.

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