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Seroepidemiological Study of Human Parechovirus 1

Shinichi Takao*, Yukie Shimazu, Shinji Fukuda, Masahiro Noda and Kazuo Miyazaki

Division of Microbiology II, Hiroshima Prefectural Institute of Health and Environment, Minami-machi 1-6-29, Minami-ku, Hiroshima 734-0007

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Human parechovirus 1 (HPEV1), previously known as echovirus 22, was recently reclassified as *Parechovirus*, a new genus distinct from the *Picornavirus* genus on the basis of its exceptional molecular and biological properties (1). HPEV1 was originally isolated in 1956 during an epidemic of summer diarrhea (2). Previous studies reported that the predominant clinical manifestations caused by HPEV1 were infantile diarrhea and respiratory illness (3-7), but more serious symptoms such as myocarditis and encephalitis have also been reported (3). Reports on infections and the epidemiology of this virus, however, have been few, probably because infection with this virus is rare compared with other enteroviruses such as echovirus 30 (8). In this study, we investigated the antibody prevalence of HPEV1 in order to elucidate the extent of its infection in Hiroshima Prefecture.

A total of 195 sera, collected in October 2000 from residents living in Hiroshima Prefecture aged between 5 months and 79 years old, were measured for neutralizing antibody titer against HPEV1. A recent isolate of HPEV1 (HA00-243 strain: isolated in Hiroshima Prefecture in July 2000) (9) was used as an antigen, and the method used was the standard micro-neutralizing technique for enteroviruses with some modifications. Neutralizing antibody titers were expressed as a reciprocal of the highest serum dilution which inhibited the cytopathic effect by 50%. Titers exceeding 1:4 were considered to be positive.

Neutralizing antibody titers and the antibody-positive rate of age groups against HPEV1 are shown in Fig. 1. Significant levels of antibody against HPEV1 were found in almost all the sera tested (178/195; 91%), with the titers ranging from 1:4 to 1:512 or more. The number of antibody-positive sera began to increase from 6 months of age, and then rapidly increased further. Almost all the children aged between 1 and 15 years old had antibody against HPEV1 (1-5 years old: 78%, 6-10: 100%, 11-15: 89%, respectively). Additionally, the geometric mean of neutralizing titers in these age groups was higher than in other age groups (Fig.1). Similar seroepidemiological findings on HPEV1 have been reported by others. In a study in Finland, 72 of 79 individuals aged over 1 year (91%) had neutralizing antibodies against HPEV1 (5), and studies carried out in the 1960s in Japan showed that almost all the children aged over 1 year had antibody against HPEV1 (6,10). These results indicate that the seroconversion for HPEV1 antibodies occur in almost all children shortly after 1 year of age. Furthermore, HPEV1 was most commonly found in children aged under 1 year: according to the WHO data from 1967 to 1974, 61% of HPEV1 infections reported

*Corresponding author: Fax: +81-82-254-1908, E-mail: takao @urban.ne.jp

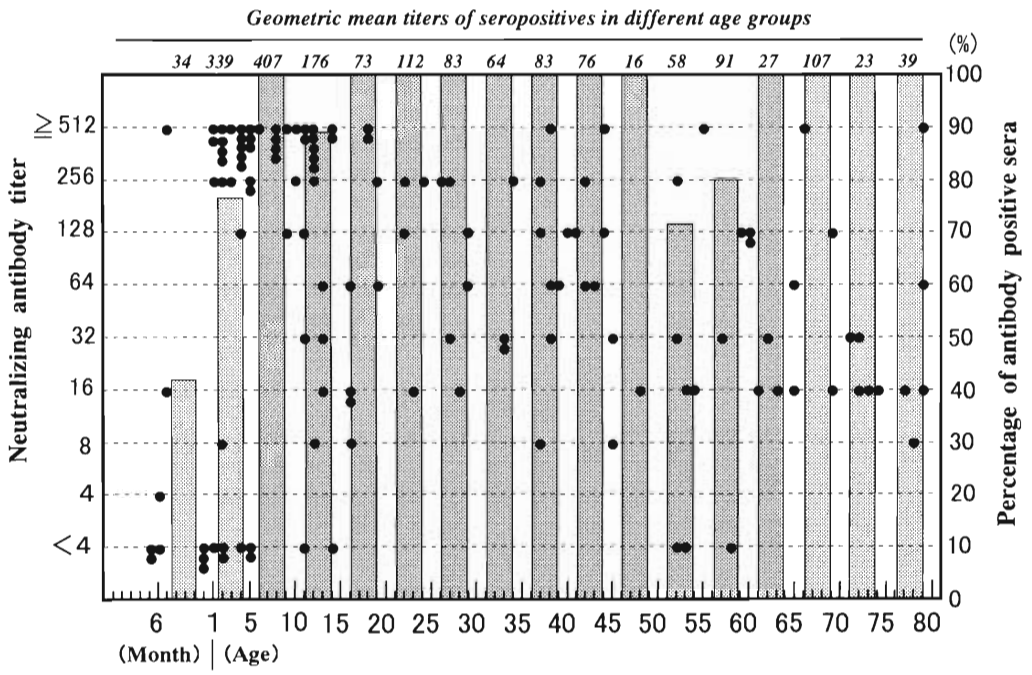

Fig. 1. Age-related distribution of neutralizing antibody and antibody-positive rate in age groups against HPEV1 isolate. Neutralizing antibody titer of 195 sera collected from residents of Hiroshima Prefecture in October 2000 were determined using the microplate technique. Sera were heated at 56°C for 30 min before testing. Two-fold serial dilutions from 1:4 to 1:512 of the sera were tested against approximately 100 $TCID_{50}$ of HPEV1 (HA00-243 strain). Serum-virus mixture were incubated for 2 h at 37°C before inoculation into 96-well microplate in which BGM cells were grown. The plates were incubated in a CO_2 incubator at 34°C for 4 days. Neutralizing antibody titers were expressed as a reciprocal of the highest serum dilution in which 50% protected the cytopathcic effect, and were plotted against age on the graph. The percentages of antibody-positive sera (neutralizing titer of 1:4 or more) were determined every 6 months (under 1 year of age) or every 5 years of age, and are shown as bars on the graph. Geometric mean titers of each age group are shown on the top of bars corresponding to the groups.

were seen in children under 1 year old, and 97% (566 out of 581 cases) were observed in children aged under 15 years (11). In a retrospective study in Sweden, a total of 109 cases of HPEV1 infection have been reported during a 25-year period from 1966 to 1990, and, of all the patients from whom HPEV1 was isolated, 72% were aged under 1 year (4). We also previously reported that all of the patients from whom HPEV1 was isolated were under 2 years of age (9). Thus, we consider that HPEV1 is a common human pathogen whose infection occurs in the early years of life.

We compared the neutralizing antibody titers of the sera against the recent isolate (HA00-243 strain) and the prototype HPEV1 (Harris strain, which was isolated in 1959) (2). Antibody titers against the Harris strain were essentially the same for those of the HA00-243 strain (data not shown), which suggests that the antigenic epitope that induces the production of neutralizing antibody may be relatively well conserved among HPEV1 as described by Joki-Korpela et al. (12). We are currently planning a phylogenetic analysis among HPEV1 to make clear the molecular epidemiology of HPEV1 infection.

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