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Comparison of Rotavirus Immunoglobulin G Titers in Sera Collected in Japan and Kenya

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Key words : rotavirus, ELISA, IgG, seroepidemiology

Abstract

ELISA for detecting IgG antibody against human rotavirus was carried out on the sera collected in Kumamoto and Nyeri, Kenya. In both areas, most of the infants acquired antibody by 3 years of age and kept high level thereafter. The antibody prevalence among age groups in the two areas showed almost same patterns. Correlation was seen between ELISA titers and neutralization titers.

Introduction

Human rotavirus (HRV) is an etiological agent of infantile gastroenteritis [1,2]. In temperate climate, HRV infection is common during winter [3], occupying 70-90% of infantile diarrhea [4]. However, the situation in tropical climate was not clear. We developed ELISA for detecting HRV antigens in feces [5]. Using this system, epidemiological survey of HRV infection was conducted in Kenya, east Africa, and found the seasonal prevalence of infection [6].

In this report, we set up ELISA for detecting IgG antibody against HRV in human serum. Using this system, we tested sera collected in Kumamoto and Kenya and compared antibody prevalence.

Materials and Methods

Cells and viruses : MA 104 cells, a cell line of monkey kidney, were used for the growth of the viruses. Tissue culture adapted human rotavirus type 1 (K8 strain), type 2

(S2 strain) and type 3 (YO strain) were kindly provided by Dr. Urasawa, Sapporo Medical College. They were propagated in MA104 cells in the presence of trypsin [7].

Purification of antigen : Human rotavirus (K8 strain) was multiplied in MA104 cells in roller culture bottle in the presence of trypsin (0.8 μg/ml). The purification procedures were essentially the same as described before [8]. Purified antigen was used for the ELISA coating.

Serum specimens : Following sera were obtained; 190 specimens were collected in Kumamoto Prefecture during July through October 1981[a], 91 specimens were collected in Nyeri area, Kenya, during January and February 1982. The sera were heat inactivated at 56°C for 30 min and assayed.

Neutralization test (NT) : Serial 3-fold dilutions of serum specimens (start from 1 : 100 to 1 : 2,700) were mixed with equal volume of virus containing 20 $TCID_{50}$ (in this case, $TCID_{50}$ was determined by antigen-ELISA) [5]. The mixtures were left for 1 hr at 37°C, then inoculated onto tube cultures of MA104 cells. After the adsorption of 1 hr at 37°C, the cells were washed twice with PBS, fed with Eagle's medium containing 0.8μg/ml of trypsin. The cultures were incubated at 37°C for 3 days in the roller culture apparatus. After the incubation period, the cells were frozen and thawed twice and culture media were assayed for rotavirus antigen by ELISA [5].

IgG-ELISA : Indirect micro-ELISA was employed[10]. The procedures were essentially as described before for the anti−Japanese encephalitis virus antibody assay[11]. Briefly, polystylene microtiter plate was coated with 100 μl of purified HRV per well by incubating at 37°C for 2.5 hr. The plate was rinsed 3 times with PBS (pH 7.2) containing 0.05% tween 20 (PBS-t) and emptied. Test or standard sera diluted in PBS-t were added to the plate (100 μl per well) and incubated at 37°C for 1 hr. The plate was then rinsed 3 times as above. Peroxidase-conjugated anti-human IgG (Cappel lab., U.S.A.) diluted in PBS-t (1 : 2,000) was applied to each well and incubated at 37°C for 1 hr. After rinsing the plate, substrate solution containing 0.5 mg/ml of o-phenylenediamine 2HCl (Wako Pure Chem. Co.) and 0.02% H_2O_2 in 0.05M citrate-phosphate buffer (pH 5.0) was reacted for 30 min at room temperature in the dark. The reaction was stopped by adding 75 μl per well of 4N H_2SO_4 and the optical density (OD) at 500 nm was recorded by microplate photometer (Corona Electric Co.). ELISA titer of the test specimen was estimated by comparing its OD with those on the standard curve obtained from a serial dilution of a standard positive serum, which was run in parallel[11]. Preliminary experiments revealed that, under conditions employed, ELISA titers over 160 was considered antibody positive.

Results

Comparison of ELISA titers with NT : Eighty-three sera collected in Kumamoto were tested both by ELISA and NT. NT titers were expressed as the highest neutralizing titers against the challenge of 3 different serotypes of human rotaviruses. Lineal correlation between logarithm of the titers obtained by ELISA and NT was observed (Fig. 1). The equation of linear regression was $Y = 154 + 0.86X$ with correlation coefficient of 0.69 ($p < 0.01$).

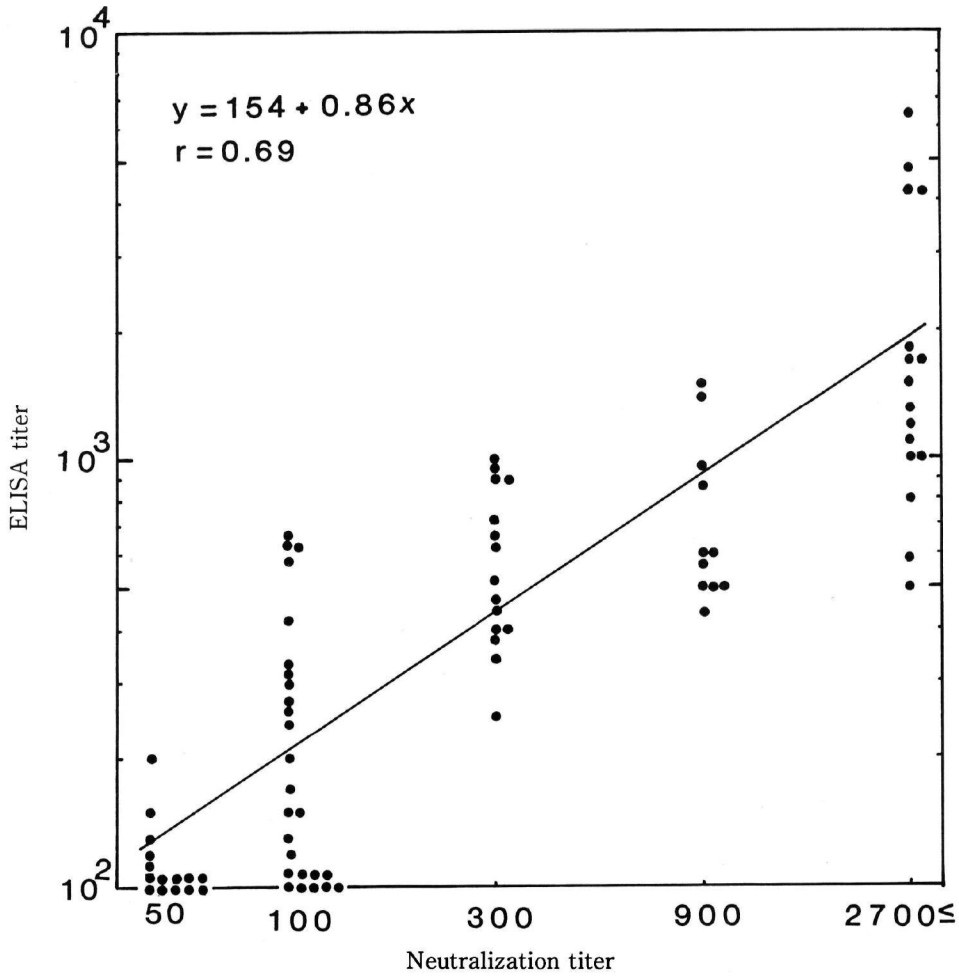

Fig. 1 Comparison of ELISA titer with neutralization titer

IgG-ELISA titers in sera of various age groups collected in Kumamoto were plotted (Fig. 2). Twelve of the 15 sera aged less than one year-old was negative at 1 :100 dilution. The geometric mean titer of this age group was 150. Whereas, the sera taken from the age group older than one year contain variety of rotavirus antibody titers : between less than 1 : 100 and over 6,400. The mean geometric titers among the age groups were between 330 and 1,100.

ELISA titers in sera taken in Kenya were shown in Fig. 3. Although the number of sera tested was not sufficient, mean titers among the age groups varies from 195 up to 950 which were almost the same levels seen in Kumamoto. However, some sera of the age group less than one year old, contain high antibody titers. Although some of this age group may possess maternal antibody, such high titers as over 1,000 may reflect recent rotavirus infection. Mean titer of this age group was 250.

Rotavirus antibody prevalence among age groups in both area was compared (Fig. 4). Antibody prevalence in Kenya and Kumamoto revealed almost same pattern. Namely, most of the children in both areas obtained antibody by 3 years and kept high positive rate in older age groups.

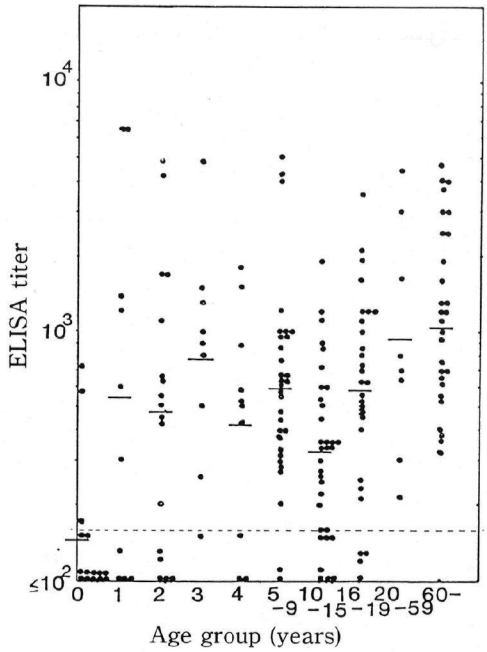

Fig. 2 Prevalence by age of serum antibody to human rotavirus detected by ELISA
(Kumamoto)

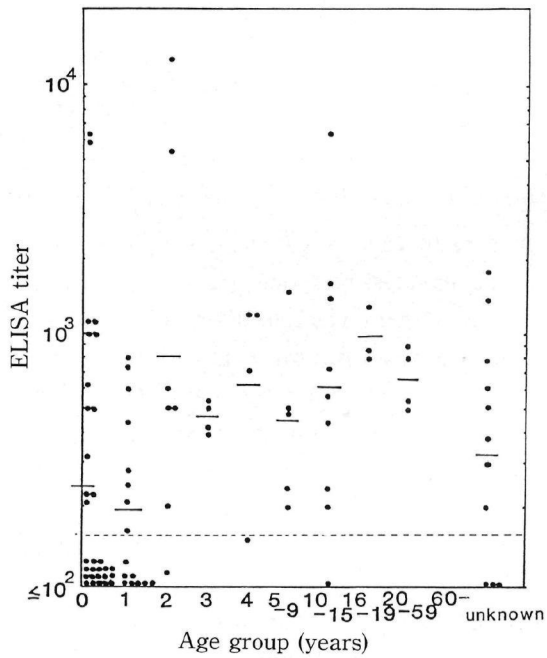

Fig. 3 Prevalence by age of serum antibody to human rotavirus detected by ELISA
(Kenya)

Discussion

It is conceivable that antibody detected by ELISA may not be the same one as detected by NT[12]. In fact, the antigens used in this experiment are mainly single shelled particles when observed by electron microscope whereas the antigen related to neutralization is thought to be present on outer capsid layer of the virion[13]. However, ELISA titer was well correlated with NT titer.

The geometric mean titer of sera collected in Kumamoto was low in age group below one-year-old and the titer went up by the age. On the other hand, a part of sera of this age group (i.e. below one-year-old) collected in Kenya showed high titers which made the mean titer high, although the incidence was not much different between Japan and Kenya (Fig. 4). HRV infection has been prevailed during winter season in Japan[3]. In Kenya, where there is no winter, HRV infection was seen all the year round, being more prevalent during dry season[6]. Infants less than 6-month-old were commonly infected with this virus[6]. While in Japan, the highest frequency of the illness was seen in the 6- to 18-month-old infants[3]. In both areas, most of the children acquired antibody by the age of 3 years. This is true of other temperate countries including United States[3]. It is surprising that although environmental sanitation in both areas is remarkably different, antibody prevalence is not much different. The critical measures for blocking the transmission of the virus seem to remain unearthed.

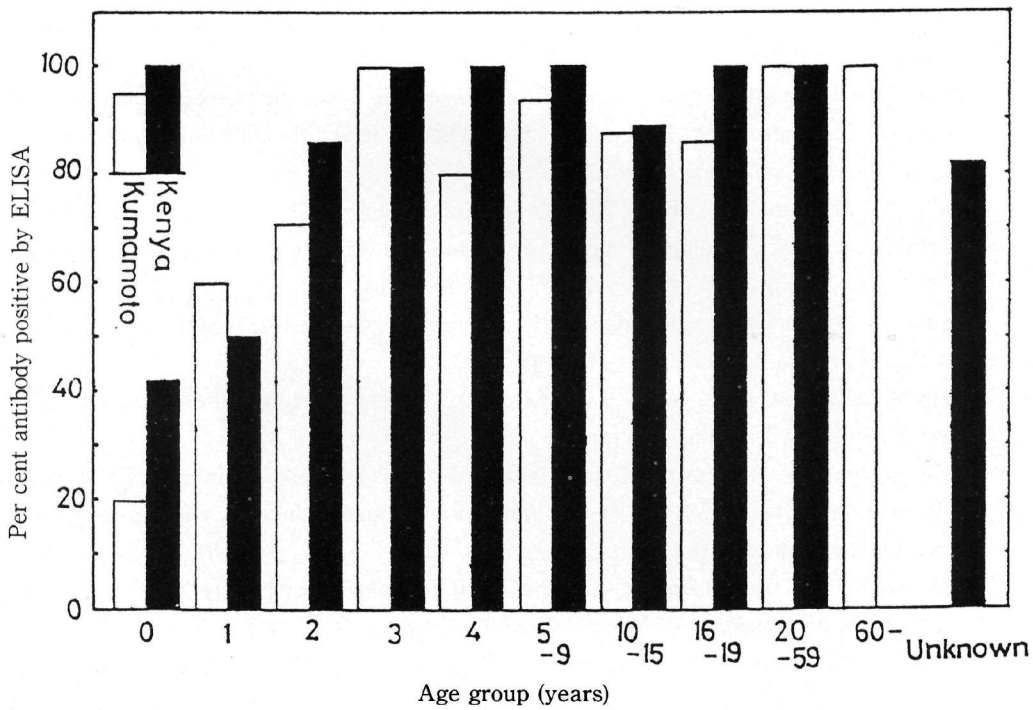

Fig. 4 Age distribution of rotavirus antibody prevalence.

The results of neutralization test of sera collected in Kumamoto revealed that the neutralizing antibody against serotype 3 was detected in high frequency over serotype 1 and 2 (data not shown). It is not clear whether one serotype is predominantly circulating or several subtypes are concomitantly present in the same community.

It has been speculated that secretory IgA may play an important role in protecting against homotypic rotavirus infection[14].Studies on the ELISA system for detecting IgA as well as IgM antibody against rotavirus are being carried out.

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