Development of Class Specific Antibodies in Sera Following Naturally Acquired Human Rotavirus Infections as Measured by Enzyme−linked Immunosorbent Assay (ELISA)

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Abstract: Temporal distributions of class specific antibodies to human rotavirus in sera from patients with infantile gastroenteritis were studied. For the detection of IgG antibody, indirect ELISA was used, while for the IgM and IgA antibody detection, antibody−capture ELISA using labeled antigen was employed. In most cases, rapid seroconversions of IgG antibody were observed. The mean IgG titer reached 160, which was the upper limit of negative value, on the 8th day of illness and increased thereafter. IgM antibody was detected on the 3rd day of illness and reached maximum level between 8 and 14 days after the onset of illness and decreased thereafter. However, low level of IgM antibody (e.g. titer 1:100) was detected for relatively longer period of time (i.e., positive at 33 and 53 days after onset of illness). Serum IgA antibody level varied among individuals. Although elevation of antibody titers were generally observed in the convalescent sera, their rises were relatively slow and mild. The results indicate that the detection of anti−rotavirus IgG and IgM antibodies by ELISA is of diagnostic value, while IgA antibody detection appeared to be of limiting diagnostic value.

Key words: Human rotavirus infection, ELISA, Class specific immunoglobulin, Temporal course

Introduction

Human rotavirus (HRV) is an etiological agent of infantile gastroenteritis (Flewett

and Woode, 1978). The lesion of HRV infection is confined to the intestinal mucosa. Several serotypes can be distinguished by neutralization test (Sato *et al.*, 1982; Urasawa *et al.*, 1982; Hoshino, 1984). Reinfection and/or infection due to the different serotypes in the presence of low level of serum antibody have been reported (McNulty, 1978; Rodriguez *et al.*, 1978). However, few studies have been done on the immune response of class specific immunoglobulins after the naturally acquired HRV infection and on the persistence of protective efficacy of the antibody against re—infection. Enzyme—linked immunosorbent assay (ELISA) is highly sensitive and has ability to detect antibodies of different immunoglobulin classes. We reported that IgG—ELISA titers were well correlated with neutralization titers (Makino *et al.*, 1984). We further attempted to detect serum IgM and IgA antibodies to HRV by antibody—capture ELISA using labeled antigen. This paper describes the temporal appearance of serum IgG, IgM and IgA antibodies to HRV following naturally acquired rotavirus infections.

MATERIALS AND METHODS

Serum specimens: A total of 137 sera (36 paired and 65 single sera) were obtained at a private pediatric clinic in Nagasaki City, from the patients with infantile gastroenteritis (aged between 1— and 26—months—old) during a period of December 1982 through February 1983, by filter paper method (Guimaraes *et al.*, 1978). The filter papers were dried at room temperature and stored at $-20°C$. When tested, the sera were eluted in PBS—T (phosphate buffered saline containing 0.05% Tween 20), inactivated at 56°C for 30 min and subjected to ELISA. The fecal specimens of the patients were also taken at the same time and examined for the rotavirus antigens by Rotazyme kit (Abbott lab).

Antigen: Strain K8 (serotype 1, subtype 2) of HRV (Urasawa *et al.*, 1982) was kindly supplied by Dr. Urasawa, Sapporo Medical College. The virus was propagated in MA104 cells in the presence of trypsin (0.8 μg/ml), concentrated by 8% polyethylene glycol and centrifugation (Espejo *et al.*, 1980).

Conjugation of antigen with horseradish peroxidase: Method described by Wilson and Nakane (1978) was followed with modifications. A mixture of 5 mg antigen at alkaline pH (pH 9.5 in 0.01M carbonate buffer) and 4 mg of activated peroxidase (PO: type VI, Sigma) was incubated at room temperature for 2 hrs. $NaBH_4$ (0.4mg) was then added to the mixture and incubated overnight at 4°C, dialyzed against phosphate buffered saline (PBS, pH7.2) overnight at 4°C. Free PO was removed by ammonium sulfate precipitation and dialysis. Aliquots were dispensed and stored at $-80°C$ until used.

IgG—ELISA: Indirect ELISA (Voller *et al.*, 1976) was employed with modifications (Makino *et al.*, 1984). Partially purified K8 antigen was immobilized on the polystylene microplate (Immulon). The plate was then reacted in the order of serum specimen (1:100), peroxidase conjugated anti—human IgG (H&L chain specific; Cappel lab.) and substrate (0.5 mg o—phenylenediamine·2HCl [Sigma] in citrate phosphate buffer, pH 5.0 plus 0.01% H_2O_2). Between each step, the plate was washed 3 times with

PBS−T. After 30 min of incubation period in the dark, the reaction was stopped by the addition of 4N H_2SO_4. Absorbance of the reaction was determined with a photometer (Corona microplate photometer) at 500 nm. Negative and serially 2−fold diluted positive standard sera were also included in each assay. ELISA titers of the test specimens were estimated by comparing its O.D. with those on the standard curve obtained from the serial dilutions of a standard positive serum running in parallel (Igarashi *et al.*, 1981; Bishop *et al.*, 1984).

IgM−ELISA: Antibody−capture ELISA using labeled antigen was employed (Duer-myer *et al.*, 1979). Brifly, goat anti−human IgM (mu−chain specific, Cappel lab.) was immobilized on polystylene microplate. After washing as above, the plate was reacted in the order of serum specimen (1:100), labeled antigen (1:50), and substrate. Subsequent steps were the same as IgG−ELISA. Preliminary experiment using sucrose gradient frac-tionated sera (Caul *et al.*, 1978) revealed that IgM−ELISA reacted only with IgM frac-tion and not with IgG fraction.

IgA−ELISA: This was the same as IgM−ELISA, except that anti−human IgM was replaced with anti−human IgA (alpha chain specific, Cappel lab.).

RESULTS

During a period examined, a total of 137 sera were obtained from the infants with gastroenteritis (pair and single sera were included). The results of the fecal examination of these patients by Rotazyme test were summarized (Table 1). The sera from patients with rotavirus antigens positive in feces were defined as "positive sera" and those with negative in feces were defined as "negative sera". (It should be noted that "Negative sera" group contained two pairs of sera which were suspected to be positive for HRV in-fection by the subsequent serological test, although Rotazyme test was negative which is discussed later.)

Twenty−four paired positive sera were tested by IgG−ELISA (Fig. 1). Four−fold or more rise of antibody titers (or sero−conversions) were commonly observed when the second sera were taken later than 10 days after the onset of illness. However, there are 7 cases in which the titers were only slightly elevated in

Table 1. Results of fecal examination for rotavirus antigens in pa-tients whose sera were taken

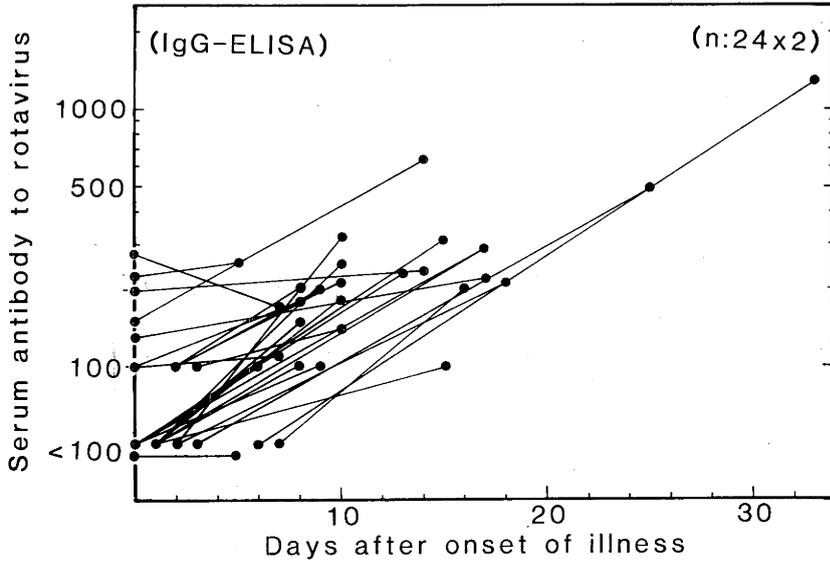| Serum	Rotavirus antigens in feces	
	Positive	Negative
Pair	48	24
Single	32	33
Total	80	57

Fig. 1. Temporal distribution of serum IgG antibody in patients with HRV infection. Solid lines indicate paired sera.
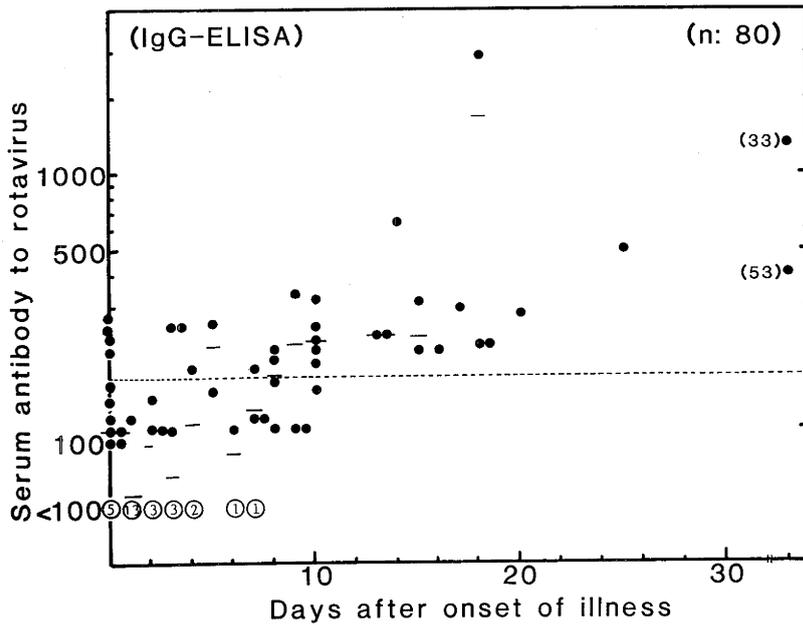

Fig. 2. Temporal distribution of serum IgG antibody in patients with HRV infection. Numbers in the circles indicate the number of specimens with the titers less than 100. Numbers in the parentheses indicate days after onset of illness that the sera were taken. Bars indicate geometric mean titers. Broken line indicates cut−off titer (e.g titer:160).

the second sera. Four of them were taken as early as 5 to 7 days of illness. One case showed decreased titer in the second serum which was discussed later.

IgG−ELISA was performed on 80 positive sera (including paired and single sera) and temporal course of antibody level was depicted (Fig. 2). IgG−ELISA titers over 160 were defined as positive in our ELISA (Makino *et al.*, 1984). Geometric mean titer reached 160 in 8 days after onset of illness and increased thereafter during a course examined.

Twelve paired "negative sera" were tested by the IgG−ELISA (Fig. 3). All the paired sera except two showed almost the same level between the first and second sera. There were two cases in which the second sera showed either seroconverted or antibody elevation, suspecting the recent HRV infection. During a course of HRV infection, large quantities of viral antigens in the feces can be detected rather in a short period and soon turned into negative by Rotazyme test, although small amount of virus continue to be excreted into the feces, thus failed to detect HRV antigen.

Temporal course of IgM antibody titers was depicted (Fig. 4). Preliminary experiments, using fractionated IgM antibody of the positive samples, revealed that IgM−ELISA titers over 100 were considered positive in our system. The IgM titer increased on the 4th days of illness and reached the maximum level in 4 to 10 days and gradually decreased thereafter. In one serum, the titer remained 100 even 53 days after the onset of illness.

Next, an attempt was made to detect serum anti−HRV IgA antibody using IgA−capture ELISA. Twenty−four paired "positive sera" were examined (Fig. 5). A variety of antibody titers were obtained in the temporal course of illness. Although in most cases, antibody rises were observed in the second serum, no such rapid seroconversions
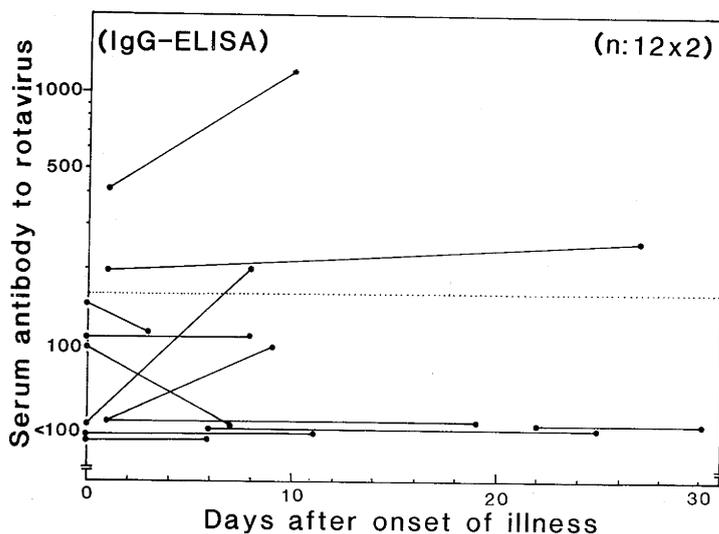

Fig. 3. Temporal distribution of serum IgG antibody in patients that were negative for HRV antigens in the feces by Rotazyme test. Dotted line indicates cut−off titer (e.g titer:160).
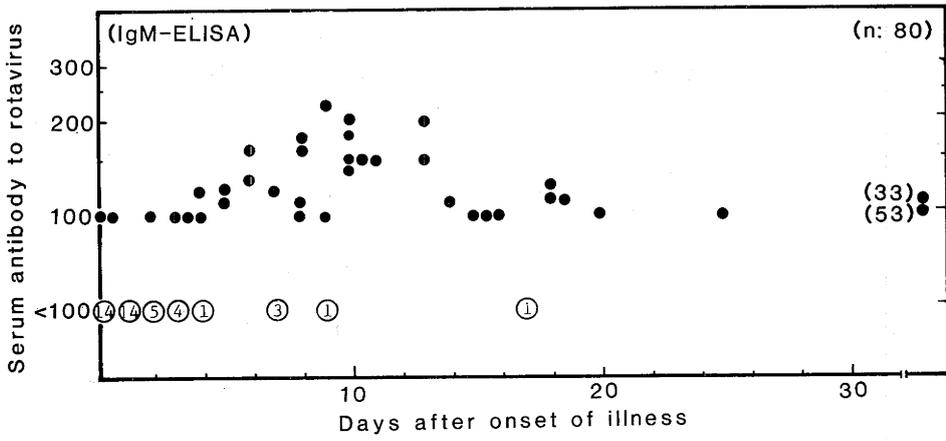
Fig. 4. Temporal distribution of serum IgM antibody in patients with HRV infection. Numbers in the circles indicate the number of specimens with the titers less than 100.
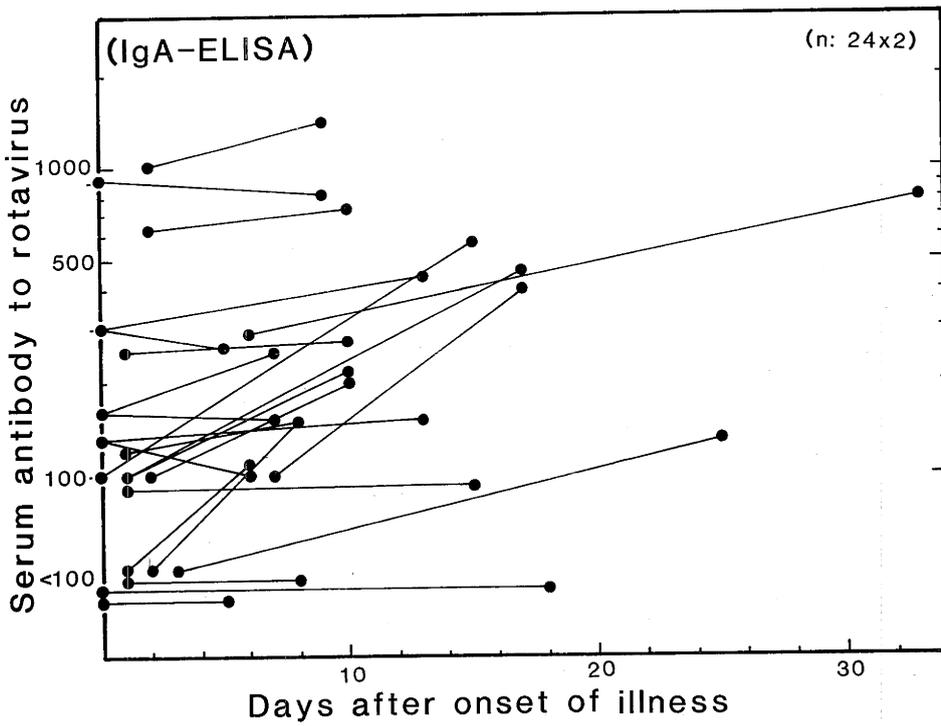

Fig. 5. Temporal distribution of serum IgA antibody in patients with HRV infection. Solid lines indicate paired sera.

as seen in IgG−ELISA was observed in IgA−ELISA. Five sera showed high titers (over 300) at an early stage of illness. All these sera also contained low levels of IgG antibodies as well, suggesting that these patients had experienced previous rotavirus infection.

DISCUSSION

In this study, we tried to detect class specific rotavirus antibodies by ELISA. For the IgG−ELISA, indirect method was employed, since this method appeared to be the best assay for IgG (Summers et. al., 1984). While, for IgM− and possibly IgA−ELISA, antibody−capture method is superior to the indirect method (Heinz et. al., 1981). In this study, antibody−capture ELISA using labeled antigen was employed. This method has been used for the detection of IgM antibody to cytomegalovirus (van Loon et. al., 1981a), herpes simplex virus (van Loon et. al., 1981b) and arboviruses (Schmitz and Emmerich, 1981). It is more simple than ordinary ELISA of four step reactions and still possesses high sensitivity and specificity.

In the IgG−ELISA of paired "positive sera", seroconversions were observed in most cases. Four−fold or more rise of the IgG titer in the convalescent serum is generally considered HRV infection. However, there were one case in which the antibody titer went down in the second serum. In this case, the patient was secreting maximum amounts of rotaviruses in the feces when the second serum was taken. Reduced serum IgG antibody response in association with severe or prolonged rotavirus enteritis has been reported (Riepenhoff−Talty et. al., 1981). In our case, serum IgA level was also reduced in the second serum, while IgM level was still negative. Therefore, it appeared that in such case, pre−existing serum IgG might be consumed or lost through the intestinal lesion where a large quantity of antigens were being produced. The results indicated that IgG− and IgM−ELISA proved its diagnostic usefulness.

The titers of IgA−ELISA varied in individuals. Some showed quite high titers from an early stage of infection. In the cytomegalovirus infection, IgA response closely resembled the IgM response (van Loon et. al., 1984). However, in the case of adenovirus, influenza A and B viruses infections, IgA antibody in the serum was able to be detected in less than a half of the cases. Moreover, in case of respiratory viruses, a number of control sera appeared positive for IgA (van Loon et. al., 1984). In case of RS virus infection, in some cases, the persistence of IgA antibodies followed that of the IgM antibodies, but in others, the IgA remained high up to the end of the follow−up (Meurman et. al., 1984). These reports and our data indicate that IgA−ELISA (for the detection of serum IgA) may be a limiting usefulness for the serodiagnosis. Recently, IgA antibody in feces has been titrated by ELISA. In feces, there exist two different IgA's; 11S antibody with secretory component and 7S antibody, presumably Fab (Inouye et. al., 1984). The significance of secretory IgA in local immunity has been discussed. However,

the role of serum IgA remains unearthed.

In this experiment, we measured serum class specific antibodies to HRV. It will be important to elucidate how long serum antibodies and coproantibodies exist after the natural HRV infections and how long they provide protection against reinfection of the same serotype or different serotype.

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酸素免疫測定測 (ELISA)　によるヒトロタウイルス自然感染後のクラス別抗体価の推移

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　乳児急性下痢症患者血清中のヒトロタウイルスに対するクラス別特異抗体価の経時的変動を ELISA で調べた. 抗原は MA 104細胞で増殖させたヒトロタウイルスK8株を部分精製し用いた.　IgG の測定は ELISA 間接法で,　IgM および IgA の測定は標識抗原を用いた抗体捕捉法で行った.　IgG 抗体の平均値は発症後8日目に陰性の上限値（1：160）に達し, 以後測定期間中, 陽性値を示した.　IgM 抗体は発症3日目から検出され, 8～14日目にピークに達し以後減少したが, 発症後33日目および53日目でも低値ながら検出された. 一方,　IgA 抗体価の変動は個々の症例で差が見られ, その上昇も IgG,IgM に比べ緩やかであった. このことは血中 IgA の測定は IgG,IgM の測定に比べ診断的価値は制限されるものと考えられる.