Complement-Fixing Immunoglobulin M Antibody Response in Patients with Infantile Gastroenteritis

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Complement-fixing immunoglobulin M antibody to infantile gastroenteritis virus (a rotavirus) was detected with highest sensitivity when the antibody-antigen-complement mixture was incubated at 37°C for 1 h prior to the addition of sensitized sheep erythrocytes. Sucrose gradient centrifugation of sera collected sequentially from four patients after infection detected 19S complement-fixing antibody up to 5 weeks, with highest titers at 1 week, after the onset of illness. Treatment of the whole sera with 2-mercaptoethanol decreased complement-fixing titers only up to 2 weeks after onset of illness.

Complement-fixing (CF) antibody (Ab) responses in paired sera from patients with infantile gastroenteritis (IG) were described by Kapikian et al. (7), and early-appearing CF Ab was found to be 2-mercaptoethanol (2ME) sensitive (8). We report here on the sedimentation patterns of CF Ab activity in sera sequentially collected after infection.

Since we found that 19S fractions of the sera from IG patients fixed complement (C) very weakly in the CF test, we first sought the most sensitive way to detect the 19S CF Ab activity. Four different incubation procedures were compared for the Ab-antigen (Ag)-C reaction in the CF test prior to the addition of sensitized erythrocytes: (i) incubation of the Ab-Ag-C mixture at 4°C overnight, i.e., the conventional procedure; (ii) the same as (i) except that the mixture was thereafter incubated at 37°C for 1 h; (iii) Ab-Ag mixture was incubated at 4°C overnight, then at 37°C for 1 h with the addition of C; (iv) Ab-Ag-C was incubated at 37°C for 1 h only. Procedure (ii) gave the best results for the detection of the 19S CF activity, and procedure (i) was the least sensitive. In procedures (iii) and (iv), CF activity of 19S fractions was slightly weaker than in (ii), and 7S Ab fixed less C than in (i) and (ii) (Fig. 1). The same results were obtained on other serum samples. Therefore, we adopted procedure (ii) in the CF tests for the following studies.

Figure 2 shows CF Ab titers of both control and 2ME-treated sera sequentially collected from four infants after infection. Within 2 weeks after the onset of disease, the CF titers were

FIG. 1. Effect of different incubation procedures on the CF activity of 7S and 19S Ab's. Serum from an infant 13 days after the onset of illness was diluted 1:3 and incubated at 56°C for 30 min; then 0.5 ml was layered on a 4.5-ml 12.5 to 37% (wt/vol) sucrose gradient in Veronal-buffered saline (pH 7.3) and centrifuged in a Spinco SW50.1 rotor at 35,000 rpm for 16 h at 4°C. As 7S and 19S markers, rabbit hemolysin was centrifuged in another gradient at the same time. After centrifugation, the gradients were fractionated

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FIG. 2. CF Ab titer response of the whole serum. CF Ab titers were determined on serum specimens sequentially collected from four patients after infection. For 2ME treatment, 0.4 ml of serum was mixed with an equal volume of Veronal-buffered saline and incubated at 56°C for 30 min, then mixed with 0.8 ml of 0.2 M 2ME and incubated at 37°C for 1 h. After overnight dialyzing against Veronal-buffered saline at 4°C, the CF test was carried out. Reciprocals of the serum dilutions that gave 50% hemolysis were taken as end points. (●—●) Control; (○—○) 2ME treatment.

decreased by 2ME treatment. Figure 3 shows the sedimentation patterns of CF Ab activity in the control sera. 19S CF activity was detected up to 5 weeks after the onset of illness. Maximum 19S activity occurred at about 1 week after onset. When the 2ME-treated sera were centrifuged, no CF activity was detected at the 19S fractions (data not shown).

In some microbial and parasitic infections, the host responds to produce rheumatoid factor (immunoglobulin M [IgM] Ab to IgG) (13). IG virus Ag's prepared from feces contain immunoglobulins (11, 12). Thus, we considered the possibility that the 19S Ab activity shown in Fig. 3 was directed not against the virus Ag but against IgG

molecules complexed with the virus. However, we could not detect any agglutination of IgG-coated latex particles (Hyland) in the sera of the four patients studied. Accordingly, we conclude that the 19S CF activity was due to virus-specific IgM Ab.

To abolish the anticomplementary activity of sera, we heated the sera at 56°C for 30 min before centrifugation. We found that without this pretreatment anticomplementary activity was detected at the highest titers in the sucrose gradient fractions just behind the 19S marker, and that the anticomplementary activity of the gradient fractions was not destroyed by heating (unpublished data). Heating of sera at 56°C might cause some aggregation of IgG. We measured the IgM and IgG concentrations of each fraction by single radial immunodiffusion with Hyland immunoplates and detected only IgM or IgG in the 19S and 7S fractions, respectively. IgG aggregates, if present, would be heterogeneous in their sedimentation rates and would have been distributed broadly in the gradient.

It has been reported that IgM Ab fixes C most effectively when an Ag of large size with repeating determinants is used (3, 6) and the Ab-Ag-C mixture is incubated at 37°C (3, 10). In the case of such small-sized viruses as picornaviruses and flaviviruses, IgM Ab does not fix C (1, 4, 9). In contrast, rotavirus would be large enough for the IgM Ab to fix C. Thus, when detecting IgM CF Ab to IG virus it is recommended to use the

using a peristaltic pump as described previously (5). Each fraction was then tested in microplates for its CF activity in four different incubation procedures using 2 units of Ag and 5 50% hemolytic units of C. The IG virus Ag was prepared from feces by treating with fluorocarbon, concentrating with polyethylene glycol, and then pelleting through 45% sucrose (2). The control Ag was prepared from normal feces in the same way as above. Whenever the reaction mixtures were incubated at 37°C, the microplates were put into a water bath after sealing with pressure-sensitive film (Falcon). After the addition of sensitized sheep erythrocytes, the plates were incubated at 37°C for 30 min and then centrifuged at 350 × g for 3 min. 1, 2, 3, and 4 indicate 75, 50, 25, and 0% hemolysis, respectively. ON, Overnight.

FIG. 3. Sedimentation patterns of CF Ab activity in the serum after IG virus infection. The same serum samples as shown in Fig. 2 were centrifuged in sucrose gradients as described in the legend to Fig. 1. All the samples from one patient were centrifuged at the same time. D, Days after the onset of disease.

particulate Ag free of "soluble" components and to incubate the Ab-Ag-C mixture at 37°C before the addition of sensitized erythrocytes.

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