Serum Antibody Response to Recombinant Major Inner Capsid Protein following Human Infection with Group B Rotavirus

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Recombinant major inner capsid protein (VP6) of the IDIR strain of group B rotavirus (GBR) was incorporated in a solid-phase immunoassay to access antibody response to infection in humans. Expression of VP6 in insect cells permitted design of a highly sensitive assay that avoided the contaminants present in GBR antigens obtained from fecal specimens. Among patients infected with the ADRV strain of GBR in China, increased reactivity with recombinant VP6 was observed in convalescent-phase sera in comparison with sera obtained shortly after infection ($P = 0.0084$). Anti-VP6 antibodies were detectable as soon as 7 days after onset of gastrointestinal symptoms, and serum reactivity persisted in specimens drawn more than 1 year after infection. Solid-phase immunoassay with recombinant VP6 was next employed in order to assess anti-GBR antibody in 513 serum specimens obtained from 423 Maryland residents (ages, 7 months to 96 years; median age, 42 years). Four individuals (<1%) exhibited serum antibodies directed against the recombinant VP6 (ages, 54 to 95 years; mean age, 77 years). Examination of 129 additional serum specimens including some from other geographic regions of the United States failed to reveal the presence of anti-GBR antibody. Anti-GBR antibody was also not detected in any of 131 serum specimens from 60 staff and residents of a nursing home in Switzerland. While infection of humans with GBR has been uncommon in these locations outside of China, the detection of serum antibodies in older individuals in the United States either indicated an unknown, age-related risk factor or may have indicated infection in the more distant past. The availability of these reagents should allow surveys for GBR infection among additional populations that have not previously been investigated.

Group B rotaviruses (GBR) have been associated with large outbreaks of gastroenteritis in China, with attack rates ranging from 5 to 51% (3, 8, 9, 11, 14, 15, 19). Gastroenteritis was initially reported among adults, and the infecting GBR strain was termed ADRV (adult diarrhea rotavirus). However, infection among all age groups has subsequently been noted, including at least one outbreak in a newborn nursery (3). Illness has been characterized by vomiting, abdominal cramps, and severe watery diarrhea without blood. Fever has not been commonly noted, and the mortality rate has generally been low. Blocking immunoassays with fecal isolates of GBR have clearly detected rises in antibody titer following ADRV infection in Chinese patients, but the peak titers have generally been much lower than after infection with group A rotavirus (GAR) (7, 10, 13–15). Counterimmunoelectrophoresis with fecal GBR antigens has indicated anti-ADRV antibody in 12 to 41% of serum samples from various locations in China (11).

Despite the many thousands of cases in China, little is known about human exposure to GBR in many other regions of the world. Nakata and coworkers found reactivity with ADRV with only 1 serum sample of 155 from the United States and with only 2 of 40 serum samples from Kenya, Australia, and Thailand (13). Peñaranda and collaborators also evaluated 60 serum samples from Burma and 60 serum samples from Thailand by testing antibody pools containing samples from 10 individuals. In that study, only one pool (contain-

ing 10 serum samples) from each country was positive (14). Among 110 veterinarians and farm workers and 100 blood donors in the United Kingdom, Brown and coworkers found blocking antibody to ADRV in 4 and 10% of specimens, respectively (2).

The ability to conduct more extensive seroepidemiologic investigations in additional locations would greatly aid in defining the spectrum of GBR infection. However, evaluations have been restricted because GBR have not grown well in tissue culture, antigenic reagents obtained from fecal specimens have been limited, and these reagents have been difficult to purify (6, 16, 18). In order to devise more convenient and useful detection of anti-GBR antibody, we have recently developed GBR reagents from alternate sources, based on the IDIR agent (infectious diarrhea of infant rats) originally isolated in Baltimore (12, 17). This virus was first identified in a colony of laboratory rats, but infection of a small number of humans with the IDIR agent has also been documented in Maryland (5). Cloning of gene 6 of the IDIR agent and expression of the gene in a baculovirus recombinant resulted in large-scale synthesis of GBR major inner capsid protein (4, 12). In previous studies, this recombinant VP6 was specifically recognized by antisera directed against heterologous GBR strains obtained from a variety of mammalian species (12). GBR VP6 appeared to encode group-specific epitopes in a manner similar to that formerly demonstrated for GAR. No reactivity with antisera directed against other rotavirus groups, including those GAR strains which have commonly been associated with winter-time epidemics of infantile diarrhea, has been observed.

We now wish to report the reactivity of human sera with

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recombinant GBR VP6 in a solid-phase immunoassay as well as an immunoblot format. The use of recombinant VP6 avoided the contaminants often encountered with GBR antigens prepared from fecal specimens. These assays proved useful in evaluating the time course and persistence of the serum antibody response following diarrhea episodes in China with the ADRV strain of GBR. Sera were then tested in order to evaluate the prevalence of human exposure outside of China. The availability of these reagents should allow future surveys for GBR infection among additional populations that have not previously been investigated.

Solid-phase immunoassay detection of human antibody directed against GBR was performed in a manner similar to that reported for animals (12). Immulon-2 plates (Dynatech, Chantilly, Va.) were coated with 100 μ l of monoclonal antibody 15-S3 directed against the major inner capsid protein of the IDIR virus per well (20). The monoclonal antibody was used at a dilution of 1:20,000 in phosphate-buffered saline (PBS). Coated plates were then kept overnight at 4°C and subsequently washed with PBST (PBS containing 0.05% [vol/vol] Tween 20). Alternate wells of the microtiter plates were next incubated with protein lysates of *Spodoptera frugiperda* (Sf-9) insect cells infected with either AcBB (control recombinant baculovirus) or AcBB-IDIR6 (recombinant baculoviruses containing IDIR virus gene 6) (12). Each well received 100 μ l of lysate containing 0.5 μ g of protein diluted in PBST-gel (PBST with 0.5% [wt/vol] gelatin). After 1 h of incubation at 37°C, the plates were again washed with PBST. Human test sera diluted 1:100 in PBST-gel were next added to wells containing AcBB or AcBB-IDIR6 and incubated for 1 h at 37°C. After the wells were washed, 100 μ l of goat anti-human antibody conjugated to horseradish peroxidase (Zymed, South San Francisco, Calif.) in a 1:10,000 dilution in PBST-gel was placed in each well for 1 h at 37°C. After the plates were washed with PBST, ABTS [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)] substrate (Kirkegaard & Perry, Gaithersburg, Md.) was added to each well and reactivity was detected after 30 min by measurement of A_{405} with a kinetic microplate reader (Molecular Devices, Menlo Park, Calif.). Mean background reactivity in control wells was approximately 0.233 optical density (OD) units for sera from Maryland, 0.267 OD units for Swiss sera, and 0.480 OD units for Chinese sera in tests at 1:100 dilution. A specific activity was calculated for each serum specimen by subtracting the mean measured absorbance in control wells containing AcBB from the mean value measured in wells containing AcBB-IDIR6. Positive reactions were identified by specific OD measurements greater than 3 standard deviations above the mean value for repeated assays on human sera which did not contain antibody directed against GBR.

For immunoblot analysis, lysates of Sf-9 insect cells were prepared following infection with either AcBB or AcBB-IDIR6, and the cellular and viral proteins were separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels (10% polyacrylamide). Proteins were then electroblotted (4°C, 1 h, 100 V) onto 0.2- μ m pore-size nitrocellulose with a Mini Trans-Blot Electrophoretic Transfer Cell (Bio-Rad Laboratories, Melville, N.Y.). The transfer buffer consisted of 15.6 mM Tris and 120 mM glycine, pH 8.1 to 8.4 (1). Blots were incubated in blocking buffer of 1% nonfat milk in 10 mM Tris-HCl and 150 mM NaCl (TN buffer) on a rotary shaker at room temperature for 30 min and then at 4°C overnight. The following day, blots were incubated at room temperature for 30 min and then washed three times for 10 min each time with TN buffer. Blots were incubated (seal bag, 125 rpm, room temperature, 1 h) with test serum in TNT-milk (TN buffer with 0.25% [wt/vol] non-fat milk-0.3% [wt/vol] Tween 20) and then

FIG. 2. Solid-phase immunoassay for anti-GBR antibody among paired human serum samples obtained following ADRV outbreaks in China. Sera were evaluated for the presence of antibody directed against the major inner capsid protein of GBR as described in the text. These values are indicated by the OD measurements displayed along the y axis. Broken line indicates results from serum from an uninfected individual.

antibody reactivity between first and second specimens was noted, but the first specimen from that patient was not obtained until 31 days after the onset of gastroenteritis.

Serial dilutions of sera from ADRV-infected patients were also evaluated for reactivity with GBR VP6 in the solid-phase immunoassay. Fifteen serum samples were initially reactive at a dilution of 1:100 (Table 1). Anti-VP6 antibodies were still detected at 1:500 dilutions for eight of these specimens. Reactivity with GBR VP6 was detectable in one specimen even after a 1:12,500 dilution. That specimen was obtained approximately 2 weeks after acute infection and demonstrated the most reactivity of any of the sera tested at 1:100 dilution.

The results of the solid-phase immunoassay were subsequently compared with reactivity in an immunoblot format. Serum samples were initially tested at the same concentration employed for screening in the solid-phase immunoassay (1:100 dilution). However, at that concentration reactions with other cellular and baculovirus proteins prevented assessment of reactivity with recombinant VP6 and necessitated the use of more-dilute serum specimens. At dilutions of 1:1,000, an intense signal was visible in the immunoblot format with two serum samples that had previously exhibited among the highest reactivities in the solid-phase immunoassay. Reaction was not detected with 1:1,000 dilutions of sera which had exhibited lower reactivity in the solid-phase immunoassay. No reactivity was observed with acute-phase sera or sera from an uninfected individual.

Of note, post-infectious-phase serum specimens from five

TABLE 2. GBR antibody human sera, Maryland

Specimen no.	Patient	Sex ^a	Age (years)	Specimen date ^b	Date of onset of GI symptoms ^c	ELISA OD ₄₀₅ at 1:100 serum dilution ^d
111146	A	M	54	4/92	(None)	0.493
111139	B	M	66	4/92	(None)	0.530
103879	C	M	93	2/92	(None)	0.409
118402	D	F	95	5/1/92	4/30/92	0.621
127252	D	F	95	5/27/92	4/30/92	0.713

^a M, male; F, female.

^b Month/year or month/day/year.

^c GI, gastrointestinal.

^d ELISA, enzyme-linked immunosorbent assay.

individuals were obtained between 243 and 507 days after acute illness, and in all five cases, reactivity with recombinant GBR antibody was demonstrated by solid-phase immunoassay (Fig. 1). These findings demonstrated persistent serum reactivity with recombinant GBR VP6 for extended periods after infection and indicated that the assay would be useful for epidemiologic studies. Assessment of sera at later times after acute infection might provide additional data about persistence of anti-GBR antibodies, but this was not done. Such evaluations would be valuable, since a decrease in antibody titers over time could potentially lead to an underestimate of human exposure to GBR.

The reactivities of 379 random serum specimens from 331 Maryland residents were tested by solid-phase immunoassay with recombinant GBR VP6. Serum samples were obtained from the laboratories of the Department of Health and Mental Hygiene from among samples submitted for evaluation of antibodies directed against a wide variety of pathogens. Aliquots of these sera were coded and then evaluated for antibody directed against GBR. Tests for anti-GBR antibody were also conducted on an additional 134 serum samples obtained from 92 patients in five Maryland nursing homes following investigations of respiratory or gastrointestinal disease. Initial evaluations had failed to identify a specific causative agent in the nursing home outbreaks. These 423 individuals ranged in age from 7 months to 96 years, with a mean age of 47 years. Fifty-nine percent of the individuals were female.

Five specimens from four Maryland residents were reactive with recombinant GBR VP6 in the solid-phase immunoassay in which sera were tested at dilutions of 1:100 (Table 2). Repeated testing of these samples by solid-phase immunoassay confirmed the initial findings. Specimens from two patients (A and B, Table 2) remained positive in the assay following dilution to 1:160. These two serum samples (from males, aged 54 and 66 years) had originally been submitted for testing for Lyme disease antibodies, and both specimens were nonreactive with *Borrelia* antigens. The third unpaired serum specimen was obtained from a 93-year-old male during an investigation of a respiratory outbreak in a nursing home. The remaining two positive serum samples were both obtained from a 95-year-old female (patient D) both before and after a 27-h episode of diarrhea, vomiting, and abdominal cramps. This illness occurred during the course of an outbreak of diarrhea at her nursing home. Both serum specimens from this patient remained reactive in the solid-phase immunoassay after dilution to 1:800. No reactivity with GBR was noted among nine acute-phase and eight convalescent-phase serum samples obtained from other residents of the same nursing home who were also ill during the outbreak.

TABLE 1. GBR antibody human sera

Geographic source ^a	No. of persons tested	No. of serum samples reactive with GBR VP6/total no. of serum samples
China ^b	12	15/25
Maryland	423	5/513
United States	129	0/129
Switzerland	60	0/131

^a As described in the text.

^b Acute-phase and convalescent-phase sera obtained during two outbreaks of SDRV gastroenteritis.

The five Maryland serum samples that had exhibited reactivity with GBR VP6 in the solid-phase immunoassay were further tested by immunoblot at dilutions of 1:1,000. Reactivity was observed with the two serum samples that had displayed the highest reactivities in the solid-phase assay (the paired serum samples obtained from the 95-year-old nursing home resident).

In a previous report, reactivity of human sera with the IDIR agent had been evaluated in our laboratory by immunoprecipitation of a polypeptide generated by *in vitro* transcription and translation of gene 8 of the IDIR virus (6). Of these 129 specimens from Maryland, Ohio, and Massachusetts, three immunoprecipitated the gene 8 product. Reexamination of these specimens by solid-phase immunoassay or immunoblot analysis failed to demonstrate reactivity with recombinant GBR VP6.

Forty-four specimens from 23 staff and 87 specimens from 37 patients at a Swiss nursing home were also tested with GBR VP6 by means of solid-phase immunoassay. These specimens were kindly provided by André Zwahlen as part of an investigation of an outbreak of gastroenteritis that occurred in February 1990. No reactivity between these sera and recombinant GBR VP6 was detected.

The low prevalence of serum reactivity against recombinant GBR VP6 in the current study was consistent with the results of earlier surveys which employed ADRV reagents prepared from fecal specimens (13, 14). While these studies have indicated marked differences between GBR exposure in China and other geographic regions, a number of questions concerning the epidemiology of GBR infection remain unanswered. Why GBR should be so common in China and not in other areas is puzzling. Transmission of GBR can apparently occur through infected water supplies as well as through person-to-person contact (8, 10). Differences in the handling of drinking water and waste disposal might account for variation in the prevalence of human infection with GBR. However, travel of ill persons could still allow dissemination of GBR by person-to-person spread. There is no apparent reason to believe that person-to-person transmission to other areas could not occur in the future.

The detection of anti-GBR antibody in four patients from Maryland indicated that exposure to GBR had occurred in a small portion of the population (<1%). Serum from one of the patients was obtained during the course of an investigation of a diarrhea outbreak at a nursing home, but the reactivities in acute-phase and convalescent-phase specimens indicated that GBR was not the cause of disease in that case. While the seropositive Maryland residents may have been exposed to GBR during travel outside the state, this information could not be obtained as part of the current study. More likely, the presence of anti-GBR antibody in these cases represented exposure to a virus endemic to the local geographic region. A small outbreak of human infection with the IDIR strain of GBR has previously been documented in Baltimore (5). Recurrences of this type of exposure remain possible, since the urban rat population in Baltimore remains a focus for this infection (3a). The current study did not permit definition of the type of exposure which resulted in synthesis of anti-GBR antibodies. Additional investigations should be performed in order to understand the clinical syndromes associated with these seroconversions.

Of note, the Maryland residents with anti-GBR antibody were older than the test population. In two cases, reactivity was noted in serum from individuals in their 10th decade of life. Anti-GBR antibody was not detected in any individual younger than 54 years of age, although the median age of the test group

was 47 years. These findings were consistent with several possible interpretations. The results may have indicated an age-related risk of exposure to GBR. Such age-related differences have been observed in at least one outbreak of ADRV in China (8). The results might also have been noted if younger individuals became infected but were less likely than adults to mount an antibody response. Alternatively, exposure to GBR may have only occurred in the more distant past in Maryland, and only older individuals possessed anti-GBR antibody in their sera. Future experiments should be directed at distinguishing among these possible hypotheses. Examination in other regions will also be important in order to define more fully the geographic range of human infection with GBR. The production of GBR VP6 in insect cells and incorporation of this antigen into screening and confirmatory immunoassays should be a substantial aid in these investigations.

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