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Homotypic Serum Antibody Responses to Rotavirus Proteins following Primary Infection of Young Children with Serotype 1 Rotavirus

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The class-specific antibody responses to serotype 1 rotavirus structural proteins were examined by immunoblotting with sera obtained from young children hospitalized with acute rotavirus diarrhea caused by serotype 1. All were believed to be primary infections. Three consecutive serum samples were obtained from 16 patients during the acute and convalescent phases of the disease and then approximately 4 months later. Immunoglobulin G (IgG)-class antibody responses to two inner capsid proteins (VP_2 and VP_6) and to the major homologous outer capsid protein (VP_7) were detected in all patients. Antibody responses to VP_6 were rapid, increased in intensity during 20 to 40 days after the onset of symptoms, and persisted for more than 4 months. Responses to VP_2 and VP_7 were more delayed, were maximal in convalescent-phase sera, and decreased markedly in intensity 4 months after the onset of symptoms in the majority of children. Two patients with evidence of mixed infection showed persisting high levels of antibody to VP_7. Responses to the outer capsid protein VP_4 were detected in 67% of patients, peaked at 20 to 40 days after the onset of symptoms, and were no longer detected at 4 months in the majority of patients. It is likely that the immunoblotting technique underestimated responses to VP_4. Acute- and convalescent-phase sera (known to contain antirotavirus IgM or IgA measured by enzyme immunoassay) were also examined by immunoblotting. IgM- and IgA-class antibody responses to viral proteins VP_2, VP_4, and VP_7 appeared to be qualitatively identical to those observed for IgG in the same serum samples.

The importance of rotavirus as a major cause of acute infantile gastroenteritis has long been recognized. Epidemiological studies support the need for an effective rotavirus vaccine strategy. Several animal rotavirus strains are being or have been evaluated as human vaccine candidates in field trials, including the NCDV bovine strain (RIT 4237), the rhesus rotavirus simian strain (MMU-18006), and the WC3 bovine strain (2, 16, 26). However, these vaccine candidates have had various successes in different areas of the world and in different age groups of vaccinees, and the mechanisms responsible for these successes and failures are not understood (9, 13, 15).

In order to design a successful vaccine strategy, the identification of the proteins involved in the development of immunity following infection is of primary importance. To date, only limited data are available concerning the contribution of each individual rotavirus protein in inducing an immune response during a naturally acquired rotavirus infection.

There is general agreement on six rotavirus structural proteins and five nonstructural proteins (6). The core and inner capsid of the rotavirus particle are composed of VP_1 (125,000 daltons [Da]), VP_2 (94,000 Da), VP_3 (88,000 Da), and VP_6 (42,000 Da), while VP_4 (84,000 Da) and VP_7 (34,000 Da) comprise the outer capsid layer. The five nonstructural proteins range in molecular size from 53,000 to 26,000 Da.

Common antigenic determinants are found on most (if not all) of the structural proteins (7). The major polypeptide of the inner capsid (VP_6) carries nonneutralizing epitopes that define group (A, B, C, etc.) and subgroup antigens (14). Group A rotaviruses are classified into subgroup I or II on the basis of these antigenic determinants. Within a serogroup, rotaviruses are further classified into serotypes on the basis of identification of the two outer capsid proteins (VP_4 and VP_7) that induce antibodies with neutralizing activity. Since VP_7 comprises a greater percentage of the rotavirus outer capsid of purified particles than VP_4 does, the predominant neutralizing antibody reaction in hyperimmune serum has been thought to be against VP_7. Eleven serotypes of rotavirus based on the antigen specificity of the glycoprotein VP_7 have been defined by neutralization assays with hyperimmune sera (6). Serotypes 1 to 4, 8, and 9 infect humans. Classification of rotavirus serotypes based on VP_4 (also associated with hemagglutination activity) has not yet been defined. However, in general, reactivities with VP_4 are detected when two viruses generate a one-way cross-neutralization with hyperimmune antiserum (6).

There are few data at present on serum antibody that is formed in response to individual proteins during the course of natural rotavirus infections in humans. Convalescent-phase serum responses to VP_1, VP_2, VP_4 (VP_3), VP_6, and a nonstructural protein (NS2) following primary human rotavirus infections of four initially seronegative children has been demonstrated by radioimmunoprecipitation (20). No responses to VP_7 of subgroups I and II rotaviruses (strains DS-1 and Wa, respectively) were identified. Ushijima et al. (25), using the simian rotavirus strain SA11 and an immunoblotting technique, found that anti-VP_2 and anti-VP_6 immunoglobulin G (IgG) antibodies were present in all acute- and convalescent-phase sera collected from four children with rotavirus infections. Only one convalescent-phase serum sample from one patient showed a strong reaction to VP_7. Antibodies of the IgM and IgA classes, which reacted to peptides VP_2 and VP_6, respectively, were shown to develop 2 to 4 weeks after the onset of gastroenteritis in all cases,

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although these responses were poor compared with the IgG response. Neither of these studies identified the serotypes of the strains that were responsible for infection. It seems likely that the relative absence of detectable VP_7 responses in both studies may have been explained by the inadvertent use of heterologous rotavirus strains in the reaction systems.

The aim of the present study was to investigate the antibody responses to specific rotavirus proteins in sera obtained from young children following a severe primary rotavirus infection. Three consecutive serum samples were collected from 16 children admitted to the hospital with serotype 1 rotavirus infections. The homologous IgG antibody responses to individual proteins of a subgroup II serotype 1 rotavirus strain (RV4) were analyzed. In addition, IgM and IgA responses to RV4 were studied in selected serum samples from the same children. The results showed similar IgG, IgM, and IgA antibody responses to four rotavirus proteins (VP_2, VP_4, VP_6, and VP_7) and illustrated that immune responses measured by enzyme immunoassay (EIA) are probably due to persisting antibodies to the major constituent protein (VP_6) of the rotavirus particle.

MATERIALS AND METHODS

Patients. Serum samples were collected from a total of 16 patients (10 males; 6 females; age, between 2 and 39 months; mean age, 19.4 months; median age, 21 months) who were admitted to the Royal Children's Hospital, Melbourne, Victoria, Australia, with acute rotavirus diarrhea in the years 1984 and 1985. All patients were considered to be experiencing a primary rotavirus infection. All had been previously shown to develop an IgM-class rotavirus antibody response in sera and in intestinal contents, as measured by EIA (12). Two infants (ages, 2 and 3 months) showed rotavirus IgG antibodies in acute-phase sera.

Rotavirus infection was diagnosed within a few hours of admission to the hospital by electron microscopy of negatively stained concentrated fecal homogenates. The group and serotype antigens of the infecting rotavirus were determined by an EIA with monoclonal antibodies (5). All of the 16 children were infected with a serotype 1 rotavirus strain, although one patient was found to have a mixed rotavirus infection of serotypes 1 and 4. By using the pattern of reaction with a panel of three anti-serotype 1 neutralizing monoclonal antibodies directed to different epitopes on the VP_7 protein (4, 24), serotype 1 strains could be subdivided further. EIA reactions with this panel of monoclonal antibodies showed that 5 children were infected with monotype 1a rotavirus, 1 child was infected with monotype 1b rotaviruses, and 10 children were infected with monotype 1c rotaviruses.

Three specimens of blood were collected from each of the 16 patients by venipuncture. Acute-phase sera were obtained at 3 to 7 days (median, 6 days), convalescent-phase sera were obtained at 26 to 42 days (median, 33.5 days), and follow-up (4 months) sera were obtained at 113 to 148 days (median, 122.5 days) after the onset of symptoms. Immediately after collection, blood was allowed to clot at room temperature and centrifuged within 4 h of collection at 750 × *g* for 5 min, and the serum samples were stored at −70°C until they were assayed.

Virus. Human rotavirus strain RV4 was isolated in our laboratory (1) and identified as subgroup II, serotype 1, monotype 1a. Monotype identity was assigned by EIA reactions with a panel of VP_7-specific neutralizing monoclonal antibodies developed in our laboratory (4). Strain RV4 was propagated in MA104 cells grown on Cytodex 3 beads (Pharmacia, Uppsala, Sweden) in spinner cultures in the presence of porcine trypsin type IX (10 μg/ml to activate virus; 1 μg/ml in maintenance medium; Sigma Chemical Co., St. Louis, Mo.). Rotavirus-infected or mock-infected cells were harvested by freeze-thawing when a 90% cytopathic effect was evident or after 4 to 5 days. Virus was prepared by fluorocarbon extraction and then concentrated 100-fold by ultracentrifugation (18). Tris-buffered saline (0.02 M, pH 7.2) with 10 mM calcium chloride was used to stabilize the outer capsid layer (3).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Purified human rotavirus strain RV-4 proteins were solubilized by boiling them for 4 min in sample buffer containing a final concentration of 5% (vol/vol) 2-mercaptoethanol and 2% sodium dodecyl sulfate. A 0.5-ml volume of the reduced proteins in sample buffer was loaded into one large well spanning the width of the 4% acrylamide stacking gel. The proteins were separated in a 10% acrylamide resolving gel under reducing conditions by using a discontinuous buffer system (17). All gels were 1.0-mm thick and were run at a constant current of 30 mA for approximately 3 h.

Immunoblotting. Proteins resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis were electrophoretically transferred (30 V for 16 h) from the gel to a 0.45-μm-pore-size nitrocellulose membrane (Schleicher & Schuell, Dassel, Federal Republic of Germany), by the procedure of Towbin et al. (23). Following transfer, the nitrocellulose membrane containing blotted rotavirus proteins was cut into a number of 6-mm-wide strips. All incubation steps and washings of these strips were performed on a rocking platform at room temperature. One of these strips was stained with a colloidal gold reagent (Aurodye forte; Janssen Biotech N.V., Beerse, Belgium) to detect all transferred viral proteins. The remaining strips were immediately placed in a Tris-buffered saline solution (10 mM Tris [pH 8.0], 150 mM NaCl) containing 0.05% Tween 20 (TBST) and 5% skim milk powder for 2 h in order to block excess protein-binding sites. Human sera were diluted 1:50 in a 5-ml volume of TBST–5% skim milk powder and were then incubated with the nitrocellulose strips for 2 h. Sequential serum samples collected from each patient were tested alongside one another on strips of nitrocellulose paper containing blotted proteins originating from the same gel. One strip was incubated without serum to act as the negative control. Another strip was incubated with hyperimmune anti-RV4 rabbit antibody at a 1:200 dilution. This latter strip was included in every immunoblot assay as a standard reference. Following three-5-min washes with TBST, the strips were incubated for 1 h with an affinity-purified, alkaline phosphatase-conjugated goat anti-human IgG antibody (Promega Biotech, Madison, Wis.) at a 1:7,500 dilution, goat anti-human IgM antibody (Promega) at a 1:1,000 dilution, or sheep anti-human IgA antibody (Silenus) at a 1:1,000 dilution in TBST. Affinity-purified, alkaline phosphatase-conjugated goat anti-rabbit IgG antibody (Promega) was used for hyperimmune rabbit antibody at a 1:7,500 dilution in TBST. After three washes as described above, the strips were developed with freshly prepared 5-bromo-4-chloro-3-indolyl phosphate (BCIP)-Nitro Blue Tetrazolium substrate (Promega) according to the instructions of the manufacturer. The reaction was allowed to proceed for 20 min and was stopped by washing the strips with distilled water.

Molecular weights of rotavirus proteins that were reactive with serum antibodies were estimated by comparing their migrations relative to those of standard proteins with known

molecular weights that were run on the same gel (Pharmacia). The identity of the band representing VP_7 was confirmed by reacting strips (after immunoblotting and inoculation with serum) with concanavalin A-peroxidase by using 4-chloro-naphthol as the substrate. The glycosylated protein VP_7 band stained in this manner proved to be identical to the band stained with alkaline phosphatase (as described above).

Densitometry. Densitometry was performed on all strips by using a densitometer (Quick Scan R & D; Helena Laboratories) linked to an integrator (Quick Quant III; Helena Laboratories). Since the nitrocellulose strips were opaque, readings were made by reflection (as opposed to transmission). Some viral proteins were detected by densitometry as broad diffuse bands, while others were well defined. Therefore, in order to quantify the recognition of viral proteins by densitometry, the area beneath the peaks rather than the height of the peaks was determined for all experiments. That is, the area beneath the peak was proportional to both the stain intensity and the width of the band on the nitrocellulose strip. The gain of the densitometer was set so that the areas that were integrated were comparable between series of readings and between the different viral proteins.

RESULTS

Characterization of the Western immunoblot. To test the assay for reproducibility, a positive control hyperimmune serum sample was included in each of the immunoblots. By using densitometry, this standard yielded only small intertest variations in peak areas corresponding to antibodies against the major inner capsid group-specific protein of rotavirus, VP_6 (mean area beneath peak, 2,946.3 ± 68.4 U). Prominent bands corresponding to proteins VP_2, VP_4, and VP_7 also appeared when this serum sample was used, but the intensity of these latter bands fluctuated in different immunoblots. This was possibly due to variations in denaturation of the proteins because of the reducing nature of the gel and to variations in the efficiency of transfer, especially of the higher-molecular-weight proteins. In order to minimize run-to-run variations, all three serum samples collected from each patient were analyzed on nitrocellulose strips from the same immunoblot.

The specificity of each assay was assessed by reacting sera with mock-infected cells that were blotted onto nitrocellulose paper and by processing one nitrocellulose strip from each blot without serum. No reactivity on the nitrocellulose strips was evident by either of these two control procedures (data not shown).

Qualitative antibody responses to individual proteins. Hyperimmune rabbit serum prepared against RV4 showed IgG antibody reactions with VP_1, VP_2, VP_4, VP_5, VP_6, VP_7, and VP_8 (Fig. 1).

A total of 16 acute-phase serum samples, 15 convalescent-phase serum samples, and 16 follow-up serum samples (collected approximately 4 months after the onset of symptoms) were tested at a 1:50 dilution for IgG against RV4 proteins. The proteins recognized by these human sera included VP_2, VP_4, VP_6, and VP_7. The qualitative analysis of IgG antibody responses to these four viral proteins is shown in Table 1 for all sera. Figure 1 illustrates typical reactions of three consecutive serum samples collected from one patient.

Antibody responses to VP_6, the major inner capsid polypeptide, were detected in all acute-phase, convalescent-phase, and 4-month serum samples tested. Responses to VP_2, an inner capsid protein, and to VP_7, the major outer capsid serotype-specific glycoprotein, were less common in

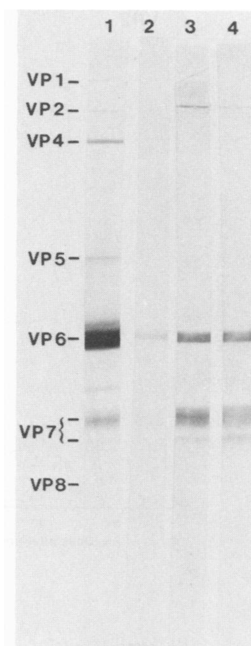

FIG. 1. Immunoblotting reaction of IgG antibody with RV4 proteins in hyperimmune rabbit serum (lane 1) and acute-phase serum, convalescent-phase serum, and serum obtained 4 months after the onset of symptoms (lanes 2, 3, and 4, respectively) from one patient following a natural infection with serotype 1 rotavirus. Virus proteins were identified by comparison of estimated molecular weights with known molecular weights of standard proteins.

the acute-phase sera but were evident in all convalescent-phase sera and remained highly prevalent in the serum samples obtained 4 months after the onset of symptoms. In comparison, IgG antibody responses to VP_4, another outer capsid serotype-specific protein, were most commonly detected in the convalescent-phase sera, while the number of acute-phase and 4-month serum samples with a detectable response was very low.

Quantitative antibody responses to individual proteins. There was considerable variation in the intensity of the reactions, not only toward each individual protein but also between each of the three serum samples collected from each of the 16 patients (Fig. 1). Quantitative analysis with the densitometer to score the intensity of reaction of each serum sample to each protein is shown in Fig. 2. The scales of intensity of reaction in each panel of Fig. 2 are relative to one another. In general, responses to VP_6 were most intense, those to VP_2 and VP_7 were of similar and lesser intensities, and those to VP_4 were of very low intensity.

In all patients, IgG responses to VP_2 were highest in the

TABLE 1. Immunoreactivity by IgG immunoblotting of sera to four rotavirus proteins

Rotavirus protein	No. (%) of serum samples showing reaction		
	Acute phase (3–7 days)	Convalescent phase (26–42 days)	Four mo (113–148 days)
VP_2	5/16 (31)	15/15 (100)	15/16 (94)
VP_4	2/16 (13)	10/15 (67)	5/16 (31)
VP_6	16/16 (100	15/15 (100)	16/16 (100)
VP_7	7/16 (44)	15/15 (100)	14/16 (88)

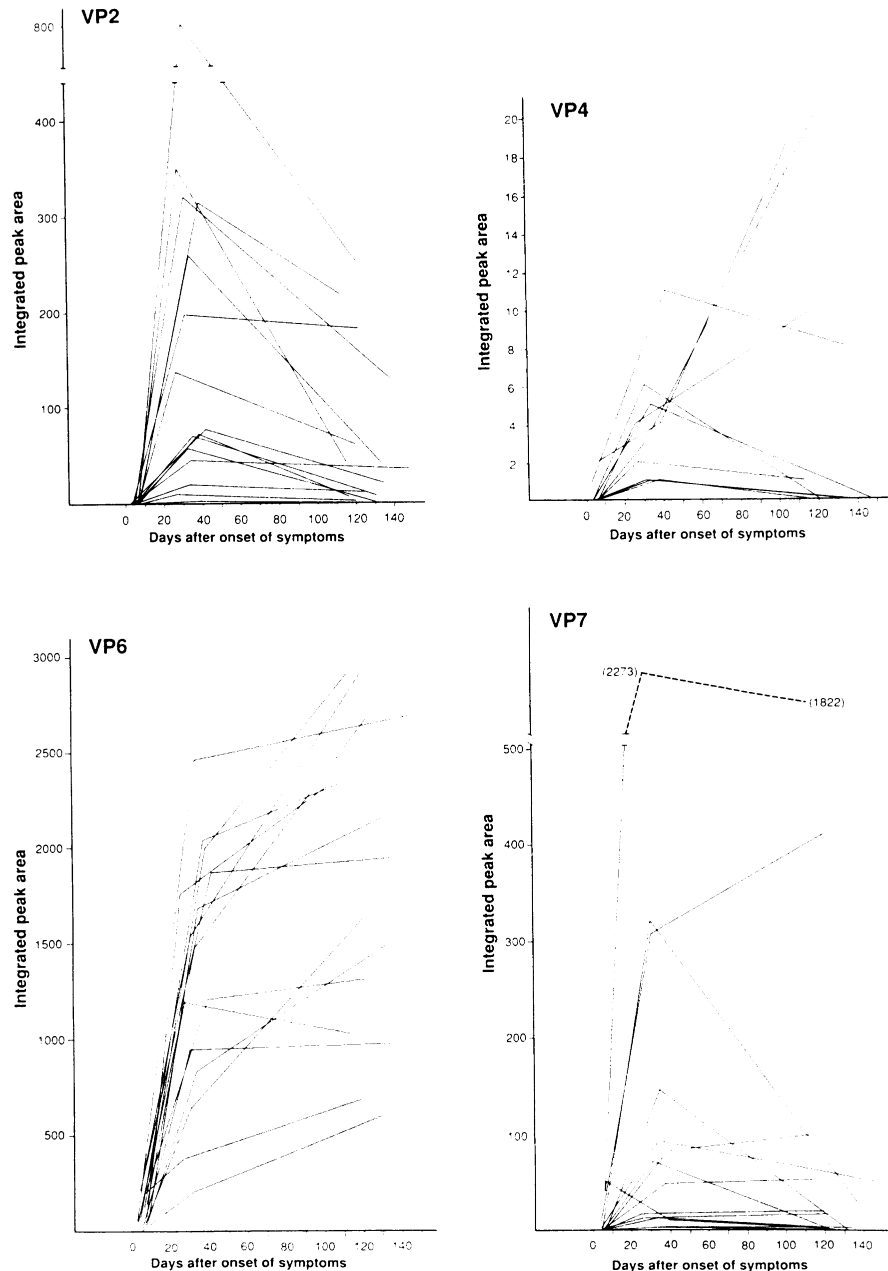

FIG. 2. Immunoblotting reaction intensities of IgG antibody with rotavirus proteins VP_2, VP_4, VP_6, and VP_7 in each of three consecutive serum samples collected from each of 16 patients included in the study. Integrated peak area is a measure of both the stain intensity and the width of the band on the nitrocellulose paper.

convalescent-phase serum sample, and a response in the acute-phase serum sample, if present, was very low in intensity. In the serum samples collected 4 months after infection, intensities of reaction of anti-VP_2 IgG antibody had returned to normal in some patients or had decreased markedly from the peak values in the other serum samples.

IgG antibody responses to VP_4 were very weak compared with the IgG responses to VP_2, VP_6, and VP_7, and differences in the intensity of responses between sera were very slight. Serum samples from five patients showed no detectable responses to VP_4. The majority of anti-VP4 responses showed trends similar to those of the anti-VP_2 responses with respect to the timing of peak reactions. In all but three patients, IgG to VP_4 showed the strongest reaction in the second serum sample and decreased either to an undetectable level or to a lower level by 4 months. The remaining three patients showed increasing anti-VP_4 IgG antibody levels (associated with sustained or increasing anti-VP_7 IgG levels in two of the three patients) over the three serum samples collected. One of these patients was found to have had a second rotavirus infection (caused by an untypeable strain) 6 weeks after the onset of symptoms of the primary infection. This patient also showed moderate sustained levels of anti-VP_7 IgG. The other two patients had previously been shown to have a fourfold increase in EIA anti-rotavirus IgG antibody titers between the convalescent-

phase and 4-month serum samples (12) and may also have been reinfected with rotaviruses. Both had been in contact with family members who had vomiting and diarrheal symptoms during that period of time.

The patterns of IgG antibody responses to VP_6 were markedly different from those to any other protein. In all patients except one, the anti-VP_6 IgG antibody response increased with time. That is, the 4-month serum sample collected from 15 of the 16 patients had a greater IgG antibody response to VP_6 compared with the acute-phase and convalescent-phase serum responses to the same protein. Sera collected from one patient showed a minimal decline in anti-VP_6 IgG response in the 4-month serum sample.

Responses of IgG to VP_7 showed similar patterns of development in each patient. Usually, IgG to VP_7 was very low in the acute-phase serum sample, increased in the convalescent-phase serum sample, and then decreased markedly in the 4-month serum sample. Three patients showed atypical results. One patient showed a low to moderate level of anti-VP_7 IgG in the acute-phase serum sample which gradually decreased in intensity with time. Two patients showed high anti-VP_7 IgG levels in their convalescent-phase serum sample that remained highly elevated in the serum sample collected at 4 months postinfection. No anti-VP_4 IgG was detectable in the 4-month serum samples from either patient. One patient had excreted serotype 1 and 4 rotaviruses on admission to the hospital. The other patient had evidence from neutralizing antibody assays (data not shown) of a mixed serotype 1 and 4 infection.

Anti-RV4 IgM and IgA antibody responses. IgM and IgA antibodies were also examined in acute- and convalescent-phase serum samples, respectively, from seven patients in whom high EIA titers of IgM or IgA were measured previously (12). Figure 3 illustrates the IgG, IgM, and IgA antibody responses to individual proteins in sera collected from one of these patients. In all patients examined, the spectrum of viral proteins detected by IgM and IgA antibodies was qualitatively identical to the spectrum detected for IgG antibodies in the same serum specimen.

DISCUSSION

The immunoblotting technique used in this study has several limitations. Since the antigens separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and used in immunoblotting were denatured and reduced, only reactions of antibodies which bound to epitopes of protein that were not dependent on conformation could be detected. Differences in the intensities of reaction of serum samples with different proteins may also reflect the degree of conservation of antigenic epitopes within each protein. In addition, labile epitopes of the proteins may be lost during immobilization of proteins on the nitrocellulose paper. The sensitivity of immunoblotting also depends on the amount of transferred antigen available for reaction with each preparation, as well as on the sensitivity of the dilution antibody and its associated substrate. Despite these limitations, immunoblotting is a useful technique since it detects IgM and IgA antibody subclass responses that are difficult to detect by other techniques. Immunoblotting can detect quantitative changes in sera obtained sequentially from the same patient.

The results of the immunoblot assay of serum obtained from young children following naturally acquired primary rotavirus infections indicate the development of antibody to at least four structural proteins (VP_2, VP_4, VP_6, and VP_7).

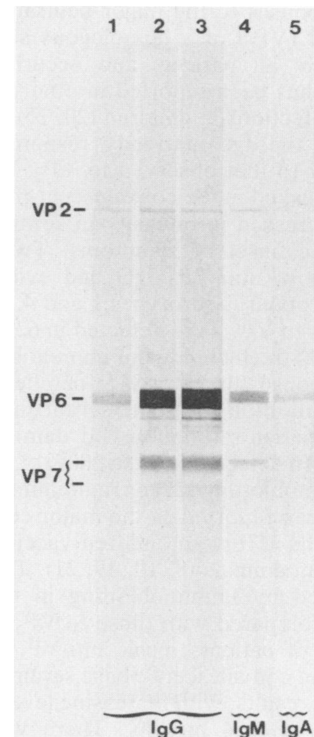

FIG. 3. Immunoblotting reaction of RV4 proteins with IgG antibody in acute-phase serum (lane 1), convalescent-phase serum (lane 2), and serum obtained 4 months after the onset of symptoms (lane 3); with IgM antibody in acute-phase serum (lane 4); and with IgA antibody in convalescent-phase serum (lane 5) collected from one patient following a natural infection with serotype 1 rotavirus.

All responses measured in this study were homotypic (with respect to VP_7 at least) to the infecting serotype 1 viruses.

The most immunogenic of the rotavirus proteins, as determined by the number of responses detected (100%) and the rapidity and duration of those responses, was VP_6. This subgroup-specific protein (11) makes up approximately 80% of the inner capsid, is the main component of the visible morphological units, and has been found to be very immunogenic in previous studies (20, 21, 25). IgG responses to VP_6 were rapid. They were detected at low levels in all acute-phase sera obtained approximately 3 to 7 days after the onset of symptoms, even in sera in which IgG-class antibodies were not detected by EIA (12). With the exception of two children (ages, 2 and 3 months) who had pre-existing maternal antibody (as judged by EIA), it was considered that antibody to VP_6 measured by immunoblotting developed as a response to primary infection. IgG responses to VP_6 increased dramatically and remained at sustained high levels beyond the convalescent phase in all patients. A sustained high level of rotavirus antibodies has also been documented previously by EIA of these sera (12). This high level of reaction of IgG to VP_6 is likely to be the main component of the immune response measured by EIA 4 months or more following infection.

The second inner capsid polypeptide, VP_2, which may also be associated with subgroup specificity (22), was highly immunogenic with respect to the number of IgG antibody responses detected (100%). Reactions appeared to be more delayed for VP_2 than they were for VP_6, with peak values occurring in convalescent-phase sera followed by declining levels 4 months postinfection.

Antibody responses to the major neutralizing antigen on the outer capsid (VP_7) of a homologous serotype 1 strain were detected in all patients and occurred much more frequently than has been reported in other studies of naturally acquired infections in children (20, 25) and adults (27). The pattern of the IgG antibody response to VP_7 was generally similar to that observed to VP_2. Responses were often delayed, peaked in the convalescent-phase serum, and then usually decreased in intensity in serum collected at 4 months after the onset of symptoms. Two patients with persisting levels of anti-VP_7 IgG had evidence of mixed infections with rotavirus serotypes 1 and 4.

IgG responses to VP_4 were detected in 62% of patients. It was apparent that the technique of immunoblotting underestimated the responses to VP_4, since parallel tests by radioimmunoprecipitation on the same serum samples identified anti-VP_4 in all patients (unpublished data). VP_4 (VP_3) responses have been detected in 25 to 100% of patients in other studies by immunoblotting (25) or radioimmunoprecipitation (20) and have been reported as the major component of the response in adults (27) or in children vaccinated with rotavirus strains from animals (8, 10, 19, 21). The intensities of reaction detected by immunoblotting in this study were extremely low compared with those to VP_2, VP_6, and VP_7. In the majority of patients, peak anti-VP_4 antibody levels were measured in convalescent-phase serum. Three patients showed atypical results, with increasing levels of anti-VP_4 in serum obtained after 4 months. There was evidence of reinfection in these three patients within the time period studied, as judged by clinical symptoms and by coproantibody levels.

Responses to the nonstructural proteins of rotavirus (if they were present) could not be detected since the method of preparation of the antigen used in the immunoblot assay excluded all nonstructural proteins of rotavirus. Antibody responses to VP_1 and VP_3, two minor inner capsid structural proteins, were not observed with any of the sera examined. Responses to VP_3 may not be detectable with this assay since none were visualized by using hyperimmune sera.

By using the immunoblot assay, reactions of IgG-, IgM-, and IgA-class antibodies to the individual proteins of rotavirus could be measured separately. Anti-rotavirus IgM antibodies in acute-phase sera and IgA antibodies in convalescent-phase sera reacted with the same spectrum of proteins recognized by IgG antibodies. However, the intensities of reaction of these IgM and IgA antibodies to individual viral proteins were less than those of IgG antibodies to the same proteins. This result may reflect the amounts of the different immunoglobulin classes in serum or may be due to the reactivities of the different secondary antibody conjugates in the immunoblot assay.

Our results indicate that during a naturally acquired primary rotavirus infection, the major serum antibody responses (detected by immunoblotting) appear to be to the nonneutralizing polypeptides (VP_2 and VP_6). However, we showed that both the two outer capsid serotype-specific proteins, VP_4 and VP_7, also contribute to the homotypic immune response. This finding is in general agreement with those of vaccine studies, but indicates a more consistent response to human rotavirus proteins than to animal rotavirus proteins. By using the immunoblotting technique, the IgG response to VP_7 appears to be more common and of greater intensity than does that to VP_4. This is probably an artifact of the technique because of the different amounts of each protein in the virus preparation or, alternatively, the different amounts of undenatured protein available for reaction with serum antibodies (or both). A longitudinal study with heterologous rotavirus strains used as antigens in assays may help to elucidate which proteins are important components of a heterotypic immune response and, furthermore, may indicate which proteins should be included in a reassortant rotavirus vaccine for humans. In addition, since the pathological process of rotavirus infection consists of viral colonization of the intestinal epithelial cell layer, other classes of immunoglobulins, and especially secretory (fecal) IgA, might be more relevant to protection. The involvement of this class of immunoglobulin in the patient response to the individual rotavirus proteins deserves further evaluation.

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