Characterization of Human Serotype 1 Astrovirus-Neutralizing Epitopes

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Astroviruses are important agents of pediatric gastroenteritis. To better understand astrovirus antigenic structure and the basis of protective immunity, monoclonal antibodies (MAbs) were produced against serotype 1 human astrovirus. Four MAbs were generated. One MAb (8G4) was nonneutralizing but reacted to all seven serotypes of astrovirus by enzyme-linked immunosorbent assay (ELISA) and immunoperoxidase staining of infected cells. Three MAbs were found to have potent neutralizing activity against astrovirus. The first (5B7) was serotype 1 specific, another (7C2) neutralized all seven human astrovirus serotypes, while the third (3B2) neutralized serotypes 1 and 7. Immunoprecipitation of radiolabeled astrovirus proteins from supernatants of astrovirus-infected cells showed that all three neutralizing antibodies reacted with VP29. MAb 5B7 also reacted strongly with VP26. A competition ELISA showed that all three neutralizing antibodies competed with each other for binding to purified astrovirus virions, suggesting that their epitopes were topographically in close proximity. None of the neutralizing MAbs competed with nonneutralizing MAb 8G4. The neutralizing MAbs were used to select antigenic variant astroviruses, which were then studied in neutralization assays. These assays also suggested a close relationship between the respective epitopes. All three neutralizing MAbs were able to prevent attachment of radiolabeled astrovirus particles to human Caco 2 intestinal cell monolayers. Taken together, these data suggest that the astrovirus capsid protein VP29 may be important in viral neutralization, heterotypic immunity, and virus attachment to target cells.

Astrovirus is a small (28 to 40-nm) single-stranded RNA virus which has been assigned its own family, the *Astroviridae*. The genome consists of three open reading frames (ORFs) which encode a viral protease (ORF1a), a polymerase (ORF1b), and a capsid precursor (ORF2) (reviewed in reference 1). ORF2 is also found in the cytoplasm of infected cells as a subgenomic RNA species (1, 11, 12). The viral particle consists of the single positive-sense genomic RNA strand and an icosahedral capsid consisting of three to five major proteins with molecular masses of between 35 and 22 kDa. The capsid proteins are all believed to be derived from a 88- to 90-kDa polypeptide encoded by ORF2 (11, 16). Recent studies of serotype 2 astrovirus demonstrated three capsid proteins of 26, 29, and 34 kDa (16). N-terminal amino acid sequence data suggesting that VP26 and VP29 were products of alternative trypsin cleavage at amino acids 362 and 395 of the precursor protein were present. Thus, VP26 most likely represents a truncated version of VP29.

Astrovirus is increasingly recognized as an important cause of diarrhea in young children (reviewed in reference 3). Despite a growing understanding of astrovirus epidemiology and molecular biology, little is known about its antigenicity and the nature of astrovirus immunity. There are seven serotypes of human astrovirus as determined by immunoelectron microscopy, immunofluorescence, and enzyme-linked immunosorbent assay (ELISA) using rabbit antiserum (8, 9, 13, 14). In stool specimen serotyping studies (8, 13, 14) and in serologic surveys, serotype 1 appears to be the most common pathogen, infecting up to 90% of children younger than 5 years of age (7). It is not known whether infection with one serotype confers heterologous immunity against any of the other serotypes.

To further understand the antigenic structure of human astroviruses and the basis of protective immunity, we have isolated four distinct monoclonal antibodies (MAbs) from mice immunized with purified astrovirus serotype 1. We have characterized them by examining their ability to stain intracellular astrovirus antigen in infected cells and neutralize the seven known human serotypes of astrovirus, their reactivities with viral proteins by immunoprecipitation and Western blotting, their antigenic resolution of the viral capsid structure by competitive ELISA, and their mechanism of neutralization.

MATERIALS AND METHODS

Cells and viruses. Caco 2 cells were obtained from the American Type Culture Collection. They were grown in RPMI medium supplemented with penicillin, streptomycin, and 10% fetal bovine serum at 37°C in a 5% CO₂ incubator. Astrovirus serotypes 1 to 7 adapted to tissue culture by Kurtz and Lee (8, 9) were the kind gift of S. Matsui, Stanford University. Astroviruses were propagated as previously described in Caco 2 cells in RPMI without fetal bovine serum and in the presence of trypsin (10 µg/ml; Sigma type IX) (18). Virus was purified by differential centrifugation as described previously (16), followed by cesium chloride gradient centrifugation (18). Positive fractions were identified visually and/or by immunodot blot, initially using 8E7 a previously described group specific antiastrovirus MAb developed by Hermann et al. (6), also provided by S. Matsui. In later viral purifications, a similar MAb, 8G4 (described below), was used for astrovirus antigen detection. Variant viruses were selected by growth in medium containing the designated MAb ascites (1 µl/ml) for three passages in Caco 2 cells.

Production of antibodies. For production of MAbs, BALB/c mice were immunized intraperitoneally with 25 µg of purified serotype 1 astrovirus. Initial immunizations were with Freund's complete adjuvant, followed by two monthly boosts with incomplete adjuvant. Five days prior to fusion, mice received 50 µg of sucrose-purified astrovirus without adjuvant. Fusion of mouse splenocytes with FOX NY myeloma cells and growth of hybridomas were as previously described (5). Growing hybridomas were screened by immunoperoxidase staining of serotype 1 astrovirus-infected Caco 2 cells in a 96-well format. Caco 2 cells were infected overnight with dilute viral stock in the absence of additional trypsin. They were then fixed in cold 100% methanol. Fifty microliters of hybridoma supernatant was added to each well for 1 h at 37°C followed by anti-mouse peroxidase and aminoethyl carbazole substrate as previously described (17). Hybridomas were also screened by neutralization of serotype 1 astrovirus

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on 96-well plates of Caco 2 cells as previously described (17). Briefly, 50 μ l of supernatant was mixed with 50 μ l of serotype 1 human astrovirus and incubated at 37°C for 1 h. The mixture was then added to Caco 2 monolayers for overnight incubation. The following morning, monolayers were washed with phosphate-buffered saline (PBS), fixed with cold methanol, and then immunoperoxidase stained with a 1:5 dilution of MAb 8G4 supernatant as described above. Neutralization is defined as greater than 80% reduction in cells staining positive for astrovirus antigen. MABs of interest were subcloned at least three times by limiting dilution, isotyped by using a kit from Sigma, and amplified as ascites fluid in pristane-primed BALB/c mice.

Polyclonal rabbit antibody against human serotype 1 astrovirus was prepared by injecting 100 μ g of cesium chloride-purified virus emulsified in Freund's adjuvant subcutaneously three times (primary immunization with complete adjuvant and subsequent boosts with incomplete adjuvant) at monthly intervals in New Zealand rabbits.

Competition ELISA. MABs were purified by protein A chromatography, using a kit from Sigma. 7C2, an immunoglobulin M (IgM) MAB, was purified by ammonium sulfate precipitation and gel filtration (2). Protein concentration of purified antibody was determined by a bicinchoninic acid assay (Pierce Chemical, Rockford, Ill.). Purified MABs were biotinylated with *N*-hydroxysuccinimide ester of biotin (Sigma) as previously described (17). Both biotinylated and purified MABs were initially titrated in ELISAs using purified serotype 1 astrovirus as the solid phase. For the competition ELISA, each of well 96-well Costar enzyme immunoassay plates was first coated with 50 μ l of sucrose-purified astrovirus (10 μ g/ml) for 3 h at 37°C and then blocked with 3% bovine serum albumin (BSA) overnight at 4°C. Serial dilutions of 50 μ l, starting at 5 μ g/ml of unlabeled MAB, in triplicate were added to the plate for 30 min, followed by biotinylated MAB at a concentration predetermined to provide a reading of approximately 1.00 optical density unit. For the biotinylated MABs 3B2, 8G4, and 5B7, this was at saturating concentration. For biotinylated 7C2, this was at 1/2 saturating concentration. After incubation at 37°C for an additional 60 min, the plate was washed six times with PBS. Avidin-peroxidase (Sigma) at a 1:5,000 dilution was then added for 1 h at 37°C. After six more washes, TMB substrate (Kierkegaard & Perry) was added for 10 min before the reaction was stopped with 1 M phosphoric acid. Absorbance was determined on an ELISA reader at 450 nm.

Immunoprecipitation. Caco 2 cells were infected with serotype 1 astrovirus at a multiplicity of infection of 5 for 12 h at 37°C. The medium was replaced with methionine-free Dulbecco modified Eagle medium containing trypsin (10 μ g/ml) and ³⁵S-labeled methionine (Translabel; 50 μ Ci/ml; ICN). The infection was allowed to proceed for 12 more h at 37°C. Supernatants were harvested by centrifugation at 2,000 $\times$ g for 10 min. High-speed supernatants were obtained by further centrifugation at 50,000 rpm for 1 h in a Beckman SW60 rotor. Two microliters of MAB ascites or rabbit serum was added to 200 μ l of supernatant for 1 to 2 h followed by 1 μ l of goat anti-mouse IgM for 1 h (in the case of 7C2). Complexes were collected on protein A-agarose beads (Sigma), washed three times with cold lysis buffer (1% Nonidet P-40 [NP-40], 1% deoxycholic acid, 2 mM phenylmethylsulfonyl fluoride, 100 U of aprotinin per ml, 10 mM Tris [pH 7.0]) and resolved on sodium dodecyl sulfate (SDS)-15% polyacrylamide gels prior to fluorography. In some experiments (Fig. 1C), PBS containing 1% NP-40 was used as a wash buffer.

Cell binding. Radiolabeled astrovirus was prepared exactly as described for the immunoprecipitation except that the infection was allowed to proceed for a total of 48 h. The resulting material was then purified by differential centrifugation through sucrose as previously described (16) and resuspended in serum-free RPMI medium with 1% BSA. Caco 2 cells in 24-well clusters were cooled to 4°C, washed, and then inoculated with 300 μ l of radiolabeled astrovirus virus (100,000 cpm, 5,000 PFU) in Dulbecco modified Eagle medium containing 1% BSA with or without appropriately diluted antibody. After 1.5 h of incubation at 4°C, the monolayers were washed extensively with cold PBS and then harvested with Laemmli sample buffer, boiled, and run on SDS-15% polyacrylamide gels prior to fluorography.

RESULTS

MABs. Screening of our initial fusion resulted in isolation of a single clone, 8G4, which reacted with astrovirus-infected cells in an immunoperoxidase staining. This MAB, isotype IgG1, reacted with human astrovirus serotypes 1 to 7 in immunoperoxidase staining of infected cells, in dot blots of infected cell culture supernatants, and in ELISA of cell culture supernatants or known positive stool specimens (data not shown). MAB 8G4 does not exhibit neutralization activity, nor does it detect viral proteins in immunoprecipitation or Western blot assays (data not shown). Thus, this MAB is quite similar to the previously described 8E7 (6).

Using 8G4 as probe of viral infection in an immunoperoxidase microneutralization assay against serotype 1 astrovirus,

TABLE 1. Neutralization of astrovirus serotypes 1 to 7

Anti-body	Titer ^a of indicated astrovirus serotype						
	1	2	3	4	5	6	7
5B7	512,000	4,000	<500	<500	<500	1,000	16,000
7C2	128,000	128,000	128,000	128,000	128,000	128,000	128,000
3B2	128,000	16,000	2,000	500	500	500	64,000
Rabbit	256,000	1,000	2,000	1,000	1,000	2,000	4,000

^a Reciprocal of antibody (ascites or serum) dilution causing 80% reduction in peroxidase focus neutralization assay.

we were subsequently able to isolate and subclone three additional MABs which were capable of neutralizing serotype 1 astrovirus. Subtyping showed that MABs 3B2, 5B7, and 7C2 were IgG1, IgG3, and IgM antibodies, respectively.

Neutralization assays. To determine the serotype specificities of our neutralizing MABs, we performed neutralization assays with the three MABs and our polyclonal rabbit serum against all seven human serotypes. Results, summarized in Table 1, show that 5B7 and the polyclonal rabbit serum were largely serotype 1 specific, while one MAB (7C2) neutralized all seven astrovirus serotypes. MAB 3B2 exhibited high titers of neutralizing activity against serotype 1 and 7 astrovirus and lower titers against serotype 2. We also observed that MAB 5B7 and our polyclonal antiserum have moderate activity against the serotype 7 strain, suggesting that astrovirus serotypes 1 and 7 are antigenically related.

Immunoprecipitation. To determine which specific astrovirus capsid proteins each MAB reacts with, we performed immunoprecipitation analysis using radiolabeled viral proteins expressed by astrovirus-infected Caco 2 cells. Although the exact number and molecular weights of astrovirus capsid proteins have varied in different studies (reviewed in reference 4), in our laboratory we identify major bands of approximately 34, 29, and 26 kDa in purified preparations of serotype 1 astrovirus (Fig. 1A, lane V; see also Fig. 4, lanes C and 8G4). Figure 1 shows results from typical immunoprecipitation experiments using radiolabeled supernatants of astrovirus-infected Caco 2 cells. The left panel of Fig. 1A shows control immunoprecipitations from mock-infected Caco 2 cell radiolabeled supernatants, while the right panel show immunoprecipitations from low-speed supernatants of astrovirus-infected cells. MAB 5B7 (Fig. 1A, right panel, lane 5B7) and our polyclonal antibody (lane Rab) consistently immunoprecipitate the 26-kDa capsid protein as well as the 29-kDa protein from low-speed supernatants of infected cells. MAB 5B7 and the polyclonal antiserum also immunoprecipitate a 52-kDa protein (Fig. 1A, right panel) which probably represents a precursor or oligomer. Under the same conditions, MABs 3B2 and 7C2 immunoprecipitate the 29-kDa protein (Fig. 1A, right panel). The immunoprecipitation of a single capsid protein from the low-speed supernatant suggests that our extremely low salt detergent wash buffer is able to disassociate viral capsids. 3B2 occasionally precipitates a 60-kDa astrovirus protein (not seen in this experiment). We have occasionally observed an approximately 5-kDa protein in immunoprecipitations with 3B2 and 7C2. In high-speed supernatants, free of infectious virions, 5B7 and the rabbit serum precipitate the 26-kDa protein and minimal amounts of VP29 (Fig. 1B, lanes 5B7 and 4Rab), while 3B2 and 7C2 fail to precipitate any significant amount of VP29 (lanes 7C2 and 3B2). We conclude that the VP29 antigens recognized by MABs 7C2 and 3B2 do not exist in abundance in a freely soluble form and are probably mainly present on assembled virions. Figure 1C depicts immunoprecipitation of

FIG. 1. Immunoprecipitation of astrovirus serotype 1-infected Caco 2 cell supernatants. Supernatants were prepared from [35 S]methionine-labeled supernatants 24 h after infection or mock infection as described in Materials and Methods. In panel A, low-speed supernatants were used. The first four lanes used mock-infected Caco 2 cells. Antibodies used are indicated above the lanes. Rab is hyperimmune rabbit serum to serotype 1 astrovirus. Lane V contains purified radiolabeled astrovirus. Molecular weights are indicated in thousands. In panel B, the supernatant underwent ultracentrifugation (50,000 rpm in an SW60 rotor for 1 h) prior to immunoprecipitation. Antibodies used are indicated above the lanes. Rab is hyperimmune rabbit serum to serotype 1 astrovirus. In panel C, low- and high-speed supernatants (sup) were used as indicated, but the immune complexes were washed with a mild wash buffer (PBS–1% NP-40). Antibodies used are indicated above the lanes. Rab is hyperimmune rabbit serum to serotype 1 astrovirus. Lane 191 contains MAb 191, a control antirotavirus IgG3 MAb. Viral capsid proteins are indicated by >.

infected Caco 2 cell supernatants performed with a mild washing protocol using PBS–1% NP-40. Under these milder conditions, all three neutralizing MAbs precipitated various amounts of the 34-, 29-, and 26-kDa peptides which we believe to be components of intact virions (designated by > in Fig. 1C). If cell lysates are used for immunoprecipitation, both 5B7 and the rabbit serum immunoprecipitate several additional high-molecular-mass bands ranging from 60 to 90 kDa, while 3B2 and 7C2 do not (data not shown).

In Western blot analysis, 7C2 reacts with the 29-kDa viral protein either from sucrose-purified virus as shown in Fig. 2 or from crude viral lysates (not shown). As with the immunoprecipitations, the 29-kDa antigen is not detected if high-speed supernatants are used for Western blots. The other MAbs fail to react in Western blots. The rabbit antiserum reacts mainly with the 26- and 29-kDa viral proteins (Fig. 2).

Competition ELISA. To further characterize the antigenic topology of astrovirus, we performed competition ELISA to determine whether any of the MAb epitopes overlapped. As seen in Fig. 3C, none of the neutralizing MAbs competed with nonneutralizing MAb 8G4. MAb 7C2 competed with 3B2 and 5B7 (Fig. 3A and B). MAb 3B2 competed slightly with 7C2 (Fig. 3D), and 5B7 competed strongly with both 7C2 and 3B2 (Fig. 3A and D). Overall, it appears likely that these three neutralizing MAbs bind to antigens which are spatially closely related on the virion surface. An alternative explanation of our competitive binding results could be that binding of some of the MAbs produces secondary changes in topographically distant parts of the viral capsid. Additionally, 7C2 is an IgM MAb

and as a very large molecule may be able to block a variety of surface antigens through its mass effect.

Viral variants. The competitive ELISA may overestimate the proximity of epitopes due to steric and allosteric factors. To further map the antigenic structure of the astrovirus particle, we selected for antigenic variants by growing serotype 1 astrovirus in the presence of substantial amounts of neutralizing MAb. After each passage, each variant virus was compared with the original serotype 1 strain for neutralization by all three neutralizing MAbs. 5B7 selection yielded a large amount of 5B7-resistant virus after one passage. After three such passages, all of the variant viruses had become phenotypically at least 10 times less susceptible to neutralization by the selecting antibody as the original strain (Table 2). The cross-neutralization studies showed that variants v3B2 and v7C2, selected by two MAbs, were quite similar in their reactions with the other neutralizing MAbs. Both of these variants were reciprocally inhibited in their neutralization to these two MAbs, and neither variant had a significant change in its neutralization titer to 5B7. v5B7 was much less reactive with all three neutralizing MAbs than the original serotype 1 astrovirus.

Neutralizing antibodies inhibit binding of astrovirus to Caco 2 monolayers. To examine possible mechanisms by which the MAbs and neutralizing rabbit serum inhibit astrovirus infection, we performed binding studies on chilled Caco 2 monolayers. Preliminary experiments showed that sucrose-purified, metabolically labeled astrovirus bound to Caco 2 cells in a dose-dependent manner which correlated with infection of the cells if they were warmed to allow infection to proceed (data not shown). Furthermore, if labeled virus was allowed to bind to the cells, the bound viral proteins could be visualized by fluorography after the monolayer was dissolved in Laemmli sample buffer. We determined the amount of MAb ascites sufficient to inhibit 90% of astrovirus infection of the monolayers under these conditions (high multiplicity of infection) and then preincubated the antibodies with purified virus for 1 h prior to exposing the mixtures to Caco 2 cells at 4°C. The results are shown in Fig. 4. All three neutralizing MAbs

FIG. 2. Western blot analysis of astrovirus serotype 1 proteins. Sucrose-purified astrovirus was resolved by SDS-polyacrylamide gel electrophoresis prior to transfer to nitrocellulose. After blocking, the strips were exposed to polyclonal rabbit antiastrovirus serum (Rab) or MAb 7C2 (7C2) followed by alkaline phosphatase-labeled anti-rabbit and anti-mouse IgM, respectively. Strips were developed in 5-bromo-4-chloro-3-indolylphosphate–nitroblue tetrazolium (Bio-Rad). Molecular weights are indicated in thousands.

FIG. 3. Epitope mapping by competitive binding ELISA. A fixed amount of biotinylated MAb (shown above each panel) was mixed with decreasing concentrations of unlabeled astrovirus MAb competitors for binding to solid-phase-purified astrovirus serotype 1 as described in the text. The vertical axis represents percent competition; the horizontal axis represents competitor antibody concentration ([aby]).

strongly inhibited viral attachment to the cells, while nonneutralizing MAb 8G4 did not. This observation supports our competition ELISA data in suggesting that all three neutralizing MAbs bind to topographically nearby epitopes on the capsid in such a way that they prevent viral attachment to target cells.

It is interesting that although VP29 binding was completely inhibited by the MAbs, some residual VP26 is observed in the MAb-treated lanes (Fig. 4, lanes 5B7, 3B2, and 7C2). This finding might suggest that some VP26 molecules, which copurify with astrovirus virions, may not be strongly associated with the virus particle. The weak association between the 24- to 26-kDa protein and purified viral capsids has been previously noted (18).

TABLE 2. Neutralization of astrovirus serotype 1 antigenic variants by MAbs

MAb	Virus titer ^a			
	Astro1*	v5B7	v3B2	v7C2
5B7	256,000	8,000	256,000	256,000
7C2	128,000	16,000	4,000	8,000
3B2	128,000	16,000	8,000	16,000

^a Mean of triplicate neutralizations. Results varied by 1 dilution or less. Astro1 indicates the original astrovirus serotype 1 stock. Variant viruses were selected by passage three times in 1:1,000 dilution of the respective MAb ascites prior to testing in microneutralization assays.

DISCUSSION

The nature, number, and antigenic structures of astrovirus structural proteins have not been well established. Previous investigators have described from one to five capsid proteins in astrovirus particles derived from various species (reviewed in reference 4). The capsid proteins are believed to be derived from a single 88-kDa precursor which is encoded by the 3' terminus of the genome and the subgenomic RNA which is found in infected cell cytoplasm (11). Most recent studies of human astrovirus agree to three major capsid proteins with

FIG. 4. Inhibition of [³⁵S]methionine-labeled astrovirus serotype 1 binding to Caco 2 cells by MAbs. Purified radiolabeled virus with or without (control [C]) the designated MAbs was allowed to bind to monolayers for 1.5 h at 4°C. The monolayers were washed extensively and then dissolved in Laemmli sample buffer prior to electrophoresis and fluorography. MAb dilutions of ascites were determined by first performing microneutralization assays with the labeled virus to determine the minimum concentration of antibody to prevent 90% of infection. Dilutions used: 5B7, 1:10,000; 3B2, 1:2,000; 7C2, 1:5,000; 8G4, 1:500. Molecular weights are indicated in thousands.

approximate molecular masses of 24 to 26, 29 to 30, and 31 to 34 kD by SDS-gel electrophoresis (16, 18). To date, it is unclear how the precursor protein is modified and what role viral, cellular, and extracellular (trypsin) proteases play in this processing. Monroe et al. reported that trypsin alone could convert the 88-kDa precursor into three capsid components (11).

Previously only a single neutralization epitope on astrovirus particles has been described (16). Sanchez-Fauquier and colleagues (16) described a neutralizing MAb raised against serotype 2 human astrovirus which reacted with the 26- and 29-kDa capsid proteins. N-terminal amino acid sequence from the 26-kDa protein showed that it coincided with sequence in the precursor protein after residue 394 of the 88-kDa precursor protein, following an arginine residue, suggesting it could be a product of trypsin digestion. The 29-kDa viral protein had an N-terminal amino acid sequence corresponding to residues in the precursor polypeptide after arginine 361. This difference in N termini accounts for virtually all of the 3- to 4-kDa difference in apparent molecular mass, suggesting that the C termini of the two proteins are similar if not identical. These data suggest that the 26- and 29-kDa proteins both contain this neutralizing epitope.

We set out to isolate MAbs to provide reagents for the detection of astrovirus particles in clinical specimens and to gain a better understanding of the neutralizing epitopes of the astrovirus particle. The first MAb that we isolated (8G4) reacts with a group antigen which allows it to react with all seven serotypes of human astrovirus by ELISA and immunostaining of infected cells. We are currently using this antibody in conjunction with our polyclonal antiserum in an ELISA for detecting fecal astrovirus excretion in children in an epidemiologic study (unpublished data). We also used 8G4 to devise a microneutralization peroxidase assay to screen other hybridomas for neutralizing antibodies against serotype 1 astrovirus.

Our newly isolated neutralizing MAbs reveal several previously undescribed features of the antigenic structure of astroviruses. Two of them, 7C2 and 3B2, immunoprecipitate only the 29-kDa astrovirus capsid protein (Fig. 1A, lanes 1 and 3), showing that epitopes which are present exclusively on VP29 may be important in protective immunity. In fact, all of our neutralizing antibodies reacted with VP29 (Fig. 1A) as does the previously reported neutralizing MAb PL-2 (16). Because the VP29 (but not VP26) could be cleared from viral lysates by high-speed centrifugation (compare Fig. 1A and B), we infer that VP29 may exist almost exclusively as a viral capsid component.

MAb 7C2, our only IgM MAb, neutralizes all seven serotypes of human astrovirus. Such cross-reactivity could be a basis for heterologous protection from other serotypes after a primary infection. Although the extent of such protection in clinical settings is not known, a strong anamnestic response to a heterologous serotype in an adult volunteer challenged with serotype 5 astrovirus has been described (10). Interestingly, 7C2 is not only the single MAb demonstrating significant heterologous neutralization; it is also the only MAb which reacts in Western blot analysis. This finding suggests that it represents a more linear, less conformationally dependent epitope which might be a candidate for a peptide or expression vector vaccine strategy. The MAb 3B2 epitope maps closely to 7C2 by immunoprecipitation (Fig. 1, lanes 1 to 3), competitive ELISA (Fig. 2), and antigenic variant neutralization (Table 2). MAb 3B2 has a distinct neutralization profile, however. It neutralizes serotypes 1 and 7 and to a lesser degree serotype 2 (Table 1).

MAb 5B7 has characteristics similar to those described for the anti-serotype 2 MAb PL-2 (16). It immunoprecipitates the 26-kDa capsid peptide and the 29-kDa capsid peptides (Fig.

FIG. 5. Proposed schematic map of serotype 1 neutralization antigens as defined by MAbs 5B7, 7C2, and 3B2 and the N-terminus peptide sequence reported by Sanchez-Fauquier et al. (16). The capsid protein VP26 is depicted a subset of VP29. MAb 5B7 binds to an epitope common to both capsid proteins, while the 7C2 and 3B2 epitopes bind to a nearby domain which is exclusively present on VP29.

1A, lane 5B7). Also like PL-2, with it also immunoprecipitate some high-molecular-weight precursors or oligomers from infected cells (Fig. 1A, lane 5B7). 5B7 is quite serotype 1 specific in its neutralization profile (Table 1).

The data from our competitive ELISA assay (Fig. 2) suggest that the viral epitopes which interact with our neutralizing MAbs are topographically very closely linked on the viral surface. This is consistent with the observation that all of the described neutralizing MAbs react with the same capsid protein VP29. Because these competitive binding experiments may not offer very fine resolution of viral capsid topology, particularly when one of them (7C2) is an IgM antibody, we also generated three viral variants which were resistant to neutralization of each of our MAbs. Neutralization studies of these variants also suggested that the viral antigens defined by the MAbs were closely related (Table 2). Two MAbs (7C2 and 3B2) selected variants which had essentially identical neutralization profiles which were reciprocal. The third variant (v5B7) had striking change in sensitivity to MAbs 7C2 and 3B2 as well as the selecting MAb.

Figure 5 schematically represents a possible antigenic map of astrovirus-neutralizing epitopes based on our data and those of Sanchez-Fauquier et al. (16). Note that the antigenic domain of VP26 represents a subset of VP29 as determined by the previously published N-terminal amino acid sequence (16). Ongoing studies of nucleic acid sequences of these astrovirus variants should allow even finer resolution of these epitopes.

Antibody-mediated viral neutralization can occur by a variety of mechanisms, including aggregation of infectious particles, inhibition of cell binding and/or penetration, and prevention of viral uncoating. We found that all three of our neutralizing MAbs inhibited ³⁵S-labeled astrovirus attachment to Caco 2 cells. For rotavirus, it has been shown that neutralizing MAbs directed against the viral attachment protein, VP8*, but not neutralizing MAbs directed against other viral capsid components are able to inhibit viral binding to target cells (15). Therefore, our data also support the notion that the three neutralizing MAbs bind to topographically closely spaced epitopes which may be closely related to the viral attachment protein.

In summary, four new MAbs to human astrovirus have been described. Based on analysis of our own and previously reported MAbs (16), we can conclude that astrovirus VP29 is

very important in neutralization of human astrovirus and that it may be the viral attachment protein. Neutralization of human astroviruses in vitro may be homotypic or heterotypic. Understanding the importance of VP29 in both homotypic and heterotypic immunity may be important in future vaccine design.

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