Arch Virol (1987) 93: 199–211

Archives of Virology
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Non-neutralizing Monoclonal Antibodies to a Trypsin-sensitive Site on the Major Glycoprotein of Rotavirus Which Discriminate Between Virus Serotypes

By

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With 3 Figures

Accepted August 14, 1986

Summary

Monoclonal antibodies were derived to a human rotavirus purified from stools. Three of the antibodies immunoprecipitated the rotavirus outer capsid glycoprotein gp 34 and were non-neutralizing. These antibodies reacted by enzyme immunoassay with cultivable rotaviruses showing the "long" RNA electropherotype but were inefficient as detectors of "long" RNA pattern rotaviruses in stools. Treatment of SA 11 rotavirus with 7.5 µg/ml porcine trypsin for 30 minutes at 37° C irreversibly reduced binding of the antibodies to SA 11 rotavirus in enzyme immunoassays by 50 per cent. Binding was abolished in the presence of rotavirus-negative faecal extracts. These results indicate that non-neutralizing sites on gp 34 of rotaviruses can vary with RNA electropherotype and serotype, and that levels of trypsin currently in use to assist growth of rotaviruses in cell culture may alter the serological profile of the viruses.

Introduction

Monoclonal antibodies to rotavirus have been of considerable benefit in detection and analysis of the viral antigens capable of eliciting neutralizing (6, 7, 14, 22) and non-neutralizing (12, 22, 23) antibodies. Certain mono-

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clonal antibodies have also proven valuable as diagnostic reagents for the detection of the rotavirus group antigen (4, 24, COULSON *et al.*, submitted for publication), subgrouping (12, 24) and serotyping (COULSON *et al.*, submitted for publication) by enzyme immunoassay (EIA) in stools.

When serotyping rotaviruses directly in stools by EIA with monoclonal antibodies it would be an advantage to be able to include a control monoclonal antibody which detects the viral outer capsid component, gp 34, of all rotaviruses. This is the glycoprotein recognized by the serotype-specific monoclonal antibodies used at present (6, 7, COULSON *et al.*, submitted for publication). Characterization of such an antibody has not been reported.

Antirotaviral monoclonal antibodies described to date have resulted from fusion of mouse myeloma cells with splenocytes of mice immunized with virions grown in cell culture. As cultivation of rotaviruses from stools is not routine and requires a large number of infectious virions, it might be possible to widen the range of rotavirus isolates against which monoclonal antibodies can be derived by immunizing mice with rotavirus purified from a human stool.

In this paper we report the production and characterization of monoclonal antibodies to a faecally-derived human rotavirus. These antibodies, although non-neutralizing, bound to the outer capsid protein gp 34.

Materials and Methods

Viruses and Cells

The cultivable rotaviruses RV-1, RV-3 (serotype 3), RV-4 (serotype 1), RV-5 and RV-6 (serotype 2) were isolated in our laboratory (1). The origins of the remaining cultivable rotaviruses used in this study have been described previously (6). The serotypes represented are 1 (Wa), 2 (DS-1), 3 (P, SA 11, RRV-2, K 9), 4 (ST-3) and the UK bovine rotavirus.

The above viruses were propagated in MA 104 cells in the presence of 1 µg/ml porcine trypsin type IX (Sigma) as described previously (19).

Faecal Samples

Faecal specimens were collected from children with acute gastroenteritis admitted to the Royal Children's Hospital (R.C.H.) Melbourne from 1974 to 1981. The presence of rotavirus in these samples was detected by electron microscopy (EM) as described previously (21). Of 43 rotavirus-containing stools, 41 showed a "long" RNA electropherotype (L), and 2 had "short" RNA patterns (S). As negative controls, 17 stools negative for rotavirus by EM were included. Rotavirus-positive faeces collected for epidemiological studies in Indonesia (21) and Papua New Guinea (2) were also available. Four L and two S rotavirus isolates from Indonesia, and 14 S rotaviruses from Papua New Guinea were included in this study.

RNA Gel Electrophoresis

Electropherotyping of rotaviral RNA was performed on faecal extracts and on all stocks of cultivable rotaviruses. Viral RNA was prepared by phenol-chloroform extraction and polyacrylamide gel electrophoresis of the RNA was performed with the Laemmli buffer system at 15 mA for 18 hours at 4° C. The RNA was visualized with silver stain (10).

Immunization of Mice

One child admitted to R.C.H. in 1975 with acute gastroenteritis produced 5 g stool containing 10^9 L rotavirus particles per g stool which serotyped as 4 by EIA (COULSON *et al.*, submitted for publication). Following fluorocarbon extraction, ultracentrifugation and banding in a caesium chloride gradient (18), 0.2 ml of the double-shelled virus was inoculated intraperitoneally, with 0.2 ml Freund's complete adjuvant, into eight-week old Balb/c mice. These mice showed rotavirus antibody titres < 100 by EIA (IgG, IgM, IgA) before inoculation. Those with at least a fourfold rise in EIA titre after 10 days were boosted subcutaneously with 0.1 ml virus and the spleens harvested for fusion 5 days later.

Preparation and EIA Testing of Hybridomas

The P 3-X 63-Ag8-653 mouse myeloma cells were fused with immunized splenocytes, and resulting hybridomas tested for antibody production by an EIA as described previously (6). Briefly, rotavirus-specific antibodies were measured by reacting hybridomas supernatants with passively immobilized viral antigens, then detecting bound antibody with horseradish peroxidase conjugated anti-mouse immunoglobulins (DAKO, Denmark). EIA antigens used for screening hybridoma supernatants including the immunogen, a faecal extract containing S rotavirus, a pooled rotavirus-negative faecal extract, SA 11 rotavirus and MA 104 cell control. These EIA antigens were produced by fluorocarbon extraction and ultracentrifugation of faeces, virus-infected or mock-infected cell harvests as described previously (6). Optimal antigen dilutions were determined by chequerboard titration.

Hybridomas showing EIA activity were subcloned at least twice by limiting dilution and grown as ascites in syngeneic mice. The end-point titres of these ascities fluids to 16 cultivable mammalian rotaviruses were determined by EIA (6) using doubling dilutions of ascites fluids. Appropriate virus and conjugate controls were included, as was ascites fluid produced from a non-secreting hybridoma.

To assess the ability of the monoclonal antibodies to detect rotavirus in stools, EIA antigen was prepared from 56 rotavirus-positive stools and a pool of 10 rotavirus-negative stools by dilution to 10–20 per cent (wt/vol) in phosphate-buffered saline pH 7.2 (PBS), homogenization, then concentration $\times 5$ using polyacrylamide hydrogel (Lyphogel; Gelman Sciences, Inc.). The level of antigenically detectable rotavirus protein present was estimated with a double sandwich EIA using guinea-pig and rabbit hyperimmune antisera to SA 11 rotavirus (17). The stool extracts were diluted to give $OD_{392} = 0.6$ in the double-sandwich EIA then applied to the solid phase as antigen in the EIA for antibody measurement described above and elsewhere (6). The pooled negative control was diluted to the same extent as each rotavirus-containing stool extract. The monoclonal antibodies were reacted as a 1 : 100 dilution of ammonium-sulphate precipitated, $\times 10$ concentrated hybridoma culture supernatant fluid. Culture fluid from a non-secreting hybridoma was prepared similarly and tested in parallel.

EIA for Effect of Trypsin and Trypsin Inhibitors on SA 11 Rotavirus

Undiluted SA 11 EIA antigen was incubated with an equal volume of various concentrations of trypsin in PBS for 30 minutes at 37° C. The working solution was diluted from a stock solution of trypsin in 0.001 M HCl (1 mg/ml). The trypsin-treated virus was diluted optimally in 0.06 M carbonate-bicarbonate buffer, pH 9.6 then applied to the solid phase for the EIA which was carried out as described previously (6). In some experiments, Soybean type 1-S trypsin inhibitor (STI; Sigma) or phenyl methyl sulphonyl fluoride (PMSF; Sigma) were diluted in PBS and incubated with the trypsin-virus mixture at 37° C. Alternatively, these trypsin inhibitors were incubated for 30 minutes at 37° C with virus already treated with trypsin for 30 minutes at 37° C.

A modified EIA method was also used, in which treatments of virus with trypsin and trypsin inhibitors took place as separate steps, after the immobilization of virus on the solid phase. Virus was adsorbed as before (6) to the microtitre trays, then incubated with trypsin diluted in PBS for 30 minutes at 37° C. After washing with PBS containing 0.05 per cent (vol/vol) Tween 20 (PBS-T), a trypsin inhibitor was added for 30 minutes at 37° C. Following a further washing step, 10 × concentrated hybridoma supernatant diluted in PBS was added, for 30 minutes at 37° C. The conjugate and substrate steps have been described previously (6).

Preparation of (^{35}S) Methionine-labelled Rotavirus-infected Cell Lysates

Monolayers of MA 104 cells in 35 mm petri dishes were infected with RRV-2 rotavirus at a multiplicity of 10 fluorescent cell-forming units/cell in the presence of 10 μg/ml trypsin and 2 μg/ml Actinomycin D as described previously (20). At 8 hours post infection the cells were labelled for 1 hour with 100 μCi/ml (35_S) methionine (1300 Ci/mmol, Amersham Australia Pty. Ltd.) in methionine-free Dulbecco's modified Eagle's medium followed by a 30 minutes incubation in complete medium containing no radiolabel. The cells were then harvested and cell lysates prepared as described by LEE *et al.* (15).

Radioimmunoprecipitation (RIP)

As described by SONZA *et al.* (22), undiluted ascites fluids containing monoclonal antibody were incubated with protein A-Sepharose 4 B beads (Pharmacia) which had been incubated previously with saturating amounts of rabbit anti-(mouse immunoglobulin) serum. After washing, the labelled cell lysate was added. Adsorbed labelled viral proteins were recovered by boiling in Laemmli sample buffer, and the supernatants were applied to a 9 per cent Laemmli polyacrylamide gel as described previously (6).

Following electrophoresis, gels were fixed and processed for fluorography by immersion in a 1 M sodium salicylate solution for 30 minutes.

Virus Neutralization Tests

Neutralization by monoclonal antibodies of Wa and SA 11 rotaviruses was tested by plaque reduction (26). Neutralizing activity to RV-3, RV-4, RV-5 and ST-3 was assessed by fluorescent focus reduction (6). Antibodies were assayed as × 10 concentrated hybridoma supernatants in doubling dilutions from 1 : 20 to 1 : 5120.

Results

Derivation and Characterization of Monoclonal Antibodies

From 6 fusions (3 mice), 51 stable hybridoma clones were derived. Approximately 20 per cent of these showed EIA reactivity to the negative control faecal extract and were discarded. Most of the remaining clones produced antibody cross-reactive by EIA between all rotaviruses tested, which presumably was directed to rotaviral group antigens. However, clones 24, 34 and 38 produced antibodies (Mab 24, Mab 34, Mab 38) which reacted preferentially with L human rotaviruses by EIA. By immunodiffusion with class-specific anti-mouse immunoglobulin sera, the antibodies were all of the IgM class. As the reactions of the three clones were similar in all tests, only results obtained with clone 24 will be reported in detail here.

By radioimmunoprecipitation, the monoclonal antibodies reacted with two proteins, p 33 and gp 34, of RRV-2. This is shown for Mab 24 in Fig. 1.

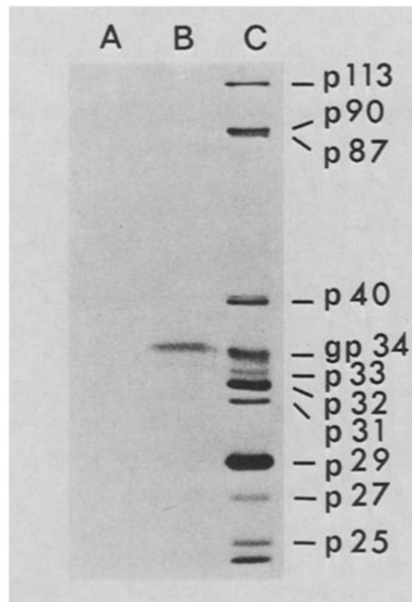

Fig. 1. SDS-polyacrylamide (9 per cent) gel electrophoresis of [^{35}S]methionine-labelled, RRV-2 infected cell lysate immunoprecipitated with monoclonal antibodies. *A* Mab P 3 (negative control: specific for phenylalanine hydroxylase); *B* Mab 24; *C* cell lysate. Molecular weights calculated from standards

Table 1. *EIA titres of monoclonal antibodies 24, 34 and 38 to a panel of cultivable mammalian rotaviruses*

Rotavirus[a]		Range of reciprocal EIA titres of antibodies $\times 10^{-3}$ [b]
Serotype	Strain	
1	RV-4	128–256
1	Wa	256
2	RV-5	1–2
2	RV-6	4
2	DS-1	2–4
3	RV-1	128
3	RV-3	128
3	P	512
3	SA 11	512
3	RRV-2	512
3	K 9	900
4	ST-3	256
Bovine	UK	512

[a] All virus stocks used in EIA were determined to contain virus of unique electropherotype by gel electrophoresis of viral RNA. The RNA extracted from EIA antigens co-electrophoresed with that of first passage virus stocks. No extra RNA bands were present

[b] Titre of mouse ascites fluids

These two proteins were also precipitated by Mab C 4/B 3 (data not shown), which was been shown to immunoprecipitate gp 34 of SA 11 (22). Hence the antibodies reacted with the equivalent of the SA 11 gp 34 in RRV-2. It has been shown that p 33 is a partially glycosylated form of gp 34 (9). These antibodies also bound to the SA 11 rotavirus gp 34 in western blots, using horseradish peroxidase-conjugated anti-mouse IgM as indicator, under both reducing and non-reducing conditions (data not shown).

None of the monoclonal antibodies had detectable neutralizing activity against serotypes 1 (Wa, RV-4), 2 (RV-5), 3 (RV-3) or 4 (ST-3) using either plaque or fluorescent focus neutralization assays. The antigenic specificity of these antibodies was investigated by determination of their EIA titres to thirteen cultivable mammalian rotaviruses (Table 1). All three monoclonal antibodies showed high titres (>128,000) to human rotaviruses of serotypes 1, 3, and 4, to simian and canine serotype 3 strains and to the UK bovine rotavirus. However, titres to human serotype 2 rotaviruses were very low (<1:4000). These monoclonal antibodies therefore showed significant levels of binding to all Group A rotaviruses tested, with the exception of human serotype 2 rotaviruses.

Evaluation of Antibodies in Detection of Rotaviruses in Stools

To test the effectiveness of these antibodies in detection of rotavirus in stools, they were reacted by EIA with antigen prepared from 63 rotavirus-positive and 17 rotavirus-negative stools. A comparison of the positive minus negative (P-N) values and detection rates with Mab 24 for cultivable rotaviruses and stool-derived rotaviruses is shown in Table 2. Mab 24 did not react with any stool negative for rotavirus by EM, or with any S RNA pattern rotavirus from stools or from cell culture. (P-N values <0.08). However, although all L RNA pattern cultivable rotaviruses were detected by Mab 24, only 13 per cent of stool L electropherotype viruses were detected. The 6 reacting stool viruses were all collected in Melbourne and

Table 2. *P-N values and rates of detection of faecally derived and cultivable rotaviruses by EIA using Mab 24*

	No (%) virus samples positive by EIA		Mean (range) P-N values[b] $\times 10^3$	
RNA electropherotype	Faecal	Cultivable	EIA negative	EIA positive
Short	0/18 (0)	0/ 3 (0)	10 (0–61)	–
Long	6/45 (13)	11/11 (100)	9 (0–80)	794 (214–1657)
–[a]	0/17 (0)	–	8 (0–16)	–

[a] Pool of 10 rotavirus negative stools, and 6 individual rotavirus-negative stools

[b] P-N value = OD_{392} (Mab 24 + SA 11)–OD_{392} (control Mab + SA 11). Samples were considered positive for P-N values >0.100

showed P-N values > 0.214. There was no correlation between a particular RNA electropherotype and EIA reactivity.

Effect of Trypsin, Trypsin Inhibitors, and Faecal Extracts on EIA Reactivity of the Antibodies to Rotavirus

One possible explanation for this low rate of rotavirus detection in faecal samples compared with cultivable rotaviruses was that factors present in the gut were altering the rotavirus structure. EIA binding of Mab 24 to SA 11 rotavirus which had been incubated with pooled rotavirus-negative faecal extract before application to the solid phase was therefore measured. As shown in Fig. 2, the EIA reaction of this antibody with SA 11 virus was eliminated by treatment with the faecal extract. The extract contained no human antirotaviral IgG, IgM or IgA detectable by EIA against SA 11 rotavirus (16). As trypsin is the major proteolytic enzyme active in the human small intestine, the effect of trypsin on the Mab 24—SA 11 interaction was also examined (Fig. 2). Purified porcine trypsin (100 μg/ml) produced a similar effect to the faecal extract treatment.

In order to determine whether trypsin was the major active component of the faecal extract, the trypsin inhibitor STI was incubated for 20 minutes at 37° C with the faecal extract, prior to addition of SA 11 rotavirus. Using 1.0 mg/ml STI, 100 per cent of the EIA binding of Mab 24 and Mab 34 to SA 11 was retained. At lower STI concentrations, the amount of binding retained was proportional to the concentration of STI.

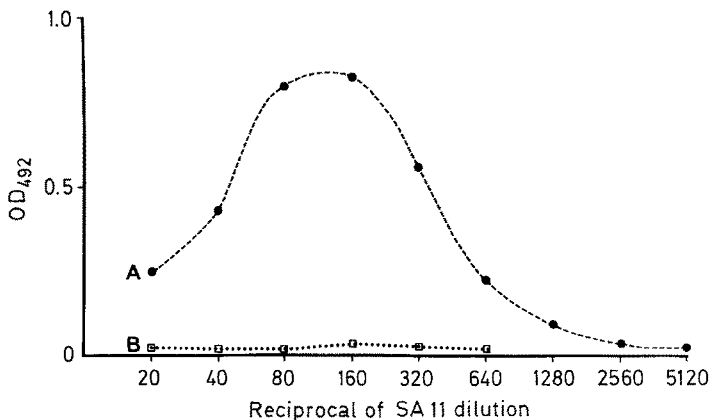

Fig. 2. Effects of trypsin, rotavirus-negative faecal extracts, and soybean trypsin inhibitor on EIA reaction of Mab 24 with SA 11 rotavirus. *A* SA 11 EIA antigen incubated with PBS for 30 or 60 minutes at 37° C before application to the solid phase; *B* SA 11 (i) incubated with negative control faecal extract 1 : 1 or with 100 μg/ml trypsin for 30 minutes at 37° C before application to the solid phase or (ii) as in (i) then incubated with 100 μg/ml soybean trypsin inhibitor for 30 minutes at 37° C. All incubations were carried out at neutral pH

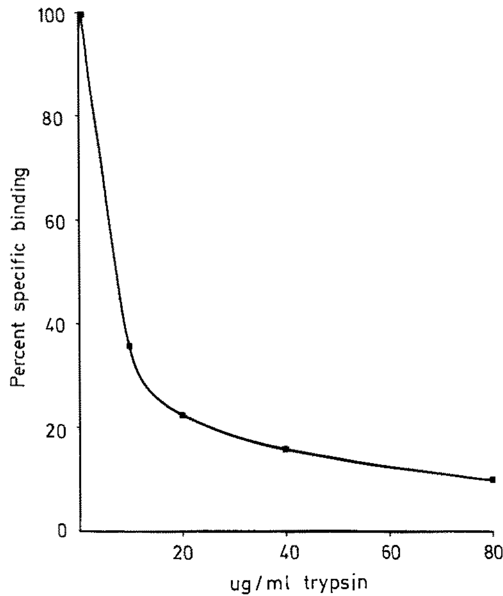

Fig. 3. Effect of trypsin concentration on the binding of Mab 24 to trypsin-treated SA 11 rotavirus. Mab 24 was used as a 1 : 1000 dilution of ascites fluid

$$\% \text{ specific binding} = \frac{OD_{392}\ (\text{SA 11} + \text{trypsin})}{OD_{392}\ \text{SA 11}} \times 100$$

The effect of various concentrations of trypsin on the binding of monoclonal antibodies to SA 11 was investigated. Incubation of SA 11, RV-1 and Wa rotaviruses with 7.5 µg/ml trypsin for 30 minutes at 37° C produced a 50 per cent reduction in EIA absorbance readings with Mab 24, Mab 34 and Mab 38. However, levels of trypsin up to 40 µg/ml had no effect on the binding of two neutralizing, serotype 3-specific monoclonal antibodies [Mab RV-3 : 1 and Mab RV-3 : 2 (6)] to SA 11. Using Mab 24 a hyperbolic reaction curve between percent specific binding and trypsin concentration was observed (Fig. 3), indicating that 80 per cent of specific binding was lost at a trypsin level of 20 µg/ml. Increases in trypsin concentration to 80 µg/ml further reduced the specific binding by only 12 per cent. The level of 50 µg/ml trypsin, which reduced specific binding to SA 11 by 90 per cent, was chosen for all further experiments.

To ascertain whether or not this effect of the rotavirus negative faecal extract on rotavirus was reversible, a faecal EIA antigen preparation known to contain L rotavirus which was detectable by EIA using polyclonal hyperimmune antiserum to SA 11 (17), but which did not react with monoclonal antibodies 24, 34 or 38, was treated with STI. Following incubation with up to 1000 µg/ml STI for 30 minutes at 37° C, EIA binding of the monoclonal antibodies to SA 11 did not return. Similarly, treatment of SA 11 virus with

rotavirus negative faecal extract, then with STI, gave no restoration of binding of the monoclonal antibodies (Fig. 2 B). These results suggested that the effect of trypsin on the binding of these monoclonal antibodies to rotavirus was irreversible, and that this effect was mimicked by faecal extracts.

The effect of trypsin inhibitors on the trypsin-SA 11 virus interaction was further investigated using this EIA system. Trypsin inhibitors were incubated with trypsin either before or after virus addition, then the mixture applied to the solid phase. This protocol was investigated both with and without trypsin inhibitors in the diluent of the detector antibody. The results using STI and PMSF, with Mab 24 and hyperimmune rabbit antiserum to SA 11 virus (anti-SA 11) as detector antibodies, are shown in Table 3. Both inhibitors reduced the effect of the trypsin on SA 11 virus, although STI produced more complete retention of binding of antibody to SA 11. Binding was retained almost completely when trypsin and STI were mixed prior to virus addition (77—90 per cent) and partially retained (33—81 per cent) when STI was added to trypsin-treated virus. Hence digestion of SA 11 virus by trypsin continued to a limited degree following virus application to the solid phase, and the digestion of the virus was confirmed to be an irreversible process. Addition of STI and PMSF to the antibody did not appear to affect binding of antibody to virus. Hence, the trypsin was acting on the virus alone and did not damage the rotavirus antibodies. Trypsin treatment of SA 11 virus reduced its EIA reaction with polyclonal antibodies to a lesser degree (27 per cent) than with monoclonal antibodies (89 per cent). This suggests that the trypsin effect is site-specific rather than a non-specific rotavirus degradation.

Table 3. *Efficiency of trypsin inhibitors STI and PMSF in preventing or modifying the effect of trypsin on the site of binding of Mab 24, and on the binding of polyclonal antiserum to SA 11*

		% retention of EIA binding to SA 11 virus[a]			
Inhibitor present[b]	Antibody[c]	STI + trp, then SA 11	trp + SA 11, then STI	PMSF + trp, then SA 11	trp + SA 11, then PMSF
SP	Mab 24	80	33	54	25
	anti-SA 11	90	80	82	73
SP + AD	Mab 24	77	44	54	21
	anti-SA 11	85	81	88	75

[a] $$\% \text{ retention} = \frac{(OD_{392}\ SA\ 11 + trp + TI) - (OD_{392}\ SA\ 11 + trp)}{(OD_{392}\ SA\ 11) - (OD_{392}\ SA\ 11 + trp)} \times 100$$

using 50 µg/ml trypsin, 100 µg/ml STI or PMSF, and SA 11 antigen diluted 1 : 500. % specific binding of antibody to SA 11 with trypsin treatment only: Mab 34, 11; anti-SA 11, 32 (means of 6 determinations). % specific binding calculated as described for Fig. 3

[b] *SP* solid phase; *AD* antibody diluent

[c] Mab 24 as 1 : 1000 dilution of ascites fluid; rabbit anti-SA 11 hyperimmune serum as 1 : 500 dilution

The above results were confirmed in a modified EIA in which the trypsin and STI treatments of SA 11 virus were included as additional incubations following virus application to the solid phase. Treatment of immobilised SA 11 virus with 50 μg/ml trypsin reduced the EIA reaction with monoclonal antibodies by 97 per cent. No regain in binding was evident from an STI incubation (50–500 μg/ml) immediately following the trypsin treatment, but prior to reaction with antibody. Treatment of virus with STI alone under the same conditions did not alter binding.

Discussion

The derivation of monoclonal antibodies to human rotavirus purified from faeces was shown to be practicable in this study. Although most hybridomas produced antibody cross-reactive between all rotaviruses tested, antibody from clones 24, 34 and 38 proved to react by EIA with cultivable rotaviruses of serotypes 1, 3 and 4 (human), serotype 3 mammalian strains and the UK bovine virus. Although directed to the major outer capsid glycoprotein gp 34, which carries antigenic sites for virus neutralization, these antibodies did not neutralize cultivable human rotaviruses representing serotypes 1, 3 and 4. It therefore appears that Mab 24, 34 and 38 recognize an antigenic region on gp 34 distinct from its neutralization sites. Although cross-reactive monospecific antisera (3) and a non-neutralizing monoclonal antibody (22) to gp 34 have been described previously, their specificities were not studied extensively.

The site recognized by Mab 24, 34 and 38 is shared between L rotaviruses, but absent on the S isolates which are human serotype 2 viruses (25). These two different types of RNA patterns result from a shift in the relative mobility of segment 11 of a L electropherotype such that the homologous gene runs as segment 10 in a S electropherotype in polyacrylamide gels (8). However, the reason for the observed correlation between segment 10 mobility and serotyping as 2 (encoded by segment 7, 8 or 9 gene product) is not clear. It is conceivable that these proteins may tend to cosegregate, as gp 34 and the outer capsid protein p 84 may tend to do in serotypes 2 (7) and 3 (6). Cosegregation of the inner capsid protein, p 94 and p 42 has also been postulated (23). Human serotype 2 rotaviruses are distinguishable from other human serotypes by reaction of antisera or monoclonal antibodies with antigens present on the major inner shell protein, p 42 (subgrouping). Serotype 2 rotaviruses subgroup as 1, as do most animal rotaviruses (25) however, so this reaction is distinct from RNA electropherotyping. The monoclonal antibodies 24, 34 and 38 therefore recognize a site on gp 34 which might, provide a marker for L rotaviruses, and perhaps be used in classification of rotaviruses as L or S by EIA.

From a comparison of the published gene sequences of rotavirus glyco-

protein genes, several regions of the deduced protein sequences would qualify as potential binding sites for these gp 34-specific monoclonal antibodies which distinguish between L and S rotavirus electropherotypes. The sequence comparisons of both DYALL-SMITH and HOLMES (10) and GUNN *et al.* (13) suggest that the protein sequence of S rotaviruses differs most widely from that of L viruses at residues 236 to 244 and 281 to 284. Neutralizing antibodies could not be elicited against a peptide containing the latter residues (13). It is possible that the gp 34 monoclonal antibodies reported here recognize sites related to one of these regions of gp 34.

These antibodies to gp 34 might have been useful as a control for the amount of gp 34 present in faecal samples assayed by serotyping EIA. However, when reacted with human stools, the antibodies, although retaining specificity for L rotaviruses reacted with only 13 per cent of L virus strains. Our experiments showed this was due to degradation of the viral antigen to which the antibodies bound, by constituent(s) of faeces, mainly trypsin, and that this effect on the virus was irreversible. It appears that the faecally derived rotavirus which was used to produce these monoclonal antibodies, fell amongst the 13 per cent of faecal isolates in which this antigen was undegraded by trypsin.

Using partially purified SA 11 rotavirus and Mab 24 as a model, 80 per cent of binding of monoclonal antibody to virus was lost upon treatment of virus with 20 μg/ml porcine trypsin for 30 minutes at 37° C. The experience of our Department is that normal levels of trypsin in duodenal juice collected from children aged 7 to 28 months, range from 80 to 200 μg/ml. The rotaviral determinant recognized by Mab 24 is likely to be destroyed in this environment.

The binding to SA 11 virus of two neutralizing, serotype 3-specific monoclonal antibodies, Mab RV-3 : 1 and RV-3 : 2 (6), was unaffected by trypsin treatment of the virus. This suggests that the trypsin effect detected with Mab 24 is site-specific and affects only one region of gp 34.

The reaction of polyclonal anti-SA 11 serum to SA 11 virus was also reduced by trypsin treatment of the virus, although to a much lesser extent. Trypsin activation of rotaviruses, necessary to produce infectious virions, results in cleavage of p 84 into two smaller polypeptides (11). This process occurs after treatment of purified rotavirus for 20 minutes at 37° C with 10 μg/ml trypsin (5), which is a level comparable with the 20–50 μg/ml trypsin used to treat semi-purified SA 11 virus in this report. Although the loss in EIA reactivity observed with the polyclonal antiserum may relate to the cleavage of p 84 by trypsin, it is clear that other effects of trypsin, not previously described, such as that detected with Mab 24, may also be occurring. It is possible that the activation of rotaviruses with 10 μg/ml trypsin, currently used in a number of laboratories to aid in yields of human rotavirus strains, may be altering the serological profile of the viruses.

Acknowledgements

We are most grateful for the assistance of Wendy McAdam, Leanne Unicomb, and John Albert with this research, for the secretarial services provided by Val Williams and Jane Lee, and the photography of Anneke Veenstra.

This project was supported by the Royal Children's Hospital Research Foundation and the National Health and Medical Research Council of Australia.

References

1. ALBERT MJ, BISHOP RF (1984) Cultivation of human rotaviruses in cell culture. J Med Virol 13: 377–384
2. ALBERT MJ, BISHOP RF, SHANN FA (1983) Epidemiology of rotavirus diarrhoea in the highlands of Papua New Guinea, in 1979, as revealed by electrophoresis of genome RNA. J Clin Microbiol 17: 162–164
3. BASTARDO JW, MCKIMM-BRESCHKIN JL, SONZA S, MERCER LD, HOLMES IH (1981) Preparation and characterization of antisera to electrophoretically purified SA 11 virus polypeptides. Infect Immun 34: 641–647
4. BEARDS GM, CAMPBELL AD, COTTRELL NR, PEIRIS JSM, REES N, SANDERS RC, SHIRLEY JA, WOOD HC, FLEWETT TH (1984) Enzyme-linked immunosorbent assays based on polyclonal and monoclonal antibodies for rotavirus detection. J Clin Microbiol 19: 248–254
5. COHEN J, LA PORTE J, CHARPILIENNE A, SCHERRER R (1979) Activation of rotavirus RNA polymerase by calcium chelation. Arch Virol 60: 177–186
6. COULSON BS, FOWLER KJ, BISHOP RF, COTTON RGH (1985) Neutralizing monoclonal antibodies to human rotavirus and indications of antigenic drift among strains from neonates. J Virol 54: 14–20
7. COULSON BS, TURSI JM, MCADAM WJ, BISHOP RF (1986) Derivation of neutralizing monoclonal antibodies to human rotaviruses and evidence that an immunodominant neutralization site is shared between serotypes 1 and 3. Virology (in press)
8. DYALL-SMITH ML, HOLMES IH (1981) Gene-coding assignments of rotavirus double-stranded RNA segments 10 and 11. J Virol 38: 1099–1103
9. DYALL-SMITH ML, HOLMES IH (1981) Comparisons of rotavirus polypeptides by limited proteolysis: close similarity of certain polypeptides of different strains. J Virol 40: 720–728
10. DYALL-SMITH ML, HOLMES IH (1984) Sequence homology between human and animal rotavirus serotype-specific glycoproteins. Nucleic Acids Res 12: 3973–3982
11. ESTES MK, GRAHAM DY, MASON BB (1981) Proteolytic enhancement of rotavirus infectivity: molecular mechanisms. J Virol 39: 879–888
12. GREENBERG H, MCAULIFFE V, VALDESUSO J, WYALL R, FLORES J, KALICA A, HOSHINO Y, SINGH N (1983) Serological analysis of the subgroup protein of rotavirus, using monoclonal antibodies. Infect Immun 39: 91–99
13. GUNN PG, SATO F, POWELL KFH, BELLAMY AR, NAPIER JR, HARDING DRK, HANCOCK WS, SIEGMAN LJ, BOTH GW (1985) Rotavirus neutralising protein VP 7: antigenic determinants investigated by sequence analysis and peptide synthesis. J Virol 54: 791–797
14. LAZDINS I, SONZA S, DYALL-SMITH ML, COULSON BS, HOLMES IH (1985) Demonstration of an immunodominant neutralization site by analysis of antigenic variants of SA 11 rotavirus. J Virol 56: 317–319
15. LEE PWK, HAYES EC, JOKLIK WK (1981) Characterization of anti-reovirus immunoglobulins secreted by cloned hybridoma cell lines. Virology 108: 134–146

16. McLean B, Sonza S, Holmes IH (1980) Measurement of immunoglobulin A, G, and M class rotavirus antibodies in serum and mucosal secretions. J Clin Microbiol 12: 314–319
17. Roberton DM, Harrison M, Hosking CS, Adams LC, Bishop RF (1979) Rapid diagnosis of rotavirus infection: comparison of electron microscopy and enzyme linked immunosorbent assay (ELISA). Aust Paediatr J 15: 229–232
18. Rodger SM, Schnagl RD, Holmes IH (1975) Biochemical and biophysical characteristics of diarrhea viruses of human and calf origin. J Virol 16: 1229–1235
19. Sato K, Inaba Y, Shinozaki T, Fujii R, Matumoto M (1981) Isolation of human rotavirus in cell cultures. Arch Virol 69: 155–160
20. Smith ML, Lazdins I, Holmes IH (1980) Coding assignments of double-stranded RNA segments of SA 11 rotavirus established by *in vitro* translation. J Virol 33: 976–982
21. Soenarto Y, Sebodo T, Ridho R, Alrasjid H, Rohde JE, Bugg HC, Barnes GL, Bishop RF (1981) Acute diarrhoea and rotavirus infection in newborn babies and children in Yogyakarta, Indonesia, from June 1978 to June 1979. J Clin Microbiol 14: 123–129
22. Sonza S, Breschkin AM, Holmes IH (1984) The major surface glycoprotein of simian rotavirus (SA 11) contains distinct epitopes. Virology 134: 318–327
23. Taniguchi K, Urasawa T, Urasawa S (1986) Reactivity patterns to human rotavirus strains of a monoclonal antibody against VP 2, a component of the inner capsid of rotavirus. Arch Virol 87: 135–141
24. Taniguchi K, Urasawa T, Urasawa S, Yasuhara T (1984) Production of subgroup-specific monoclonal antibodies against human rotaviruses and their application to an enzyme-linked immunosorbent assay for subgroup determination. J Med Virol 14: 115–125
25. Thouless ME, Beards GM, Flewett TH (1982) Serotyping and subgrouping of rotavirus strains by the ELISA test. Arch Virol 73: 219–230
26. Urasawa S, Urasawa T, Taniguchi K (1982) Three human rotavirus serotypes demonstrated by plaque neutralization of isolated strains. Infect Immun 38: 781–784

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Received July 4, 1986