Passive Protection against Rotavirus-Induced Diarrhea by Monoclonal Antibodies to Surface Proteins vp3 and vp7

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Monoclonal antibodies directed against two rotavirus surface proteins (vp3 and vp7) as well as a rotavirus inner capsid protein (vp6) were tested for their ability to protect suckling mice against virulent rotavirus challenge. Monoclonal antibodies to two distinct epitopes of vp7 of simian rotavirus strain RRV neutralized RRV in vitro and passively protected suckling mice against RRV challenge. A monoclonal antibody directed against vp3 of porcine rotavirus strain OSU neutralized three distinct serotypes in vitro (OSU, RRV, and UK) and passively protected suckling mice against OSU, RRV, and UK virus-induced diarrhea. The role of vp3 in eliciting protection against heterotypic rotavirus challenge should be considered when developing a vaccine with cloned rotavirus genes. Alternatively, immunization with a reassortant rotavirus containing vp3 and vp7 from two antigenically distinct rotavirus parents might protect against diarrhea induced by two or more rotavirus serotypes.

Rotaviruses are an important cause of acute gastroenteritis in infants and young children in developed countries (6, 16). In less developed countries, rotavirus infections often result in diarrhea, dehydration, and death (3, 33).

Parenteral immunization of animals with rotavirus strains heterotypic to a challenge virus has been found to protect against rotavirus-induced gastroenteritis (20, 27, 36-38). Large-scale clinical trials with human infants have demonstrated protection against illness with virulent human strains by oral immunization with a heterotypic bovine rotavirus (30, 31). Little is known about the molecular basis of protection against rotavirus-induced diarrhea evoked by immunization with strains homotypic or heterotypic to the challenge virus. This information may be relevant to the development of a successful live virus vaccine or a vaccine which utilizes cloned rotavirus genes.

To determine which rotavirus proteins stimulate protective immunity in vivo, we evaluated monoclonal antibodies directed against two rotavirus surface proteins (vp3 and vp7) as well as an inner capsid protein (vp6) for their ability to protect suckling mice against virulent rotavirus challenge (Table 1). Genetic studies using reassortant rotaviruses have found that only gene segments which code for vp3 and vp7 cosegregate with neutralization specificities (15, 18). Monoclonal antibodies were administered orally, since high titers of circulating rotavirus-specific antibodies fail to protect suckling mice against rotavirus challenge and resistance to clinical illness is best correlated with the presence of rotavirus-specific antibodies at the intestinal cell surface (19). Also, oral administration of monoclonal antibodies closely approximates the passive transfer of colostrum and milk antibodies found in breast-fed neonates.

Studies were performed by using a previously developed murine model for oral infection with a tissue culture-adapted primate rotavirus (simian strain SA11) (21). We recently found that suckling mice orally inoculated with the simian rotavirus strain RRV, the porcine strain OSU, or the bovine strain UK developed gastroenteritis similar to that found after oral inoculation with SA11 virus (data not shown) (12).

Five-day-old CD-1 mice were orally inoculated with 100 μ l of various dilutions of monoclonal antibodies derived from ascitic fluids. Serum obtained from a BALB/c mouse before inoculation with hybridoma cells was used as a control. After 30 min, suckling mice were orally challenged with 6×10^6 PFU of RRV, SA11, or OSU rotavirus or with 10^7 PFU of UK rotavirus; these doses caused disease in approximately 95% of animals previously inoculated with control serum (Table 1).

Rotaviruses of six well-characterized serotypes, human strains Wa (serotype 1), S₂ (serotype 2), and Saint Thomas (serotype 4), as well as the porcine strain OSU (serotype 5), the simian strains SA11 and RRV, and the bovine strain UK (serotype 6) were used in these studies (14). The simian strains SA11 and RRV have been found to be indistinguishable from a serotype 3 human strain by plaque reduction neutralization (PRN) (14).

Production of monoclonal antibodies by immunization of BALB/c mice with RRV (simian), OSU (porcine), and Wa (human) rotaviruses, fusion of spleen and NS-1 cells, screening of culture supernatant fluids by radioimmunoassay, and production of ascitic fluids in seronegative BALB/c mice were performed as previously described (10, 11). All monoclonal antibodies were isotype immunoglobulin G1, determined by using ¹²⁵I-labeled goat anti-mouse immunoglobulin G1 (Cappel Laboratories, Malvern, Pa.). Three monoclonal antibodies (2G4, 8B3, and 7A12) which immunoprecipitated vp3 from OSU- and RRV-infected cell lysates (data not shown) were studied for their ability to protect suckling mice against challenge (Table 1). Antibody 2G4 was isolated by fusing lymphocytes obtained from mice parenterally immunized with the porcine OSU strain. Monoclonal antibody 2G4 neutralized the serotypically distinct OSU, serotype 3 (SA11, RRV), and UK strains by PRN and protected suckling mice against diarrhea induced by OSU, RRV, SA11, or UK virus challenge (Table 1). The neutralizing activity of monoclonal antibody 2G4 in vitro against RRV and SA11 was greater than that against OSU. Our finding of a monoclonal antibody (2G4) with neutralizing activity against a heterotypic strain greater than that against the immunizing virus has been described in other systems (34, 35). Mono-

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TABLE 1. Protection against RRV-, SA11, or OSU-induced diarrhea in suckling mice by monoclonal antibodies derived from ascitic fluids^a

Monoclonal antibody designation	Immunizing antigen	Structural specificity ^b	RIA ^c	PRN ^d titer against the following viruses:							In vivo protective titer ^e against the following viruses:			
				Wa	S ₂	RRV	SA11	ST	OSU	UK	RRV	SA11	OSU	UK
2G4	OSU	vp3	1.4 × 10 ⁶	<50	<50	156,250	156,250	<50	1,250	1,250	2,500	3,000	2,500	1,000
8B3	RRV	vp3	4.0 × 10 ⁵	<50	<50	31,250	<50	<50	<50	<50	<20	<20	<20	<20
7A12	RRV	vp3	4.0 × 10 ⁵	<50	<50	31,250	<50	<50	<50	<50	<20	<20	<20	<20
255/60	RRV	vp6	2.0 × 10 ⁵	<50	<50	<50	<50	<50	<50	<50	<20	<20	<20	<20
159-H11	RRV	vp7	1.2 × 10 ⁶	<50	<50	>3,900,000	>3,900,000	<50	<50	<50	1,200	2,000	<20	<20
4F8	RRV	vp7	1.0 × 10 ⁶	<50	<50	700,000	130,000	<50	<50	<50	6,250	2,500	<20	<20
4C3	RRV	vp7	1.2 × 10 ⁶	<50	<50	3,900,000	2,000,000	<50	<50	<50	1,000	500	<20	<20
5H3	RRV	vp7	1.0 × 10 ⁶	<50	<50	156,250	<50	<50	<50	<50	500	<20	<20	<20
2C9	WA	vp7	3.0 × 10 ³	31,250	<50	<50	<50	<50	<50	<50	<20	<20	<20	<20
129-B8	WA	vp7	1.0 × 10 ⁶	<50	<50	<50	<50	<50	<50	<50	<20	<20	<20	<20
60-F2	WA	vp7	1.0 × 10 ⁶	<50	<50	<50	<50	<50	<50	<50	<20	<20	<20	<20
Serum control			<1.0 × 10 ²	<50	<50	<50	<50	<50	<50	<50	<20	<20	<20	<20

^a Conventionally bred BALB/c and pregnant CD-1 mice obtained from Charles River Breeding Laboratories, Inc. (Wilmington, Mass.) were housed in isolation units. Mice were bled on arrival by capillary plexus puncture, and sera were tested by RIA using SA11 virus as previously described (22). Seronegative BALB/c mice and litters from seronegative CD-1 dams were used in these studies. Viral growth, purification, quantitation, and infectivity titration by plaque assay were performed as previously described (23).

^b For a determination of the structural specificities of monoclonal antibodies, [³⁵S]methionine-labeled RRV-infected cell lysates and radioimmunoprecipitation of labeled proteins with hybridoma culture fluid supernatants were performed as previously described (11). Monoclonal 2C9, 4F8, and 4C3 did not immunoprecipitate vp7 efficiently. The structural specificities of these monoclonal antibodies were demonstrated by hemagglutination inhibition using reassortant rotaviruses or by viral variant analysis as previously described (11).

^c RIA, Radioimmunoassay. The radioimmunoassay using either RRV or Wa (2C9) as the detecting antigens was performed as previously described (22). Titers are expressed as the reciprocal of the positive ascitic fluid dilution.

^d The PRN assay was a modification of the technique described by Matsuno et al. (17), and was performed as previously described (22). Titers are expressed as the reciprocal of the ascitic fluid dilution showing a 50% reduction in the mean plaque count for each virus. ST, Saint Thomas.

^e The reciprocal of the dilution of the ascitic fluid at which 50% of newborn mice were protected against RRV, SA-11, OSU, or UK rotavirus challenge.

clonal antibodies 8B3 and 7A12, isolated from mice immunized with RRV, efficiently neutralized RRV in vitro, but failed to protect suckling mice against RRV challenge; the basis for the failure of these antibodies to protect in vivo awaits further study. Competition antibody binding assays and viral variant analysis indicated that monoclonal antibody 2G4 recognized an epitope distinct from that recognized by monoclonal antibodies 8B3 and 7A12 (R. Shaw and H. B. Greenberg, unpublished data).

Seven monoclonal antibody preparations specific for vp7 were studied in passive protection experiments (Table 1). The structural specificities of these monoclonal antibodies were demonstrated by immunoprecipitation, reactivity with virus reassortants or viral variants, or both (10, 26) (data not shown). Three of these monoclonal antibodies (159-H11, 4F8, and 4C3) neutralized serotype 3 rotaviruses RRV and SA11 by PRN and protected suckling mice against serotype 3 (RRV and SA11) but not against OSU or UK virus challenge. One monoclonal antibody (5H3) neutralized only the RRV serotype 3 strain by PRN and protected suckling mice against RRV but not against SA11, OSU, or UK virus challenge. Competition antibody binding assays and viral variant analysis indicated that monoclonal antibody 5H3 recognized an epitope distinct from that recognized by monoclonal antibodies 159-H11, 4F8, and 4C3 (Shaw and Greenberg, unpublished data). Monoclonal antibody 2C9 neutralized only Wa rotavirus by PRN and did not protect suckling mice against heterotypic rotavirus challenge. Monoclonal antibodies 60-F2 and 129-B8 were directed against an epitope on vp7 which is not involved in neutralization in vitro (11). This epitope is shared antigenically with most group A rotavirus strains (data not shown). Passive transfer of monoclonal antibodies 60-F2 or 129-B8 failed to protect newborns against challenge.

Monoclonal antibody 255/60, isolated from lymphocytes obtained after RRV immunization, precipitated the major

inner capsid protein vp6. This monoclonal antibody has been found to react with the subgroup epitope of vp6 (10). Monoclonal antibody 255/60 did not neutralize rotaviruses by PRN or protect suckling mice against RRV challenge.

In the present study we demonstrated that rotavirus-specific monoclonal antibodies were capable of protecting newborn mice when inoculated orally 30 min before heterologous host rotavirus challenge. In addition, we found that monoclonal antibodies administered up to 2 h after challenge protected suckling mice against disease (data not shown). The viral amplification and viral shedding observed in mice infected with heterologous host strains are less than those observed in homologous host rotavirus infection (12, 21). Nevertheless, the epithelial cell histopathological changes in the small intestine and serotype-specific humoral immune response in mice infected with heterologous host rotaviruses are characteristic of rotavirus-induced gastroenteritis (21). In addition, the clinical disease induced by heterologous host rotaviruses in mice is indistinguishable from that observed in homologous host infection (12). Therefore, the molecular determinants of antibody-mediated protection observed in our studies are likely to be relevant to the determinants of protection against challenge in natural rotavirus infection. However, the degree to which our findings are predictive of the ability of passively transferred rotavirus-specific monoclonal antibodies to either ameliorate or prevent homologous host rotavirus infection awaits further study.

Protection against challenge mediated by two or more epitopes on a single protein (4, 8, 24) or by distinct epitopes on two proteins (2, 8, 24, 25, 32) has been found in orthomyxovirus, paramyxovirus, herpesvirus, and alphavirus infections. We found that monoclonal antibodies directed against two distinct rotavirus surface proteins were capable of protecting suckling mice against challenge. A vp3-specific monoclonal antibody passively protected against diarrhea induced by three distinct rotavirus

serotypes (OSU, RRV, and UK). Previous investigators found that monoclonal antibodies specific for vp3 of human rotaviruses neutralized several human rotavirus serotypes in vitro (29). The epitope or epitopes recognized by monoclonal antibody 2G4 and other neutralizing anti-vp3 monoclonal antibodies might in part provide the structural basis for protection against rotavirus-induced diarrhea evoked by immunization with rotavirus strains heterotypic to a challenge virus (20, 27, 30, 31, 36–38). On the other hand, we found that monoclonal antibodies specific for two epitopes of vp7 of serotype 3 rotaviruses protected newborn mice only against challenge with serotype 3 strains. A comparison of the mechanisms by which antibodies directed against vp3 and vp7 neutralize rotaviruses in vitro and protect against challenge in vivo awaits further study.

In this initial study, a monoclonal antibody specific for vp3 was capable of protecting suckling mice against challenge with three rotavirus serotypes. If vp3 is serotypically less diverse than vp7, this protein may represent a more rational target for vaccine strategies which use cloned rotavirus genes (1, 5, 7, 9). Alternatively, immunization with a reassortant rotavirus containing vp3 and vp7 from two antigenically distinct rotavirus parents might protect against diarrhea induced by two or more rotavirus serotypes.

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LITERATURE CITED

- Arias, C. F., S. López, J. R. Bell, and J. H. Strauss. 1984. Primary structure of the neutralization antigen of simian rotavirus SA11 as deduced from cDNA sequence. *J. Virol.* 50:657–661.
- Balachandran, N., S. Bacchetti, and W. E. Rawls. 1982. Protection against lethal challenge of BALB/c mice by passive transfer of monoclonal antibodies to five glycoproteins of herpes simplex virus type 2. *Infect. Immun.* 37:1132–1137.
- Black, R. E., M. H. Merson, A. S. M. M. Rahman, M. Yunis, A. R. M. A. Alim, I. Huq, R. H. Yolken, and G. T. Curlin. 1980. A two-year study of bacterial, viral and parasitic agents associated with diarrhea in rural Bangladesh. *J. Infect. Dis.* 142:660–664.
- Boere, W. A. M., B. J. Benaissa-Trouw, M. Harmsen, C. A. Kraaijeveld, and H. Snippe. 1983. Neutralizing and non-neutralizing monoclonal antibodies to the E₂ glycoprotein of Semliki Forest virus can protect mice from lethal encephalitis. *J. Gen. Virol.* 64:1405–1408.
- Both, G. W., J. S. Mattick, and A. R. Bellamy. 1983. Serotype-specific glycoprotein of simian SA-11 rotavirus: coding assignment and gene sequence. *Proc. Natl. Acad. Sci. USA* 80:3091–3095.
- Davidson, G. P., R. F. Bishop, R. R. W. Townley, I. H. Holmes, and B. J. Ruck. 1975. Importance of a new virus in acute sporadic enteritis in children. *Lancet* i:242–246.
- Dyall-Smith, M. L., and I. H. Holmes. 1984. Sequence homology between human and animal rotavirus serotype-specific glycoproteins. *Nucleic Acids Res.* 12:3973–3982.
- Gerhard, W., J. W. Yewdell, D. Lopes, A. J. Caton, and G. C. Brownlee. 1983. Point mutations in the hemagglutinin molecule of influenza virus: their frequencies, phenotypic characteristics and biological relevance, p. 227–251. In L. M. de la Maza and E. M. Peterson (ed.), *Medical virology II*. Elsevier Science Publishing, Inc., New York.
- Glass, R. I., J. Keith, O. Nakagomi, T. Nakagomi, J. Askaa, A. Z. Kapikian, R. M. Chanock, and J. Flores. 1985. Nucleotide sequence of the structural glycoprotein vp 7 gene of Nebraska calf diarrhea virus rotavirus: comparison with homologous genes from four strains of human and animal rotaviruses. *Virology* 141:292–298.
- Greenberg, H., V. McAuliffe, J. Valdesuso, R. Wyatt, J. Flores, A. Kalica, Y. Hoshino, and N. Singh. 1983. Serological analysis of the subgroup protein of rotavirus using monoclonal antibodies. *Infect. Immun.* 39:91–99.
- Greenberg, H. B., J. Valdesuso, K. van Wyke, K. Midthun, M. Walsh, V. McAuliffe, R. G. Wyatt, A. R. Kalica, J. Flores, and Y. Hoshino. 1983. Production and preliminary characterization of monoclonal antibodies directed at two surface proteins of rhesus rotavirus. *J. Virol.* 47:267–275.
- Greenberg, H. B., P. T. Vo, and R. Jones. 1986. Cultivation and characterization of three strains of murine rotavirus. *J. Virol.* 57:585–590.
- Greenwood, F. C., W. M. Hunter, and J. S. Glover. 1963. The preparation of ¹²⁵I-labeled human growth hormone of high specific radioactivity. *Biochem. J.* 89:114–123.
- Hoshino, Y., R. G. Wyatt, H. B. Greenberg, J. Flores, and A. Z. Kapikian. 1984. Serotypic similarity and diversity of rotaviruses of mammalian and avian origin as studied by plaque-reduction neutralization. *J. Infect. Dis.* 149:694–702.
- Kalica, A. R., H. B. Greenberg, R. G. Wyatt, M. M. Sereno, A. Z. Kapikian, and R. M. Chanock. 1981. Genes of human (strain Wa) and bovine (strain UK) rotaviruses that code for neutralization and subgroup antigens. *Virology* 112:385–390.
- Kapikian, A. Z., H. W. Kim, R. G. Wyatt, and W. L. Cline. 1976. Human reovirus-like agent as the major pathogen associated with "winter" gastroenteritis in hospitalized infants and young children. *N. Engl. J. Med.* 294:965–972.
- Matsumo, S., S. Inouye, and R. Kono. 1977. Plaque assay of neonatal calf diarrhea virus and the neutralizing antibody in human sera. *J. Clin. Microbiol.* 5:1–4.
- Ofit, P. A., and G. Blavat. 1986. Identification of the two rotavirus genes determining neutralization specificities. *J. Virol.* 57:376–378.
- Ofit, P. A., and H. F. Clark. 1985. Protection against rotavirus-induced gastroenteritis in a murine model by passively acquired gastrointestinal but not circulating antibodies. *J. Virol.* 54:58–64.
- Ofit, P. A., and H. F. Clark. 1985. Maternal antibody-mediated protection against gastroenteritis due to rotavirus in newborn mice is dependent on both serotype and titer of antibody. *J. Infect. Dis.* 152:1152–1158.
- Ofit, P. A., H. F. Clark, M. J. Kornstein, and S. A. Plotkin. 1984. A murine model for oral infection with a primate rotavirus (simian SA11). *J. Virol.* 51:233–236.
- Ofit, P. A., H. F. Clark, and S. A. Plotkin. 1983. Response of mice to rotaviruses of bovine or primate origin assessed by radioimmunoassay, radioimmunoprecipitation, and plaque reduction neutralization. *Infect. Immun.* 42:293–300.
- Ofit, P. A., H. F. Clark, W. G. Stroop, E. M. Twist, and S. A. Plotkin. 1983. The cultivation of human rotavirus, strain "Wa," to high titer in cell culture and characterization of the viral structural polypeptides. *J. Virol. Methods* 7:29–40.
- Orvell, C., and M. Grandien. 1982. The effects of monoclonal antibodies on biological activities of structural proteins of Sendai virus. *J. Immunol.* 129:2779–2787.
- Rector, J. T., R. N. Lausch, and J. E. Oakes. 1982. Use of monoclonal antibodies for analysis of antibody-dependent immunity to ocular herpes simplex virus type 1 infection. *Infect. Immun.* 38:168–174.
- Shaw, R., D. L. Stoner-Ma, M. K. Estes, and H. B. Greenberg.

1985. Specific enzyme-linked immunoassay for rotavirus serotypes 1 and 3. *J. Clin. Microbiol.* **22**:286-291.
27. Sheridan, J. F., C. C. Smith, M. M. Manak, and L. Aurelian. 1984. Prevention of rotavirus-induced diarrhea in neonatal mice born to dams immunized with empty capsids of simian rotavirus SA-11. *J. Infect. Dis.* **149**:434-438.
28. Stuker, G., L. S. Oshiro, and N. J. Schmidt. 1980. Antigenic comparisons of two new rotaviruses from rhesus monkeys. *J. Clin. Microbiol.* **11**:202-203.
29. Taniguchi, K., S. Urasawa, and T. Urasawa. 1985. Preparation and characterization of neutralizing monoclonal antibodies with different reactivity to human rotaviruses. *J. Gen. Virol.* **66**:1045-1053.
30. Vesikari, T., E. Isolauri, A. Delem, E. D'Hondt, F. E. Andre, G. M. Beards, and T. H. Flewett. 1985. Clinical efficacy of the RIT 4237 live attenuated bovine rotavirus vaccine in infants vaccinated before a rotavirus epidemic. *J. Pediatr.* **107**:189-194.
31. Vesikari, T., E. Isolauri, E. D'Hondt, A. Delem, F. E. Andre, and G. Zissis. 1984. Protection of infants against rotavirus diarrhea by RIT 4237 attenuated bovine rotavirus strain vaccine. *Lancet* **i**:977-980.
32. Walsh, E. E., J. J. Schlesinger, and M. W. Brandriss. 1984. Protection from respiratory syncytial virus infection in cotton rats by passive transfer of monoclonal antibodies. *Infect. Immun.* **43**:756-758.
33. Walsh, J. A., and K. S. Warren. 1979. Selective primary health care: an interim strategy for disease control in developing countries. *N. Engl. J. Med.* **301**:967-974.
34. Webster, R. G., and W. G. Laver. 1980. Determination of the number of non-overlapping antigenic areas on Hong Kong (H3N2) influenza virus hemagglutinin with monoclonal antibodies and the selection of variants with potential epidemiological significance. *Virology* **104**:139-148.
35. Wiktor, T. J., and H. Koprowski. 1978. Monoclonal antibodies against rabies virus produced by somatic cell hybridization: detection of antigenic variants. *Proc. Natl. Acad. Sci. USA* **75**:3938-3942.
36. Wyatt, R. G., A. Z. Kapikian, and C. A. Mebus. 1983. Induction of cross-reacting serum neutralizing antibody to human rotavirus in calves after in utero administration of bovine rotavirus. *J. Clin. Microbiol.* **18**:505-508.
37. Wyatt, R. G., C. A. Mebus, R. H. Yolken, A. R. Kalica, H. D. James, Jr., A. Z. Kapikian, and R. M. Chanock. 1979. Rotaviral immunity in gnotobiotic calves: heterologous resistance to human virus induced by bovine virus. *Science* **203**:548-550.
38. Zissis, G., J. P. Lambert, P. Marbhant, D. Marissens, M. Lobmann, P. Charlier, A. Delem, and N. Zygraich. 1983. Protection studies in colostrum-deprived piglets of a bovine rotavirus vaccine candidate using human rotavirus strains for challenge. *J. Infect. Dis.* **149**:1061-1068.