JOURNAL OF VIROLOGY, Dec. 1990, p. 6325–6328
0022-538X/90/126325-04$02.00/0
Copyright © 1990, American Society for Microbiology
Vol. 64, No. 12

Rotavirus-Specific Cytotoxic T Lymphocytes Passively Protect against Gastroenteritis in Suckling Mice

PAUL A. OFFIT* AND KARLA I. DUDZIK

Division of Infectious Diseases, The Children's Hospital of Philadelphia, 34th Street and Civic Center Boulevard, and The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104*

Received 25 June 1990/Accepted 13 September 1990

Suckling mice are protected against murine rotavirus-induced gastroenteritis after adoptive transfer of splenic lymphocytes from immunized animals. Adoptive transfer of Thy1$^+$-depleted or CD8$^+$-depleted lymphocytes abrogated protection against challenge. (We previously found that depletion of Thy1$^+$ or CD8$^+$ lymphocytes from rotavirus-immunized mice decreased rotavirus-specific cytotoxic activity in vitro.) Protection against disease occurred in the absence of rotavirus-specific neutralizing antibodies in the sera of suckling mice. Rotavirus-specific cytotoxic T lymphocytes may be important in either amelioration of acute infection or protection against reinfection.

Rotaviruses are an important cause of gastroenteritis in infants and young children worldwide (2, 9). Rotaviruses induce disease by replicating in mature villus epithelial cells at the intestinal mucosal surface (30). Studies of the immunologic determinants associated with protection against rotavirus challenge have focused primarily on virus-specific humoral immunity, including secretory immunoglobulin A (sIgA) (12, 31). However, the intestine is also a rich source of T lymphocytes (4, 15, 23, 24). About 50 to 60% of lymphocytes which reside among villus epithelial cells (intraepithelial lymphocytes) have surface markers consistent with the functions of cytotoxicity or suppression; less than 10% of intraepithelial lymphocytes are B cells (4, 23, 24). In addition, large numbers of T cells reside in the lamina propria and Peyer's patch (14, 23). However, little is known about the importance of T lymphocytes in protection against rotavirus infection. We recently found that virus-specific cytotoxic T lymphocytes (CTLs) appear at the intestinal surface acutely after rotavirus infection (20). In experiments discussed in this paper, we found that rotavirus-specific lymphocytes passively protected suckling mice against rotavirus-induced diarrhea; protection was abrogated by depletion of Thy1$^+$ or CD8$^+$ cells. Rotavirus-specific CTLs may in part mediate protection against rotavirus disease.

Suckling mice develop gastroenteritis after oral inoculation with homologous (murine strain JMV) or heterologous (simian strain RRV) host rotaviruses. To determine the capacity of homologous or heterologous host rotaviruses to induce diarrhea in inbred suckling mice, 7-day-old C57BL/6 (*H-2^b*) and BALB/c (*H-2^d*) mice obtained from Taconic Breeding Laboratories (Germantown, N.Y.) were orally inoculated with simian rotavirus strain RRV (obtained from N. Schmidt, Berkeley, Calif.) or murine rotavirus strain JMV (obtained from H. Greenberg, Palo Alto, Calif.) at doses known to induce diarrhea in 90% of inoculated outbred mice (Table 1) (8, 21). Virus growth in MA-104 cells and quantitation by plaque assay were performed as previously described (18). Similar to studies of Greenberg and coworkers (8), the dose required to induce disease was approximately 500,000-fold greater for heterologous than for homologous host strains. In addition, mice inoculated with RRV developed diarrhea 1 to 2 days earlier than those inoculated with JMV.

Murine rotaviruses are well adapted to growth in the murine intestinal tract; small quantities of infectious virus are logarithmically amplified in the intestinal tract after multiple cycles of virus replication (8). Nonmurine rotavirus strains, on the other hand, do not undergo multiple cycles of replication in murine intestinal epithelial cells (8, 17), and the mechanism by which nonmurine rotavirus strains induce disease in suckling mice remains unclear. The large quantities of infectious nonmurine rotaviruses required to induce disease in suckling mice are consistent with the hypothesis that intestinal epithelial cell function may be impaired early in the infectious cycle prior to production of infectious virus progeny.

Thy1$^+$ CD8$^+$ splenic lymphocytes from rotavirus-inoculated adult mice passively protect suckling mice against rotavirus challenge. To determine whether rotavirus-specific T lymphocytes protect against rotavirus challenge, 7-day-old C57BL/6 or BALB/c mice were challenged with RRV or JMV prior to passive transfer of splenic lymphocytes from adult C57BL/6 or BALB/c mice inoculated with RRV or JMV (Table 2). Adult mice were inoculated intraperitoneally with 10^7 PFU of RRV or 10^5 PFU of JMV. Splenic lymphocytes taken 5 to 7 days after inoculation were used in adoptive transfer assays. Suckling mice were challenged with either RRV or JMV at a dose known to cause disease in approximately 90% of inoculated animals. At 12, 24, and 48 h after rotavirus challenge, suckling mice were inoculated intraperitoneally with 10^7 splenic lymphocytes from RRV- or JMV-inoculated adult mice. Suckling mice were examined daily for the presence of diarrhea by gentle palpation of their abdomens.

Suckling mice challenged with JMV were protected against disease after passive transfer of lymphocytes from major histocompatibility complex (MHC)-compatible but not MHC-incompatible adult animals inoculated with either RRV or JMV (Table 2). (Both JMV and RRV are serotype 3 rotaviruses [data not shown].) However, suckling mice challenged with RRV were not protected against disease after passive transfer of lymphocytes from MHC-compatible animals inoculated with RRV. Sera obtained 5 days after rotavirus challenge from each group shown in Table 2 did not contain antibodies which neutralized either RRV or JMV at

* Corresponding author.

TABLE 1. Suckling mice develop gastroenteritis after oral inoculation with homologous (murine strain JMV) or heterologous (simian strain RRV) host rotavirus[a]

Species of mice inoculated	Inoculum	No. and percentage[b] of mice with diarrhea at various intervals (days) after inoculation				
		1	2	3	4	5
C57BL/6	JMV	0/20 (0)	0/20 (0)	4/20 (20)	17/20 (85)	16/20 (80)
C57BL/6	RRV	2/18 (11)	18/18 (100)	18/18 (100)	4/18 (22)	ND
BALB/c	JMV	0/17 (0)	0/17 (0)	2/17 (12)	17/17 (100)	17/17 (100)

[a] Seven-day-old suckling mice were orally inoculated with 5×10^5 PFU of RRV or 1 PFU of JMV by proximal esophageal intubation. Mice were inspected daily for the presence of diarrhea by gentle palpation of their abdomens. Percentage of mice with diarrhea was not determined (ND) for all groups.
[b] Number of mice with diarrhea/number of mice inoculated with rotavirus; percentages are shown in parentheses.

a dilution of 1:50 as determined by plaque reduction neutralization assay performed as previously described (18).

To determine the surface markers of cells associated with protection against JMV challenge, lymphocytes were either untreated or treated as previously described (19) with either anti-Thy1.2-specific IgM (HO-13-4; American Type Culture Collection, Rockville, Md.) (13) plus guinea pig complement (Cappel Laboratories, Malvern, Pa.), anti-CD8 (29) plus complement, or complement alone prior to passive transfer (Table 3). Percentages of cells containing Thy1.2 (13), CD8 (29), or immunoglobulin were determined by fluorescence-activated cell sorter analysis using fluorescein isothiocyanate-conjugated, affinity-purified, mouse anti-rat IgG $F(ab')_2$ (Pel-Freez Biologicals, Rogers, Ariz.) as previously described (20). B-cell surface markers were detected by using fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Cappel Laboratories). Treatment of lymphocytes with anti-Thy1 or anti-CD8 plus complement decreased the percentage of $Thy1^+$ or $CD8^+$ lymphocytes, respectively, by approximately fivefold. Reduction in the percentage of adoptively transferred $Thy1^+$ or $CD8^+$ cells was associated with ablation of protection against challenge. We found as previously reported that treatment of splenic lymphocytes with anti-Thy1 plus complement or anti-CD8 plus complement ablated cytotoxic activity in a rotavirus-specific ^{51}Cr-release assay (19).

Using a monoclonal antibody preparation directed against CD4 plus complement, we were unable to adequately decrease the percentage of $CD4^+$ cells by a factor greater than twofold. Although adoptive transfer of splenic lymphocytes treated with anti-CD4 plus complement protected suckling mice against JMV challenge (data not shown), our inability to successfully reduce numbers of $CD4^+$ cells limited our ability to assign a role to these cells in protection against rotavirus challenge.

Our finding that adoptive transfer of $Thy1^+$-depleted or $CD8^+$-depleted lymphocytes abrogated protection against challenge is consistent with several previous observations. First, protection against rotavirus challenge is not clearly explained by the humoral immune response. Oral active immunization of animals can induce protection against heterotypic challenge (3, 33). However, rotavirus neutralizing antibody titers in both serum and feces do not always correlate with a protective response (3, 33). In addition, immunization of infants and young children with bovine rotavirus strain WC3 (serotype 6) protected against disease caused by serotype 1 rotavirus; protection occurred in the absence of serotype 1 virus-specific neutralizing antibodies in the serum (5). Although mice orally inoculated with WC3 also do not develop serotype 1 rotavirus-specific neutralizing antibodies, they do develop virus-specific CTLs which broadly lyse target cells infected with different rotavirus serotypes (including serotype 1) (22). Second, because rotaviruses cause disease by replicating in mature intestinal epithelial cells (30), rotavirus-specific CTLs would probably have to act at the intestinal surface to limit production and spread of infectious virus. The intestine is a rich source of $Thy1^+$ $CD8^+$ lymphocytes (4, 15, 23, 24). We recently found that rotavirus-specific CTLs are detected at the intestinal surface of mice 6 days after oral inoculation with simian strain RRV (20). Third, Dharakul and coworkers recently found that shedding of murine rotavirus was ablated in mice with severe combined immunodeficiency after passive transfer of $CD8^+$ lymphocytes from immunocompetent, MHC-compatible mice previously inoculated with murine rotavirus (6). Fourth, virus-specific CTLs have been found to correlate with protection against influenza virus (14) and cytomegalovirus (25, 27) infections in humans. Fifth, adoptive transfer of $Thy1^+$ $CD8^+$ lymphocytes has been found to protect against disease caused by herpes simplex virus (11), cytomegalovirus (26), influenza virus (32), and Sendai virus (10) in experimental animal models.

TABLE 2. MHC-restricted protection of suckling mice against rotavirus challenge by splenic lymphocytes from parenterally immunized animals[a]

Lymphocytes for transfer		Adoptive transfer of lymphocytes into suckling mice prior to rotavirus challenge						
Species	Immunizing virus	Species challenged	Challenge virus	No. and percentage[b] of mice with diarrhea at various intervals (days) after challenge				
				1	2	3	4	5
C57BL/6	JMV	C57BL/6	JMV	0/21 (0)	0/21 (0)	2/21 (10)	2/21 (10)	2/21 (10)
C57BL/6	RRV	C57BL/6	JMV	0/18 (0)	0/18 (0)	1/18 (6)	1/18 (6)	1/18 (6)
BALB/c	RRV	BALB/c	JMV	0/22 (0)	0/22 (0)	3/22 (14)	3/22 (14)	3/22 (14)
C57BL/6	RRV	BALB/c	JMV	0/20 (0)	0/20 (0)	8/20 (40)	20/20 (100)	20/20 (100)
BALB/c	RRV	C57BL/6	JMV	0/20 (0)	0/20 (0)	8/20 (40)	16/20 (80)	16/20 (80)
C57BL/6	RRV	C57BL/6	RRV	0/16 (0)	16/16 (100)	16/16 (100)	4/16 (25)	ND

[a] Seven-day-old C57BL/6 or BALB/c mice were challenged with heterologous host (simian strain RRV) or homologous host (murine strain JMV) rotaviruses as described in the text.
[b] Number of mice with diarrhea/number of mice challenged with rotavirus; percentages shown in parentheses. ND, Not determined.

TABLE 3. Thy1$^+$ CD8$^+$ lymphocytes are responsible for protection against rotavirus challenge[a]

Lymphocyte treatment	% Adoptively transferred cells with surface markers			No. and percentage[b] of suckling mice with diarrhea at various intervals (days) after challenge				
	Thy1	CD8	Ig	1	2	3	4	5
None	51	23	42	0/15 (0)	0/15 (0)	1/15 (7)	1/15 (7)	1/15 (7)
Complement	48	20	40	0/10 (0)	0/10 (0)	1/10 (10)	1/10 (10)	1/10 (10)
Anti-Thy1 + complement	9	7	52	0/12 (0)	0/12 (0)	4/12 (33)	10/12 (83)	4/12 (83)
Anti-CD8 + complement	35	4	45	0/10 (0)	0/10 (0)	6/10 (60)	10/10 (100)	10/10 (100)

[a] Seven-day-old C57BL/6 mice were challenged with murine rotavirus strain JMV as described in the text. Ig, Immunoglobulin.
[b] Number of mice with diarrhea/number of mice challenged with rotavirus; percentages shown in parentheses.

Compared with other studies of adoptive transfer of lymphocytes in experimental animals (10, 11, 26, 32), large numbers of lymphocytes were required to protect suckling mice against rotavirus-induced diarrhea. There are several possible explanations of this finding. Rotavirus-specific lymphocytes for adoptive transfer were obtained directly from the animal and not amplified in vitro by stimulation with virus-infected antigen presenting cells. Frequencies of virus-specific CTLs are probably 5- to 10-fold greater after in vitro stimulation (28). In addition, adoptively transferred lymphocytes leave the circulation and enter the intestinal tract after binding to specific receptors (vascular addressins) located on specialized capillary endothelial cells (1). However, decreased expression of vascular addressins in the lamina propria or Peyer's patches of suckling mice may not allow for adequate uptake of virus-specific lymphocytes from the circulation (E. C. Butcher and P. R. Streeter, personal communication).

JMV- or RRV-specific CTLs protected animals against homologous (JMV) but not heterologous (RRV) host rotavirus challenge. This is most likely associated with the observation that JMV and RRV exhibit markedly different patterns of gastrointestinal tract virulence when inoculated orally into suckling mice. JMV probably induces gastroenteritis after multiple cycles of replication and viral amplification over several days. RRV, on the other hand, appears to induce disease after a single cycle of replication 8 to 12 h after inoculation without viral amplification. In addition, greater numbers of villus epithelial cells are initially infected after inoculation of mice with 5×10^5 PFU of RRV than after inoculation with 1 PFU of JMV (H. B. Greenberg, personal communication). Therefore, to prevent disease, greater numbers of rotavirus-specific CTLs are probably initially needed to lyse villus epithelial cells 24 to 48 h after RRV infection than 24 to 48 h after JMV infection.

Recovery of suckling mice from rotavirus-induced gastroenteritis is probably not mediated by a virus-specific immunologic response. Athymic (*nu/nu*) mice recover from murine rotavirus-induced gastroenteritis without development of virus-specific antibodies or a functional T-cell response (7). Therefore, the suckling mouse model cannot be used to accurately define the immunologic determinants of amelioration of acute disease. In addition, the immunologic immaturity of suckling mice, as well as the narrow window of susceptibility, has not allowed for studies of determinants of the active immune response associated with protection against rotavirus disease. Studies of the importance of virus-specific antibodies (16, 21) and virus-specific T lymphocytes (6) in protection against disease in mice have been limited to passive transfer experiments. Although passive transfer experiments do afford information on the relative importance of different effector arms of the immune system, the degree to which our findings are predictive of events occurring in the human gastrointestinal tract remains to be determined.

This work was supported in part by The Thomas B. McCabe and Jeannette E. Laws McCabe Fund, The Lederle Biologicals Young Investigator Award in Vaccine Development, The University of Pennsylvania Research Foundation Award, and Public Health Service grants 1 R01 AI26251-01A1 and 1K04AI00889-01A1 to P.A.O. from the National Institutes of Health.

LITERATURE CITED

1. **Berg, E. L., L. A. Goldstein, M. A. Jutila, M. Nakache, L. J. Picker, P. R. Streeter, N. W. Wu, D. Zhou, and E. C. Butcher.** 1989. Homing receptors and vascular addressins: cell adhesion molecules that direct lymphocyte traffic. Immunol. Rev. **108**:5–18.
2. **Black, R. E. M., M. H. Merson, A. S. S. 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.
3. **Bridger, J. C., and G. Oldham.** 1987. Avirulent rotavirus infections protect calves from disease with and without inducing high levels of neutralizing antibody. J. Gen. Virol. **68**:2311–2317.
4. **Cerf-Bensussan, N., D. Guy-Grand, and C. Griscelli.** 1985. Intraepithelial lymphocytes of human gut: isolation, characterisation and study of natural killer activity. Gut **26**:81–88.
5. **Clark, H. F., F. E. Borian, L. M. Bell, K. Modesto, V. Gouvea, and S. A. Plotkin.** 1988. Protective effect of WC3 vaccine against rotavirus diarrhea in infants during a predominantly serotype 1 rotavirus season. J. Infect. Dis. **158**:570–587.
6. **Dharakul, T., L. Rott, and H. B. Greenberg.** 1990. Recovery from chronic rotavirus infection in mice with severe combined immunodeficiency: virus clearance mediated by adoptive transfer of immune CD8$^+$ T lymphocytes. J. Virol. **64**:4375–4382.
7. **Eiden, J., H. M. Lederman, S. Vonderfecht, and R. Yolken.** 1986. T-cell-deficient mice display normal recovery from experimental rotavirus infection. J. Virol. **57**:706–708.
8. **Greenberg, H. B., P. T. Vo, and R. Jones.** 1986. Cultivation and characterization of three strains of murine rotavirus. J. Virol. **57**:585–590.
9. **Kapikian, A. Z., H. W. Kim, R. G. Wyatt, W. L. Cline, J. O. Arrobio, C. D. Brandt, W. J. Rodriguez, D. A. Sack, R. M. Chanock, and R. H. Parrott.** 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.
10. **Kast, W. M., A. M. Bronkhorst, L. P. deWaal, and C. J. M. Melief.** 1986. Cooperation between cytotoxic and helper T lymphocytes in protection against lethal sendai virus infection: protection by T cells is MHC-restricted and MHC-regulated; a model for MHC-disease associations. J. Exp. Med. **164**:723–738.
11. **Larsen, H. L., R. G. Russell, and B. T. Rouse.** 1983. Recovery from lethal herpes simplex virus type 1 infection is mediated by cytotoxic T lymphocytes. Infect. Immun. **41**:197–294.
12. **Losonsky, G. A., M. B. Rennels, Y. Lim, G. Krall, A. Z. Kapikian, and M. M. Levine.** 1988. Systemic and mucosal immune responses to rhesus rotavirus vaccine MMU 18006.

Pediatr. Infect. Dis. J. **7**:388–393.

13. **Marshak-Rothstein, A., P. Fink, T. Gridley, D. H. Raulet, M. J. Bevan, and M. L. Gefter.** 1979. Properties and applications of monoclonal antibodies directed against determinants of the Thy 1 locus. J. Immunol. **122**:2491–2497.
14. **McMichael, A. M., F. M. Gotch, G. R. Noble, and P. A. S. Beare.** 1983. Cytotoxic T-cell immunity to influenza. N. Engl. J. Med. **309**:13–17.
15. **McWilliams, M., M. E. Lamm, and J. M. Phillips-Quagliata.** 1974. Surface and intracellular markers of mouse mesenteric and peripheral lymph node and Peyer's patch cells. J. Immunol. **113**:1326–1333.
16. **Offit, 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.
17. **Offit, P. A., H. F. Clark, M. K. Kornstein, and S. A. Plotkin.** 1984. A murine model for oral infection with a primate rotavirus (simian strain SA11). J. Virol. **51**:233–236.
18. **Offit, P. A., H. F. Clark, and S. A. Plotkin.** 1983. Response of mice to rotaviruses of bovine or primate origin as assessed by radioimmunoassay, radioimmunoprecipitation, and plaque reduction neutralization. Infect. Immun. **42**:293–300.
19. **Offit, P. A., and K. I. Dudzik.** 1988. Rotavirus-specific cytotoxic T lymphocytes cross-react with target cells infected with different rotavirus serotypes. J. Virol. **62**:127–131.
20. **Offit, P. A., and K. I. Dudzik.** 1989. Rotavirus-specific cytotoxic T lymphocytes appear at the intestinal mucosal surface after rotavirus infection. J. Virol. **63**:3507–3512.
21. **Offit, P. A., R. D. Shaw, and H. B. Greenberg.** 1986. Passive protection against rotavirus-induced diarrhea by monoclonal antibodies to surface proteins vp3 and vp7. J. Virol. **58**:700–703.
22. **Offit, P. A., and Y. M. Svoboda.** 1989. Rotavirus-specific cytotoxic T lymphocyte response of mice after oral inoculation with candidate rotavirus vaccine strains RRV or WC3. J. Infect. Dis. **160**:783–788.
23. **Parrott, D. M. V., C. Tait, S. MacKenzie, A. M. Mowat, M. D. J. Davies, and H. S. Micklem.** 1983. Analysis of the effector functions of different populations of mucosal lymphocytes. Ann. N.Y. Acad. Sci. **409**:307–320.
24. **Petit, A., P. B. Ernst, A. D. Befus, D. A. Clark, K. L. Rosenthal, T. Ishizaka, and J. Bienenstock.** 1985. Murine intestinal intraepithelial lymphocytes. I. Relationship of a novel $Thy1^-$, $Lyt1^-$, $Lyt2^+$ granulated subpopulation to natural killer cells and mast cells. Eur. J. Immunol. **15**:211–215.
25. **Quinnan, G. V., Jr., N. Kirmani, A. H. Rook, J. F. Manischewitz, L. Jackson, G. Moreschi, G. W. Santos, R. Saral, and W. H. Burns.** 1982. Cytotoxic T cells in cytomegalovirus infections: HLA-restricted T-lymphocyte and non-T-lymphocyte cytotoxic responses correlate with recovery from cytomegalovirus infection in bone marrow transplant recipients. N. Engl. J. Med. **307**:6–13.
26. **Reddehase, M. J., W. Mutter, K. Munch, H. J. Buhring, and U. H. Koszinowski.** 1987. CD8-positive T lymphocytes specific for murine cytomegalovirus immediate-early antigens mediate protective immunity. J. Virol. **61**:3102–3108.
27. **Rook, A. H., G. V. Quinnan, W. J. R. Frederick, J. F. Manischewitz, N. Kirmani, T. Dantzler, B. B. Lee, and C. B. Currier.** 1984. Importance of cytotoxic lymphocytes during cytomegalovirus infection in renal transplant recipients. Am. J. Med. **76**:385–392.
28. **Rouse, B. T., and H. Wagner.** 1984. Frequency of herpes simplex virus-specific cytotoxic T lymphocyte precursors in lymph node cells of infected mice. Immunology **51**:57–64.
29. **Sarmiento, M., A. L. Glasebrook, and F. W. Fitch.** 1980. IgG or IgM monoclonal antibodies reactive with different determinants in the molecular complex bearing Lyt2 antigen block T cell-mediated cytolysis in the absence of complement. J. Immunol. **125**:2665–2672.
30. **Starkey, W. G., J. Collins, T. S. Wallis, G. J. Clarke, A. J. Spencer, S. J. Haddon, M. P. Osborne, D. C. A. Candy, and J. Stephen.** 1986. Kinetics, tissue specificity and pathological changes in murine rotavirus infection of mice. J. Gen. Virol. **67**:2625–2634.
31. **Ward, R. L., D. I. Bernstein, R. Shukla, E. C. Young, J. R. Sherwood, M. M. McNeal, M. C. Walker, and G. M. Schiff.** 1989. Effects of antibody on protection of adults challenged with a human rotavirus. J. Infect. Dis. **159**:79–88.
32. **Wells, M. A., F. A. Ennis, and P. Albrecht.** 1981. Recovery from a viral respiratory infection. II. Passive transfer of immune spleen cells to mice with influenza pneumonia. J. Immunol. **126**:1042–1046.
33. **Woode, G. N., S. Zheng, B. I. Rosen, N. Knight, N. E. Gourley, and R. F. Ramig.** 1987. Protection between different serotypes of bovine rotavirus in gnotobiotic calves: specificity of serum antibody and coproantibody responses. J. Clin. Microbiol. **25**:1052–1058.