T-Cell-Deficient Mice Display Normal Recovery from Experimental Rotavirus Infection

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Rotaviruses are common causes of diarrhea in animals and humans. Little is known, however, about the components of the host response to these viruses. Rotavirus infection was studied in athymic mice experimentally infected with murine rotavirus. Neonatal T-cell-deficient mice experienced a self-limited gastrointestinal infection which was identical to that observed in age-matched immunocompetent mice. Adult T-cell-deficient seronegative mice and age-matched normal mice showed a similar extent of resistance to symptomatic rotavirus infection. In both cases, the infection was resolved without the generation of antirotavirus antibody. These studies indicate that host defense against murine rotavirus requires neither functional T-lymphocytes nor specific antiviral antibody.

Rotaviruses are double-stranded RNA viruses which are important etiologic agents of diarrhea in human infants and the young of other animals (2, 3, 7). There is currently an intensive effort directed toward the development of an effective vaccine for the prevention of rotavirus infection in humans (4, 14). It can be expected that a detailed understanding of the role of the immune system in the prevention of and recovery from rotavirus infection will be useful in developing effective vaccine strategies. Although passively administered immunoglobulin can prevent disease in experimental animals (8, 11), little is known about the relative importance of other components of the host immune system in preventing or resolving rotavirus infection. We therefore used congenitally athymic (*nu/nu*) and neonatally thymectomized mice to investigate the role of T and B lymphocytes in rotavirus infection.

Purified murine rotavirus was prepared from homogenates of infected mouse intestine (13), and the 100% infective dose of the preparation was established by per os inoculation of suckling mice. Diarrhea in suckling mice was assessed by inspection of individual animals and confirmed by examination of the colonic contents of representative mouse pups after cervical dislocation (1). Rotavirus antigen in fecal contents was detected by an enzyme-linked immunosorbent assay (17). Antibody in serum and intestinal contents was assayed by an enzyme immunoassay blocking test (18). The blocking assay detects immunoglobulin G (IgG), IgM, and IgA and is more sensitive than complement fixation or fluorescent antibody assays in the detection of antirotavirus antibody (18). The presence of antirotavirus antibody in a specimen was defined by the ability of the specimen to block >40% of the binding of rotavirus antigen to antirotavirus antibody.

T lymphocyte function was assessed in a mitogen-driven proliferation assay (12). Spleen cells (2×10^5) were cultured for 72 h in RPMI 1640 medium containing fetal calf serum plus concanavalin A (Pharmacia, Inc., Piscataway, N.J.), then pulsed with 1 mCi of [3 H]thymidine (6.7 mCi/mg) for an additional 4 h. [3 H]thymidine incorporated into DNA was

measured and expressed as mean counts per minute in three replicate cultures for each mouse.

The course of rotavirus infection was initially investigated in congenitally athymic BALB/c suckling mice (Harlan-Sprague-Dawley Laboratories, Indianapolis, Ind.). Before infection, neither the infant mice nor their mothers had any detectable antirotavirus antibody in serum. The absence of functional T lymphocytes was tested by measuring lymphocyte proliferation in response to mitogen. Athymic mice had less than 1% and control mouse pups had less than 5% of the normal adult mouse T-cell response to concanavalin A. Mouse pups were inoculated per os at 7 days of age with a single 100% infective dose of murine rotavirus. Diarrhea was noted 3 days postinoculation in all 13 immunodeficient and in all 10 normal mice. Individual mice were then sacrificed, and gastroenteritis was confirmed by inspection of the intestinal contents. Rotavirus antigen was detected in every mouse with diarrhea that was sacrificed. Immunodeficient mouse pups and normal controls excreted similar amounts of rotavirus antigen (Fig. 1). Rotavirus antigen was undetectable in fecal specimens from either group by day 13 postinfection. No differences in recovery were noted in the clinical course of the two groups; diarrhea had resolved in both athymic mice and normal controls by day 13 postinoculation. However, a serum antibody response was observed in normal mice but not in congenitally athymic mice (four of four normal mice versus zero of eight athymic mice; $P < 0.01$, Fisher's exact test). In addition, intestinal antibody was not detected in any of the immunodeficient pups after infection, although it was present in normal mouse pups.

Rotavirus infection was also investigated in fully grown BALB/c mice (more than 7 weeks old) which were either congenitally athymic (*nu/nu*), neonatally thymectomized (10), or normal. Both the athymic and thymectomized mice had <4% of the normal mouse T lymphocyte response to mitogen. All the immunodeficient mice and normal controls were also seronegative for rotavirus antibody before infection. Since normal adult mice have been reported to be resistant to rotavirus infection (1, 15, 16), mice were inoculated per os with a murine rotavirus preparation containing

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FIG. 1. Rotavirus antigen excretion and serum antiviral antibody in newborn mice inoculated per os with murine rotavirus. Symbols: □, congenitally athymic mice; ●, normal controls. The numbers in the box above the graph indicate number positive/number tested for each time point.

10^4 times the 100% infective dose, for normal suckling mice. Even with this large inoculum, both immunodeficient and control animals were resistant to symptomatic infection. Rotavirus antigen excretion was detected in three of seven athymic mice, zero of three thymectomized mice, and 8 of 11 normal mice (Fig. 2). No diarrhea was observed in any of the adult mice after inoculation with rotavirus. The amount of rotavirus antigen shed by the immunodeficient mice after

infection did not differ significantly from that shed by normal mice at comparable times after infection (Fig. 2). However, infection resulted in a detectable serum antibody response in 8 of 11 normal control animals; in contrast, serum or intestinal antibody to rotavirus was not detectable in any of the immunodeficient mice (0 of 10; $P < 0.01$, Fisher's exact test).

These experiments indicate that neither host antibody nor functional T lymphocytes are necessary for neonatal mice to recover from rotavirus infection. Older mice were also resistant to rotavirus infection even when these host defenses were absent. These results are consistent with those for experimental infection of athymic mice with other members of the *Reoviridae* family (5). Congenitally athymic mice clear reovirus infection as quickly as normal controls, and there is evidence that macrophages are responsible for this activity (6). Macrophages or other host immune functions (e.g., complement or phagocytes) might similarly account for the experimental results reported here. Alternatively, nonimmune host defenses may be primarily responsible for prevention or resolution of rotavirus infection. For example, steroids decrease the age at which mice become resistant to rotavirus (16), presumably because of steroid-induced maturation of intestinal cells. Intestinal enterocytes from adult mice have also been found to bind fewer rotavirus particles in vitro than neonatal enterocytes do (9). These factors could explain the age-dependent susceptibility of mice to rotavirus infection. Additional investigations will be needed to define precisely which host defenses are responsible for resistance to rotavirus infection and recovery from disease. An understanding of these defenses will be valuable in developing effective measures for immunoprophylactic control of rotavirus infection in humans and other animals.

FIG. 2. Fecal rotavirus antigen excretion in T-cell-deficient (□) and normal (●) adult mice inoculated per os with murine rotavirus. Each data point represents assay results for a single mouse or more than one mouse (the number is shown in parentheses beside the symbols).

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LITERATURE CITED

1. Eydeloth, R. S., S. L. Vonderfecht, J. F. Sheridan, L. D. Enders, and R. H. Yolken. 1984. Kinetics of viral replication and local and systemic immune responses in experimental rotavirus infection. *J. Virol.* **50**:947-950.
2. Flewett, T. H., and G. N. Woode. 1978. The rotaviruses. *Arch. Virol.* **57**:1-23.
3. Kapikian, A. Z., H. W. Kim, R. G. Wyatt, W. L. Cline, J. P. 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.
4. Kapikian, A. Z., R. G. Wyatt, H. B. Greenberg, A. R. Kalica, H. W. Kim, C. D. Brandt, W. J. Rodriguez, R. H. Parrott, and R. M. Chanock. 1980. Approaches to immunization of infants and young children against gastroenteritis due to rotaviruses. *Rev. Infect. Dis.* **2**:459-469.
5. Letvin, N. L., R. S. Kauffman, and R. Finberg. 1981. T-lymphocyte immunity to reovirus: cellular requirements for generation and role in clearance of primary infections. *J. Immunol.* **127**:2334-2339.
6. Letvin, N. L., R. S. Kauffman, and R. Finberg. 1982. An adherent cell lyses virus-infected targets: characterization, activation, and fine specificity of the cytotoxic cell. *J. Immunol.* **129**:2396-2401.
7. McNulty, M. S. 1978. Rotaviruses. *J. Gen. Virol.* **40**:1-18.
8. 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.
9. Riepenhoff-Talty, M., P. C. Lee, P. J. Carmody, H. J. Barrett, and P. L. Ogra. 1982. Age dependent rotavirus-enterocyte interactions. *Proc. Soc. Exp. Biol. Med.* **170**:146-154.
10. Sjodin, K., A. P. Dalmasso, J. M. Smith, and C. Martinez. 1963. Thymectomy in newborn and adult mice. *Transplantation* **1**:521-525.
11. Snodgrass, D. R., and P. W. Wells. 1976. Rotavirus infection in lambs: studies on passive protection. *Arch. Virol.* **52**:201-205.
12. Strong, D. M., A. A. Ahmed, G. B. Thurman, and K. W. Sell. 1973. *In vitro* stimulation of murine spleen cells using a microculture system and a multiple automated sample harvester. *J. Immunol. Methods* **2**:279-283.
13. Theil, K. W., C. M. McClosky, L. J. Saif, D. R. Redman, E. H. Bohl, D. D. Hancock, E. M. Kohler, and P. D. Moorhead. 1981. Rapid, simple method of preparing rotaviral double-stranded ribonucleic acid for analysis by polyacrylamide gel electrophoresis. *J. Clin. Microbiol.* **14**:273-280.
14. Vesikari, T., K. Isolauri, A. Delem, E. D'Houdt, T. E. André, and G. Zissis. 1983. Immunogenicity and safety of live oral attenuated bovine rotavirus vaccine strains RIT 4237 in adults and young children. *Lancet* **ii**:807-811.
15. Wilsnack, R. E., J. H. Blackwell, and J. C. Parker. 1969. Identification of an agent of epizootic diarrhea of infant mice by immunofluorescent and complement fixation titers. *Am. J. Vet. Res.* **30**:1195-1204.
16. Wolf, J. L., G. Cukor, N. R. Blacklow, R. Dambrauskas, and J. S. Trier. 1981. Susceptibility of mice to rotavirus infection: effects of age and administration of corticosteroids. *Infect. Immun.* **33**:565-574.
17. Yolken, R. H. 1982. Enzyme immunoassays for the detection of infectious antigens in body fluids: current limitations and future prospects. *Rev. Infect. Dis.* **4**:35-68.
18. Yolken, R. H., R. G. Wyatt, B. A. Barbour, H. W. Kim, A. Z. Kapikian, and R. M. Chanock. 1978. Measurements of rotavirus antibody by enzyme-linked immunosorbent assay blocking assay. *J. Clin. Microbiol.* **8**:283-287.