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Development of Immunity to Porcine Rotavirus in Piglets Protected from Disease by Bovine Colostrum

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Bovine colostrum with rotavirus-neutralizing activity was fed for 10 days to two groups of piglets, one of which was inoculated intranasally with a rotavirus of porcine origin. A third group, which did not receive colostrum, was also inoculated with the virus, and these piglets developed diarrhea, excreted rotavirus in the feces, and died 6 days after infection. In contrast, the infected piglets fed with bovine colostrum remained healthy, although they developed antibody to rotavirus. Twenty-seven days after the primary inoculation, piglets in the colostrum-fed groups were inoculated intranasally with virus. Those in the previously unexposed group became clinically ill and excreted rotavirus, whereas those which had experienced a previous subclinical infection (the colostrum-fed, virus-inoculated group) remained healthy. It was concluded that bovine colostrum protected piglets from the clinical effects of a porcine rotavirus and that these animals developed an immunity which prevented subsequent disease.

Rotaviruses have been associated with diarrhea in piglets both at and before weaning (3). Experimental evidence shows that, in calves and lambs, protection from the clinical effects of infection can be achieved by feeding specific antibody provided by colostrum from the homologous species (4, 12). First-day bovine colostrum is produced in large quantities and commonly possesses high levels of bovine rotavirus-neutralizing activity (19), and Lecce et al. (7) suggested that it could reduce the severity of the clinical symptoms associated with rotavirus infections in pigs. Since it has been shown by cross-neutralization tests that rotaviruses from bovine and porcine populations share some antigens (15), a study of the protective effect of bovine colostrum was made.

Seroconversion to rotavirus has been demonstrated in animals protected passively from clinical illness (10, 11), but no information is available to show whether, in these circumstances, animals are protected from a subsequent rotavirus infection. Thus, experiments were designed to establish (i) whether bovine colostrum could protect piglets from clinical illness caused by a porcine rotavirus and (ii) whether infection in these circumstances would confer immunity to a subsequent infection.

MATERIALS AND METHODS

Viruses. Porcine rotavirus (SW20/21 [18]) was obtained from an outbreak of diarrhea in pigs and had been passaged serially seven times in gnotobiotic piglets. For piglet inoculation, it was used as a 0.45-μm fecal filtrate, and 10^4 to 10^5 50% tissue culture doses (TCD_{50}) was administered intranasally per piglet. The bovine rotavirus used was the tissue culture-adapted Compton-UK virus (4).

Animals. Gnotobiotic piglets were produced and reared as described previously (14). *Streptococcus* sp. was detected in groups 1 and 3 at 13 days of age.

Bovine colostrum. Colostrum was collected within 24 h of parturition from five cows in the institute's herd. After pooling, it was sterilized by irradiation and fed as 40% of the piglets' diet three times a day. Colostral wheys were prepared by centrifugation at 90,000 × *g* for 1 h, followed by filtration through 0.45-μm filters.

Virus infectivity assays. Ten percent suspensions of feces to be assayed were made in Eagle minimal essential medium containing 0.1% sodium bicarbonate, 100 U of benzylpenicillin, 100 μg of streptomycin sulfate, and 25 μg of mycostatin per ml of culture fluid (Eagle medium). After centrifugation at 8,000 × *g* for 30 min, the supernatant fluids were filtered through clarifying and 0.45-μm filters. Serial 10-fold dilutions were made in Eagle medium, and 0.1 ml was inoculated per well onto confluent LLC-MK_2 cells grown for 48 h in microtiter trays. Each sample was assayed in duplicate. Inoculated cultures were centrifuged at 300 × *g* for 1 h, incubated for 18 h at 37°C, and then stained and read by immunofluorescence (6).

Virus neutralization test. A method similar to that of Thouless et al. (15) was followed. Tenfold dilutions of sera and wheys were made in Eagle medium and heated at 56°C for 30 min, and doubling dilutions (50 μl in 50 μl) were prepared in duplicate. Fifty-microliter volumes of a virus suspension containing 100 to 200 fluorescent focus-forming units per 5 μl were added to the serum dilutions and incubated for 2 h at 37°C. Ten-microliter volumes of the virus-serum mixtures were inoculated onto 48-h-old confluent monolayers of LLC-MK_2 cells maintained with 0.1 ml of Eagle medium. The infected monolayers were cen-

trifuged, incubated, and stained as described for infectivity assays. End points were expressed as the reciprocal of the dilution giving a 50% reduction of the fluorescent foci found in control monolayers.

Immunofluorescence test. Sera were tested at a dilution of 1:10 by an indirect immunofluorescence test, as described previously (2).

Virus detection by electron microscopy. Virus pellets from up to 5 g of feces were prepared by centrifugation as described previously (5).

RESULTS

Experimental design. Pairs of piglets were arranged in three groups (Table 1). Groups 1 and 3 were fed bovine colostrum for 10 days, beginning 2 days before the primary inoculation. Piglets in groups 2 and 3 were inoculated intranasally with porcine rotavirus for the first time at 6 days of age, that is, on the 3rd day of colostrum feeding. At 30 days of age, the surviving piglets were bled for serum; at 33 days they were challenged with the same porcine rotavirus. Twenty-one days after challenge, further serum samples were obtained.

Bovine colostrum. Whey prepared from the pool of irradiated bovine colostrum neutralized both the porcine and bovine rotaviruses at a dilution of 1:640.

Primary inoculation. The feces of both piglets in group 2 became diarrheic within 24 h of inoculation; that is, there was a marked change in the color and consistency of the feces compared with preinoculation samples. The piglets were visibly ill on the 2nd day postinoculation (p.i.), lost body weight, and died at 6 days p.i. (Table 1, Fig. 1). In contrast, piglets in groups 1 and 3 remained healthy and their body weights showed a steady increase. Little change was seen in the color or consistency of the feces during or after colostrum feeding.

During the period of colostrum feeding, virus was not detected by infectivity assays in the feces of group 3 piglets, whereas up to 10^6 TCD_{50} per g was found in the feces of animals in group 2 (Fig. 2). The former did experience a rotavirus infection, however, since when colostrum feeding was discontinued on day 7 p.i., rotavirus was detected in the feces of one piglet for 2 days. At this time, rotavirus was not found by infectivity assays in the feces of the second piglet in group 3, but aggregates of rotavirus particles were seen in its feces at 4 days p.i. by electron microscopy.

Immunofluorescent and neutralizing activities to porcine rotavirus developed in the sera of group 3 piglets, whereas they were absent in group 1 animals (Table 2). Although convalescent sera of group 2 piglets were not available in this instance, piglets from other experiments, some of which survived infection with this porcine rotavirus, had levels of neutralizing antibody in their sera similar to those found in the sera of group 3 animals. Convalescent sera from group 3 piglets failed to neutralize the Compton-UK bovine rotavirus at a 1:10 dilution.

Challenge inoculation. Twenty-seven days after the primary inoculation, piglets in groups 1 and 3 were challenged with porcine rotavirus (Table 1). Only those in group 1 which had not experienced a previous infection became clinically ill; they were depressed and anorexic on days 2 and 3 p.i., and the feces first became diarrheic on day 2 p.i. Rotavirus was excreted in the feces for at least 4 days (Fig. 3), and neutralizing activity to porcine rotavirus appeared in the sera for the first time (Table 2).

In contrast, piglets in group 3 remained clinically normal. Virus was not detected in the feces by infectivity assays, although a small number of rotavirus particles were seen at 4 days p.i. in one of six fecal samples prepared for electron microscopy. Neutralizing activity to porcine rotavirus in the convalescent sera showed a 32-fold increase when compared with levels after the primary inoculation (Table 2).

DISCUSSION

Piglets fed bovine colostrum at the time of exposure to porcine rotavirus failed to show signs of disease and, 3 weeks later, were clinically

TABLE 1. *Summary of experimental design and results*

Group	Study design[a]								
	Primary inoculation					Challenge inoculation			
	Colostrum fed	Virus inoculated	Clinical illness	Virus excretion	Antirotavirus activity in serum	Virus inoculated	Clinical illness	Virus excretion	Antirotavirus activity in serum
1	+	−	−	NT	−	+	+	+	+
2	−	+	+	+	NA	NA	NA	NA	NA
3	+	+	−	+	+	+	−	±	+

[a] NT, Not tested; NA, data not available since piglets died. Two piglets were allocated to each group.

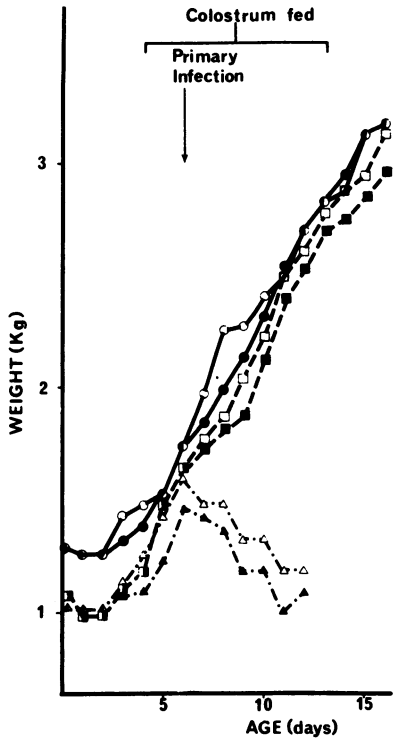

FIG. 1. *Effect of bovine colostrum on the body weight of piglets after primary inoculation. Symbols: ◨, Group 1; Δ, group 2; ◑, group 3. Open and closed symbols represent the results from the two animals in each group.*

immune to homologous challenge. These results may explain the finding that many farm animals which have not experienced clinical disease have antibody to rotavirus after passive maternal immunity has waned. Bovine colostrum, or an active derivative of it, could be used to control rotavirus-associated disease not only in calves but also in weaned piglets without diminishing their ability to mount a protective immune response.

After the primary infection, virus multiplication in the colostrum-fed piglets (group 3) was confirmed by the presence of neutralizing activity to porcine rotavirus in the convalescent sera in contrast to its absence in the colostrum-fed controls (group 1). Serum antibody responses to oral inoculations in the presence of passively acquired antibody in the gut lumen have been observed previously, for example, with inactivated bacteria (1, 8) and human and ovine rotaviruses (10, 11). When colostrum feeding ceased at 7 days p.i., one piglet in group 3 excreted virus subclinically for 1 day in amounts comparable to those detected in one of the unprotected piglets (group 2), indicating that this level of rotavirus is not necessarily associated with overt clinical disease. Excretion of infectious virus by this group 3 piglet could possibly have been prevented by feeding colostrum for a longer period.

The difference in the clinical signs observed in piglets inoculated with virus for the first time at 5 or 33 days of age is likely to be due to increasing resistance with age, which has been observed previously (7). The 32-fold increase in serum antibody levels after challenge of group 3 piglets may suggest that virus multiplication occurred in these immune piglets, but it is im-

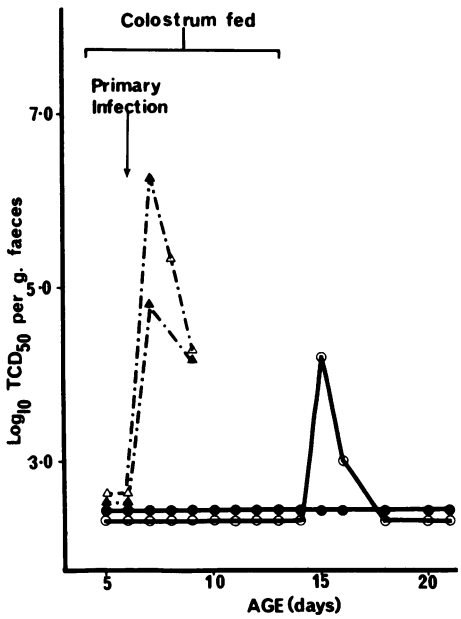

FIG. 2. *Rotavirus excretion after the primary inoculation as determined by infectivity assays. Symbols: Δ, Group 2; ◐, group 3. Open and closed symbols represent the results from the two piglets in each group. The assay was incapable of detecting virus at or below $10^{2.5}$ TCD_{50} per g of faeces; negative samples are indicated by symbols at $10^{2.5}$.*

TABLE 2. *Neutralizing activity to porcine rotavirus in the sera of piglets after the primary and challenge inoculations*

Group	Piglet	Primary inoculation (24 days p.i.)	Challenge inoculation (21 days p.i.)
1	A	<10	640
	B	<10	640
2	A	NA[a]	NA
	B	NA	NA
3	A	320	10,240
	B	320	10,240

[a] NA, Data not available since piglets died.

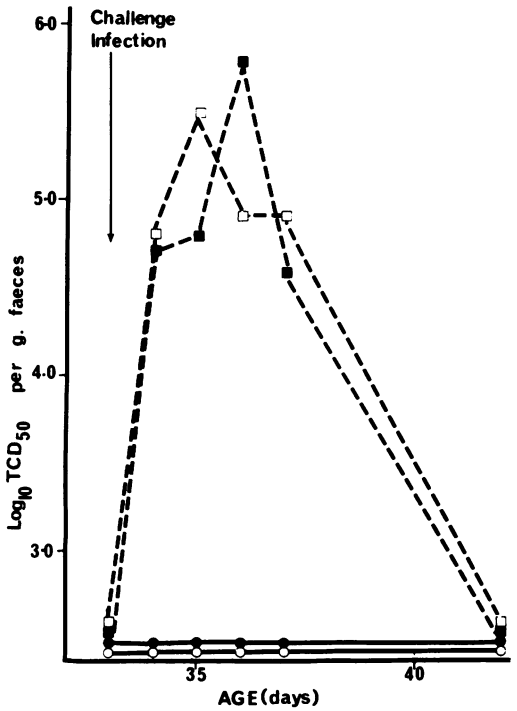

FIG. 3. *Rotavirus excretion after the challenge inoculation as determined by infectivity assays. Symbols: ■, Group 1; ◑, group 3. Open and closed symbols represent the results from the two piglets in each group. The assay was incapable of detecting virus at or below $10^{2.5}$ TCD_{50} per g of faeces; negative samples are indicated by symbols at $10^{2.5}$.*

possible to rule out the possibility that the increased antibody levels were due to a continuing response to primary inoculation and that the virus particles identified in the feces after challenge were a result of prolonged excretion after the primary infection.

This experiment did not establish whether active immunity would develop in the presence of systemic as well as local passively acquired antibody, as would be found in most natural circumstances, but previous studies have suggested that, with some ingested antigens (bovine serum albumin and *Escherichia coli*) passively acquired circulating antibody does not affect the development of active immunity (9, 16).

The level of neutralizing activity for porcine rotavirus in the bovine colostrum pool was higher than expected from the 8- to 16-fold difference observed previously in the level of cross-reactivity between antiserum to the Compton-UK bovine rotavirus and porcine rotavirus (15). Subsequent studies showed that in addition to the Compton-UK virus, a second rotavirus was present in the bovine population from which the colostrum was obtained. This second bovine isolate shows a strong cross-relationship with the porcine virus used in this study, and it is possible that it was the source of the high antibody levels to porcine rotavirus found in the bovine colostrum. A detailed investigation of the rotaviruses present in bovine and porcine populations is under way. The pool of colostrum used in this study was not unique, since two other pools from different sources have been shown to neutralize bovine and porcine rotaviruses equally well. In the United States, Lecce et al. (7) found that piglets were only partially protected from the effects of rotavirus infection by feeding bovine colostrum but, since neither the dose of virus nor the relationship between the virus and colostrum was established, it is impossible to speculate on the reasons for the difference between the two studies.

Vaccination of dams to boost the amount and duration of antibody excreted in colostrum and milk is a method which has been suggested to combat rotavirus disease in unweaned calves and lambs but, in both vaccinated and control dams, the level of milk antibody declines after parturition (13, 17). Since 10- to 16-week-old gnotobiotic calves are susceptible to clinical disease caused by the Compton-UK isolate of bovine rotavirus (J. C. Bridger, personal observation), vaccination of dams to boost milk antibody may only delay the onset of clinical symptoms in neonates. The experiment reported here indicated that boosting maternal antibody could be useful in eliminating clinical disease, so long as animals at risk are exposed to virus while protected passively. The relative infrequency of clinical illness due to rotaviruses compared with the frequency of antibody suggests that a similar process commonly occurs naturally. With bacterial antigens, it has been shown that intestinal secretion of antibodies can interrelate with declining passive antibody to maintain an almost continuous level of intestinal antibody (1).

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