Prevention of Human Rotavirus–Induced Diarrhea in Gnotobiotic Piglets using Bovine Antibody

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The efficacy of passively administered bovine antibody for preventing human rotavirus (HRV)–induced diarrhea was investigated using a gnotobiotic pig model. Cows were immunized with inactivated HRV serotypes 1 (Wa) and 2 (S2) and simian rotavirus serotype 3 (SA11), and immune colostrum and milk were collected. Antibody concentrates derived from these materials were fed to germ-free piglets that were subsequently inoculated with HRV Wa. Both viral shedding and diarrhea were effectively reduced or eliminated in a dose-dependent manner as a result of HRV immune antibody feeding. A quantitative virus-neutralizing (VN) antibody method permitted assessment of the functional antibody dose required to achieve a 50% reduction of disease (PD_{50}). PD_{50} dose levels of 15.8 and 19.5 $\times$ 10^6 VN antibody units were determined for inhibition of diarrhea and viral shedding, respectively. Studies reported here provide new information on the quantitative relationship between protective antibody dose and diarrheal disease response.

Human rotavirus (HRV)–induced gastroenteritis is a well-characterized, potentially severe disease that is endemic worldwide. Since its identification as a cause of infant diarrhea in 1973 [1], HRV has been identified as the most common cause of severe childhood diarrhea in developed countries, and HRV ranks second only to enterotoxigenic *Escherichia coli* as a cause of severe childhood diarrhea in developing countries [2–4]. Rotaviruses also have been identified as a major cause of diarrheal outbreaks in pediatric hospital nurseries [5–7] and day care centers in industrialized nations [8].

Research directed at both active and passive immunoprophylaxis against HRV-induced diarrhea has been reported. Efforts to develop a satisfactory HRV vaccine have been extensive [9]. Passive antibody approaches have been attempted in piglets [10, 11], cows [12–14], lambs [15], and humans [16–18] with variable success. Prevention of HRV infection and spread in human infants by the feeding of colostrum from cows hyperimmunized with noninactivated monovalent [16] or inactivated tetravalent HRV [18] has been reported. Treatment of infants with acute HRV disease using bovine milk immunoglobulin containing HRV-specific antibody apparently reduces HRV excretion time but not the duration of illness [17]. Although not specifically addressed in these reports [16–18], antibody dose is likely to be of critical importance.

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Studies reported here examined the quantitative relationships involved in the prevention of HRV-induced diarrheal disease by passively administered bovine antibody. The gnotobiotic piglet model was used because of the reproducibility of HRV infection and diarrheal disease induction in that system [19].

Materials and Methods

Piglets. Gnotobiotic piglets were obtained by hysterectomy and placed in isolators using methods previously described [20].

Viruses and cells. HRV serotype 1 Wa (HRV Wa) was initially obtained from R. G. Wyatt (National Institutes of Health, Laboratory of Infectious Diseases, Bethesda, MD) as a virus isolate from a pediatric fecal specimen [21] and was maintained by in vivo passage in gnotobiotic pigs. HRV serotype 2 (S2) was obtained from J. Hughes (Children's Hospital, Columbus, OH), who originally obtained it from T. Urasawa (Sapporo, Japan) [22]. The SA11 strain (serotype 3), obtained from J. Hughes as passage 8, originally was obtained from H. H. Malherbe [23].

Preparation of challenge virus, infection with HRV Wa, and harvest from cecum and intestinal contents of infected piglets was as described earlier [20, 21, 24, 25]. Briefly, piglets were challenged orally with 2 ml of 10^5–10^7 fluorescent focus units (FFU)/ml virulent HRV Wa [25]. The animals were euthanized and the intestinal contents were collected near the onset of diarrhea (~48–72 h after challenge). The titer of HRV Wa chosen (2 ml of 4 $\times$ 10^5 FFU/ml) produced diarrhea in 100% of the challenged piglets in preliminary studies (unpublished data).

For cow immunization and use in virus neutralization assays, pools of plaque-purified Wa, S2, or SA11 were grown in MA-104 cells (continuous rhesus monkey kidney cell line) in 850-mm^2 plastic roller bottles using Eagle's MEM (Whittaker Bioproducts, Walkersville, MD) with 10% fetal calf serum (Hyclone Laboratories, Logan, UT). Viruses harvested from infected MA-

104 cell culture fluids were concentrated by ultrafiltration, purified by a proprietary method involving cesium chloride and sucrose density gradient centrifugation, and inactivated using binary ethyleneimine [26].

Cow immunizations. Groups of cows were immunized with purified trivalent inactivated immunogen preparations according to methods previously described [26, 27]. Briefly, cows were immunized using a combination of intramuscular and intramammary inoculations with 10 ml of trivalent HRV immunogen emulsified in Freund's incomplete adjuvant.

Antibody preparations. Colostrum and milk pools were collected and converted to whey by rennin treatment [25] or by acid precipitation. Whey was then either stored frozen in aliquots or purified further to increase the proportion of IgG1 protein. Control materials were prepared in the same manner using colostrum or milk pools from nonimmunized cows. To prevent interference with the diarrheal responses in piglets due to the nonsterile nature of the test products and resulting microbial contamination, antibody concentrates and matched control materials were irradiated (0.4 Mrad) or treated with antibiotics (gentamicin, 100 μg/ml; vancomycin, 300 μg/ml; and fungizone, 20 μg/ml).

Characterization of test product potency. Quantitation of virus-neutralizing (VN) antibody in the test product was accomplished using an infected cell reduction assay in microtiter plates, modified from that of Gerna et al. [28]. Briefly, serial twofold dilutions of antibody were mixed with equal volumes of trypsin-activated HRV Wa appropriately diluted to yield 500–1000 MA-104 cell infectious units per well in 96-well microtiter plates. After incubation (1 h at room temperature), the virus-antibody reaction mixtures were added to MA-104 cell monolayers in microtiter plates and incubated for 12–14 h at 37°C in 5% CO_2. After incubation, the monolayers were fixed with ethanol and stained for detection of infected cells using an immunoperoxidase method consisting of the sequential addition of bovine anti-HRV antibody, horseradish peroxidase–conjugated rabbit anti-bovine IgG, and diaminobenzidine substrate. Residual infectivity was determined at each dilution of test antibody, and the VN antibody titer was calculated at a virus survival of 37% [29]. Antibody potency (VN antibody units [VNU] per milligram of IgG1) was determined as the VN antibody titer per unit volume of antibody (0.05 ml) in the reaction mixture (0.10 ml) divided by the IgG1 concentration. Bovine IgG1 concentrations were determined using radial immunodiffusion (ICN Biomedicals, Costa Mesa, CA) and used for the determination of bovine immunoglobulin specific activity. Standardized preparations of infectious virus and reference HRV-immune bovine antibody were used to maintain consistent antigen and antibody equivalents [29] and hence optimize assay accuracy. VN antibody determinations on piglet sera were made using a plaque-reduction assay [25].

Piglet feeding regimen and challenge studies. Protection studies were conducted in litter sizes of eight piglets. Within each litter, four or five piglets were fed HRV-specific antibody, two or three were fed control bovine immunoglobulin, and at least two were used as challenge controls and did not receive any bovine immunoglobulin. Piglets were fed ~70 ml of Similac with iron (SWI; Ross Laboratories, Columbus, OH) three times per day on the first and second days after birth.

Feeding of test preparations began on day 3, followed by three test preparation feedings daily for various intervals (5, 8, or 16 days). Test preparation feedings (12–30 ml, three times/day) were supplemented with SWI for a total volume of 250–360 ml/day. Piglets were challenged orally at age 4 days with 2 ml of an intestinal contents suspension of virulent HRV Wa (4×10^5 FFU/ml). Rectal swabs were taken and the piglets were observed for signs of disease daily [25]. Stool characteristics were scored as normal (brown solid feces), mild diarrhea (light-colored, semisolid feces), or profuse diarrhea (copious yellow, watery stools). Blood samples were obtained from all piglets before HRV challenge and at ~2 and 3–5 weeks of age. Rectal fluids were examined for infectious virus (viral shedding) using an infected-cell assay in microtiter plates (infected cells detected using immunofluorescence) [30].

Seroconversion. Serum samples were examined for HRV Wa–specific neutralizing antibody activity by plaque-reduction assay [25].

Statistical methods. Least-squares linear regression was used to determine the relationship between antibody-fed (dose) and diarrhea/viral shedding (response) in all piglets. A two-sided Student's *t* test was used to determine significance. The ranges determined for protective dose levels yielding 50% effect (PD_{50}) were calculated using a fiducial limits method [31].

Results

Immunoglobulin preparations and feeding regimen. Eleven colostrum or milk HRV-immune antibody preparations and four preparations from nonimmunized cows (table 1) were examined for their ability to prevent HRV-induced diarrhea in piglets. Immune preparations 2 and 8 were irradiated colostrum–derived products, whereas all others consisted of antibiotic-treated milk immunoglobulin concentrates (table 1). Fed antibody dose levels ranged from a high of 89.3×10^6 VNU/day contained in 1.72 g of IgG1 to 3.6×10^6 VNU in 70 mg of IgG1. Nonimmune control preparations were fed to within-litter controls at IgG1 dosing levels similar to the HRV-immune dosing levels tested.

Bovine immunoglobulin preparations were analyzed for IgG1 and VN antibody activity and the concentrations adjusted to the desired dose before feeding. Characterization of the HRV-specific and control nonimmune materials and feeding regimen (dose and duration of feeding) are shown in table 1. The HRV Wa–specific VN antibody potency levels of the γ-irradiated colostral whey antibody (immune) preparations 2 and 8 were the lowest of all preparations tested (9.4 and 5.5×10^6 VNU/mg of IgG1, respectively). All other preparations were milk-derived immunoglobulin concentrates with VN antibody potency levels (HRV-specific) ranging from 35 to 70×10^6 VNU/mg of IgG1 (mean ± SD, $50.5 \pm 13.8 \times 10^6$). Nonimmune control immunoglobulin preparations had a mean potency of 5.1×10^6 VNU/g of IgG1, 7- to 14-fold lower than the immunoglobulin concentrates from HRV-immunized cows.

Table 1. Human rotavirus (HRV)–specific bovine immunoglobulin preparations and feeding regimen in gnotobiotic piglets.

Preparations (feeding groups)*	No. of days fed	HRV Wa VN antibody specific activity†	Dose fed/day	
			IgG1 (g)	VN antibody units × 10^6
Immune				
1	16	43.3	1.72	89.3
2	5	9.4	6.17	58.0
3	16	35.0	1.57	55.0
4	8	42.8	1.13	48.0
5	5	36.9	1.21	44.6
6	16	61.1	0.50	30.5
7	8	42.8	0.56	24.0
8	5	5.5	3.35	18.3
9	16	71.5	0.23	16.5
10	16	70.0	0.11	7.7
11	16	51.3	0.07	3.6
Nonimmune				
1	5	2.8	2.90	8.2
2	5	3.6	1.00	3.6
3	16	6.7	0.50	3.3
4	16	7.0	0.12	0.8

NOTE. VN, virus-neutralizing.

* Immune preparations 2 and 8 were derived from the pooled colostral whey. All other immune preparations were milk-derived. Nonimmune preparation 1 was derived from pooled colostral whey. Other nonimmune preparations were milk-derived.

† Specific activity = VN antibody units per milligram of IgG1.

Disease response in control piglets. The HRV-induced diarrheal disease response using the gnotobiotic piglet model was characterized by determining the occurrence of viral shedding and diarrhea in control piglets (figure 1). The profile of the occurrence of HRV shedding and diarrhea was determined for the 2-week interval following inoculation with HRV Wa. Infectious virus was shed by 100% of the 11 control piglets on postchallenge days 2 and 3 and continued to be shed in most of the piglets through days 6 and 7. Likewise, the occurrence of diarrhea (mild, profuse) approached 100% during this interval.

To compare the disease responses among various feeding groups, the interval from day 1 through day 6 after virus challenge was analyzed in subsequent experiments for the occurrence of infectious viral shedding and the occurrence of diarrhea. This interval corresponded with the prevalence of diarrheal disease and therefore provided the best opportunity to compare disease responses between groups. Diarrheal disease was scored as mild or profuse on the basis of fecal color, consistency, and volume of the feces compared with normal. Profuse diarrhea occurred with highest frequency during postchallenge days 3–7 (figure 1).

Disease response in HRV Wa–challenged, passive antibody–fed piglets. Diarrheal disease responses for three groups of piglets fed HRV-immune antibody, one fed nonimmune bovine immunoglobulin, and one fed unsupplemented SWI are shown in table 2. Antibody from nonimmunized cows (nonimmune 1) slightly delayed the appearance of diarrheal disease and HRV shedding compared with SWI-fed controls. An immune antibody dose containing fivefold higher VNU than the control material (immune 5, 44.6 × 10^6 VNU/day for 5 days) appeared to further delay the onset of disease (table 2). The feeding of a slightly higher dose (immune 2) eliminated viral shedding and diarrhea until well after the antibody feeding had stopped on postinfection day 3 (mild diarrhea at day 11). Infectious HRV shedding and diarrhea were completely eliminated by feeding the high-VN antibody dose (immune 1, 89.3 × 10^6 VNU/day) for all 16 days of observation.

Results obtained for all piglets examined in these studies are summarized in table 3. Protection from HRV Wa challenge for piglets observed 1–6 days after challenge is shown for piglets fed at different dose levels calculated as a percent-

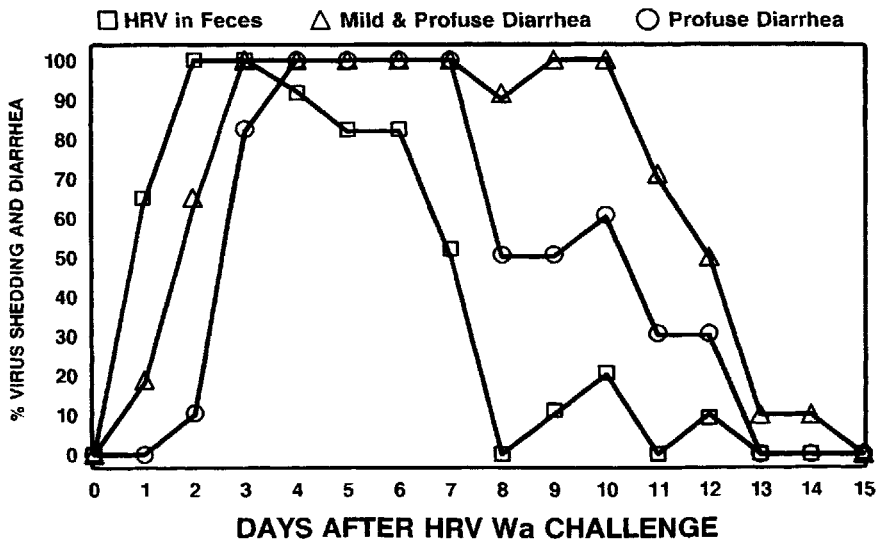

Figure 1. Viral shedding and diarrhea in human rotavirus (HRV)–challenged piglets (n = 11) fed nonimmune control diet (Similac with iron). Percentage of animals with detectable virus in feces, mild and profuse diarrhea, and profuse diarrhea are plotted for each of 15 days after HRV strain Wa challenge.

Table 2. Viral shedding and diarrheal repsonses in human rotavirus (HRV) Wa–challenged gnotobiotic piglets fed immune or nonimmune bovine IgG1 or control material.

Group	Feeding regimen: No. of days treated*	Dose/day (VN antibody units × 10^6)	Animal no.	Viral shedding and diarrheal response by days after exposure to HRV Wa challenge: −1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Immune																			
1	16	89.3	1–5																
2	5	58.0	1													M	M	M	
			2													M	M	M	
			3													M			
			4													M			
5	5	44.6	1								P+	P+	P+	P	P+		M	M	
			2					+		P	P+	P	P+	P	P	M	M		
			3										P+	P	P	M	P		
			4						+	+	+		P+	P	P	M	P		
Nonimmune																			
1	5	8.2	1						P+	P+	P	P							
			2					+	P+	P+	P	P	P						
			3				+	+	P+	P	P+	P	P	P	P				
			4				+	+	P	P+	P	P	P	P	P				
			5					M+	P+	P+	P	P+	P	M					
			6					M+	P	P+	P+	P+	P+	P	P	M		M	M
SWI control		0	1			+	P+	P+	P+	P+	P	P	M	M	P	P	P		
			2			+	+	P+	P+	P	P	P	M	M	P	P	P		
			3			M	M+	P+	P+	P+	P+	P	P	P	P	M	M	M	M
			4			+	M+	P+	P+	P+	P+	P+	P	P	P	M			

NOTE. SWI, Similac with iron; VN, virus-neutralizing; M, mild diarrheal response; P, profuse diarrheal response; +, positive HRV in feces.
* Piglets were fed immune or nonimmune test material beginning on the day before challenge (−1) and continuing for a total of either 5 or 16 days as shown.

age of the control group disease response (diarrhea and viral shedding). Low-level protection was observed in piglets fed IgG1 from nonimmunized cows (table 3) and was roughly equivalent to that observed with the feeding of similar VN antibody doses from immunized cows.

Protection from disease and viral shedding appeared to be clearly influenced by the total VN antibody dose of the bovine immunoglobulin fed. In addition, moderate levels of bovine antibody (16.3 and 30.5 × 10^6 VNU/day) fed for a longer period (16 days) reduced the diarrheal disease response (50% and 16.7% of diarrhea days, respectively; table 3). These doses likely provided effective prevention of infectious virus breakthrough. Short-term feeding for 5 days (1 day before to 3 days after challenge) with a slightly higher VN antibody dose (44.6 × 10^6 VNU/day) prevented diarrheal disease during antibody feeding and delayed the disease response to HRV challenge (tables 2 and 3).

Figure 2 summarizes the dose-response data observed in all piglets studied and presents viral shedding and diarrheal disease responses in immune-fed (10 groups), non-immune-fed (2 groups), and control piglets. For this analysis, the response to HRV challenge is presented for the period 2–6 days after challenge. Results are similar to those for days 1–6, but with a higher incidence of disease due to elimination of the first day after challenge. Determination of the antibody dose–disease response relationship provided the opportunity to calculate protective dose levels required to lower the incidence of HRV-induced diarrhea or viral shedding by 50%. Antibody dose levels yielding PD_{50} were determined by regression analysis using disease response and viral shedding data for days 1–6 (figure 3). Linear models were used for determining the relationships between disease and antibody dose. The null hypothesis (no VN antibody dose effect) was rejected at a significance level of $P = .0001$ for both viral shedding (R^2 = 86%) and diarrhea (R^2 = 85%). The PD_{50} for HRV shedding was similar (19.5 × 10^6 VN antibody units) to that for diarrhea (15.8 × 10^6 VN antibody units).

Seroconversion studies. The ability of antibody-fed and control piglets to respond immunologically to virulent HRV Wa challenge was tested by determining the serum VN antibody titers before (1–4 days of age) and after challenge (table 4). All piglets, including high-dose antibody-fed piglets, seroconverted after challenge. Some reduction in VN antibody response to challenge was apparent in the piglets receiving high antibody dose levels.

Discussion

Rotaviruses are highly stable [32–34], are infectious at very low concentrations [35], and can be continuously shed by chronically infected, asymptomatic individuals [36]. Con-

Table 3. Disease responses in human rotavirus (HRV)–challenged gnotobiotic piglets fed IgG from HRV-immunized or unimmunized cows or control material.

Group	Feeding regimen: Dose/day (VN units × 10^6)	Feeding regimen: Piglets per group	Feeding regimen: No. of days fed	Response to challenge, no. of days (%)*: Viral/shedding	Response to challenge, no. of days (%)*: Diarrhea	% protection†
Immune						
1	89.3	5	16	0/30	0/30	100
2	58.0	4	5	0/24	0/24	100
3	55.0	4	16	1/24 (4)	0/24	97.4
4	48.0	2	8	3/12 (25)	0/12	83.9
5	44.6	4	5	6/24 (25)	3/24 (12.5)	75.8
6	30.5	2	16	5/12 (41.7)	2/12 (16.7)	62.3
7	24.0	2	8	4/12 (33.3)	0/12	78.5
8	18.3	4	5	7/24 (29.2)	13/24 (54.2)	46.2
9	16.3	2	16	8/12 (66.7)	6/12 (50)	24.7
10	7.7	2	16	8/12 (66.7)	9/12 (75)	8.5
11	3.6	2	16	9/12 (75)	9/12 (75)	3.2
Nonimmune						
1	8.2	6	5	17/36 (47.2)	20/36 (55.6)	33.6
2	3.6	2	5	8/12 (66.7)	8/12 (66.7)	13.9
3	3.3	2	16	8/12 (66.7)	8/12 (66.7)	13.9
4	0.8	2	16	10/12 (83.3)	10/12 (83.3)	0
SWI control	0	11	0	85/102 (83.3)	73/102 (71.6)	0

NOTE. VN, virus-neutralizing; SWI, Similac with iron.

* Response between days 1–6 after challenge with virulent HRV Wa. Data are group values: days of viral shedding or diarrhea/total days observed expressed as percentage.

† % protection = {[% days (viral shedding + diarrhea) of antibody-fed group]/[% days (viral shedding + diarrhea) of control group]} × 100 − 100.

sequently, eradication of group A rotaviruses is not likely, and exposure to these viruses is inevitable [37]. Effective active or passive (or both) immune prevention methods are therefore essential to reduce the morbidity of rotaviral diarrhea. Development of live, attenuated HRV vaccine continues to offer significant hope for providing this protection, but extensive efforts to date have not resulted in an acceptable vaccine [38].

Passive immune protection methods may prove to be useful for at-risk populations, providing the needed margin of safety against rotaviral diarrhea. Reports of successful passive antibody immunoprophylaxis in hospitalized patients [16, 18] suggests that this supportive procedure may significantly reduce the incidence of nosocomial HRV infection. As described by Davidson et al. [18], the value of such an antibody preparation is broad, with potential for use in hospi-

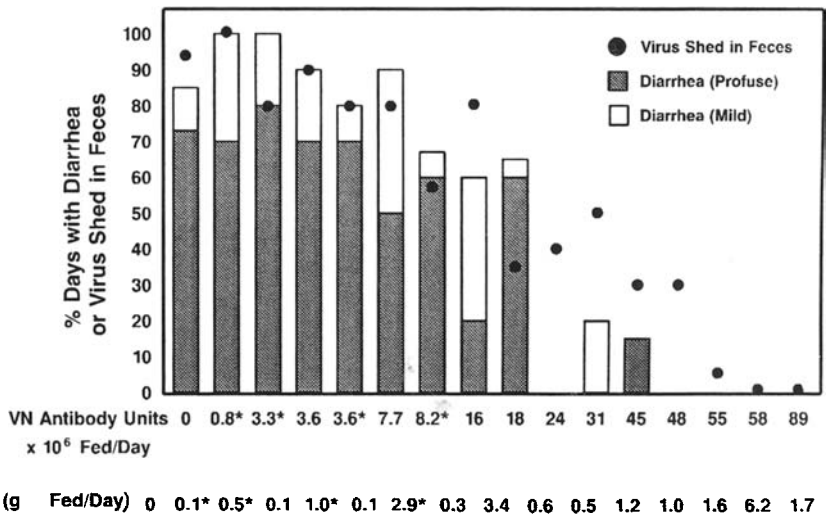

Figure 2. Disease (virus shed in feces and diarrhea: mild and profuse) for groups of gnotobiotic piglets fed human rotavirus (HRV)–immune and nonimmune (*) bovine immunoglobulin. Daily antibody dose (virus-neutralizing [VN] units fed/day × 10^6) and IgG1 (g fed/day) for each group tested. Mild diarrhea, profuse diarrhea, and virus shed in feces are shown as percentage of days (2–6) observed.

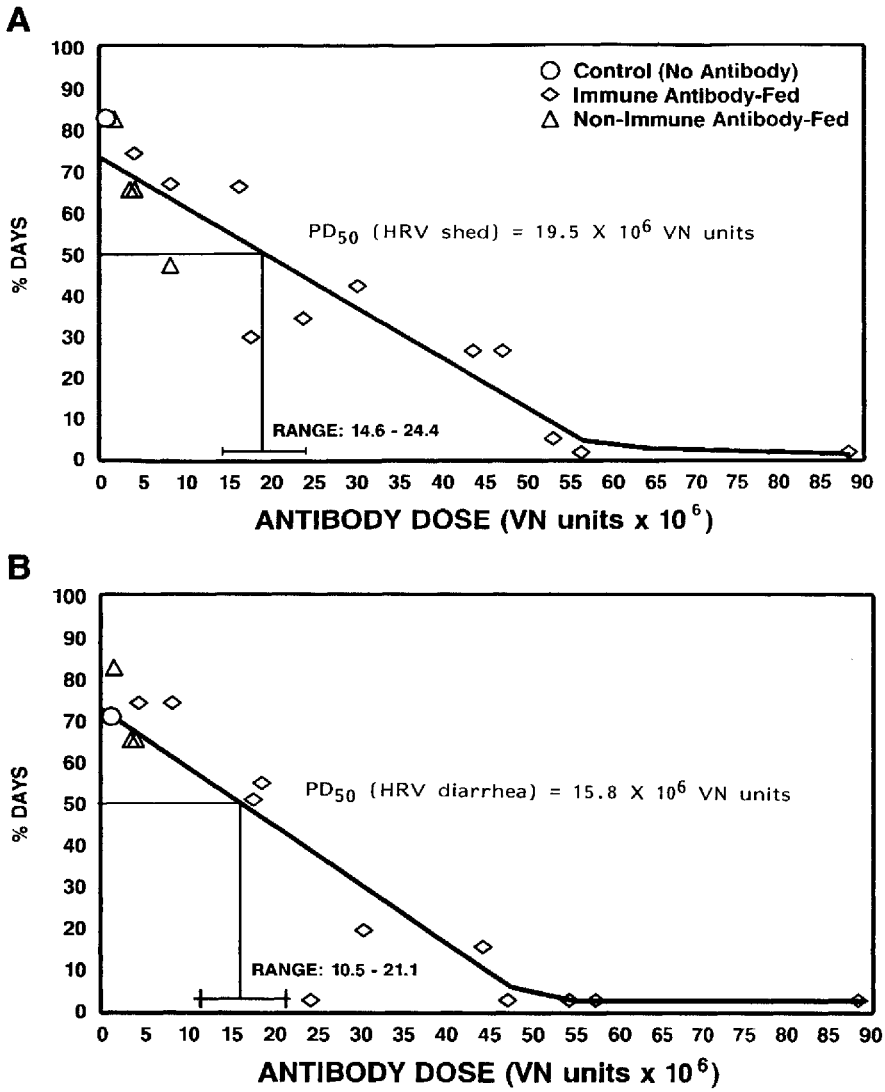

Figure 3. Determination of 50% of protective dose (PD_{50}) for human rotavirus (HRV)–immune bovine immunoglobulin concentrates in HRV strain Wa–challenged gnotobiotic piglets (16 feeding groups, total $n = 62$). The prevalence of viral shedding (**A**) or diarrhea (**B**), observed from days 1–6 (percentage of days), are plotted for each daily antibody dose level tested. PD_{50} levels of 15.8 (range, 10.5–21.1) and 19.5 (range, 14.6–24.4) $\times$ 10^6 virus-neutralizing (VN) units fed/day were determined for diarrhea and viral shedding, respectively. End points for each range were determined from the 95% confidence limits of the response regressions at VN antibody dose = PD_{50}. Each linear model was fit omitting the response at VN antibody dose = 89.3 $\times$ 10^6 VN units. Significance levels were $P = .0001$ for both models. Data from the nonimmune-antibody-fed groups, immune antibody-fed groups, and control (Similac with iron–fed) group were all used in the regression analyses.

tal nurseries, in day care centers, and at home. Other at-risk populations, including the elderly and immunosuppressed, might also benefit from such a prevention strategy. Of critical importance, however, is the need for controlled dosing studies to identify effective and consistent levels of antirotavirus antibody administration.

The gnotobiotic piglet represents a useful model for understanding these dose-response relationships. Gnotobiotic piglets are susceptible to challenge by HRV serotypes 1 (Wa) [21] and 3 (M strain) (unpublished data), yielding diarrhea by a similar mechanism and with similar kinetics to that shown in the human disease. This model represents a considerable advantage, as it is possible to test prototype HRV-specific antibody materials by challenging with HRV strains. Piglet gut physiology (e.g., lack of permeability to intact antibody) and diarrheal response are also similar to that of the human infant [39].

Results of studies reported here show the dose-dependent, passive antibody protection from infectious HRV challenge (viral shedding and diarrhea) in piglets fed bovine IgG concentrates from immunized cows. A clear relationship between fed antibody dose (as measured using an in vitro VN method) and diarrheal disease response was established. Because of the variability in antibody titer observed in colostrum and milk immunoglobulin preparations, the conversion to specific activity (VNU per milligram of IgG1) provided a convenient means of standardizing antibody potency. The value of this conversion appears to be confirmed by the relationship observed between antibody dose and disease response. This correlation between in vivo protection and in vitro–determined VN antibody potency will be helpful in planning and implementing clinical investigations with these antibodies.

Because of multiple passage in gnotobiotic piglets, the HRV Wa strain used in these studies may show increased virulence for the porcine intestinal tract. This would then

Table 4. Virus-neutralizing (VN) antibody seroconversion of human rotavirus (HRV)–challenged gnotobiotic piglets fed IgG from HRV immunized and unimmunized cows.

Group	Dose/day (VN units × 10^6)	Response (% days)*		VN antibody response by days after challenge†		
		Viral shedding	Diarrhea	−3 to 0	14	21 to 30
Immune						
1	89.3	0	0	<5 (5)	11 (5)	26 (5)
2	58.0	0	0	<5 (4)	ND	104 (4)
3	55.0	4	0	<5 (4)	ND	52 (4)
4	48.4	25	0	<5 (2)	21 (2)	75 (2)
5	44.6	25	12.5	<5 (4)	150 (2)	87 (2)
6	30.5	41.7	16.7	<5 (1)	ND	147 (2)
7	24.0	33.3	0	<5 (2)	85 (2)	356 (2)
8	18.3	29.2	54.2	<5 (4)	63 (4)	33 (1)
9	16.5	66.7	50	<5 (2)	ND	87 (2)
10	7.7	66.7	75	<5 (2)	310 (1)	258 (2)
11	3.6	75	75	<5 (2)	30 (1)	120 (1)
Nonimmune						
1	8.2	47.2	55.6	<5 (6)	ND	ND
2	3.6	66.7	66.7	<5 (2)	ND	280 (1)
3	3.3	66.7	66.7	<5 (2)	270 (1)	629 (2)
4	0.8	83.3	83.3	ND	ND	ND
SWI control	0	83.3	71.6	ND	ND	337 (10)

NOTE. SWI, Similac with iron; ND, not determined.

* Response between days 1–6 after challenge with virulent HRV Wa. Data are group values for the fraction (days of viral shedding or diarrhea/total days examined) expressed as percentage.

† Plaque reduction neutralization expressed as geometric mean titer (number of piglets tested at each interval).

represent a particularly severe test of passive antibody efficacy in the piglet model. As shown here, clear protection end points can be seen in pigs that received sufficient amounts of immune bovine IgG.

Our results suggested that some reduction in seroconversion might have occurred at the highest antibody levels administered. This is not unexpected given the substantial decrease in viral antigen shedding associated with the bovine antibody dose fed. Moderately high levels of HRV VN antibody were seen in sera of piglets that were protected from disease with high-dose bovine antibody, reflecting the presence of sufficient immunogenic HRV antigen likely representing progeny virus from infected intestinal epithelium. Similar seroconversion levels were shown to be correlated with protection in related studies using porcine rotavirus challenge [10] and in human infants [16] fed rotavirus-immune bovine colostrum. It is doubtful that passively administered bovine antibody is responsible for VN antibody in piglet serum, as gut closure in the piglet occurs within the first 24 h after birth.

The extent of diarrheal disease and viral shedding was influenced by both VN antibody dose and the duration of feeding. The data presented here suggest that maximum protection is achieved as long as the immune antibody is present in the gastrointestinal tract during the period of risk and during the interval corresponding to the normal course of the disease.

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