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Immunization of Swine with Heat-Stable *Escherichia coli* Enterotoxin Coupled to a Carrier Protein Does Not Protect Suckling Pigs Against an *Escherichia coli* Strain That Produces Heat-Stable Enterotoxin

HARLEY W. MOON,[1]* ALBERT L. BAETZ,[1] AND RALPH A. GIANNELLA[2]

National Animal Disease Center, Agricultural Research, United States Department of Agriculture, Ames, Iowa 50010,[1] and Division of Digestive Diseases, College of Medicine, University of Cincinnati, and Veterans Administration Hospital, Cincinnati, Ohio 45267[2]

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Pregnant swine were immunized parenterally with purified heat-stable *Escherichia coli* enterotoxin that was made antigenic by coupling it to bovine immunoglobulin G. Immunized swine had high titers of antitoxin in serum and colostrum as measured by radioimmunoassay. However, the heat-stable enterotoxin neutralizing titers of the serum and colostrum from immunized swine were comparatively low. Newborn pigs suckling their immunized dams were not protected against challenge with porcine enterotoxigenic *E. coli* that produce heat-stable toxin but do not produce heat-labile toxin.

Escherichia coli enterotoxins are designated as heat-labile (LT) or heat-stable enterotoxins (10). LT preparations from porcine and human enterotoxigenic *E. coli* (ETEC) isolates are antigenically related to each other and to cholera enterotoxin (1, 6). Pregnant swine vaccinated with purified LT or cholera enterotoxin antigens subsequently protected their suckling newborn pigs (passive colostral immunity) against fatal diarrhea caused by infection with LT^+ porcine ETEC (2, 4). In contrast to LT, heat-stable toxin is not antigenic in its native form (10). However, heat-stable toxin of the type that causes intestinal secretion in infant mice (STa) has been made antigenic by coupling it to protein carriers (3, 5). Radioimmunoassays (RIAs) based on antibodies raised against these coupled STa antigens demonstrated that STa preparations from different porcine and human ETEC isolates were all antigenically related (3, 5). Rats actively immunized with protein-coupled STa antigens were protected against challenge with STa and viable cultures of LT^-/STa^+ ETEC (7, 8).

The objective of the experiment reported here was to determine whether vaccination of pregnant swine with STa coupled to protein would protect against porcine STa^+ ETEC infection in suckling pigs born to vaccinated dams. For this purpose nine primiparous swine (gilts) were selected from a single herd and randomly assigned to immunized or control groups. Gilts in both groups were vaccinated subcutaneously three times during pregnancy (at 30 to 35, 72 to 77, and 93 to 98 days of gestation). Gilts were vaccinated with STa coupled to bovine immunoglobulin G (IgG; coupling ratio, 6.5:1) (immunized group) and bovine IgG (control group). STa for the vaccine was purified from the human ETEC strain 18D (serotype O42:K86:H37) as reported previously (11). The procedure for coupling purified STa to IgG and for vaccination (initially with Freund adjuvant plus 7.8 mg of IgG per animal and subsequently with 3.9 mg of IgG per animal, each dose distributed to four separate sites per animal) were carried out as reported for the production of STa antisera in goats (5). Serum was collected from the gilts before the initial vaccination and 10 days after the final vaccination, and colostral whey was collected at parturition (about the 114th day of gestation). The samples were frozen at −70°C until they were tested for antibody. Pigs were challenged intragastrically with the porcine ETEC strain 431 (O101:K30, K99:H^-:STa^+) at 10^{10} viable *E. coli* per pig when they were 1 to 7 h old. Previous studies indicated that this strain and dose constituted a 50% lethal dose (in 5 days) for pigs suckling control gilts (9). The STa produced by this strain demonstrated complete RIA cross-reactivity with that from the vaccinal strain 18D (5). The procedures used to challenge the pigs and to evaluate their response were reported previously (9), except that the pigs were allowed to suckle the gilts at will from birth. One day after challenge, a pig from each litter was selected randomly, killed, and necropsied to provide tissues for histological and bacteriological evaluation. Gilts were killed 5 days after parturition,

TABLE 1. Geometric mean titers of antitoxin to *E. coli* STa in fluids from swine before and after vaccination with bovine IgG or IgG coupled to STa

Gilts	Antitoxin titer[a]							
	RIA				Neutralization			
	Serum		Colostrum	Ileum	Serum		Colostrum	Ileum
Vaccine (no.)	Before	After			Before	After		
IgG (3)	0	0	0	0	3 (2–4)	3 (2–4)	4 (2–8)	8 (2–32)
STa-IgG (6)	0	1,062 (294–15,385)	3,351 (555–20,000)	0	3 (2–8)	18 (4–128)	14 (4–256)	3 (0–16)

[a] Values are reciprocals of the dilution giving 50% ^{125}I-labeled STa binding or of the highest dilution neutralizing 2.2 mouse 100% effective doses of STa. Ranges are given in parentheses. Colostrum and ileum titers were measured after vaccination.

and fluid was harvested from their ileal contents, brought to 0.5% Formalin, heated to 56°C for 30 min, and frozen until tested for antibody. Titrations of antibody against STa by RIA and by the neutralization test in mice were carried out as described previously (5, 9). STa from the human ETEC strain 18D was used as the antigen in the RIA and STa from the porcine ETEC strain 431 was used for the neutralization test in mice.

There was no RIA detectable antitoxin in serum before vaccination or in fluids from control (IgG) gilts after vaccination (Table 1). Gilts had high titers of RIA antitoxin in serum and colostrum after vaccination with STa-IgG. The serum titers attained were comparable to those in goats vaccinated according to this protocol (5). There was also some increased STa-neutralizing activity of serum and colostrum after vaccination with STa-IgG (Table 1). However, the neutralizing titers of serum and colostrum from immunized gilts were much lower than their binding (RIA) titers. A similar disparity between RIA and neutralizing titers was observed in antisera from goats immunized according to this protocol (reference 5; R. A. Giannella, unpublished data). Possible reasons for the disparity include: (i) greater sensitivity of the RIA system, (ii) antibody binding to portions of the STa molecule other than the active site, and (iii) disruption of antigen-antibody complexes in the gut of the mouse.

Immunized gilts did not demonstrably protect their pigs against challenge. There was a high incidence of fatal diarrhea and of weight loss among survivors in both groups (Table 2). The lower portions of the small intestines of pigs in both groups were intensively colonized with *E. coli* adherent to the villi. Mortality (Table 2) was lower in the immunized group than in the control group. This difference was significant (p = 0.017) according to chi-square analysis. Mortality varied within litters from 0 to 75% in the immunized group and from 40 to 100% in controls. When this litter-to-litter variation was considered in the analysis (arcsine transformation of percentages), the difference in mortality between groups was not significant (P = 0.16).

In apparent contrast to the results reported here, rats actively immunized by combined parenteral and oral routes with STa coupled to porcine IgG or LT responded to Sta$^+$ human ETEC, in ligated intestinal loops, with less secretion than did control rats (7, 8). There are several possible reasons for this apparent difference. (i) The rats may have produced antitoxin with greater neutralizing activity than that reported here. (ii) Oral immunization of rats may have produced antitoxin of the IgA class that may be more effective in the intestinal lumen than that (presumably mostly IgG) reported here. (iii) Adult rats may be inherently more resistant (and thus more easily protected) to human ETEC than newborn pigs are to porcine ETEC. The affinity of STa (from a porcine ETEC strain) for enterocyte brush border preparations from pig small intestine is 10 times greater than that for similar preparations from rat small intestine (D. C. Robertson, personal communication). (iv) Immunization of gilts may have resulted in a degree of protection too small to be demonstrable with the severe challenge and limited number of pigs used in this study. The increased toxin-neutralizing activity of serum and colostrum and the apparent trend toward reduced mortality in the immunized group are both consistent with this latter possibility.

Previous studies demonstrated that vaccinated gilts can protect (by antibacterial immunity) suckling newborn pigs against the challenge strain and dose used here (9). Furthermore, studies with purified LT and choelra enterotoxin antigens have demonstrated that passively acquired (colostral) antitoxic immunity engendered by parenteral vaccination can reduce diarrhea and death in newborn pigs given an approximate 50% lethal dose challenge with LT$^+$ porcine ETEC (2). Thus, antitoxic immunity alone can protect in this system. However, vaccination with STa-IgG apparently stimulated the production of antitoxin with high binding activity but with low neutralizing, and thus low protective, activity in this system. In view of the

TABLE 2. Effect of vaccination of pregnant swine with STa coupled to bovine IgG on the response of their newborn suckling pigs to challenge with the porcine enterotoxigenic *E. coli* strain 431 (LT^-/STa^+)

Newborn pigs vaccine group (no.)	Clinical signs (%)				*E. coli* present at necropsy on day 1[a]					
	Diarrhea		Weight[b] change	Died[c]	Jejunum (upper)		Jejunum (lower)		Ileum	
	Day 1	Day 5			No. of bacteria	Adherent bacteria	No. of bacteria	Adherent bacteria	No. of bacteria	Adherent bacteria
IgG (25)	100	43	−14	68	7.0	0/3	8.9	3/3	9.4	2/3
STa-IgG (48)	100	54	−10	37	7.0	2/6	9.4	2/6	9.5	6/6

[a] One pig per litter was killed and necropsied 1 day after challenge. The numbers of bacteria are presented as $\log_{10}$ the mean number of viable bacteria per 10 cm of intestine. The adherent bacteria were determined as the number of sections with layers of bacteria adhering per number of sections examined.

[b] Mean change from initial (day 0) body weight in pigs surviving to day 5.

[c] Died of diarrheal disease by day 5.

potential of STa in vaccine development, it seems useful to attempt to prepare antigens that stimulate more neutralizing antibody than the preparations used here.

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