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Passive Immunity in Transmissible Gastroenteritis of Swine: Immunoglobulin Characteristics of Antibodies in Milk After Inoculating Virus by Different Routes[1]

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Pregnant swine were exposed to transmissible gastroenteritis (TGE) virus by different routes, and their serum, colostrum, and milk were examined for titer and immunoglobulin (Ig) class of antibodies. When 2 to 4 days old, the litters of most of these animals were challenged with virulent TGE virus to determine the effectiveness of passive immunity. After two oral/intranasal exposures to attenuated virus, none of the six pregnant animals became sick. TGE antibodies in milk were primarily or solely of the IgG class, although low levels of IgA antibodies were detected in three animals. Pigs in the five challenged litters received some passive immunity, the mortality being 25%. After intramuscular injection of six pregnant swine with virulent virus, two types of clinical and immunological responses were observed, presumably dependent on whether the gut was infected by an hematogenous spread of the virus. Three became sick, showing typical clinical signs of TGE, and their immunological response was characterized by the occurrence in milk of antibodies of the IgA class. A good degree (0% mortality) of passive immunity occurred upon challenge of the suckling pigs. In contrast, in three pregnant animals that did not sicken, antibody in milk was primarily of the IgG class, and poor (69% mortality) passive immunity occurred. After intramammary injections of three pregnant swine with virulent virus, no sickness was observed and the immunological response was characterized by the occurrence in colostrum of high titers of TGE antibodies that were primarily or solely of the IgG class; good (0% mortality) passive immunity occurred. The occurrence in milk of TGE antibodies of the IgA class was associated with an intestinal infection, whereas antibodies of the IgG class resulted from a parenteral antigenic stimulation. The role of antigenic stimulation of the intestinal tract for providing antibodies in milk of the IgA class is discussed. Passive immunity against intestinal infection with TGE virus was generally more complete in pigs ingesting antibodies of the IgA than of the IgG class.

In previous investigations, we have established the importance of specific antibodies of the immunoglobulin (Ig) A class in milk for providing passive immunity against transmissible gastroenteritis (TGE), an intestinal viral disease of swine (3, 4, 12). These results have indicated that in serum, colostrum, and milk from pregnant sows vaccinated intramuscularly (IM) or intramammarily (IMm) with live attenuated TGE virus, TGE antibody activity was associated primarily, if not entirely, with the IgG class. In contrast, milk of sows experimentally or naturally infected with live virulent TGE virus contained TGE antibodies predominantly of the IgA class.

However, our results have been at variance with the results of other investigators who have demonstrated antibodies of the IgA class in milk of swine (1), rabbits (7), and sheep (9) after intramammary injections of various antigens.

The purpose of the present investigation was to further investigate the occurrence of Ig classes of TGE antibodies in sow serum, colostrum, and milk as a result of being stimulated by different types of TGE viral preparations administered by different routes. Also, an evaluation was made on the probable role of Ig

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classes of antibodies for providing passive immunity against enteric infection with TGE virus.

MATERIALS AND METHODS

Experimental animals. The methods used in the inoculation and challenge of swine were similar to those described previously (4). All sows used for virus inoculation were from herds that had not recently shown clinical signs of TGE and were serologically negative for TGE antibodies by the plaque reduction test.

Viruses. The TGE viral preparations used in this report have been described in detail previously (4). The live attenuated TGE virus used for inoculation was a high-passaged Purdue strain that had been serially passaged 114 times in porcine kidney cell cultures, and is referred to as the P-114 strain. It contained approximately 2×10^6 plaque-forming units per ml. The virulent strain of TGE virus used, and referred to as Miller no. 3 (M-3) strain, had a titer of about 10^5 pig-infective doses per ml. It was of gut origin.

Inoculation with attenuated virus. P-114 was given orally and intranasally (O/IN) to six pregnant swine. Two 10-ml doses (5 ml by each route) were given with the use of a syringe with a long blunt needle. The first dose was administered at approximately 5 to 7 weeks and the second at 2 to 3 weeks prepartum (see Table 1).

Inoculation with M-3. M-3 was given IM to six pregnant swine and IMm to three pregnant swine. IM inoculations of 5 ml per dose were given one to three times into the ham or behind the ear during a prepartum period of 46 to 7 days (see Table 4). IMm inoculations were made either into the milk cistern or the adjacent mammary tissue at the base of the teats. Five milliliters of virus was inoculated into three glands on the left side of the udder at 5 to 7 weeks prepartum, and the same procedure was repeated about 2 weeks prepartum (see Table 5).

Challenge of suckling pigs. Piglets were orally challenged with 1 ml of a 1:1,000 dilution of M-3 representing about 100 pig-infective doses, as previously described (4).

Collection of specimens. Methods used for the collection and processing of serum, colostrum, and milk samples were the same as those described previously (4, 12). As used in this report, colostrum refers to mammary secretions obtained within 24 h after farrowing.

Antibody titrations. A plaque reduction test was used for the detection of TGE virus-neutralizing antibodies (4). Antibody titers were expressed as the reciprocal of the sample dilution resulting in an 80% reduction in plaques.

Antisera and absorption tests. The monospecific rabbit anti-porcine IgM, anti-IgG, and anti-IgA used in this study were prepared as described previously (12). Only a single line was evident when each antiserum was tested against porcine serum and colostral whey by immunodiffusion and immunoelectrophoresis.

Absorption tests of serum and colostral and milk whey were conducted as previously reported to determine the Ig classes of TGE antibodies (12). One milliliter of a diluted milk or serum sample was mixed with monospecific anti-IgG or anti-IgA and then incubated, first at 37 C for 30 min, and then at 4 C for 24 to 48 h. Controls, treated in the same manner, contained saline instead of specific antisera. Precipitates that formed were removed by centrifugation. The absorptions were repeated on the supernatants until all of a particular class of Ig was precipitated from the sample by the monospecific antisera, as determined by the absence of a line on immunodiffusion against the antiserum.

After absorption, samples were filtered through 0.45-μm membrane filters (Millipore Corp., Bedford, Mass.) and subsequently tested for virus-neutralizing antibodies. Virus neutralization titers were expressed in terms of the final dilutions of the absorbed samples and saline controls. A reduction in antibody titer of less than twofold after absorption with antisera was considered of little, if any, significance because of the many steps involved in the procedure and the limitation in obtaining precise reproducible results by the neutralization test. All anti-IgA and anti-IgG sera used for absorption were tested for TGE antibody activity and were negative.

A few of the samples that had TGE antibody activity in the IgA and/or IgM portions of the gel filtration runs were absorbed with anti-IgM in a manner similar to that just described. However, little or no reduction occurred in antibody titer when compared with the controls.

Immunodiffusion. Double immunodiffusion using 0.9% agarose in 0.05 M sodium barbital buffer, pH 8.6, was performed by the micromodification of Ouchterlony's method (16).

Gel filtration. Gel filtration using Sephadex G-200 (Pharmacia, Uppsala, Sweden) and 0.1 M tris(hydroxymethyl)aminomethane-0.2 M NaCl buffer, pH 8, was conducted as described in an earlier investigation (12). Three-milliliter fractions were collected and the optical density (OD) of the fractions at 280 nm was determined.

RESULTS

O/IN inoculation with P-114. Antibody titers in serum, colostrum, and milk collected periodically from six O/IN-exposed pregnant swine are summarized in Table 1. All swine remained healthy after inoculation. Antibody titers in colostrum were higher than in the corresponding serum samples. However, titers in milk declined promptly after farrowing, so that by 4 to 5 days post-farrowing (DPF), they were usually several-fold less than in the corresponding serum samples and in the colostrum samples that had been previously collected. This was especially evident in sow 2, who had titers of 4 in milk and 230 in serum at 18 DPF, when her pigs were challenged with M-3 (Table 1).

Pigs in five litters were challenged when 3 days old, and all had diarrhea within 24 to 48 h, with a morbidity of 100% and a mortality of 25% (Table 2). Pigs in one litter (sow 2) were challenged when 18 days old and all developed diarrhea, but there were no deaths. After challenge of their suckling pigs, all of the sows except one (sow 7-7) were sick, with some or all

TABLE 1. *TGE antibody titers in serum and milk of swine after oral and intranasal exposure to P-114 and after challenge of their suckling pigs*

Sow no.	Days pre-farrowing exposed	Age of litter at challenge[a] (days)	Specimen	Antibody titers[b]				
				13–19 days pre-farrowing	0–1 days post-farrowing	4–5 days post-farrowing	18–29 days post-farrowing	44–74 days post-farrowing
2	45, 17	18	Serum	80 (17)[c]		360	230 (18)	3,300 (74)
			Milk		1,300(0)	29	4 (18)	1,100 (74)
43	44, 16	3	Serum	38 (16)		400		3,500 (44)
			Milk		750(.5)	125		5,400 (44)
61-5	45, 15	3	Serum	110 (15)	390(.5)	360	5,400 (29)	
			Milk		2,000(.5)	100	1,050 (29)	
38-4	43, 13	3	Serum	256 (13)		320	8,300 (29)	
			Milk		2,700(0)	76	5,100 (29)	
11-10	44, 15	3	Serum	38 (15)		94	4,400 (20)	
			Milk		340(0)	100		
7-7	32, 13	3	Serum	256 (13)	270(1)	360	1,750 (23)	
			Milk		320(1)	220	2,500 (23)	

[a] Litters were orally challenged with M-3 at the age indicated.

[b] Expressed as reciprocal of the sample dilution resulting in an 80% reduction in plaque number in neutralization test.

[c] Number in parentheses indicates the actual day when the sample was collected.

TABLE 2. *Transfer of passive immunity to suckling pigs challenged with M-3*

Experimental group	Sow no.	Age of litter at challenge[a] (days)	Morbidity			Mortality		
			No./ total	%	Mean % (after last challenge)	No./ total	%	Mean % (after last challenge)
O/IN-inoculated	43	3	5/5	100		2/5	40	
(P-114 strain)	61-5	3	6/6	100		0/6	0	
	38-4	3	5/5	100	100 (20/20)	0/5	0	25 (5/20)
	11-10	3	2/2	100		2/2	100	
	7-7	3	2/2	100		1/2	50	
IM-inoculated	71[b]	3	5/5	100	100 (13/13)	1/5	20	69 (9/13)
(M-3 strain)	211[b]	3	8/8	100		8/8	100	
	133[c]	3	0/7	0		0/7	0	
		6	0/7	0		0/7	0	
		12	3/7	43		0/7	0	
	455[c]	3	0/7	0	56 (10/18)	0/7	0	0 (0/18)
		17	7/7	100		0/7	0	
	108[c]	3	0/4	0		0/4	0	
		7	0/4	0		0/4	0	
IMm inoculated	64-9	11	4/4	100		0/4	0	
(M-3 strain)	58-3	4	0/5	0		0/5	0	
		11	5/5	100	100 (17/17)	0/5	0	0 (0/17)
	55-5	3	0/8	0		0/8	0	
		11	8/8	100		0/8	0	

[a] Some litters were challenged a second or third time, as indicated.

[b] Sows 71 and 211 did not become sick after IM inoculations with M-3, and TGE antibodies in milk were primarily or solely of the IgG class.

[c] Sows 133, 455, and 108 became sick after IM inoculation with M-3, and TGE antibodies in milk were primarily of the IgA class.

of the following clinical signs: anorexia, diarrhea, and agalactia. Subsequently, all six sows had marked increases (5- to 60-fold) in antibody titers in both serum and milk (Table 1).

Gel filtration studies on colostrum and 4- or 5-day milk samples from all sows indicated that TGE antibody activity was primarily or solely associated with IgG. In two sows (2 and 43), antibody could be detected only in the IgG region (second peak fractions) of the chromatogram, as illustrated by the colostrum and 4-DPF milk sample from sow 43 (Fig. 1a and b). In the colostrum from the other four sows, antibody was primarily in the IgG region, although low levels were also detected in the IgA region of the chromatogram (first shoulder peak). The 4- to 5-DPF milk from three of these sows contained similar titers of TGE antibodies in the IgA and IgG regions of the chromatograms. These results are illustrated in the G-200 profiles shown for colostrum and 5-day milk from sow 61-5 (Fig. 2a and b).

Milk and serum samples (29 to 74 DPF) obtained from the sows after challenge of their litters were also examined by gel filtration. In all these milk samples, TGE antibody activity was associated with both IgA and IgG fractions, with more of the activity in the IgA peak fractions (Fig. 1c and 2c).

To confirm the immunoglobulin classes of TGE antibodies suggested by the gel filtration studies, samples were absorbed with monospecific antisera to remove a particular class of immunoglobulin. A summary of the virus neutralization titers of these absorbed samples and their saline controls is presented in Table 3. Gel filtration studies of colostrum and milk from sows 38-4 and 61-5 had suggested these samples contained both IgG and IgA TGE antibodies. Absorption of the colostrum from these two sows with anti-IgA reduced titers by 1.1- and 1.5-fold, respectively, whereas absorption with anti-IgG correspondingly reduced titers by >8- and 13-fold. This indicates that most of the antibody in the colostrum of these two sows was of the IgG class, with only a small amount of IgA, and confirms the gel filtration results. When the 5-day milk samples from these two sows were similarly absorbed with anti-IgA, titers were reduced by 2.6- and 3.3-fold, and

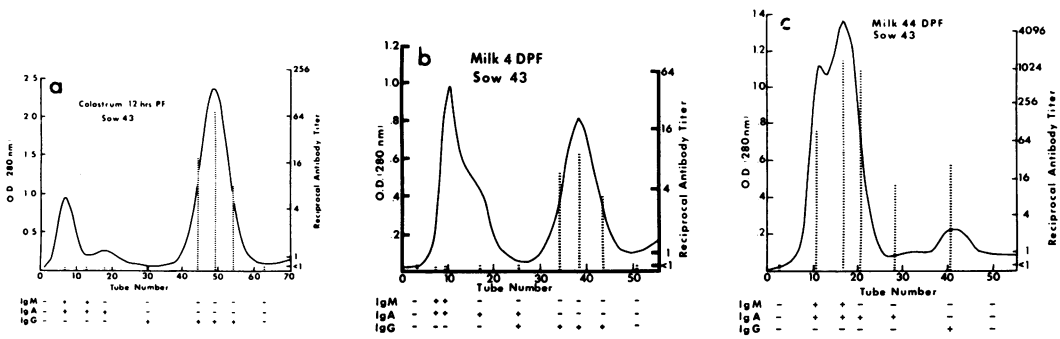

FIG. 1. *Gel filtration on Sephadex G-200 of 12-h post-farrowing colostral whey (a), 4-DPF milk whey (b), and 44-DPF milk whey (c) from O/IN inoculated sow 43. (The 44-DPF sample was collected 41 days after the sow's litter was challenged.) Vertical bars represent TGE-neutralizing antibody titers in individual unconcentrated fractions. Immunoglobulins were detected in these fractions by means of monospecific antisera.*

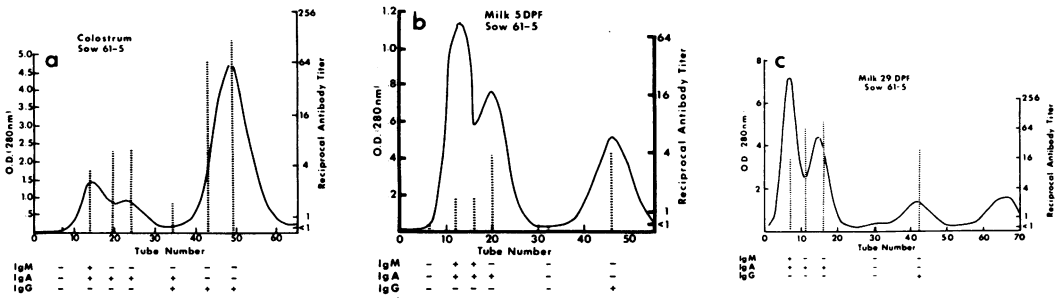

FIG. 2. *Gel filtration on Sephadex G-200 of colostral whey (a), 5-DPF milk whey (b), and 29-DPF milk whey (c) from O/IN-inoculated sow 61-5. The 29-DPF sample was collected 26 days after the sow's litter was challenged. See Fig. 1 for legend.*

TABLE 3. *Comparison of TGE antibody titers of paired samples after absorption with monospecific antisera or saline*

Sow no.	Route of exposure to TGE	Sample	Antibody titer[a] after absorption with		Fold reduction	Antibody titer[a] after absorption with		Fold reduction
			Saline control	Anti-IgA		Saline control	Anti-IgG	
38-4	O/IN	Colostrum	2,457	2,268	1.1	2,400	<300	>8
		5-DPF milk	92	36	2.6	66	29	2.3
		29-DPF serum	17,600	16,800	1.1	25,000	1260	19.8
43	O/IN	Colostrum	495	990	0	624	<120	>5.2
		44-DPF milk	5,400	20	270	4,800	1,720	2.8
		44-DPF serum	2,820	3,000	0	1,800	560	3.2
61-5	O/IN	Colostrum	2,880	1,935	1.5	2,626	202	13
		5-DPF milk	150	45	3.3	84	23	3.7
		29-DPF milk	1,230	150	8.2	1,880	376	5
71	IM	Colostrum	1,825	1,650	1.1	3,200	200	16
133	IM	4-DPF milk	540	48	11.3	625	195	3.2
58-3	IMm	Colostrum	7,875	12,800	0	7,800	572	13.6
55-5	IMm	Colostrum	4,920	5,040	0	5,000	<200	>25

[a] See footnote *b*, Table 1.

with anti-IgG by 2.3- and 3.7-fold. Therefore, approximately similar amounts of the antibody activity in these two milk samples were associated with IgA and IgG.

In contrast, gel filtration studies had indicated that the colostrum and the 4-DPF milk from sow 43 contained TGE antibodies only of the IgG class. Absorption of colostrum with anti-IgA did not reduce the titer, whereas absorption with anti-IgG reduced the titer by 5.2-fold. This confirms the gel filtration results that antibody in the colostrum was primarily of the IgG class.

Absorption of 29- to 44-DPF milk samples from these sows (after exposure to virulent virus) indicated the association of TGE antibodies with both classes of Ig (Table 3). Absorptions with anti-IgA resulted in greater reductions in TGE antibody titers (8.2- to 270-fold) than did absorption with anti-IgG (2.8- to 5-fold). However, in the corresponding serum samples most of the TGE antibody activity was associated with IgG, since absorption with anti-IgG reduced titers by 3.2- to 19.8-fold, whereas absorption with anti-IgA reduced titers by only 0- to 1.1-fold.

IM inoculation with M-3. TGE antibody titers in serum, colostrum, and milk collected periodically from six IM-inoculated pregnant swine are summarized in Table 4. The clinical and immunological responses of these animals can be divided into two distinct types as follows.

(i) Three animals (278, 71, and 211) remained healthy after IM inoculations. Antibody titers in milk decreased several-fold within a few days after farrowing and became lower than in the corresponding serum samples (Table 4). This was especially notable in sow 278, whose milk and serum antibody titers were 2 and 23, respectively, at 14 DPF. Gel filtration of colostrum and 4-DPF milk from sows 71 and 211 indicated that TGE antibodies were primarily associated with IgG, as illustrated for sow 71 (Fig. 3a and b). Absorption tests on the colostrum from sow 71 also indicated that most of the antibody was of the IgG class, since absorption with anti-IgG reduced the titer by 16-fold, whereas with anti-IgA only a 1.1-fold reduction occurred (Table 3). After challenge at 3 days of age, all the pigs of sows 71 and 211 developed typical clinical signs of TGE and the mortality was 69% (Table 2). One (20%) of the five pigs from sow 71 died, whereas all eight pigs from sow 211 died. Subsequently, both sows 71 and 211 developed clinical signs of TGE and both had a marked rise in antibody titer in serum (Table 4). Gel filtration of the 23-DPF milk from sow 71 (3 weeks after challenge of her litter) revealed that TGE antibodies were present in both IgA and IgG, whereas before challenge they were primarily associated with IgG (Fig. 3c). The litter of sow 278 was not challenged, so that the decline in antibody levels in milk could be followed as lactation progressed (Table 4).

(ii) The responses in the other three sows (133, 455, and 108) were quite different. All three developed typical clinical signs of TGE after IM inoculation with virulent virus. All had higher antibody titers in milk than in serum (Table 4). Gel filtration of colostrum and 2- to

TABLE 4. *TGE antibody titers in serum and milk after IM inoculation of pregnant swine with M-3 and after challenge of their suckling pigs*

Sow no.	Days pre-farrowing injected	Age of litter at challenge[a] (days)	Specimen	Antibody titers[b]					
				7-21 days pre-farrowing	0-0.4 days post-farrowing	1-4 days post-farrowing	12-16 days post-farrowing	18-28 days post-farrowing	41-43 days post-farrowing
278	30, 16	NC	Serum	4 (16)[c]	92	62 (1)	23 (14)	25 (28)	
			Milk		200	33 (1)	2 (14)	<1 (28)	
71	46, 24	3	Serum	5 (24)		98 (4)		3,300 (24)	
			Milk		3,600	37 (4)		410 (24)	
211	35, 21, 7	3	Serum	200 (7)		400 (4)		5,000 (18)	
			Milk		2,400	80 (4)			
133	43, 21	3, 6, 12	Serum	130 (21)		210 (4)	150 (12)	82 (27)	
			Milk		1,150	360 (4)	180 (12)	330 (27)	
455	34	3, 17	Serum			256 (2)	240 (16)		270
			Milk		2,000	1,100 (2)	350 (16)		1,200
108	42	3, 7	Serum		107	76 (3)	26 (14)		26
			Milk		3,200	720 (3)	146 (14)		76

[a] See footnote *a*, Table 1. NC, Not challenged.
[b] See footnote *b*, Table 1.
[c] See footnote *c*, Table 1.

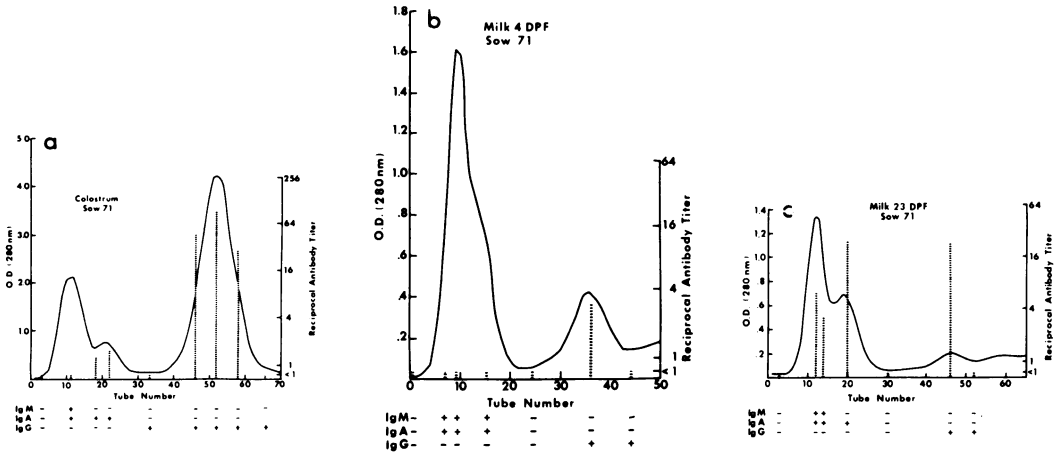

FIG. 3. *Gel filtration on Sephadex G-200 of colostral whey (a), 4-DPF milk whey (b), and 23-DPF milk whey (c) from IM-inoculated sow 71. The 23-DPF sample was collected 20 days after the sow's litter was challenged. See Fig. 1 for legend.*

4-DPF milk samples from sows 133 and 455 indicated that TGE antibody was associated with both IgA and IgG but primarily with the former, as illustrated for sow 133 (Fig. 4a and b). Absorption tests on the 4-DPF milk from sow 133 also indicated that most of the antibody was of the IgA class, since absorption with anti-IgA reduced the titer by 11.3-fold, whereas with anti-IgG a 3.2-fold reduction occurred (Table 3). Pigs in all three litters were initially challenged when 3 days old and all remained healthy (Table 2). All the pigs from sow 455 became sick after the second challenge at 17 days of age. Three of seven pigs from sow 133 became sick after the third challenge at 12 days of age, and none of the pigs from sow 108 became sick after the second challenge at 7 days of age (Table 2). None of the pigs in these three litters died. All three sows remained healthy after their litters were challenged, and none had a rise in serum antibody titer, although sows 133 and 455 had about a two- or threefold rise in milk antibody (Table 4). Gel filtration of the 27-DPF milk from sow 133 (Fig. 4c) and the

41-DPF milk from sow 455 resulted in TGE antibodies being detected only in the IgA fractions.

IMm inoculation with M-3. TGE antibody titers in serum, colostrum, and milk collected periodically from three IMm-inoculated pregnant swine are summarized in Table 5. None of the animals had clinical signs of TGE after inoculation. Titers observed in the colostrum from these sows ranged from 1,500 to 6,600 and were generally several-fold greater than those in the colostrum of sows from the other experimental groups (Tables 1 and 4). Moreover, antibody titers were usually slightly higher in colostrum and 4- to 5-DPF milk from injected glands on the left side of the udder than from noninjected glands on the right side of the udder. Within a few days after farrowing, milk antibody levels had declined markedly in all three sows, and in sows 64-9 and 58-3 were several times less than the corresponding serum titers. This situation was especially pronounced at 11 to 12 DPF, with titers in milk ranging from 6 to 34, whereas the titers in the analogous serum samples were much greater, ranging from 360 to 1,200. After challenge of their litters at 11 DPF, an anamnestic response was evident at 26 to 46 DPF in both serum and milk samples from the

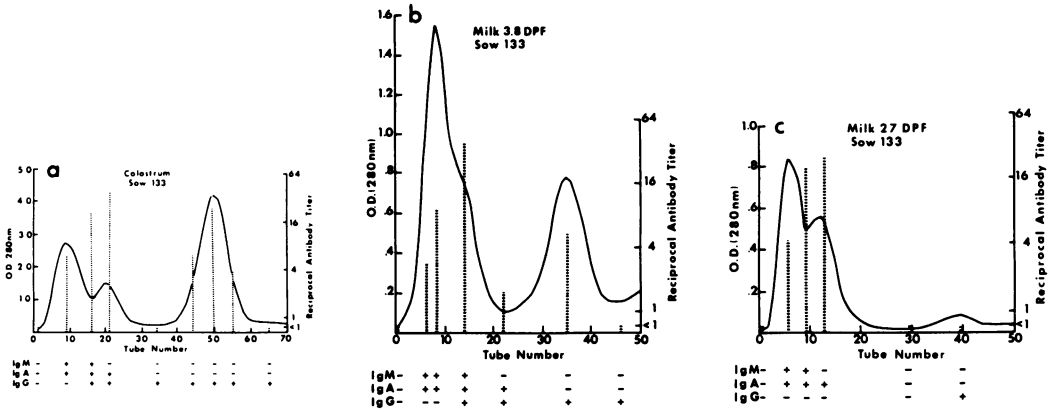

FIG. 4. *Gel filtration on Sephadex G-200 of colostral whey (a), 4-DPF milk whey (b), and 27-DPF milk whey (c) from IM-inoculated sow 133. The 27-DPF sample was collected 24 days after the sow's litter was initially challenged. See Fig. 1 for legend.*

TABLE 5. *TGE antibody titers in serum and milk after IMm inoculation of pregnant swine with M-3 and after challenge of their suckling pigs*

Sow no.	Days pre-farrowing injected	Age of litter at challenge[a] (days)	Specimen	Antibody titers[b]				
				9–16 days pre-farrowing	0 days post-farrowing	3.8–5 days post-farrowing	11–12 days post-farrowing	26–46 days post-farrowing
64-9	43, 16	11	Serum	5 (16)[c]	560	570	380	5,400 (46)
			Milk					
			Igl[d]		2,900	90	6	370 (46)
			NIgl[e]		1,500	76	7	
58-3	43, 9	4, 11	Serum	19 (9)		1,500	1,200	5,400 (26)
			Milk					
			Igl		6,400	110	34	220 (26)
			NIgl		5,800	95	34	195 (26)
55-5	42, 17	3, 11	Serum	18 (17)		380	360	4,400 (33)
			Milk					
			Igl		6,600	500	20	140 (33)
			NIgl		6,100	330	15	160 (33)

[a] See footnote *a*, Table 1.
[b] See footnote *b*, Table 1.
[c] See footnote *c*, Table 1.
[d] Igl, Milk from injected glands on left side of udder.
[e] NIgl, Milk from noninjected glands on right side of udder.

three sows. Serum titers increased to 4,400 to 5,400, an increase of 4- to 13-fold.

When pigs in the litters from sows 55-5 and 58-3 were initially challenged at 3 and 4 DPF, respectively, no morbidity or mortality occurred (Table 2). However, after challenge of all three litters at 11 DPF, all the pigs developed diarrhea after an incubation period of 3 to 4 days. All three sows also became sick and developed some or all of the following clinical signs: depression, anorexia, diarrhea, and agalactia.

Gel filtration of colostrum and milk samples from the injected and noninjected glands of the three sows showed similar results. As exemplified by colostrum and 4.5-DPF milk whey from sow 58-3 (Fig. 5a and b), antibody activity was highest in the IgG portion of the chromatogram, with little activity apparent in the IgA region. However, in the 26-DPF milk from this sow (approximately 2 weeks after the second challenge of her litter), TGE antibody activity was present in both the IgA and IgG portions of the chromatogram (Fig. 5c). Similar results were seen in the 33- and 46-DPF milk from sows 64-9 and 55-5, respectively. Thus, contact exposure of the sows to M-3 resulted in the production of antibodies in milk of the IgA class.

Absorption tests performed on colostrum samples from the injected glands of sows 58-3 and 55-5 also indicated that TGE antibodies were mainly of the IgG class, confirming the results obtained from gel filtration studies. In both colostrum samples, absorptions with anti-IgG reduced the antibody titers by 13.6- to >25-fold, whereas absorption with anti-IgA did not decrease the titers (Table 3).

DISCUSSION

After O/IN exposure of six pregnant swine to live attenuated TGE virus, none became sick. Presumably, this was due to an absence of, or an inadequate, infection of the epithelial cells of the small intestines. In two of the six sows, TGE antibodies in colostrum and milk appeared to be entirely of the IgG class. In the other four sows, although IgG TGE antibodies were predominant, there was evidence of low levels of IgA TGE antibodies. The absence, or the presence of only low levels, of IgA TGE antibodies in milk was thought to be due to an inadequate antigenic stimulation of the gastrointestinal tract. We have previously reported that the occurrence of IgA TGE antibodies in milk appears to be a result of a prior infection or antigenic stimulation of the gastrointestinal tract (3, 4). The lower antibody titers in milk than in the corresponding serum can be explained on the basis that antibodies in milk were primarily of the IgG class, since it is known that the level of IgG in milk of sows declines rapidly within a few days postpartum and then throughout lactation remains less than that in serum (6, 11). The passive immunity resulting from challenging 3-day-old suckling pigs was only partial since there was a 100% morbidity and a 25% mortality. Five of the six sows became sick, some with severe diarrhea, from contact with their challenged pigs, and all six had marked rises in antibody titers in both serum and milk. Thus, it appears that the small intestines of the pregnent swine were inadequately infected by the prior exposure with P-114, so that active immunity did not result. It also indicates that circulating antibodies provided little, if any, immunity against either intestinal infection with TGE virus or clinical signs.

Whereas in this group of O/IN-infected animals IgG was the primary class of TGE antibody in milk collected before challenge, IgA had become the predominant class of TGE antibody in milk samples collected after challenge (Fig. 1 and 2). It is unlikely that the production of IgA

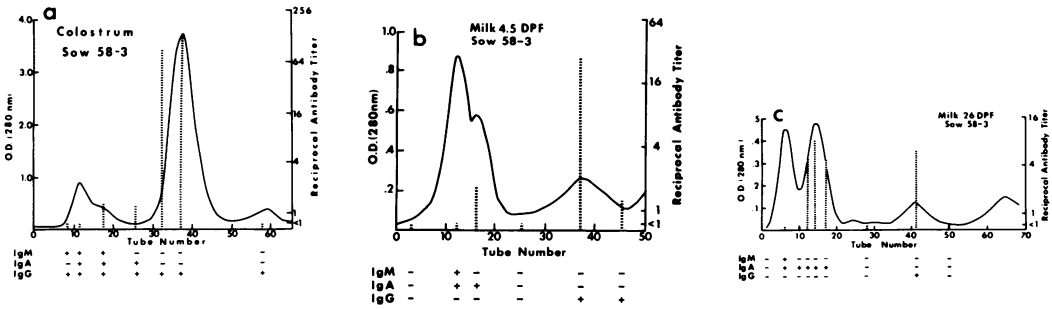

FIG. 5. *Gel filtration on Sephadex G-200 of colostral whey (a), 4.5-DPF milk whey (b), and 26-DPF milk whey (c) from IMm-injected sow 58-3. The 26-DPF sample was collected 22 days after the sow's litter was first challenged and 15 days after second challenge. See Fig. 1 for legend.*

TGE antibody in milk in these animals after challenge was due to a viral antigenic stimulation of the IgA-producing cells already localized in the mammary gland since, at time of challenge, these animals had TGE-neutralizing antibodies in both serum and milk. Thus, the hematogenous spread of the virus from the intestinal tract to the mammary gland would be highly improbable. However, the presence of TGE-neutralizing antibodies in serum should not interfere with relocation of virus-sensitized, IgA-producing cells from the intestinal tract to the mammary gland, a mechanism we have previously suggested (3, 4).

Immunization of pregnant swine by the use of an attenuated TGE virus given orally and/or intranasally, so as to provide passive immunity to suckling pigs, is worthy of further investigations. In this report, although only a limited number of litters and pigs were challenged, the mortality was only 25% which is considerably less than would be expected in nonimmune pigs. For example, for pigs that were similarly challenged and were nursing susceptible sows, the reported mortality figures have been 71% (4) and 92% (14), whereas for pigs nursing immune sows (previously infected with virulent virus), the figures have been 5% (4) and 0% (14). Ideally, such a viral vaccine should (i) be sufficiently virulent that it would infect the intestinal tract of adult swine so as to result in the production of IgA TGE antibodies in milk, but (ii) be sufficiently attenuated that it would remain stable and produce no, or only mild, sickness in neonatal pigs. However, the selection of a viral strain that would accomplish both objectives might be very difficult if not impossible to attain.

After IM inoculation of six pregnant swine with virulent TGE virus, two distinct types of clinical and immunological responses occurred, apparently based on whether an intestinal infection occurred from a hematogenous transfer of the virus. Three animals remained healthy, and their immunological response was characterized by the production of antibodies in milk that were primarily of the IgG class. As a result, antibody titers in milk decreased to low levels and became less than in serum within a few days postpartum. The 3-day-old litters from two of these sows were challenged, resulting in a 100% morbidity and a 69% (9 of 13) mortality. Subsequently, these sows became sick and had a marked rise in antibody titer in serum, allowing the conclusion that the intestinal tract had not previously been infected by the IM injection of M-3.

In contrast, the other three IM-inoculated swine developed typical clinical signs of TGE, and their immunological response was characterized by the production of antibodies in milk that were primarily of the IgA class. We believe these results can best be interpreted as due to the hematogenous spread of the virus, leading to an infection of the epithelial cells of the small intestine. Antibody titers in milk were at relatively high levels and remained higher than those in serum. The suckling pigs of these sows were provided with good passive immunity when challenged. None of the three sows became sick from exposure to their challenged pigs, nor were there any significant increases in serum antibody titers. This information indicated that the sows were immune to reinfection, apparently due to a previous infection of the intestinal tract resulting from the IM inoculation of virulent virus. However, in two animals (133 and 455; Table 4) there was a two- or threefold increase in the antibody titers in the milk collected several weeks post-challenge. This may have been a reflection of an increase in the concentration of IgA, the level of which rises in milk late in lactation (L. Saif and E. Bohl, unpublished observations).

After IMm inoculation of three pregnant swine with M-3, none became sick and the immunological response was characterized by the production of high levels of TGE antibodies, predominantly of the IgG class, in serum, colostrum, and milk. Antibody titers were usually slightly higher in colostrum and 4- to 5-DPF milk from injected than from noninjected glands, and agrees with results reported previously (4) when P-114 was inoculated. This suggests either the presence of an inflammation in the injected glands, permitting an increased passage of circulating antibodies (which were of the IgG class), or the local production of IgG by the mammary gland. Gel filtration and absorption studies indicated that TGE antibodies in colostrum and milk were predominantly of the IgG class in milk from either injected or noninjected glands. These results are similar to those we previously reported after the IMm inoculation of attenuated TGE virus (4, 12), but are in contrast to those of Abou-Youssef and Ristic, who reported that TGE antibodies of the IgA class were produced in colostrum and milk after IMm inoculation of virulent virus (1).

The passive immunity provided to the suckling pigs of the IMm-injected sows was rather effective. This was probably due to the fairly high level of antibody occurring in milk near the time of challenge, even though the antibody was primarily of the IgG class. For example, pigs of two litters were challenged when 3 or 4 days old,

and no sickness occurred. However, when all three litters were challenged at 11 days of age, all pigs had severe diarrhea, but no deaths occurred. All three sows also became sick with clinical signs typical of TGE, and all had a marked anamnestic response in both serum and milk, indicating that the intestinal tract had not been previously infected by the IMm injection of virulent virus. The susceptibility of the pigs at 11 but not at 3 days of age was probably due to the marked decline in antibody titer that occurred in milk between 3 and 11 to 12 DPF (Table 5). The decrease in antibody titer in milk during this period can be explained on the basis that antibody was primarily of the IgG class.

The greater effectiveness observed for TGE antibodies of the IgA class in contrast to those of the IgG class in providing passive immunity against intestinal infection with TGE virus may be related to (i) higher levels of IgA in milk, and (ii) increased resistance of IgA to enzymatic degradation (13, 15). In this regard, Keller and Dwyer (8) demonstrated that 11*S* IgA was the predominant Ig recovered intact from human fecal extracts that neutralized poliovirus.

A number of studies have reported that the local immunization of the mammary gland with various antigens elicited the production of IgA antibodies in colostrum in guinea pigs (10), rabbits (7), and sheep (9). It is possible that the adjuvants and the complex bacterial antigens used in these studies may have contributed to the localization of the antigen in the mammary gland tissue and thereby influenced the production of a local IgA response.

The origin of IgA TGE antibodies in milk remains obscure. In the current as well as previous studies (4, 12), the appearance of IgA TGE antibodies in milk correlated with the presence of an infection of the intestinal tract. To account for this observation, we have suggested that after a local antigenic stimulation in the intestine, IgA immunocompetent cells may relocate and colonize the mammary glands, leading to a local synthesis of antibodies of the IgA class (3, 4). A similar mechanism has been proposed by others to account for the presence of IgA antibody-producing cells in extraintestinal lymphoid tissues after oral immunization with various antigens (2, 5).

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