Isolation of Porcine Immunoglobulins and Determination of the Immunoglobulin Classes of Transmissible Gastroenteritis Viral Antibodies¹

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The porcine immunoglobulins M (IgM), A (IgA), and G (IgG) were isolated and purified and some of the properties of the porcine milk IgA were examined. Monospecific antisera which were prepared against these immunoglobulins in rabbits were then used to absorb a particular class of immunoglobulin from sow serum, colostrum, and milk in an attempt to identify the immunoglobulin classes of neutralizing antibodies to the porcine enteric virus, transmissible gastroenteritis (TGE). The results of these absorption studies suggest that in colostrum and milk from sows experimentally (orally) or naturally infected with live virulent TGE virus, IgA is the predominant immunoglobulin class of TGE antibodies. Both IgA and IgG TGE antibodies appeared to be present in the serum from these sows, but with IgG TGE antibodies predominating. In contrast, in the serum, colostrum and milk from sows vaccinated intramuscularly or intramammarily with live attenuated TGE virus, the TGE antibody activity was associated mainly with the IgG class of immunoglobulins. These results provide additional data indicating that the route of infection or vaccination markedly influences the immunoglobulin class of antibodies in colostrum and milk. Secondly, IgA antibodies in mammary secretions are probably essential for providing optimal passive immunity of nursing pigs against infection with TGE virus.

The absence of the transplacental passage of maternal antibodies in the pig has been reported in studies by Sterzl et al. (23) and Kim, Bradley, and Watson (10). Sow mammary secretions are therefore the sole source of passively acquired antibodies to the newborn piglets. Because it is during this period that piglets are most susceptible to infection with the transmissible gastroenteritis (TGE) virus, neutralizing antibodies transmitted in the milk and colostrum are of primary significance for passive immunity so as to provide protection to the epithelial cells of the small intestine (8, 9).

The results reported in our previous papers (1a, 2) based on G-200 gel filtration studies have indicated that TGE antibodies in milk from vaccinated sows were associated mainly with second

peak fractions which contained predominantly immunoglobulin G (IgG), whereas in milk from infected sows they appeared mainly in first peak fractions where immunoglobulin A (IgA) was most concentrated. Moreover, piglets from infected sows were better protected against challenge than piglets from vaccinated sows. The importance of the IgA secretory antibody system in humans in the protection of mucous membranes has been demonstrated in a number of studies (5, 22, 27) and secretory IgA has been shown to possess neutralizing-antibody activity to a number of respiratory and enteric viruses (5, 14, 22, 27). The protective role of secretory IgA in alimentary immunity against poliovirus has been reported in studies by Ogra (14, 15).

The isolation of IgA from porcine serum and secretions has only recently been reported (3, 17, 20, 24, 25). Its possible role in local infections in the pig has been suggested also in studies by Porter, Noakes, and Allen (19) who found that colostrum and milk antibodies to somatic antigens

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of *Escherichia coli* were predominantly associated with IgA. This study was undertaken to isolate the porcine immunoglobulins IgM, IgA, and IgG and to identify the immunoglobulin classes of TGE antibodies in the serum, colostrum, and milk from vaccinated and infected sows. Some of the properties of the isolated porcine milk IgA are described, including its significance as neutralizing antibody in passive immunity to TGE.

MATERIALS AND METHODS

Experimental animals. The TGE viral preparations and the methods employed in the infection, vaccination, and challenge of swine were described in the preceding report (1a).

Infection with live TGE virus. The pregnant sows, 255 and 83-1, were naturally infected with TGE virus. An outbreak of TGE occurred in the herd of sow 255 approximately 157 days preparturition and in the herd of sow 83-1 about 80 days preparturition. Sows 107 and 76-10 were experimentally infected (orally and intranasally with 5 ml of Miller no. 3, virulent strain of TGE virus) at 32 and 33 days preparturition, respectively.

Vaccination with attenuated TGE virus. Sows 5-1 and 179 were vaccinated twice intramammarily (imm) with the high cell-culture-passaged Purdue (HPP) strain of TGE virus, each time using 5 ml of inoculum divided among 3 glands on the left side of the udder. Sow 5-1 was injected at 43 and 14 days preparturition and sow 179 received imm injections at 34 and 12 days preparturition. Sow 74-1 was injected intramuscularly (im) in the ham on days 41 and 13 preparturition, using a 5-ml dose of the HPP strain. Piglets from both the infected and vaccinated sows (except the piglets of sow 107) were challenged orally with virulent TGE virus at about 3 days postparturition (DPF) as described previously (1).

Collection of specimens. Methods used for the collection and processing of serum, colostrum, and milk were the same as those reported in the preceding paper (1a). Colostrum and milk samples from imm vaccinated sows were collected from the injected glands only.

Virus neutralization test. TGE virus-neutralizing antibodies were detected using the plaque reduction test as described previously (1a). Antibody titers were expressed as the reciprocal of the sample dilution resulting in an 80% reduction in plaques.

Chromatographic methods. Gel filtration chromatography was performed on Sephadex G-200 in 0.1 M tris(hydroxymethyl)aminomethane (Tris)-0.2 M NaCl buffer adjusted to pH 8 with HCl. Two columns (2.5 by 45 cm and 2.5 by 100 cm) were connected in series and a flow rate of about 10 ml/hr was maintained with the use of a peristaltic pump (Buchler Instruments, Fort Lee, N.J.). The eluate was collected in fractions of 3 ml and the optical density (OD) at 280 nm was determined. Selected fractions were pooled and concen-

trated by ultrafiltration through an Amicon Diaflo XM-100 membrane.

Ion-exchange chromatography was performed on diethylaminoethyl (DEAE)-cellulose (Whatman DE 52) using a column 1.5 by 30 cm. Protein was eluted using a stepwise buffer elution procedure at a flow rate of 15 ml/hr. Samples used for the isolation of IgA were eluted using the following stepwise changes in the molarity of Tris-hydrochloride buffer at pH 7.4: (i) 0.02 M, (ii) 0.1 M, (iii) 0.15 M, (iv) 0.2 M, and (v) 0.4 M plus 2 M NaCl. Following a procedure similar to that outlined by Mach and Pahud (11), samples used for the isolation of IgM or IgG were eluted using the following phosphate buffers: (i) 0.01 M, pH 7.4 (ii) 0.02 M, pH 7.2; (iii) 0.06 M, pH 7; (iv) 0.1 M, pH 6; (v) 0.2 M, pH 6; (vi) 0.3 M, pH 6; and (vii) 0.4 M, pH 6 plus 2 M NaCl. Fractions of 5 ml were collected and their OD values at 280 nm were determined.

Isolation of porcine immunoglobulins. Milk IgA. Three times concentrated sow's milk whey, late in lactation (10-11 weeks postparturition), was the source of porcine IgA. A typical elution profile obtained with such a sample of milk whey on Sephadex G-200 is shown in Fig. 1. On the basis of immunodiffusion and immunoelectrophoresis using specific antisera, the protein later identified as IgA appeared to be most concentrated in fractions 2 and 3 from the apex and descending portions of the first peak. These fractions were pooled, concentrated, and applied to a DEAE-cellulose column after thorough dialysis at 4°C against the first Tris-hydrochloride buffer. Porcine IgA was eluted with the second, third, and fourth Tris-hydrochloride buffers. Fractions eluted with the third and fourth buffers contained both IgM and IgA. However, the fractions eluted with the second buffer contained no other immunoglobulins except IgA when tested by immunodiffusion and immunoelectrophoresis using specific antisera prepared against porcine IgM, IgA, and IgG and anti-porcine colostrum whey. Therefore, these fractions were pooled, concentrated and refiltered on Sephadex G-200. A single symmetrical peak was eluted after the void volume. Fractions from the apex and the descending portions of this peak were pooled, concentrated and used for the preparation of anti-IgA serum.

IgG. Porcine colostrum whey was the source of IgG, which is the predominant immunoglobulin in this secretion (17). Ten milliliters of colostrum whey were dialyzed against the first phosphate buffer and then fractionated on DEAE-cellulose. IgG was the only immunoglobulin detectable in fractions 1, 2, and 3 which were eluted with the first and second phosphate buffers (Fig. 2). The fall-through peak, fraction 1, contained mainly slow IgG (IgG₂), but with small amounts of fast IgG (IgG₁) also present. IgG₁ was present mainly in fractions 2 and 3 which contained little IgG₂. However, a complete separation of these two subclasses was not attempted. This first fraction, when concentrated and filtered on Sephadex G-200, resulted in a single highly symmetrical peak which eluted in the 7S region of the chromatogram. Peak fractions were concentrated and used for the production of anti-porcine IgG serum.

IgM. Lipoproteins were precipitated from porcine

FIG. 1. Gel filtration on Sephadex G-200 of 70-day post-farrowing (DPF) milk whey from a naturally infected sow. Vertical bars represent the TGE-neutralizing antibody titers in unconcentrated eluates from individual tubes. Immunoglobulins were identified in the concentrated fractions (four to five times) by means of specific antisera.

serum as described by Bourne (4). A euglobulin precipitate was then obtained by dialysis of the lipoprotein-free serum against distilled water for 48 hr at 4 C. This euglobulin precipitate was dissolved in the third phosphate buffer and applied to a DEAE-cellulose column equilibrated with this same buffer. IgM was the only immunoglobulin detected in fractions 10 and 11 which were eluted with the sixth and seventh phosphate buffers (Fig. 2). These fractions were concentrated and recycled twice on Sephadex G-200 to give a single sharp peak which eluted with the exclusion volume. Peak fractions were concentrated and used to prepare anti-IgM serum.

Antisera. Rabbit antisera were prepared to solutions of purified porcine IgA, IgM, and IgG and to porcine serum, colostral, and milk whey. Rabbits received a primary im injection of about 2 mg of protein (0.5 ml) emulsified in an equal volume of Complete Freund's Adjuvant. Thereafter, rabbits were given im or intravenous (iv) injections, or both, of 2 to 5 mg of protein every 2 weeks for a period of approximately 2 months. Rabbits were exsanguinated by cardiac puncture. A small quantity of rabbit anti-human myeloma IgA was kindly supplied by Karen Costa (University Hospital, Columbus, Ohio). Rabbit anti-human IgM serum was obtained from Pentex (Miles Laboratories, Kankakee, Ill.; lot 4194).

Rabbit antisera to porcine IgA, IgM, and IgG were rendered monospecific for α , μ , and γ heavy chains by absorption with soluble antigens. Anti-IgA and anti-IgM were absorbed with small samples of porcine IgG until all anti-light chain activity was removed (200 μ g of IgG/ml of anti-IgA and 400 μ g of IgG/ml of anti-IgM). Similarly, anti-IgG was ab-

sorbed with small samples of a mixture containing porcine IgM and IgA until no more anti-light chain activity was observed.

The anti-IgM antiserum contained an α -macroglobulin contaminant which was removed by absorption with precostral piglet serum (17).

Immunoelectrophoresis and immunodiffusion. Immunoelectrophoresis was performed by a slight modification of the micromethod of Scheidegger (21) in a Gelman electrophoresis apparatus. A 1% solution of purified Noble agar in 0.05 M sodium barbital buffer, pH 8.6, was used.

Double immunodiffusion using 1% Noble agar (Difco) in 1% NaCl with 1:10,000 merthiolate was performed by the micromodification of Ouchterlony's method described by Wadsworth (26).

Protein concentrations. The concentrations of solutions of purified porcine immunoglobulins were determined using the extinction coefficients for porcine IgA, IgM, and IgG reported by Curtis and Bourne (7) at 280 nm.

Sucrose density gradient ultracentrifugation. A modification of the method described by Martin and Ames (12) was used. Samples of 0.2 ml of purified porcine IgG and IgA containing 5 to 7 mg of protein/ml were applied to the top of linear 5 to 20% sucrose density gradients prepared in Tris-NaCl buffer, pH 8. Centrifugation was in a Beckman model L3-50 ultracentrifuge in an SW41 ti rotor at an average speed of 40,000 rev/min for 18 hr at 4 C. A purified sample of bovine lactoferrin with a sedimentation coefficient of 5.3 S was used as a sedimentation marker in some of the gradients. The gradients were fractionated by puncturing the bottom of the tubes and eluting with a

FIG. 2. DEAE-cellulose chromatography of porcine colostrum whey. Arrows indicate buffer changes and the numbers above the arrows represent the following phosphate buffers: (2) 0.02 M, pH 7.2; (3) 0.06 M, pH 7; (4) 0.1 M, pH 6; (5) 0.2 M, pH 6; (6) 0.3 M, pH 6; (7) 0.4 M, pH 6 plus 2 M NaCl. The fall-through peak (fraction 1) was eluted with 0.01 M phosphate, pH 7.4. Total elution volume was 1,100 ml. Immunoglobulins were detected in concentrated fractions (8 to 12 times) using specific antisera.

50% sucrose solution in an upward direction. Peaks were monitored by absorbance at 280 nm.

Absorption of colostrum and milk whey samples with heavy chain-specific antisera. Representative porcine colostrum and milk whey and serum samples were collected from vaccinated and infected sows. These milk and colostrum samples were diluted (1:4 to 1:20, respectively) and then absorbed with monospecific rabbit anti-porcine IgA, anti-IgM, or anti-IgG to remove the corresponding class of immunoglobulin. One-milliliter samples of diluted whey secretions were mixed with the specific antiserum and then incubated, first at 37°C for 30 min and then at 4°C for 24 to 48 hr. Controls incubated in the same manner contained 1 ml of the diluted whey sample and saline instead of antiserum. Precipitates which formed in the tubes containing the whey and antiserum were removed by centrifugation. The same procedures were repeated on the supernatants until all of the particular class of immunoglobulin was precipitated by the specific antiserum. Removal of an immunoglobulin class was considered to be complete when the sample no longer showed a line of precipitation on immunodiffusion against the specific antiserum. However, as shown by immunodiffusion using specific antisera, the nonhomologous classes of immunoglobulins were still present in the samples following absorption. As a further control, a sample of purified porcine milk IgA, which had a TGE-neutralizing antibody titer of 74 was absorbed with monospecific anti-IgG to determine the possible significance of nonspecific precipitation of immunoglobulins from the samples. However, no significant reduction in antibody titer was observed after absorption with anti-IgG.

After absorption, samples were filtered through 0.45 μm membrane filters and subsequently tested in

the virus neutralization test. Virus neutralization titers were expressed in terms of the final dilutions of the absorbed samples and saline controls. Differences in titers between the absorbed samples and saline controls of less than twofold were not considered significant. All immunoglobulin antisera used for absorption were first tested for TGE virus-neutralizing activity and were found to be negative.

RESULTS

Gel filtration of porcine whey and serum. When porcine colostrum and milk whey were filtered on Sephadex G-200, four peaks were observed as was shown in Fig. 1 for milk whey collected late in lactation.

Immunoglobulins were identified in the various pooled and concentrated fractions by immunodiffusion and immunoelectrophoresis using monospecific antisera. IgM was generally detected in fractions throughout the first peak, but was most concentrated in fractions from the ascending portion of the first peak. IgA was eluted in fractions throughout the first peak, often as a shoulder on the first peak, and sometimes was also detected in the second peak fractions. It appeared to be most concentrated in fractions from the apex and descending portions of the first peak. IgG was detected in second peak fractions, in colostrum whey, and often in first peak fractions as well. The third peak fractions contained mainly albumin. The milk-specific proteins (they did not cross-react with anti-porcine serum) which were

eluted in fourth peak fractions were possibly alpha lactalbumin and beta lactoglobulin.

As reported by others, IgG, which is the predominant immunoglobulin in colostrum, decreases approximately 30-fold during the first week of lactation whereas the concentration of IgA decreases only two- to threefold, becoming the predominant immunoglobulin in porcine milk (7, 17). These findings were suggested in this study by the changes observed in the G-200 elution profiles of colostrum and milk. The second peak which contains predominantly IgG was the major peak eluted from colostrum whey and the minor peak eluted from late milk whey (Fig. 1). In all the 4- to 5-DPF milk whey samples studied, the first peak (which contains IgA and IgM) was the major protein peak eluted from G-200.

In this paper, only the first two peaks are shown in most of the G-200 chromatograms of porcine whey since the immunoglobulins and TGE antibody activity were detected only in these fractions.

Gel filtration of porcine serum on Sephadex G-200 resulted in the appearance of three protein peaks. Using immunodiffusion and immunoelectrophoresis, IgM was detected throughout the first peak fractions. IgA was generally present in fractions from the descending portion of the first peak and the ascending and top portion of the second peak. IgG was detected throughout the second peak and also sometimes in first peak fractions. Third peak fractions contained albumin and other serum proteins.

Purity and identification of isolated porcine immunoglobulins. When porcine IgM, IgA, and IgG (concentration of 5 to 12 mg of protein/ml) were tested by immunodiffusion and immunoelectrophoresis against rabbit anti-porcine serum and anti-colostral whey, single precipitin arcs were observed with each immunoglobulin preparation. On immunoelectrophoresis the porcine IgA and IgM migrated in the B₂ position and the IgG in the gamma position. Rabbit antisera prepared against each purified porcine immunoglobulin were adsorbed to remove anti-light chain reactivity as described, and then examined for heavy chain specificity by immunodiffusion and immunoelectrophoresis. As shown in Fig. 3, each antiserum developed only one precipitin arc which was characteristic for each class of immunoglobulin when tested against porcine serum or colostral whey. All three antisera failed to react with the nonhomologous classes of immunoglobulins (at concentrations of 10 mg/ml) when tested by immunodiffusion.

The identity of porcine IgM and IgA was further confirmed by their cross-reactions with

anti-human IgA or IgM antisera. When analyzed by immunoelectrophoresis, porcine IgM, but not IgA or IgG, showed a precipitin arc with anti-human IgM. Similarly, porcine IgA, but not IgM or IgG, revealed an arc of precipitation against anti-human IgA.

The porcine milk IgA isolated in this study was further characterized by Ouchterlony analysis to determine its relationship to porcine serum IgA. As shown in Fig. 4, the purified porcine milk IgA, colostral, and milk whey lines spurred over the porcine serum line when tested against anti-milk IgA, indicating the presence of additional antigenic determinants on the colostral and milk IgA. When examined by sucrose density ultracentrifugation, one preparation of milk IgA showed a single symmetrical peak with a sedimentation coefficient of 11.0 S. A second IgA preparation showed a peak sedimentation coefficient of 11.0 S with a shoulder which had a sedimentation coefficient of 9.55 S. Porcine IgG sedimented as a single peak with a sedimentation coefficient of 6.5 S.

Changes in TGE antibody titers after absorption

FIG. 3. Immunoelectrophoresis of porcine colostral whey (CW) and serum (PS) developed with porcine, heavy chain-specific anti-IgM, anti-IgA, and anti-IgG, and also anti-porcine serum (anti-PS). Anode to the left.

of samples with monospecific antisera. (i) *Experimental or natural infection.* In G-200 fractionated colostrum and milk whey from naturally or experimentally infected sows, the TGE antibody titers were highest in fractions from the apex and descending portions of the first peak, where IgA is most concentrated. Antibody activity was absent or low in second peak fractions where IgG is most concentrated. These results are illustrated by the G-200 chromatograms shown for colostrum and 5-DPF milk whey from a naturally infected sow, 255 (Fig. 5). Similar results were seen after gel filtration of milk or colostrum whey from experimentally infected sows (1a). When serum from experimentally or naturally infected sows was filtered on G-200, TGE antibody activity was highest in fractions from the ascending portion and top of the second peak, where both IgG and IgA were detected by immunodiffusion. This finding is illustrated by the G-200 chromatogram of serum from sow 76-10 shown in Fig. 6.

To confirm the immunoglobulin classes of TGE antibodies suggested by these observations, samples were absorbed with heavy chain-specific antisera to remove a particular class of immunoglobulin. The virus neutralization titers of these absorbed samples and saline controls are summarized in Table 1. When colostrum whey from the infected sow 255 was absorbed with anti-IgA, the TGE antibody titer was decreased at least 70-fold (to the limit of the dilution factor) compared to the control. However, absorption of this

sample with anti-IgG or anti-IgM did not significantly reduce the antibody titer. Absorption of colostrum whey samples from infected sows 83-1, 107, and 76-10 with anti-IgA reduced the antibody titers from about seven- to twofold. When these samples were absorbed with anti-IgG, two- to threefold reductions in antibody titers were observed. This suggests the association of antibody activity with both IgG and IgA, but predominantly with the latter in these colostrum samples. Absorption of 4- to 5-DPF milk whey samples from these sows (255, 107, and 76-10) with anti-IgA resulted in 72- to 6-fold decreases in antibody titers, all of which were reduced to the level of the final dilution factors. This significant reduction in antibody activity after absorption with anti-IgA is in good agreement with the observation that absorption with anti-IgG or anti-IgM produced little or no change in the antibody titers of these samples. As expected, similar results were seen when a G-200 milk whey fraction (sow 255, G-200 Fr. 4, Fig. 5), which contained TGE antibody activity and both IgG and IgA, was absorbed with anti-IgA and anti-IgG (Table 1). Following absorption of a serum sample from sow 76-10 with anti-IgA, only a slight decrease in titer was observed, while absorption with anti-IgG resulted in about a threefold reduction. Since both absorptions resulted in reductions in antibody titers, but neither anti-IgA nor anti-IgG reduced the antibody titer completely, this suggests that both IgG and IgA TGE antibodies were present.

(ii) *im and imm vaccination.* Results of gel filtration studies of colostrum and 4- to 7-DPF milk whey and serum from both im and imm vaccinated sows suggested that TGE antibody activity was mainly associated with second peak fractions which contained predominantly IgG. A typical G-200 elution profile of colostrum and milk whey from the imm vaccinated sow 179 is shown in Fig. 7. Also shown in this figure is the G-200 elution curve of 29-DPF milk whey which was collected 26 days after the exposure of this sow's piglets to TGE virus. Whereas TGE antibody activity was highest in second peak fractions from colostrum whey and 4-DPF milk whey, in the 29-DPF milk whey, the antibody activity was highest in fractions from the descending portion of the first peak. Similar results, with the primary localization of the TGE antibody activity in second peak fractions, were observed after gel chromatography of serum from im or imm vaccinated sows. This is exemplified by the G-200 elution curve of serum from the imm vaccinated sow 5-1 (Fig. 6). Gel filtration on G-200 of colostrum and milk whey and serum from im vaccinated sows resulted in similar curves with

FIG. 4. Immunodiffusion of porcine serum (PS), colostrum whey (CW), milk whey (MW), and purified porcine milk IgA with anti-porcine milk IgA (center well) showing spurring of milk and colostrum IgA lines over serum IgA lines.

FIG. 5. Gel filtration on Sephadex G-200 of colostral whey (a) and 5-day postfarrowing (DPF) milk whey (b) from a naturally infected sow (255). Vertical bars represent the TGE-neutralizing antibody titers of unconcentrated eluate from individual fractions (a) or concentrated (five times) pooled fractions (b). Immunoglobulins were identified in concentrated pooled fractions (five times) using monospecific antisera.

FIG. 6. Gel filtration on Sephadex G-200 of serum (2 ml) from an intramammarily vaccinated sow, 5-1 (a), and an experimentally infected sow, 76-10 (b). TGE-neutralizing antibody titers in individual unconcentrated fractions are indicated by vertical bars. Immunoglobulins were identified in these fractions by means of immunodiffusion using monospecific antisera.

the highest antibody titers in second peak fractions (1a).

The results of absorption studies of samples from these sows are also summarized in Table 1. In all cases, absorption of colostral whey, 4- to 7-DPF milk and serum samples from vaccinated sows (74-1, 5-1, and 179) with anti-IgG resulted in significant reductions in antibody titers ranging from 15- to 12-fold decreases compared to the controls. In all the samples from vaccinated

sows, except colostral whey from sow 179 and 4-DPF milk whey from the imm vaccinated sows 5-1 and 179, absorption with anti-IgG reduced the titer to the limit of the final dilution factor. When the samples from sows 74-1, 5-1, and 179 were absorbed with anti-IgA or anti-IgM (sow 5-1), no significant reductions in antibody titers occurred compared with the controls. Similarly, when a G-200 colostral whey fraction (Fig. 7a, sow 179, G-200 Fr. 1) from the descending portion of the first peak which contained both IgA

TABLE 1. Comparisons of the TGE-neutralizing antibody titers of paired samples after absorption with rabbit anti-porcine heavy chain-specific sera or saline

Sow no.	Route of exposure to TGE	Sample	Antibody titer ^a after absorption with					
			Anti-IgM	Saline control	Anti-IgA	Saline control	Anti-IgG	Saline control
255	Natural infection	Colostrum whey	4,080	3,840	<41	2,800	1,800	3,370
255	Natural infection	5-DPF ^b milk whey	820	840	<13.2	950	528	680
255	Natural infection	5-DPF milk whey			<2.5	50	31	34
		G-200 Fr. 4 ^c						
83-1	Natural infection	Colostrum whey			170	950	337	1,080
107	Exptl infection	Colostrum whey			87	690	385	880
107	Exptl infection	5-DPF milk whey			<12	444	279	315
76-10	Exptl infection	Colostrum whey			400	960	478	1,320
76-10	Exptl infection	4-DPF milk whey			<16	109	80	85
76-10	Exptl infection	4-DPF serum			76	116	39	133
74-1	im Vaccination ^b	Colostrum whey			10,320	8,370	<100	14,200
74-1	im Vaccination	7-DPF milk whey			86	100	<5.8	93
5-1	imm Vaccination ^b	Colostrum whey	15,600	14,820	15,400	12,250	<97.5	14,625
5-1	imm Vaccination	4-DPF milk whey	1,184	1,152	1,088	1,200	92	1,100
5-1	imm Vaccination	6-hr PF serum ^d			3,200	2,000	<52.5	2,835
179	imm Vaccination	Colostrum whey			41,400	60,750	510	52,500
179	imm Vaccination	Colostrum whey			17	15	4	27
		G-200 Fr. 1 ^e						
179	imm Vaccination	4-DPF milk whey			846	684	39	704
179	imm Vaccination	29-DPF milk whey			34	1,024	480	688
179	imm Vaccination	29-DPF serum			3,500	3,750	<105	3,570

^a Reciprocal of the dilution resulting in 80% plaque reduction.^b Abbreviations: DPF, day postfarrowing; im, intramuscular; imm, intramammary.^c See Fig. 5.^d See Fig. 6.^e See Fig. 7.

FIG. 7. Gel filtration on Sephadex G-200 of colostrum whey (a), 4-day postfarrowing (DPF) milk whey (b), and 29-DPF milk whey (c) from an intramammarily vaccinated sow. Vertical bars represent TGE-neutralizing antibody titers in individual unconcentrated fractions. Immunoglobulins were detected in these fractions by means of monospecific antisera.

and IgG was absorbed with anti-IgG, a seven-fold reduction in titer occurred. Absorption with anti-IgA, however, did not significantly reduce the titer. An exception to these findings was the

29-DPF milk whey from sow 179, which had been collected 26 days after exposure of this sow's piglets to virulent TGE virus. Both the piglets and sow developed clinical signs of TGE.

When this sample was absorbed with anti-IgG there was only a 1.4-fold decrease in titer, whereas absorption with anti-IgA resulted in a 30-fold reduction in titer.

DISCUSSION

The porcine immunoglobulins, IgG, IgA, and IgM isolated in this study had properties similar to those described in the literature. Porcine IgG had a sedimentation coefficient of 6.5 *S* which is consistent with the $S_{20, w}$ value of 6.7 *S* reported by Porter and Allen (18). As noted by others (7, 13), at least two subclasses of IgG were observed in this study, IgG₁ and IgG₂.

Identification of porcine IgM and porcine milk IgA was based, in part, on their cross-reactivity with anti-human IgM or IgA serum. The isolated porcine milk IgA had properties similar to those described by other investigators (3, 17, 19, 20). The heterogeneity in the molecular size of IgA in milk and colostrum was indicated by its elution throughout the first peak and often in second peak fractions on Sephadex G-200 (Fig. 1, 5). The isolated porcine milk IgA had a sedimentation coefficient of 11.0 *S* which agrees well with the $S_{20, w}$ value of 11.2 *S* reported for porcine colostrum IgA by Richardson and Kelleher (20). On sucrose density gradient ultracentrifugation, one preparation of milk IgA had a shoulder on the 11 *S* peak which had a sedimentation coefficient of 9.55 *S*. This is similar to the sedimentation coefficient of 9.3 *S* reported by Porter and Allen (18) for dimeric serum IgA which lacks the secretory component. Therefore, the 11.0 *S* IgA peak might represent dimeric IgA with secretory component and the 9.55 *S* shoulder could represent the presence of dimeric IgA lacking secretory component. While there are presently no reports in the literature describing the isolation of porcine secretory component, the possibility of its presence is further suggested by the observation that porcine milk and colostrum IgA possessed additional antigenic determinants not present on serum IgA (Fig. 4). Similar findings were reported by Bourne (5), but Richardson and Kelleher (20) found no unique antigenic determinants present on colostrum IgA compared to serum IgA.

The results of the absorption studies (Table 1) using monospecific antisera indicate that in the milk and colostrum from sows infected either naturally or experimentally with live TGE virus, the TGE antibody is associated predominantly with the IgA class of immunoglobulin, although IgG TGE antibodies also were evident in colostrum. In the serum from such animals, the TGE antibody appears to be associated with

both the IgA and IgG classes, but with more of the antibody activity in the IgG class.

In contrast, in the serum, colostrum and milk from sows vaccinated im or imm with live attenuated TGE virus, the TGE antibody is associated primarily, if not entirely, with the IgG class of immunoglobulin.

Such findings suggest that following im or imm vaccination, the attenuated virus primarily stimulates the peripheral lymphoid tissues resulting in the production of antibodies predominantly if not solely of the IgG class in serum, colostrum, and milk. This suggests that the IgG TGE antibodies found in colostrum and milk may originate primarily from the serum. The higher antibody titers observed in colostrum from the injected glands than from the noninjected glands, as previously reported (1a), may be the result of an inflammation in the injected glands which might permit an increased passage of circulating antibodies (which were of the IgG class) into the colostrum of the injected glands. An alternative explanation would be that some local synthesis of IgG occurred in the injected mammary glands.

The mechanism accounting for the predominance of IgA TGE antibodies in the milk and colostrum of naturally and experimentally infected swine is not known. Studies by Ogra (14) have demonstrated the highly localized nature of the secretory IgA antibody response in the intestinal tract. Local immunization of the colon with attenuated or inactivated poliovaccine produced a secretory IgA antibody response only in the immunized segment of the gut and not in the nasopharynx. However, in this study, the predominance of IgA TGE antibodies in colostrum and milk seems to be associated with an infection of the intestinal tract with the virulent TGE virus. In other studies (1, 6), to account for the extra-intestinal IgA observed after oral administration of certain antigens, it was proposed that intestinal IgA immunocompetent cells, after being stimulated by the antigen, emigrate from the gut to colonize distant lymphoid tissues. Migration of sensitized IgA immunocompetent cells from the intestine to the mammary gland might be one explanation for the predominance of IgA TGE antibodies observed in the milk and colostrum of infected sows as was suggested in our previous studies (1a, 2). An alternate explanation would be that orally administered live virus was carried by the circulation to the mammary gland where it stimulated local production of IgA TGE antibodies which were secreted into the colostrum and milk. However, the low antibody titers of the infected sow's serum (1a) suggests that this is a less-plausible explanation.

Another explanation which has been proposed for the origin of antibodies in colostrum and milk is their transport from the serum. In the bovine, IgG₁, the predominant immunoglobulin in colostrum and milk, appears to be selectively transported to the mammary gland from the serum (16). However, there is no evidence for a selective transport of IgA or IgG in the pig, and IgG₂ seems to be the predominant subclass of IgG in porcine serum, colostrum, and milk (7).

After exposure of an imm vaccinated sow (179) to live TGE virus through contact with her challenged piglets, the immunoglobulin class of antibodies in milk changed from the predominance of IgG TGE antibodies (4-DPF milk whey) to mainly IgA TGE antibodies (29-DPF milk whey). However, in the 29-DPF serum from this sow, the results of the absorption studies indicated that IgG TGE antibodies were present with little or no antibody activity in the IgA class. This suggests that synthesis of IgA TGE antibodies by plasma cells in the mammary gland, either of local origin or relocated from the intestinal tract, may be a more plausible explanation for the origin of IgA TGE antibodies in milk than transport from the serum

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LITERATURE CITED

1. Bazin, H., G. Levi, and G. Doria. 1970. Predominant contribution of IgA antibody-forming cells to an immune response detected in extraintestinal lymphoid tissues of germ-free mice exposed to antigen by the oral route. *J. Immunol.* 105:1049-1051.
- 1a. Bohl, E. H., R. K. Paul Gupta, M. V. F. Olquin, and L. J. Saif. 1972. Antibody responses in serum, colostrum, and milk of swine after infection or vaccination with transmissible gastroenteritis virus. *Infect. Immunity* 6:289-301.
2. Bohl, E. H., R. K. P. Gupta, L. W. McCloskey, and L. Saif. 1972. Immunology of transmissible gastroenteritis. *J. Amer. Vet., Med. Ass.* 160:543-549.
3. Bourne, F. J. 1969. IgA immunoglobulin from porcine milk. *Biochim. Biophys. Acta* 181:485-487.
4. Bourne, F. J. 1969. IgA immunoglobulin from porcine serum. *Biochem. Biophys. Res. Commun.* 36:138-145.
5. Cate, T. R., R. D. Rosen, R. G. Douglas, W. T. Butler, and R. B. Couch. 1966. The role of nasal secretion and serum antibody in the rhinovirus common cold. *Amer. J. Epidemiol.* 84:352-363.
6. Crabbe, P. A., D. R. Nash, H. Bazin, H. Eyssen, and J. F. Heremans. 1969. Antibodies of the IgA type in intestinal plasma cells of germ-free mice after oral or parenteral immunization with ferritin. *J. Exp. Med.* 130:723-738.
7. Curtis, J., and F. J. Bourne. 1971. Immunoglobulin quantitation in sow serum, colostrum and milk and the serum of young pigs. *Biochim. Biophys. Acta* 236:319-332.
8. Haelterman, E. O. 1963. Transmissible gastroenteritis of swine. *Proc. 17th World Vet. Cong.* 1:615-618.
9. Hooper, B. E., and E. O. Haelterman. 1966. Concepts of pathogenesis and passive immunity in transmissible gastroenteritis of swine. *J. Amer. Vet. Med. Ass.* 149:1580-1586.
10. Kim, Y. B., S. G. Bradley, and D. W. Watson. 1966. Ontogeny of the immune response. I. Development of immunoglobulins in germfree and conventional colostrum-deprived piglets. *J. Immunol.* 97:52-63.
11. Mach, J. P., and J. J. Pahud. 1971. Secretory IgA, a major immunoglobulin in most bovine external secretions. *J. Immunol.* 106:552-563.
12. Martin, R. G., and B. N. Ames. 1961. A method for determining the sedimentation behavior of enzymes: application to protein mixtures. *J. Biol. Chem.* 236:1372-1379.
13. Metzger, J. J., and M. Fougereau. 1968. Caracterisation biochimique des immunoglobulines γ G et γ M porcines. *Rech. Vet.* 1:37-61.
14. Ogra, P. L. 1971. The secretory immunoglobulin system of the gastrointestinal tract, p. 259-279. In *The secretory immunologic system*. U. S. Government Printing Office, Washington, D.C.
15. Ogra, P. L., and D. T. Karzon. 1969. Poliovirus antibody response in serum and nasal secretions following intranasal inoculation. *J. Immunol.* 102:15-18.
16. Pierce, A. E., and A. Feinstein. 1965. Biophysical and immunological studies on bovine immune globulins with evidence for selective transport within the mammary gland from maternal plasma to colostrum. *Immunology* 8:106.
17. Porter, P. 1969. Transfer of immunoglobulins IgG, IgA and IgM to lacteal secretions in the parturient sow and their absorption by the neonatal piglet. *Biochim. Biophys. Acta* 181:391-392.
18. Porter, P., and W. D. Allen. 1972. Classes of immunoglobulins related to immunity in the pig. *J. Amer. Vet. Med. Ass.* 160:511-517.
19. Porter, P., D. E. Noakes, and W. D. Allen. 1970. Secretory IgA and antibodies to *Escherichia coli* in porcine colostrum and milk and their significance in the alimentary tract of the young pig. *Immunology* 18:245-257.
20. Richardson, A. K., and P. C. Kelleher. 1970. The 11S-sow colostrum immunoglobulin isolation and physicochemical properties. *Biochim. Biophys. Acta* 214:117-124.
21. Scheidegger, J. J. 1955. Une micromethode d'immunoelectrophorese. *Int. Arch. Allergy Appl. Immunol.* 7:103-110.
22. Smith, C. B., R. H. Purcell, J. A. Bellanti, and R. M. Chanock. 1966. Protective effect of antibody to parainfluenza type I virus. *N. Engl. J. Med.* 275:1145-1152.
23. Stertzl, J., J. Kostka, I. Riha, and L. Mandel. 1960. Attempts to determine the formation and character of γ -globulin and of natural and immune antibodies in young pigs reared without colostrum. *Folia Microbiol.* 5:29-40.
24. Vaerman, J. P., J. P. Arbuckle, and J. F. Heremans. 1970. Immunoglobulin A in the pig. II. Sow's colostrum and milk IgA-quantitative studies and molecular size estimation. *Int. Arch. Allergy* 39:323-333.
25. Vaerman, J. P., and J. F. Heremans. 1970. Immunoglobulin A in the pig. I. Preliminary characterization of normal pig serum IgA. *Int. Arch. Allergy* 38:561-572.
26. Wadsworth, C. A. 1957. A slide microtechnique for the analysis of immune precipitates in gel. *Int. Arch. Allergy Appl. Immunol.* 10:355-360.
27. Waldman, R. H., J. J. Mann, and P. A. Small. 1969. Immunization against influenza: prevention of illness in man by aerosolized inactivated vaccine. *J. Amer. Med. Ass.* 207:520-541.