Protective Effect of Immunoglobulins in Serum and Milk of Sows Exposed to Transmissible Gastroenteritis Virus

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ABSTRACT

Experimental exposure of susceptible pregnant sows by various routes to the gut-origin transmissible gastroenteritis virus stimulated production of milk and serum antibodies. These antibodies neutralized the cytopathic effect of transmissible gastroenteritis virus propagated in cell culture. This in vitro neutralizing antibody resided in the IgG and IgA immunoglobulin classes. On the other hand, protection for baby pigs resided in the IgA class of milk immunoglobulin of sows exposed orally or intramammarily but not of sows exposed intramuscularly to the virus.

RÉSUMÉ

L'injection d'une souche intestinale du virus de la gastro-entérite transmissible, par diverses routes, à des truies gestantes et susceptibles, suscita l'élaboration d'anticorps dans leur lait et leur sérum. Ces anticorps neutralisèrent l'effet cytopathogène du virus, sur cultures cellulaires, et se situaient dans les immunoglobulines IgG et IgA. Par ailleurs, la protection des porcelets résultait de la présence d'immunoglobulines IgA dans le lait des truies infectées par la voie buccale ou intra-mammaire; ces immunoglobulines ne se retrouvaient pas dans le lait des truies infectées par la voie intra-musculaire.

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INTRODUCTION

Transmissible gastroenteritis (TGE) is a viral disease of swine characterized by vomition, profuse diarrhea and dehydration with mortality up to 100% among pigs less than one week old. Protection of baby pigs against TGE virus depends on the transfer of passive immunity from the dam to the pigs. Pregnant sows recovered from natural infection (3, 8) or exposed orally to purified virulent TGE virus (15) develop adequate immunity to the virus and usually their baby pigs are protected against TGE. This protection persists only so long as the pigs receive antibody containing colostrum (7).

Although systemic active immunity can be induced in baby pigs (9, 10) it is of little value in protection against TGE. Moreover, recent studies of local immunity in baby pig small intestines demonstrated that this immune system commenced only after the first week of life (12). However, the susceptibility of newborn pigs to TGE and the rapid replication of the virus in the intestine of baby pigs offers little time for activation and expression of immune responses. Thus, major research efforts have been focused on inducing anti-TGE immunity in pregnant sows, which in turn would transfer it passively to their newborn litters.

The importance of the IgA secretory antibody system in protection of mucous membranes has been demonstrated in a number of studies (5, 11, 13, 17). Its possible role in protection of baby pigs against TGE has been suggested in studies by Bohl *et al* (4). Recently we briefly reported some of the characteristics of the protective antibodies to TGE virus in serum and milk of sows exposed to the

virus (1,2,16). This report will further elaborate on such studies and will indicate the importance of TGE antibodies of the IgA class in milk of immune sows for passive protection of baby pigs.

MATERIALS AND METHODS

Experimental Animals

Three susceptible pregnant sows were exposed to the virulent TGE virus (I11-isolant) of gut origin by oral, intramammary or intramuscular route. The orally exposed sow was given one dose of 10 x 10^6 LD-50 of the virus at five weeks before farrowing. The sows injected intramammarily or intramuscularly were each given two injections of 10 x 10^6 LD-50 at five and three weeks before farrowing. Blood serum and milk samples were collected after farrowing.

Measurement of Anti-TGE Antibody Activity

Viral neutralization tests were performed with various dilutions of whey and serum (the latter being inactivated at 56°C for 30 minutes). One volume of cell culture fluid, containing 100 cell culture ID-50 of TGE virus (Miller 108-isolant) per 0.1 ml, was added to an equal volume of the diluted whey or serum. The mixtures were incubated at 37°C for one hour. Primary pig kidney cell cultures were inoculated with 0.2 ml of the mixture. The highest serum or whey dilution which prevented development of cytopathic effect (CPE) was designated as the antibody titer.

Gel Filtration Chromatography-Isolation and Identification of Immunoglobulin Classes

Chromatography was carried out according to procedures described earlier (6). A known quantity of protein contained in the serum and in the whey portion of the colostrum and milk was fractionated by Sephadex G-200 column chromatography and 5 ml fractions were collected. Phosphate buffer saline (PBS) at pH 7.5 and at a flow rate of 15 ml/hr was used. Selected protein peaks were collected and concentrated by Diaflo ultrafiltration. Concentrated fractions were then processed through a diethylaminoethyl cellulose (DEAE) column. Elution was accomplished by following stepwise changes of phosphate molarity at pH 7.5: 0.01, 0.2, 0.05, 0.075, 0.125 and 0.2 M.

Immunoglobulin classes IgM, IgA, and IgG contained in eluates obtained by Sephadex G-200 and by DEAE column chromatography were identified by precipitation in gel using specific antisera kindly provided by Dr. Philip Porter.[1] These purified immunoglobulins were also used to produce monospecific antiserum to swine immunoglobulin classes in rabbits. The purity and quantity of each of the immunoglobulins were determined by immunoelectrophoresis and radial-immunodiffusion, respectively.

Neutralization of TGE Virus with Immunoglobulin of Different Classes

Fractions containing IgM, IgA, or IgG as the major component obtained from whey and serum were used to neutralize TGE virus *in vitro* as described above.

Protection of Three Day Old Pigs with Immunoglobulin of Different Classes

A quantum of virulent TGE virus of gut origin containing 100 baby pig ID-50 was mixed with an equal volume of the different immunoglobulins obtained from whey and serum. After incubation for one hour at 37°C, three day old pigs were used to assay protective antibody. The mixture of virus and immunoglobulin was given orally to three day old pigs kept in individual isolation units.

RESULTS

Susceptible pregnant sows exposed to the virulent gut origin TGE virus by the oral route at five weeks, and the intramammary and intramuscular routes at five and three weeks before farrowing produced antibodies against the virus. At farrowing these antibodies were demonstrated in the milk and serum of exposed sows by the *in vitro* neutralization test using cell culture TGE virus (Table I). In the milk of the sow orally exposed to the virus, the antibody detected by the *in vitro* neutralization test was distributed between the IgA and the IgG fractions (Table II and Fig. 1). Analysis

[1]Unilever Research Laboratory Shainbrook, England.

TABLE I. In vitro neutralization of cell culture TGE virus by serum and milk of sows exposed to virulent TGE virus of gut origin

Route of Exposure to TGE	Sample Tested	Neutralizing Titer
Oral Exposure	Serum	1:320
	Milk	1:640
Intramammary	Serum	1:40
	Milk	1:160
Intramuscular	Serum	1:40
	Milk	1:20

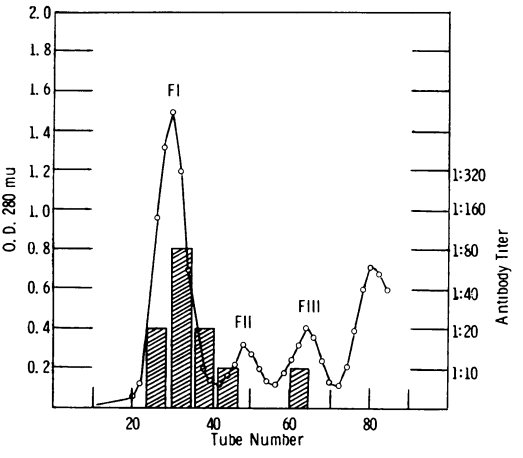

Fig. 1. Results of in vitro neutralization of TGE virus by milk whey eluates of sow exposed orally to TGE virus of gut origin. IgM and IgA were found in (fraction) F1; F11 contained IgA, and F111 contained the IgA immunoglobulin.

of the serum profile of this sow showed that the preponderance of *in vitro* neutralizing antibody was in the IgG fraction (Table II and Fig. 2).

Analysis of the immunoglobulin profile of milk from sows injected intramammarily showed that most of the *in vitro* neutralizing antibody resided in the IgA fraction and a small amount of the neutralizing antibody was in the IgG fraction (Table III and Fig. 3). The antiviral activity in the serum of these sows was found in the IgG fraction (Table III). All of the *in vitro* neutralizing antibody in the milk and serum of sows injected intramuscularly was confined to the IgG class of immunoglobulins (Table IV).

Results of baby pig protection by different immunoglobulin classes from these sows, after *in vitro* neutralizing of TGE virus, are summarized in Tables II, III and IV. It is evident that most of the baby pig protective effects resided in the IgA fraction of milk from sows exposed orally or intramammarily to the virulent TGE virus.

DISCUSSION

The present study demonstrated a great deal of variations in anti-TGE activity in milk and serum of sows exposed to TGE virus of gut origin by oral, intramammary or intramuscular routes. While oral and intramammary injection of the virus produced a high titer of protective antibody in the milk of exposed sows, the production of protective antibody was negligible in both milk and serum of the sow receiving the intramuscular injection.

TABLE II. In vitro neutralization of TGE virus (assay in baby pigs) by serum and milk immunoglobulins of sows exposed orally to TGE virus of gut origin

Sample	Immunoglobulin in Eluates[a]	Titer[b]	No. of Pigs with TGE / No. of Pigs Exposed	Time Signs of TGE Developed after Exposure
Serum.....	IgG	1:20	2/2	72 hours
	IgA	1:10	2/2	24 hours
	IgM	—[c]	2/2	24 hours
Milk.....	IgG	1:10	2/2	72 hours
	IgA	1:80	0/2	
	IgM	—	2/2	24 hours
Control.....			2/2	18-24 hours

[a]Immunoglobulin fraction was incubated for one hour at 37°C with equal volume of TGE virus of gut origin having 100 baby pig LD-50 titer, and then administered orally into two three-day-old baby pigs
[b]*In vitro* neutralizing titer against 10^2 CPE ID_{50} of cell culture propagated TGE virus
[c]— No neutralizing activity in undiluted serum or milk

TABLE III. In vitro neutralization of TGE virus (assay in baby pigs) by serum and milk immunoglobulins of sows exposed intramammarily to TGE virus of gut origin

Sample	Immunoglobulin in Eluates[a]	Titer[b]	No. of Pigs with TGE / No. of Pigs Exposed	Time Signs of TGE Developed after Exposure
Serum.....	IgG	1:40	2/2	72 hours
	IgA	—[c]	2/2	24 hours
	IgM	—	2/2	24 hours
Milk.....	IgG	1:20	2/2	48 hours
	IgA	1:80	0/2	
	IgM	—	2/2	18-24 hours
Control.....			2/2	18-24 hours

[a]Immunoglubilin fraction was incubated for 1 hour at 37°C with equal volume of TGE virus of gut origin having 100 baby pig LD-50 titer, and then administered orally into two three-day-old baby pigs
[b]*In vitro* neutralizing titer against 10^2 CPE ID-50 of cell culture propagated TGE virus
[c]—No neutralizing activity of undiluted serum or milk

TABLE IV. In vitro neutralization of TGE virus (assay in baby pigs) by serum and milk immunoglobulins of sows exposed intramuscularly to TGE virus of gut origin

Sample	Immunoglobulin in Eluates[a]	Titer[b]	No. of Pigs with TGE / No. of Pigs Exposed	Time Signs of TGE Developed after Exposure
Serum.....	IgG	1:20	2/2	72 hours
	IgA	—[c]	2/2	24 hours
	IgM	—	2/2	24 hours
Milk.....	IgG	1:10	2/2	48 hours
	IgA	—	2/2	24 hours
	IgM	—	2/2	24 hours
Control.....			2/2	18-48 hours

[a]Immunoglobulin fraction was ncubated for 1 hour at 37°C with equal volume of TGE virus of gut origin having 100 baby pig LD-50 titer, and then administered orally into two-three-day-old baby pigs
[b]*In vitro* neutralizing titer against 10^2 CPE ID-50 of cell culture propagated TGE virus
[c]—No neutralizing activities of undiluted serum or milk

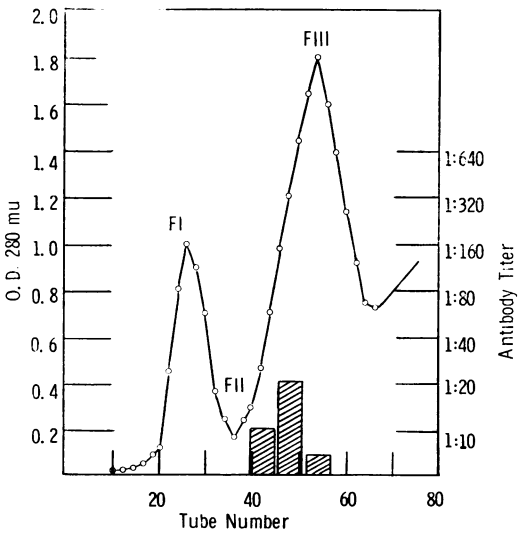

Fig. 2. Results of in vitro neutralization of TGE virus by serum eluates of sow exposed orally to TGE virus of gut origin. IgM was found in (fraction) F1, IgA was found in the valley between F1 and F11, and IgG was found in F111.

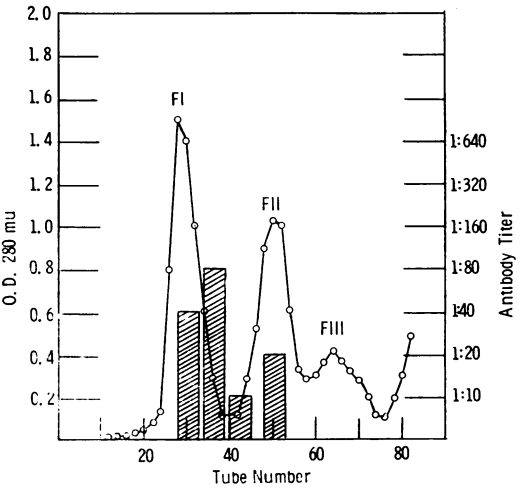

Fig. 3. Results of in vitro neutralization of TGE virus by milk whey eluates of sow exposed to TGE virus of gut origin by intramammary injection. IgM and IgA were found in (fraction) FI, and IgG was found in F11.

The existence of the three major immunoglobulins (IgM, IgA, and IgG) in swine serum and milk is well established (6, 12, 14, 18). Immunoglobulin IgA has proved to be the major immunoglobulin in most exocrine secretions in the pig, including milk, saliva, and intestinal juice (6). The role of IgA in milk is probably to provide adequate levels of immunity in the gut of the young pig until its own intestinal secretory immunoglobulin system is fully developed.

Recently, Bohl *et al* (4) have reported that sows exposed orally to TGE virus, either naturally or experimentally, protected their pigs and they suggested the protection was due to IgA antibody. The same authors have failed to produce protective antibody when they vaccinated sows intramuscularly or intramammarily with live attenuated TGE virus. These results are in agreement with our previous observations that oral and intramammary exposure of pregnant sows to live virulent TGE virus resulted in the development of protective antibody (1, 16). The results of this study give additional data indicating that the protective properties of the milk of sows exposed to TGE virus resided in the IgA immunoglobulin class.

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