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ANTIGENICITY OF STRUCTURAL COMPONENTS FROM PORCINE TRANSMISSIBLE GASTROENTERITIS VIRUS

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ABSTRACT

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Pregnant sows were inoculated with inactivated transmissible gastroenteritis virus and with preparations of virus surface projections and subviral particles derived by detergent treatment of the virus. Neutralising antibody was demonstrated in serum and colostrum from animals that received whole virus or preparations of surface projections whereas subviral particles failed to stimulate neutralising antibody formation. Similar results were obtained with serum from rabbits inoculated with whole virus and structural components. All three preparations stimulated the formation of agglutinating antibodies, as demonstrated by sedimentation analysis and filtration studies with radiolabelled virus.

The immunoglobulin classes responsible for neutralising antibody activity in sows inoculated by the intramammary route were examined. In each case where the immunoglobulin class was determined, IgG was found. One sow that received surface projections also had IgA with neutralising activity in her colostrum. In contrast, infection of sows with live whole virus resulted in neutralising antibody of the IgG, IgM and IgA classes.

INTRODUCTION

Infection of pigs with the coronavirus causing transmissible gastroenteritis (TGEV) results in disease that is most severe, frequently fatal, in young animals and of less severity in animals over 3 weeks old. Following recovery of the animals from infection, neutralising antibody can be detected in serum and secretions; transfer of this antibody, predominantly IgA, in the colostrum and milk from a convalescent dam to her offspring confers reasonable protection against disease in her piglets (Bohl et al., 1972 b; Saif et al., 1972; Stone et al., 1974).

Parenteral administration of inactivated virus stimulates circulating antibody of the IgG class (Bohl et al., 1972 a; Saif et al., 1972; Lucas et al.,

1974) but, as little or no specific IgA antibody is formed, the low level of IgG in the milk affords little protection to newborn piglets during the period that they are most susceptible. Work is in progress to investigate how an IgA response can be stimulated by inactivated virus but more information is required about the nature of the viral antigen.

We have previously demonstrated that inactivated purified TGEV is capable of inducing a primary neutralising antibody response in pigs (Garwes and Pocock, 1975 a), suggesting that a virus structural element is the antigen responsible for stimulating the neutralising antibody. There is evidence, however, that at least one soluble component from TGEV-infected swine testis cells can stimulate neutralising antibody production (Stone et al., 1976 a). Following development of techniques to separate and purify the surface profections (SP) from the internal proteins of a subviral particle (SVP) (Garwes et al., 1976) it has been possible to determine which of these components are involved in the neutralising antibody response.

This report describes the immune response in swine and rabbits inoculated with inactivated TGEV and purified subviral components.

MATERIALS AND METHODS

Virus cultivation

Antigen preparation

The FS772/70 isolate of TGEV was used after six passes through gnotobiotic piglets aged 2—5 days. A 10% (*w/v*) suspension of infected small intestine was clarified by centrifugation at 15 000 × *g* for 20 min and was then inoculated onto secondary pig thyroid (APT/2) cells as monolayer cultures in medium 199 containing galactose (Pocock and Garwes, 1975). Virus was harvested following freezing and thawing the cultures at 18 h after infection and was purified as previously described (Garwes and Pocock, 1975 b). Isolation of purified SP and SVP antigen preparations was achieved by differential centrifugation after treatment of purified virus with 1% Nonidet-P40 (Garwes et al., 1976). Each antigen preparation (whole virus, SP and/or SVP) was diluted 1 : 5 with growth medium, formaldehyde was added to 0.05% and the preparations were held at 37° C for 1 h. Following this treatment, no infectivity could be detected in any of the samples. The preparations were stored at 4° C until used. Each preparation of antigen for immunisation studies was derived from 2—5 × 10^9 pfu (sows) or 2 × 10^8 pfu (rabbits) of TGEV.

Radioactive virus production

Virus used for radio-immune assay was prepared from APT/2 cells infected with TGEV FS772/70 passaged 132 times through primary pig kidney cells. The medium contained ^{3}H-uridine (specific activity 26 Ci/mmole; The Radiochemical Centre, Amersham) at 10 μCi/ml and the virus

was harvested 18 h after infection and purified as described (Garwes and Pocock, 1975 b). Infectivity was titrated by plaque assay.

Antibody detection

Virus neutralisation test

Sera, colostra and milk samples were tested as previously described (Cartwright, 1966).

Sedimentation analysis

Purified TGEV that had been grown in the presence of ^{3}H-uridine was mixed with equal volumes of sera or 2% bovine serum albumin, and incubated at 37° C for 1 h. A sample was removed for titration of infectivity and the remainder was layered on to a linear 15—30% (*w/w*) sucrose gradient and centrifuged at 70 000 × *g* for 1 h. The gradient was fractionated by siphon into aliquots, material that had sedimented to the bottom of the tube was resuspended in distilled water and a sample was removed from each fraction for determination of radioactivity using a toluene-Triton X 100 based scintillant.

Filtration studies

Measurement of virus agglutination by antibody was accomplished by incubating 50 µl samples of ^{3}H-uridine labelled TGEV with equal volumes of test sera diluted in isotonic saline at 37° C for 2 h. To each reaction mixture was then added 100 µl of rabbit anti-porcine IgG serum (Miles Laboratories Ltd., Stoke Poges) and incubation continued for 1 h at 37° C followed by storage overnight at 4° C. The samples were passed through cellulose acetate membrane filters of 200 nm mean pore diameter (type EG, Millipore (U.K.) Ltd., London), the filters were washed with four changes of 2.5 ml distilled water and their radioactivity was determined by liquid scintillation. Earlier attempts to use membrane filters composed of mixed esters of cellulose (MF filters, Millipore (U.K.) Ltd., London) were not successful as purified TGEV is bound irreversibly to them (unpublished observations). This problem was overcome by using the cellulose acetate membranes to which the virus did not bind.

Immunoglobulin class of antibodies

Solutions of serum IgM and IgG and colostral IgA were obtained by gel filtration and ion-exchange chromatography (Porter, 1969; Vaerman and Heremans, 1970). Antisera were raised in rabbits by injection of precipitin lines made by immunoelectrophoresis of the immunoglobulin solutions against rabbit antiserum to pig serum (Goudie et al., 1966; Higgins, 1976). Sera were made class specific by absorption with glutaraldehyde cross-linked

immunoglobulin solutions (Avrameas and Ternynck, 1969). Specificity was assessed in immunoelectrophoresis and immunodiffusion tests against serum, colostrum and immunoglobulin solutions.

Sera and colostral wheys were examined by gel filtration in Sephadex G-200 (Pharmacia) in 0.1 M tris-HCl, pH 8.0, containing 1.0 M NaCl and 1 mM EDTA. Wheys were also examined by ion-exchange chromatography on DEAE-cellulose (DE-11, Whatman), using the six step buffer system described by Stone et al. (1976 b). The eluate obtained with each buffer was treated as a single fraction. Presence of IgM, IgG and IgA in the fractions was observed by microimmunodiffusion against specific antisera. Fractions were concentrated about five-fold by dialysis against polyvinylpyrrolidone and examined for neutralising antibodies to TGEV. The allocation of antibody activity to an immunoglobulin class was made by comparison of the distribution of antibody activity and immunoglobulins in the fractions.

Immunisation

Sows

Twelve pregnant sows were inoculated by the intramammary route: one with live, whole virus, three with formalin-inactivated virus, two with a mixture of purified SP and SVP, four with SP alone and two with SVP only. In addition one sow was given live virus orally and two sows were kept as uninoculated controls.

For the intramammary inoculations, 0.5 ml of the antigen preparation was injected through the skin near the teat into three mammary glands of each sow three times. The same glands were inoculated at approximately 5, 3 and 1 week before farrowing. For the first inoculation, the antigen was emulsified in complete Freund's adjuvant.

Colostrum samples were taken from inoculated and uninoculated glands separately at farrowing and milk samples were taken for several days after that. Blood samples were removed before the first inoculation and at the intervals shown in Table I.

Rabbits

Three rabbits were inoculated with TGEV antigen: one with inactivated whole virus, one with SP and one with SVP. Each rabbit received two subcutaneous inoculations of antigen emulsified in complete Freund's adjuvant at an interval of 2 weeks, followed by three intramuscular inoculations of aqueous antigen at weekly intervals. One rabbit was kept as an uninoculated control.

Blood samples were taken from each animal before the first inoculation and at intervals up to 3 months.

TABLE I

Serological response of sows to inoculation with TGE whole virus and viral components

Virus preparation	Route	Sow no.	Piglet mortality (%)	Serum titres[a]							Colostrum			
				Weeks before farr.			Farr.	Weeks after farr.			Days after farr.			
				5	3	1	0	1	2	3	0	1	3	4
Whole live virus	p/o	1	80	—	20	80	320		160	80	640		80	
Whole live virus	i/mam	2	89		—		80	160			320			
Whole virus killed	i/mam	3	80	—	—	80	40	80	320	320	80			—
		4	62.5	—	—	80	40	80	1280	320	320			—
		5	100	—	—	40	40	20	160	160	60		10	
SP + SVP	i/mam	6	100	—	—	—	—	—	160	640	—			—
		7	42.5	—	—	20	80	20	40	40	20			
SP	i/mam	8	100	—	—	—	—	20	40	160	40	10		
		9	0	—	—	80	80	160	320	160	320			—
		10	100	—	—	—	—	10	1280	80	80			
		11	100	—	—	—	—	—	640	160	10			
SVP	i/mam	12	100	—	—	—	—	—	20	10	—	—		—
		13	100	—	—	—	—	20	40	40	—		—	
None		14	100				—	—	20	40	---			
		15	100				—	—	20	40	—			

[a]Expressed as the reciprocal of the higest dilution capable of neutralising 100 $TCID_{50}$ of virus.
p/o = orally; i/mam = by the intramammary route.
— = not detected.
Those not tested are indicated by blank spaces.

Challenge of piglets

Piglets born to the experimental sows were challenged at 2 days of age by oral administration of 2 ml of a suspension of TGEV-infected intestine diluted to contain approximately 5×10^2 $TCID_{50}$ per ml (equivalent to approximately 2×10^3 LD_{50} per ml in piglets reared away from the sows). The suspension was free, as far as it was possible to determine, from moulds, bacteria and other viruses.

RESULTS

Sow inoculation

The results of neutralisation tests for serum and colostrum are shown in Table I. After intramammary inoculation of sows, antibody was detected at the time of farrowing in serum and colostrum from all of three sows given whole killed virus, from 1 out of 2 given surface projections with subviral particles, from 1 out of 4 given surface projections only and from neither of the animals given subviral particles alone. Neutralising antibody in colostrum only was detected in 3 out of the 4 given purified surface projections. There was little or no difference between antibody titres of colostrum from inoculated and uninoculated glands.

The levels of neutralising antibody in milk from sows inoculated with inactivated whole virus or viral components fell to undetectable levels between 3 to 5 days after farrowing.

Neutralising antibody titres in sera of all sows, including the uninoculated controls, rose during the first 2 weeks after farrowing. This reflects the immune response to infection of the dam by live virus administered to her piglets 2 days after farrowing.

Analysis of immunoglobulin class of antibodies

The results are shown in Table II. The immune response to infection with live virus (sow no. 1) resulted in neutralising antibodies in the three immunoglobulin classes reported previously (Ristic and Abou-Youssef, 1972; Bohl et al., 1972 b; Saif et al., 1972; Lucas et al., 1974) although in the example given IgG antibody was detected in serum but not in colostrum.

Samples from sows that received intramammary inoculation contained neutralising antibodies of the IgG class in all cases where the specific imunoglobulin could be characterised. No IgM antibodies were detected in any sample and IgA antibodies were demonstrated in the colostrum from only 1 out of 4 sows that received SP antigen.

Piglet challenge

The only litter that survived challenge was that of sow no. 9 which had

TABLE II

Immunoglobulin classes represented in the antibody response

Virus preparation	Route of inoculation	Sow no.	Immunoglobulin[a] in	
			Serum	Colostrum
Whole live	p/o	1	MAG[b]	MA
Whole killed	i/mam	3	G	G
		4	G	G
		5	—	G
SP+ SVP	i/mam	7	G	—
SP	i/mam	8	G	G
		9	G	AG

[a]Serum and colostrum taken at farrowing.
[b]M, A and G refer to immunoglobulin classes IgM, IgA and IgG respectively.
— Denotes no immunoglobulin detected.

been inoculated with SP antigen and had produced neutralising IgA antibodies in the colostrum. The other litters experiences 40—100% mortality and all piglets from the control uninoculated sows died.

Rabbit inoculation

Serum samples taken from the rabbit that had been inoculated with inactivated whole TGEV showed neutralising antibodies rising to a maximum titre of 1/640. Neutralising antibodies were detected also in the serum of the rabbit that received SP antigen; the rate of antibody production was slower, however, and the peak titre reached was 1/160. No neutralising antibody was found in serum from the rabbits inoculated with SVP antigen and the uninoculated animal remained seronegative throughout the course of the experiment.

Detection of antibodies by radioimmune assay

Sedimentation analysis

The sedimentation profiles of ^{3}H-uridine TGEV after incubation with bovine serum albumin or with sera from sows that had been inoculated with viral antigen preparations are illustrated in Fig. 1.

Virus incubated with either bovine albumin or normal pig serum sedimented as a sharp, homogenous band of radioactivity with very little material collecting at the bottom of the gradient. In contrast, however, virus incubated with sera from animals inoculated with viral antigen moved through the gradient as a broad band, suggesting heterogeneity of size and density, with a large proportion of the total radioactivity sedimented at the

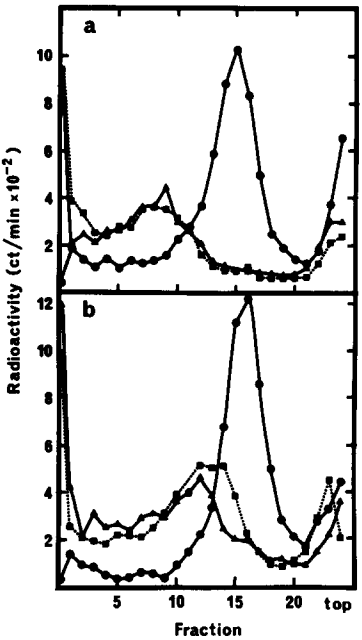

Fig. 1. Sedimentation analysis of TGEV. Purified preparations of ^{3}H-uridine labelled TGEV were incubated for 1 h at 37° C in the presence of pig serum or 1% BSA. The samples were then layered over linear 15—30% sucrose gradients and centrifuged at 70 000 × *g* for 1 h. After fractionation the bottom of each tube was washed with water to resuspend any pelleted material and samples were taken for radioactivity determination. Sedimentation was from right to left. The profiles illustrated are from virus incubation with:

(a) Serum from an uninoculated sow ●———●, sow no. 1 ■------■ and sow no. 4 ▲———▲.

(b) Serum from sow no. 9 ■------■, sow no. 13 ▲———▲ and 1% BSA ●———●.

For sow numbers, see Table I.

bottom of the tube. There were no obvious differences between the profiles obtained after treatment of virus with sera that contained neutralising antibodies (sows no. 1, 4 and 9) or with serum from an animal that received SVP antigen and which produced no neutralising antibodies (sow no. 13).

Filtration of immune complexes

Incubation of ^{3}H-uridine TGEV with sera from sows inoculated with viral antigen resulted in the formation of immune complexes that were retained by membrane filters (Fig. 2). After incubation with serum from uninoculated animals approximately 10% of the virus was retained on the filter, probably due to aggregation that occurs in purified TGEV preparations. Sera from sows inoculated with whole virus, SP antigen or SVP antigen preparations produced virus complexes that were retained most efficient

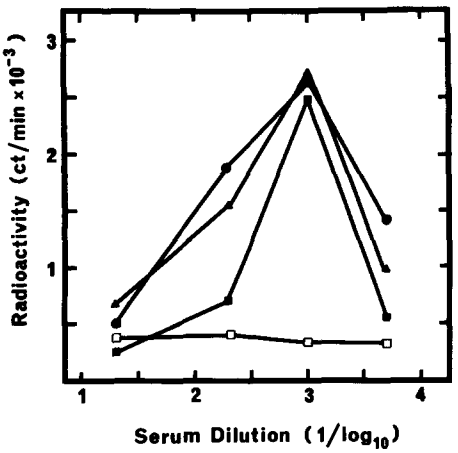

Fig. 2. Retention of TGEV by cellulose acetate filters. Samples of ^{3}H-uridine labelled TGEV containing 4000 ct/min were incubated for 2 h at 37° C in dilutions of test sera followed by incubation with rabbit anti-porcine IgG serum. After filtration through cellulose acetate membrane filters and repeated washing with isotonic saline, the retained radioactivity was determined by scintillation counting. Test sera from: sow no. 4 ▲———▲, sow no. 9 ●———●, sow no. 13 ■———■, uninoculated sow □———□.

ly at a serum dilution of 1/1000. At higher serum dilutions there was reduced retention, implying that the level of antibodies was limiting. Serum dilutions lower than 1/1000 gave suboptimal retention due, probably, to an excess of antibodies and the formation of smaller complexes. Omission of the rabbit anti-porcine IgG serum from the test resulted in poorer retention of radioactive virus at all dilutions of experimental serum and a decline in the reproducibility of the test.

DISCUSSION

We report the immune response of sows and rabbits to TGEV preparations containing whole virus, isolated surface projections or subviral particles produced by removal of the surface projections and envelope lipid from the virus by detergent treatment.

Sows receiving whole virus, isolated SP antigen and mixtures of SP and subviral particles produced antibody capable of neutralising virus infectivity; sows that received subviral particles alone, however, did not. Similarly rabbits immunised with purified whole virus or surface projections produced neutralising antibody to TGEV while administration of subviral particles produced no detectable neutralising activity. These data suggest that neutralising antibody raised during infection of swine with TGEV is directed against a structural antigen of the virion and that this antigen is associated with the surface projection, a similar finding to that obtained with other lipid enveloped viruses (Webster and Laver, 1966; Cartwright et al., 1969;

Jennings et al., 1974; Hunsmann et al., 1975). The fact that no neutralising antibody was produced in the pigs and rabbits that received subviral particles alone strongly suggests that the structural elements of these particles are not involved in virus neutralisation; conclusive proof of this, however, would necessitate the use of more animals than were available in the present study. The possibility existed that the TGEV subviral particles were not antigenic in the animals used. This seemed unlikely as we had previously shown that these structures of 60 nm diameter, readily visualised by electron microscopy, contained all the structural proteins except for the sulphated glycoprotein of the SP (Garwes et al., 1976). Standard serological tests, such as complement fixation and radial immunodiffusion, are not sensitive enough to detect the levels of antibodies present in the experimental sera. The radioimmune assay techniques described above indicated, however, that antibody directed against the surface of the virus was produced in response to inoculation of sows with whole virus or either of the structural moieties. The sedimentation analyses showed that these sera caused an increase in the sedimentation coefficient of the virus, presumably by coating the particles with antibody molecules, and brought about sufficient aggregation to pellet radioactivity to the bottom of the gradient.

Application of this serum-induced clumping of radiolabelled virus to filtration studies demonstrated that sows receiving SVP antigen were capable of producing antibodies to surface components at a similar level to those animals receiving whole virus or SP antigen. The use of this technique to quantitate agglutinating antibody is complicated by the need to incorporate rabbit anti-porcine IgG serum into the test. As discussed in the text, purified TGEV adsorbs irreversibly to membrane filters containing cellulose nitrate. This necessitated using cellulose acetate filters, for which the smallest pore diameter available is 200 nm. Assuming that virus aggregates are approximately spherical and composed of particles 70—80 nm in diameter, the smallest aggregate that sould be retained reproducibly by such filters would need to comprise 20—30 virions. The secondary antiserum to porcine immunoglobulin is required to achieve complexes of this size. The low level of retention of virus incubated with low dilutions of immune sera presumably reflects the solubility of antigen—antibody complexes in the presence of large excesses of either of the two reactants.

Analysis of the immunoglobulin classes responsible for neutralising activity after inoculation of sows by the intramammary route showed that IgG was the predominant antibody species in both serum and colostrum, consistent with previous findings with inactivated TGEV (Bohl et al., 1972 a; Lucas et al., 1974) but differing from the stimulation of IgA to ferritin reported by Bourne et al. (1975). Whether this discrepancy is due to differences in the types of antigen used is not known. It is of interest that sow no. 9, which received SP antigen, produced neutralising antibody of the IgA class in the colostrum and was the only animal capable of protecting her piglets against challenge with live virus. The possibility that this sow

encountered live TGEV prior to farrowing and that the experimental inoculation generated a secondary immune response cannot be totally ruled out, since infection with the live virus stimulates IgM and IgA antibody production (sow no. 1 above; Bohl et al., 1972 a; Lucas et al., 1974; Stone et al., 1974) and may result in limited protection of the litter. There was no other reason to believe that prior infection with TGEV had occurred, however. All the control sows remained sero-negative throughout the experimental period. The apparent lack of neutralising antibody of the IgM class in the pigs that received inactivated viral material may have resulted from the materials used and the route of inoculation; the times at which the serum samples were taken may have been too late, however, to have detected a transitory IgM response.

Most of the sows showed rising neutralising antibody titres after challenge of the piglets, probably in response to cross-infection. Those inoculated with SP antigen that had no detectable neutralising antibody at farrowing nevertheless developed titres by 2 to 3 weeks, comparable with those that were sero-positive at farrowing. The two sows inoculated with SVP antigen alone, however, as well as having no detectable serum neutralising antibody at farrowing, developed lower titres that were more comparable with those achieved by the uninoculated controls.

It has been reported that a soluble antigen from TGEV-infected swine testis cells (Stone et al., 1976 a) could induce neutralising antibody formation when injected into rabbits. From the data presented above we should anticipate that the soluble antigen concerned is likely to comprise viral SP either free in solution or bound to small fragments of membrane. Since the viral SP is characterised mainly by the molecular weight of its component glycopolypeptide and has neither haemagglutinin nor enzyme activity associated with it, the task of recognising the presence of SP in crude tissue extracts is difficult. It should be possible, however, to develop a radioimmune assay using specific antibody to purified components. Such a test would facilitate the preparation of potent antigen suspensions and work is in progress to this end.

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