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Natural Infection with the Porcine Respiratory Coronavirus Induces Protective Lactogenic Immunity against Transmissible Gastroenteritis

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ABSTRACT

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Our objective was to evaluate the level of passive protection against transmissible gastroenteritis (TGE) among 57 newborn piglets nursing from seven seropositive sows previously naturally infected with porcine respiratory coronavirus (PRCV). After challenge exposure we observed mortality rates of 44% for litters of seven PRCV-infected sows, 40% for litters of four sows orally immunized with the attenuated TGEV strain Nouzilly, and 91% for litters of seven seronegative susceptible sows. A blocking ELISA with two appropriate monoclonal antibodies distinguished serological responses of PRCV-infected sows from those of TGEV-immunized sows. The results suggest that natural infection of the sow with PRCV may induce a degree of protective lactogenic immunity against TGE.

INTRODUCTION

The gut epithelium of the newborn piglet is the main target of the transmissible gastroenteritis (TGE) coronavirus (TGEV) which is therefore a specific enteropathogenic virus (Haelterman, 1972). The tonsils and respiratory tract are considered as secondary targets (Kemeny et al., 1975; Kemeny and Woods, 1977; Furuuchi et al., 1979) and respiratory disorders caused by TGEV are unknown. Since 1984, TGEV seroconversion has been observed in the swine population of different European countries in the absence of any clinical disease. These countries include Belgium (Pensaert et al., 1986), Denmark (P. Have, personal communication, 1987), France (Jestin et al., 1987) and England (Brown and Cartwright, 1986). A porcine respiratory, non-enteric

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coronavirus related to TGEV was isolated in Belgium (Pensaert et al., 1986), in Denmark (P. Have, personal communication, 1987), in England (Garwes et al., 1988) and in France (Duret et al., 1988). This porcine respiratory coronavirus (PRCV) induces a serological response that cannot be distinguished from that of TGEV-infected pigs by seroneutralization test in cell culture. PRCV and TGEV show a close antigenic relationship and can be distinguished from each other only by use of appropriate monoclonal antibodies (Garwes et al., 1988; Laude, 1988). This shows that several epitopes located in the C and D domains of gpE2 of TGEV are absent on the gpE2 of PRCV (Laude, 1988). Our objective was to evaluate the degree of passive protection against experimental TGE challenge exposure among 57 piglets nursing from seven seropositive sows from two herds which had been infected by PRCV >1 year ago.

MATERIALS AND METHODS

Cells

The McClurkin swine testicle (ST) cell line was used for virus titration (plaque assay under agarose) and seroneutralization tests. RP-D is a pig kidney cell line previously described by Laude et al. (1981) and used for the preparation of Purdue-115 and Nouzilly virus stocks.

Virus

The highly virulent Gep-II strain of TGEV is an isolate from an acute outbreak of TGE among swine in France (Aynaud et al., 1985). A virulent virus stock (10^6 LD_{50} ml^{-1}) was prepared from the contents of the small intestine of colostrum-deprived newborn piglets inoculated with Gep-II strain and harvested 24 h post-inoculation. The virus stock was shown to be free of rotavirus by a virus enzyme-linked cell immunoassay (VELCIA) (Grom and Bernard, 1985). The Nouzilly strain is an attenuated mutant of TGEV obtained in our laboratory by serial cycles of survivor selection in the gastric juice of adult pigs. The properties of the Nouzilly strain were described previously (188-SG strain) (Aynaud et al., 1985; Nguyen et al., 1987). The high-passage Purdue-115 strain of TGEV was used for neutralizing antibody titration.

Sows

Eighteen pregnant sows used in this study were either Large Whites or Meishans. Seven seropositive sows were obtained from two herds which had seroconverted against PRCV >1 year ago without TGE symptoms (Szymansky, unpublished results, 1988). Eleven seronegative sows were obtained from a

herd free of TGE and PRCV. Each group of sows was housed in isolated units (Nouzilly) before and after parturition.

Immunization of sows

Fifteen milliliters of Nouzilly virus-infected RP-D cell supernatant (5×10^6 plaque-forming units ml^{-1}), diluted in 300 ml of McIlvaine's buffer (0.025 M, pH 4.0) were administered per os to fasting sows (no food or water during 24 h prior to vaccination). A first virus dose was administered 42–49 days before parturition and a second virus dose (booster) was administered 7–15 days before farrowing.

Evaluation of TGE immunity

When they were 2–17 days old, suckling piglets were challenged with the Gep-II strain by oral administration of 1 ml containing 1000 LD_{50}. Clinical signs and mortality rate were scored during the 15 days post-exposure. Neutralizing antibody response in serum and delipidated milk were examined using a microneutralization test in ST cells as previously described (Toma and Benet, 1976). Litters were considered to be protected if $<30\%$ of the piglets died.

Blocking ELISA

The monoclonal antibody (MAb) E4 was kindly provided by Dr. N. Juntti, SVA, Biomedicum, Uppsala, Sweden. The MAb 44-4 was kindly provided by Dr. H. Laude, INRA, 78350 Jouy-en-Josas, France (Delmas et al., 1986); it neutralizes TGEV and reacts by indirect immunofluorescence with TGEV antigens, but not with PRCV. MAb 44-4 is used as a representative reagent for enteric TGE viruses. MAb E4 strongly neutralizes both TGEV and PRCV, and is used as a representative of a common epitope located in the A domain (Delmas et al., 1986) of the peplomer glycoprotein E2. A blocking ELISA was developed in Lindholm using pelleted Purdue-115 virus as an antigen. Virus was adsorbed directly onto the plates followed by overnight incubation with dilutions of test sera in PBS–Tween 20. The tests were completed using successively a predetermined dilution of monoclonal antibody and peroxidase-conjugated rabbit anti-mouse IgG. Titers were calculated by interpolation as the dilution giving 50% inhibition (E4) or 25% inhibition (44-4) compared with a negative reference. A 50% cut-off level was selected for the E4 test on the basis of a statistical analysis of results obtained by examining 748 TGEV-seronegative samples. These samples showed, on average, 0% inhibition with a standard deviation of $\pm 11\%$ inhibition. Thus, the 50% cut-off level allows for a very specific test while maintaining a sensitivity that is, on average, four times higher than the neutralization test for serum samples (data not shown).

The 25% cut-off level for the 44-4 test was chosen primarily to obtain a

reasonable test sensitivity. On the basis of statistical analysis of 197 PRCV antibody-positive samples the 44-4 test showed, on average, 9% inhibition with a standard deviation of ±10%. With a cut-off level of 25%, this would correspond to a probability of getting a false-positive sample of $P=0.05$ (one-sided test).

RESULTS

Morbidity and mortality rate after challenge exposure

The results are presented in Table 1. All 57 piglets nursed by the seven PRCV-infected sows were challenge exposed to virulent TGEV when 6–10 days old.

TABLE 1

Passive protection against TGE challenge exposure of piglets nursing from sows naturally infected with PRCV, immunized with attenuated TGEV or that were susceptible seronegative

Sow No.	Age of litter at challenge (days)	Piglet morbidity (sick/total)	Piglet mortality	
			Died/total	%
Natural infection with PRCV				
Herd A				
44	8	10/10	2/10	25/57
30	10	0/7	0/7	(44)
113	7	7/7	2/7	
87	6	7/7	7/7	
111	6	11/11	11/11	
Herd B				
232	9	0/4	0/4	
913	6	11/11	3/11	
Orally immunized with attenuated TGEV				
1100	3	5/6	5/6	14/35
1048	3	6/9	6/9	(40)
946	7	10/10	2/10	
1045	7	10/10	1/10	
Control susceptible seronegative				
25	3	8/8	8/8	55/60
4961	17	4/4	4/4	(91)
6926	4	12/12	12/12	
108	8	10/10	5/10	
071	2	11/11	11/11	
6261	3	9/9	9/9	
191	5	6/6	6/6	

TABLE 2

Analysis of antibody response of sows with different immune status (PRCV infection, TGEV vaccination, controls) at challenge exposure of suckling piglets: comparison of neutralizing activity with E4 and 44-4 epitope reactivity in ELISA

Sow No.	Piglets mortality[1]	Neutralizing antibody titer[2]		ELISA antibody titer[3]			
		Serum	Milk	Serum		Milk	
				E4	44-4	E4	44-4
Natural infection with PRCV							
87	100	1.51	0.90	2.58	0.00	0.00	0.00
111[4]	100	0.00	0.00	0.00	0.00	0.00	0.00
113	29	0.90	0.30	1.17	0.00	0.00	0.00
913	27	2.71	1.81	3.60	0.00	1.37	0.00
44	20	1.2	0.60	2.24	0.00	0.00	0.00
30	0	1.81	1.20	2.88	0.00	0.00	0.00
232	0	2.71	1.51	3.56	0.00	1.53	0.00
Orally immunized with attenuated TGEV							
1100	83	2.41	1.81	2.65	2.32	0.87	1.50
1048	67	2.71	2.11	3.00	3.08	1.91	1.84
946	20	2.71	2.41	2.70	2.04	2.11	2.22
1045	10	2.41	2.41	2.52	2.38	1.35	1.64
Control susceptible seronegative							
25	100	<0.1	<0.1	NT[5]	NT	NT	NT
4961	100	<0.1	<0.1	NT	NT	NT	NT
6926	100	<0.1	<0.1	NT	NT	NT	NT
71	100	<0.1	<0.1	NT	NT	NT	NT
6261	100	<0.1	<0.1	NT	NT	NT	NT
191	100	<0.1	<0.1	NT	NT	NT	NT
108	50	<0.1	<0.1	NT	NT	NT	NT

[1]Percentage of piglet mortality (died/total).
[2]Neutralizing antibody titer expressed as $\log_{10}$ inverse dilution able to inhibit CPE of 200 virus doses in ST cells.
[3]Blocking ELISA titer against E4 and 44-4 epitope.
[4]Several months ago, serum from Sow 111 was positive for E4 and negative against 44-4 epitope.
[5]NT, not tested.

All litters, except 30 and 232, developed diarrhea, but variable degrees of disease were observed. Mild and delayed clinical signs were observed in litters from Sows 44, 113 and 913. In contrast, acute symptoms of typical TGE (vomiting, diarrhea, dehydration) were observed in Litters 87 and 111. Five sows out of seven protected their piglets resulting in a mortality rate of 44% within 10 days after challenge exposure.

All piglets nursing from seven susceptible seronegative sows developed acute TGE resulting in a mortality rate of 91%. Although most piglets nursed by four

sows orally immunized with the attenuated Nouzilly strain of TGEV developed diarrhea, two litters out of four were protected resulting in a mortality of 40%.

Antibody response in serum and milk of sows

TGEV neutralizing antibody response

The results are presented in Table 2. At challenge exposure, antibody was detected in serum and milk samples of all infected or vaccinated sows (except PRCV-infected Sow 111) but not in those from seven control sows. Whatever the immunization procedure no correlation was seen between antibody level and degree of passive protection transmitted to suckling piglets (correlation coefficient, $r^2=0.154$ for serum and $r^2=0.096$ for milk, $P>0.05$). All seven PRCV-infected sows were seropositive 204 and 64 days before challenge exposure (data not shown). Using the seroneutralization test, it was not possible to distinguish the serological response of TGE-vaccinated sows from that of PRCV-infected sows.

Antibody response against E4 and 44-4 epitopes of gpE2 of TGEV

The results are presented in Table 2. At challenge exposure, serum and milk samples of all but one PRCV-infected sow were positive for antibodies specific for E4 epitope and negative for 44-4.

In contrast, serum and milk samples of all four TGEV-vaccinated sows contained antibodies specific for E4 and 44-4 epitopes. Ten days after challenge exposure, 44-4 antibodies are detected in four (44, 111, 232, 913) out of seven PRCV-infected sows (data not shown).

DISCUSSION

Using a blocking ELISA with two appropriate MAbs, we demonstrated that seven seropositive sows from naturally infected herds developed an antibody response specific for PRCV. This is based on recent data from H. Laude concerning the molecular antigenic characterization of PRCV isolates (Laude, 1988). Serological differentiation between PRCV and TGEV infection is clearly possible using these monoclonal antibodies (P. Have, unpublished results, 1987; Garwes et al., 1988; Caillebaut et al., 1988).

Our results show that natural infection with PRCV induces protective lactogenic immunity against TGE. However, protection was not complete in that only two litters out of seven did not show morbidity after challenge exposure, and five out of seven sows had protected their litters. This level was comparable with that of sows vaccinated with the Nouzilly strain, and contrasts with the absence of protection by all the seronegative sows. The age of litters at challenge could be a relevant factor. Age-related resistance to TGEV is well

documented and the choice of a highly virulent TGEV for challenge of litters is crucial. We rejected the Miller strain because of its moderate pathogenicity. The mortality rate observed with the Miller strain by Moxley (Moxley, 1983) and by us does not exceed 60–70% (data not shown). In contrast, with the Gep-II strain, most (91%) of the 2- to 17-day-old control piglets died. In this experiment, it is not possible to show any difference by ANOVA analysis between age average of the three groups ($F_{2.15}=0.549, P=0.62$). In contrast, the difference in the average mortality is highly significant ($F_{2.15}=5.047, P=0.02$).

All PRCV-infected sows (except Sow 111) and all TGEV-immunized sows had antibody in serum and milk whatever the degree of passive protection that was transmitted to suckling piglets. Ten days after challenge, all TGEV-immunized sows showed an anamnestic neutralizing antibody response in serum and milk. An anamnestic antibody response specifically of E4 epitope was detectable in sera of all seven PRCV-infected sows.

Hooyberghs et al. (1988) found conflicting evidence that sows naturally infected with PRCV may not adequately protect their litters against natural TGE challenge in the field. This discrepancy could be explained by the conditions used for evaluation of protective immunity. In our case, a standardized challenge with a highly virulent strain of TGEV was carried out under experimental conditions among sows of different but known immune status. During natural TGE outbreaks, the occurrence of other enteropathogens, known for their ability to enhance the pathogenicity of TGEV (*E. coli*, Rotavirus, Coccidia), cannot be excluded. It is interesting to observe that a decrease of clinical TGE in Europe has been concomitant with development of PRCV seroconversion in the swine population. This feature can be considered as an argument in favor of cross-protection between TGEV and PRCV.

Our results show evidence of cross-protection between PRCV and TGEV, but further investigations are needed to check if TGEV vaccination or TGEV infection protects swine against respiratory infection caused by PRCV. If such complete cross-protection is confirmed, PRCV infection could be considered as an interesting and valuable experimental model to elucidate the mechanism of immunological link between the lung and the mammary gland in the sow.

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