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Induction of lactogenic immunity to transmissible gastroenteritis virus of swine using an attenuated coronavirus mutant able to survive in the physicochemical environment of the digestive tract

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ABSTRACT

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A transmissible gastroenteritis (TGE) coronavirus mutant (188-SG), selected as attenuated and resistant to acidity and proteases of the digestive tract of adult pigs, was used as vaccine ("Nouzilly strain") in sows to protect suckling piglets against a challenge exposure carried out with a highly virulent TGEV strain. The pregnant sows were immunized once (42–49 days before farrowing) or twice (42–49 and 7–15 days before farrowing) by the oral, intramuscular or conjunctival route with the 188-SG strain. Sows exposed to virulent TGEV in the field and experimentally infected sows (two oral inoculations during pregnancy) were used as positive controls leading to high protection. The neutralizing antibody response to vaccination and/or infection was studied in serum and milk. No protection against mortality was observed in the litters of (1) the nine seronegative, susceptible sows, with piglet mortality of 65/70, (2) the seven once orally vaccinated sows, with mortality of 44/54, (3) the seven sows vaccinated twice by the conjunctival route, with mortality of 55/76. Moderate protection was observed in (1) the eight sows vaccinated intramuscularly twice with piglet mortality of 36/90, (2) the seven orally and intramuscularly vaccinated sows with piglet mortality of 31/51. In contrast, improved protection was observed in (1) the 10 sows vaccinated twice orally, with piglet mortality of 23/95, (2) the four naturally infected sows with piglet mortality of 6/41, (3) the six sows experimentally infected with virulent TGEV with piglet mortality of 1/59. No correlation was found between neutralizing antibodies titers in serum and milk and protection rate of the piglets. The results indicate that relative protective lactogenic immunity against TGEV is induced only by repeated ingestion of the attenuated 188-SG strain of TGEV.

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INTRODUCTION

Transmissible gastroenteritis (TGE) of swine is a highly contagious enteric viral disease of pigs under 2 weeks of age. It is well established that sows which have recovered from TGE are able to transmit protective immunity to their suckling pigs (Hooper and Halterman, 1966; Bohl et al., 1972). Neutralization of TGE virus (TGEV) in the lumen of the gut of the newborn by antibodies continuously supplied by colostrum and milk of immune sows is believed to provide passive protection to suckling piglets. Numerous TGEV vaccines have been evaluated but their efficacy is questioned (Henning and Thomas, 1981; Bohl et al., 1982; Bohl, 1982; Moxley and Olson, 1989). These attenuated TGEV live vaccines fail to initiate the gut and mammary gland immune response essential for providing optimal immunity (Salmon, 1987). The low stability of cell culture adapted TGEV strains in gastric and gut juices (Laude et al., 1981; Aynaud and Bottreau, 1984), which may destroy the virus during transit through the gut, is probably responsible for failures of oral vaccination. Using a survivor selection process in gastric juice, we have selected an attenuated mutant of TGEV which survives in the physico-chemical environment of the digestive tract of adult pigs (Aynaud et al., 1985). Preliminary experiments carried out with a limited number of sows had indicated that this new TGEV mutant was capable of inducing a protective lactogenic immunity and that could be considered as a candidate for an oral TGEV vaccine (Aynaud et al., 1985). The purpose in the present work was to study the lactogenic immunity afforded to suckling piglets on 40 sows immunized with this new mutant using oral, intramuscular and conjunctival routes. Passive protection was assayed by exposure of piglets to a standard challenge dose of fully virulent TGEV and antibody response was evaluated by titration of neutralizing activity in serum and milk collected from immunized sows at the time of challenge exposure.

MATERIALS AND METHODS

Cells and viruses

RP.D is a pig kidney cell line previously described (Laude et al., 1981). The McClurkin swine testis ST cell line was supplied by E.H. Bohl (Wooster, Ohio, USA). Minimum essential medium (MEM) supplemented with 10% fetal calf serum was used for cell growth. The 188-SG strain of TGEV is an attenuated mutant obtained previously in our Laboratory from the virulent Gep-II strain by 188 serial cycles of survivor selection in gastric juices of adult pigs. The properties of this new TGEV strain were described previously (Aynaud and Bottreau, 1984; Aynaud et al., 1985; Bernard et al., 1986; Ngu-

yen et al., 1987abc). Attenuation has been verified by five serial back passages in fully susceptible neonatal pigs.

The high passaged Purdue-115 strain of TGEV was used for seroneutralization tests in cell culture. 188-SG and Purdue-115 were produced in RP.D cells, and virus infectivity titration was performed in ST cells using a plaque assay previously described (Aynaud et al., 1985). The fully virulent Gep-II strain isolated in an acute outbreak in France was described elsewhere (Aynaud and Bottreau, 1984; Aynaud et al., 1985; Bernard et al., 1986) and was used for challenge of suckling piglets. Virulence of Gep-II strain was titrated in 4-day-old piglets.

Swine

The 59 sows used in this study were Large-White or Meishan bred. Fifty-five were obtained from two TGEV free herds and were seronegative for TGEV. Four seropositive sows were obtained from a previously infected herd. Sows were housed in an isolation unit before and after farrowing, including the post challenge period.

Immunization of sows

188-SG strain: the supernatant of RP.D culture (5×10^6 PFU/ml) was used for the vaccination. Oral vaccination was performed in fasting sows with 15 ml of virus diluted in 300 ml of McIlvaine's buffer (0.025 M, pH 4). For other immunization routes, 15 m of viral suspension (infected cell supernatant) was used for intramuscular vaccination, and 1 ml was administered to each eye for conjunctival vaccination. Gep-II strain: 5 ml of the fully virulent Gep-II strain (10^6 LD50/ml was diluted in 300 ml of McIlvaine's buffer. Protocole of immunization: a first virus dose was administered 42–49 days before parturition in some groups, a second virus dose (booster) was administered 7–15 days before farrowing.

Evaluation of TGEV immunity

Passive protection of piglets against TGEV virulent challenge. 549 suckling piglets nursed by their own mother (59 sows) were challenged with 1000 LD50 of the virulent Gep-II strain by the oral route (1 ml) some days after birth. Clinical signs and mortality rate were scored during the following 15 days. A litter was considered protected if more than 70% of the challenged piglets survive.

Neutralizing antibody response in serum and milk of sows. Serum and milk samples were collected at different periods (vaccination, booster, challenge, 10 days after challenge) and were examined for the presence of NT antibody

using a microneutralization test in ST cells previously described (Toma and Benet, 1976). In tables, only antibody titers evaluated at challenge time and 10 days later are shown. Immunochemical analysis of antibody classes in serum and milk samples of some sows was published previously (Bernard et al., 1987; Shiraï et al., 1988).

Statistical evaluation

Total mortality rates of the sow/vaccine groups were taken into account instead of protection rate per litter due to the need to incorporate all the data without further assumption in the mortality rate to class the sows in non protected and protected groups. Comparison between routes of immunization were made by χ^2 and in the case of differences, comparison between two groups were done.

RESULTS

No clinical response was observed in sows following vaccination. Following virulent infection with Gep II strain, sows exhibited clinical reactions.

Morbidity and mortality rate of piglets after challenge exposure

Litters from control sows.

Seronegative susceptible (SS) sows (Table 1). All piglets nursing at 9 (SS) sows exhibited acute clinical signs (vomitting, diarrhea, dehydration) by 24 h after challenge exposure. Death occurred in 3 to 8 days. Sixty-five out of 70 control piglets died. The mortality rate of this group was 93%. Using, ELISA, TGEV could be shown in the diarrheic faeces (Bernard et al., 1986).

Naturally infected (NI) sows (Table 1). Studies were conducted on 4 sows from a herd which had been naturally infected with TGE 17 months ago. After challenge exposure of 41 suckling piglets morbidity was mild and delayed. The mortality rate was 15% and 3 litters out of 4 were considered to be protected.

Experimentally infected (EI) sows (Table 2). Immunization with Gep-II virulent strain was carried out twice by the oral route in six pregnant sows. Following the first oral inoculation, all sows exhibited clinical signs such as no appetite, depression and diarrhea. After challenge exposure of piglets only two litters out of six were observed slightly sick during 4 to 7 days post exposure. Only one piglet out of 59 died, resulting in a mortality rate of 1.6% and all the litters were protected.

TABLE 1

Virulent challenge exposure of control piglets nursing at either seronegative or seropositive sows – survival rate of piglets and neutralizing antibody response of mother sows

Sow no.	TGEV neutralizing activity[1] sows				Clinical (piglets)		
	Serum		Milk		Age of litter at challenge (days)	Morbidity[4] (sick/total)	Mortality (died/total)
	ch[2]	ch+10[3]	ch	ch+10			
Seronegative susceptible							
071	<4	nt[5]	<4	nt	2	11/11	11/11
25	<4	8	<4	32	3	8/8	8/8
6261	<4	32	<4	nt	3	9/9	9/9
314	<4	nt	<4	nt	3	5/5	5/5
6926	<4	16	<4	nt	4	12/12	12/12
191	<4	64	<4	32	5	6/6	6/6
62168	<4	256	<4	nt	6	5/5	5/5
108	<4	16	<4	16	8	10/10	5/10
4961	<4	16	<4	nt	17	4/4	4/4
Average	<4	29[6]	<4		5.6	70/70 (100%)	65/70 (93%±0.9)
Seropositive, natural virulent infection[7]							
360	512	256	nt	nt	2	6/12	0/12(p)[8]
231	128	64	128	512	3	8/8	0/8(p)
358	512	64	nt	nt	4	9/10	4/10
348	32	8	256	nt	4	2/11	2/11(p)
Average	181[6]	53[6]	181[6]	512	3.2	25/41 (61%)	6/41 (15%±5%)

[1]Antibody titers are expressed as the reciprocal of the highest dilution of serum or milk able to inhibit a cytopathic effect of 200 virus doses in ST cells.
[2]ch: day of challenge.
[3]ch+10: 10 days after challenge.
[4]Number of pigs with clinical signs of TGE (vomitting, diarrhea, dehydration) during 15 days after challenge exposure.
[5]nt = not tested.
[6]Geometric mean.
[7]From a herd which has been infected naturally by TGEV 17 months ago.
[8]p = protected litter.

Litters from sows vaccinated with the attenuated 188-SG strain

Oral priming without booster (Table 3). Seven sows were vaccinated. Challenge exposure of 54 piglets induced intense morbidity among all piglets. Mortality rate was 81% and no litter was protected.

Oral priming and oral booster (OR/OR) (Table 3). After challenge exposure of 95 suckling piglets (10 litters), delayed clinical reactions of varied intensity were observed but diarrhea occurred generally in most litters (except 542 and

TABLE 2

Virulent challenge exposure of piglets nursing at sows experimentally oral infected with virulent TGEV (GEP-II) – survival rate of piglets and neutralizing antibody response of mother sows

Sow no.	TGEV neutralizing activity[1] (sows)				Clinical (piglets)		
	Serum		Milk		Age of litter at challenge (days)	Morbidity[4] (sick/total)	Mortality (died/total)
	ch[2]	ch+10[3]	ch	ch+10			
2540	128	128	nt	nt[5]	3	0/7	0/7 (p)[7]
376	32	64	32	64	6	15/15	0/15 (p)
377	64	32	512	64	6	0/14	0/14 (p)
370	64	64	32	32	6	12/12	1/12 (p)
374	256	128	256	32	7	0/5	0/5 (p)
365	32	32	128	32	7	1/6	0/6 (p)
Average	72[6]	64[6]	111[6]	42[6]	5.8	28/59 (47%)	1/59 (1.6% ± 0.2)

[1]Antibody titers are expressed as reciprocal of highest dilution of serum or milk able to inhibit cytopathic effect of 200 virus doses in ST cells.
[2]ch: day of challenge.
[3]ch + 10: 19 days after challenge.
[4]Number of pigs with clinical signs of TGE (vomitting, diarrhea, dehydration) during 15 days after challenge exposure.
[5]nt = not tested.
[6]Geometric mean.
[7]p = protected litter.

62150). Twenty-three piglets out of 95 died resulting in a mortality rate of 24% during 15 days observation and seven litters out of 10 were protected.

Oral priming and oral booster with killed virus (Table 3). A sow (1099) was primed and boosted by the oral route with a vaccine dose composed of 8.10^7 PFU inactivated by ultraviolet treatment just before administration. After challenge exposure all piglets of the litter died after typical signs of TGE.

Intramuscular priming and intramuscular booster (Table 4). Eight sows were vaccinated twice by the intramuscular route. After challenge exposure of 90 piglets, morbidity was observed in all litters. The mortality rate was 40% but the response per litter was not uniform: from four litters (8, 13, 196, 224) 4 piglets out of 47 died resulting in a low mortality rate of 8.5%. From four other litters (9, 10, 107, 771) 32 piglets out of 43 died resulting in a high mortality rate of 74.4% (50% litter protection). Four litters out of 8 were protected.

Oral priming and intramuscular booster (Table 4). After challenge exposure of

TABLE 3

Virulent challenge exposure of piglets nursing at sows orally immunized with 188-SG strain of TGEV – survival rate of piglets and neutralizing antibody response of mother sows

Sow no.	TGEV neutralizing activity[1] (sows)				Clinical (piglets)		
	Serum		Milk		Age of litter at challenge (days)	Morbidity[4] (sick/total)	Mortality (died/total)
	ch[2]	ch+10[3]	ch	ch+10			
Oral vaccination							
222	512	nt[5]	256	nt	3	6/6	4/6
44	64	2048	32	512	4	6/6	4/6
223	64	2048	16	512	4	9/9	7/9
5232	128	16384	64	8192	5	9/9	9/9
5215	64	65536	1024	8192	8	8/8	7/8
318	198	1024	32	128	12	7/7	4/7
317	256	2048	64	512	13	9/9	9/9
Average	136[6]	4597[6]	78[6]	1024[6]	7	54/54 (100%)	44/54 (81%±5%)
Oral priming and oral booster							
1100	256	4096	64	4096	3	6/6	5/6
160	1024	8192	256	2048	5	2/11	2/11(p)[8]
62150	32	1024	256	2048	5	0/7	0/7(p)
1048	512	16384	128	16384	6	9/9	6/9
946	512	4096	256	4096	7	2/10	2/10(p)
1045	256	2048	256	1024	7	1/10	1/10(p)
195	64	8192	4	512	8	6/6	3/6
SN	512	4096	16	128	8	3/13	3/13(p)
159	218	4096	16	512	9	1/13	1/13(p)
542	512	4096	32	512	9	0/10	0/10(p)
Average	256[6]	4390[6]	64[6]	1351[6]	6.7	30/95 (31%)	23/95 (24%±5%)
Oral priming and oral booster with irradiated vaccine dose							
1099[7]	<4	32	<4	nt	5	10/10	10/10

[1]Antibody titers are expressed as the reciprocal of the highest dilution of serum or milk able to inhibit a cytopathic effect of 200 virus doses in ST cells.
[2]ch: day of challenge.
[3]ch+10: 10 days after challenge.
[4]Number of pigs with clinical signs of TGE (vomitting, diarrhea, dehydration) during 15 days after challenge exposure.
[5]nt = not tested.
[6]Geometric mean.
[7]This sow was orally primed and boostered with the 188-SG strain (8×10^7 PFU) inactivated by ultraviolet treatment just before administration.
[8]p = protected litter.

54 piglets (seven litters), morbidity was observed in all litters (except 155) and mortality was 57%. Two litters out of 7 were protected.

Conjunctival priming and conjunctival booster (Table 4). Seven sows were vac-

TABLE 4

Virulent challenge exposure of piglets nursing at sows diversely immunized with 188-SG strain of TGEV – survival rate of piglets and neutralizing antibody response of mother sows

Sow no.	TGEV neutralizing activity[1] (sows)				Clinical (piglets)		
	Serum		Milk		Age of litter at challenge (days)	Morbidity[4] (sick/total)	Mortality (died/total)
	ch[2]	ch+10[3]	ch	ch+10			
Intramuscular priming and intramuscular booster							
10	2048	2048	16	64	3	12/12	4/12
224	512	2048	8	64	4	13/13	0/13 (p)[7]
771	256	2048	64	nt[5]	4	8/8	8/8
107	2048	2048	64	nt	5	12/12	12/12
9	2048	4096	32	256	5	11/11	8/11
8	512	4096	16	128	7	13/13	1/13 (p)
196	512	8192	8	128	8	9/9	1/9 (p)
13	256	512	8	32	9	12/12	2/12 (p)
Average	724[6]	2435	19[6]	90[6]	5.6	90/90 (100%)	36/90 (40%±5%)
Oral priming and intramuscular booster							
43	256	8192	64	2048	4	14/14	11/14
5213	1024	8192	128	1024	4	8/8	5/8
18	2048	16384	256	4096	4	6/6	1/6 (p)
5231	4096	32768	512	16384	6	9/9	5/9
155	2048	1024	1024	512	9	0/2	0/2 (p)
5006	4096	4096	256	512	11	7/7	6/7
5216	8192	32768	512	4096	19	8/8	3/8
Average	2048[6]	9044[6]	282[6]	2048[6]	8.1	52/54 (96%)	31/54 (57%±6%)
Conjunctival priming and conjunctival booster							
681	512	16384	128	4096	1	11/11	9/11
732	256	16384	32	1024	3	11/11	11/11
679	512	8192	64	1024	5	11/11	7/11
733	512	2048	64	256	6	12/12	8/12
577	512	8192	32	512	6	17/17	12/17
377	64	4096	8	nt	6	8/8	8/8
173	128	4096	8	256	7	6/6	0/6 (p)
Average	282[6]	6720[6]	32[6]	724[6]	4.8	76/76 (100%)	55/76 (72%±5%)

[1]Antibody titers are expressed as the reciprocal of the highest dilution of serum or milk able to inhibit a cytopathic effect of 200 virus doses in ST cells.
[2]ch: day of challenge.
[3]ch+10: 10 days after challenge.
[4]Number of pigs with clinical signs of TGE (vomitting, diarrhea, dehydration) during 15 days after challenge exposure.
[5]nt=not tested.
[6]Geometric mean.
[7]p=protected litter.

cinated twice by the conjunctival route. After challenge exposure of 76 piglets, morbidity was intense in all litters an the mortality rate was high (62%). Only 1 litter (173) out of 7 was protected. Statistical analysis (chi-square) shows that the oral/oral and intramuscular/intramuscular routes were more potent than the oral/intramuscular or conjunctival/conjunctival routes. Comparing separately the conjunctival/conjunctival and non immunized sows, it appears that this route is effective ($\chi^2=10.45$, $P=0.001$). On the other hand, comparison between oral/oral and intramuscular/intramuscular by Chi-square reveals that oral/oral is more potent than intramuscular/intramuscular ($\chi^2=5.22$, $P<0.05$).

Neutralizing antibody response in serum and milk of sows

Whatever the conditions of immunization, all sows showed a seroconversion demonstrated by a significant NT antibody increase after vaccination (Tables 1–4). Antibody titers observed at the time of challenge in serum and milk of sows are similar except for sows primed and boosted by the intramuscular route where high antibody titers were seen in serum but low titers in milk. Comparing antibody levels at challenge exposure of piglets, 6 NI or EI sows out of 7 showed milk antibody titers which were equal or higher than those in serum. Conversely, whatever the immunization procedure, only 2 (5215, 155) out of 39 vaccinated sows showed a comparable situation of serum and milk. After challenge exposure antibody titers increased exclusively in vaccinated sows. Thus, only 1 sow (no. 155) out of 39 vaccinated sows did not exhibit an significant anamnestic antibody response (Table 4).

After oral priming and oral boostering (sow 1099, Table 3) with ultraviolet inactivated vaccine, NT antibody was not detectable in serum and milk at the time of challenge suggesting that multiplication of TGEV is needed for induction of a detectable humoral immune response. A slight antibody increase was observed in serum of sow 1099 and also in serum and milk of control SS sows after challenge exposure of suckling piglets (Table 1) suggesting a primary antibody response against the virulent TGEV excreted by diarrheic piglets.

DISCUSSION

Evaluation of the passive protection against TGE is largely dependant on the virulence of TGEV strain used for challenge. The virulent Miller strain used previously by several authors (Bohl et al., 1972; Bohl et al., 1975; Henning and Thomas, 1981; Bohl and Saif, 1975; Moxley and Olson, 1989) was not retained in this study because of its moderate pathogenicity observed in our experimental conditions. In our experiments the mortality rate observed with Miller strain never exceeded 68% (data not shown). In contrast, with an isolate from an acute outbreak in France (Gep-II strain) a high mor-

tality rate was regularly obtained. In the negative control experiment presented in this report, a mortality rate of 100% was obtained in 8 out of 9 susceptible litters. With the Miller strain, the age of piglets at challenge was noted by Moxley and Olson (1989) as a factor influencing the mortality rate. In our experimental conditions, a statistical analysis carried out on the 59 litters challenged with the highly virulent Gep-II strain, demonstrated no significant relationship between age of piglets at challenge and mortality rate (correlation coefficient $r^2=0.005$, $P>20\%$).

We compared the ability of the 188-SG strain given by different routes, to induce lactogenic protection. There is considerable variation among groups of vaccinated sows in regard to the mortality rate of piglets, 81% (oral route) to 24% (oral/oral route). It is evident that booster immunization is essential to induce high protection. The oral route for priming and for boostering also influenced the induction of a high protection rate (76%) against mortality similar to that observed among naturally infected sows (75%). This observation indicated that repeated oral administration of vaccine influences the development of TGEV protective immunity. Absence of detectable humoral immune response after oral vaccination with ultraviolet treated virus suggests also that repeated antigenic stimulations of the gut with an appropriate replicating antigen is needed for optimal induction of passive protection. The heterogeneous immune response among the eight sows vaccinated and boosted by the intramuscular route (four litters well protected and four fully susceptible) was similar to the observations reported by Bohl and Saif (1975) after intramuscular injection of pregnant sows with virulent TGEV. As postulated by these authors, passive protection is strongly dependent on whether the sow gut was infected by a haematogenous spread of virus. The possibility that infected macrophages (Laude et al., 1984) spread the vaccinal virus to the gut or mammary gland after intramuscular administration has been suggested. Arguing the concept of a common mucosal immune system, we postulated that conjunctival route (Montgomery et al., 1983), which has been used successfully against various pathogens (Fensterbank et al., 1985; Tannock et al., 1985; Kramer et al., 1987), would be an alternative approach to oral immunization. Unfortunately it was inefficient in our experiments in terms of protection although all vaccinated sows exhibited serum and milk neutralizing antibodies after conjunctival vaccination. The reason of this lack of success is unknown but it probably suggests that the conjunctival route is not able to induce local antigenic stimulation of gut.

All vaccinated sows developed significant antibody titers in serum and milk whatever the degree of protection transmitted to suckling piglets. Our results confirmed the lack of correlation between degree of passive protection of piglets and level of neutralizing antibodies in sow serum ($r=0.06$) and milk ($r=0.06$) at time of challenge observed by previous authors (Bohl et al., 1972;

Bohl and Saif, 1975; Moxley and Olson, 1989). However, after challenge exposure 95% of vaccinated sows showed an anamnestic antibody response, generally higher when the degree of protection of the litter was low. In contrast, all naturally or experimentally infected sows failed to show such anamnestic response. We suggest that the lack of anamnestic response observed in sows after challenge exposure of piglets could be considered as an a posteriori index of protective lactogenic immunity. These present results confirmed our previous preliminary observations (Aynaud et al., 1985; Shiraï et al., 1988). Furthermore, when sensitive immunochemical approaches (ELISA, immunoadsorbent) were used, IgA antibody was detected at time of challenge in the milk of experimentally infected sows and also in milk of orally vaccinated and boostered sows (Bernard et al., 1987). But in contrast to infected sows, the IgA antibody level was never predominant in the milk of vaccinated sows, suggesting that mechanisms involved in TGE lactogenic immunity induced by attenuated 188-SG strain could be different from those for virulent TGEV strain (Bohl et al., 1972).

188-SG strain did not induce a complete protective lactogenic immunity compared with natural or experimental virulent infection; morbidity was frequently observed in surviving litters. Nevertheless these results suggest that oral immunization of the pregnant sow with this new strain is successful in comparison with previous attenuated TGE vaccines which were of limited effectiveness (Henning and Thomas, 1981; Bohl et al., 1982; Bohl, 1982; Moxley and Olson, 1989).

The protective immunity induced by vaccination with the 188-SG strain could be partly explained by (1) higher stability of the 188-SG strain in the physico-chemical environment of the digestive tract in the sow, allowing the virus to reach the susceptible sites in gut (2) high content of structural antigens compared with Purdue-115 (Bernard et al., 1986; Nguyen et al., 1987a) and Ambico strain (data not shown), allowing the virus to survive inactivation by digestive proteases. The exact mechanism leading to induction of protective immunity following immunization with the 188-SG strain is still unknown. 188-SG strain is characterized by different in vitro properties (resistance to acidity and proteases, higher content of structural antigens, delay in viral multiplication) (Aynaud et al., 1985; Nguyen et al., 1987a) which can have possible repercussions on conditions of virus multiplication and also presentation of viral antigens at the surface of infected enterocytes. The amount of appropriate antigen produced by gut epithelial cells and processed by antigen presenting cells would perhaps be a crucial factor mainly involved in induction of optimal local immune response and the development of TGEV lactogenic immunity. Better knowledge in this field and analysis of milk immune factors connected with protection is without doubt the key of any improvement in TGEV immunization, whatever the type of vaccine.

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