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Intestinal replication of a porcine respiratory coronavirus closely related antigenically to the enteric transmissible gastroenteritis virus

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ABSTRACT

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One-week-old piglets were inoculated with the porcine respiratory coronavirus (PRCV) either intravenously or directly into the lumen of the gastrointestinal tract. Both inoculation routes resulted in the isolation of virus from the caudal small intestine. Viral replication, however, was only observed upon inoculation into the digestive tract in quantities of $\geq 10^3$ $TCID_{50}$. Replication remained limited to a few unidentified cells located in or underneath the epithelial layer at villus- or crypt-sites. Virus was excreted in the faeces for several days but infection of the respiratory tract occurred rarely in the same pigs.

The results of this study indicate that small changes in molecular structure between PRCV and transmissible gastroenteritis virus have resulted in important changes in host cell tropism.

INTRODUCTION

Transmissible gastroenteritis virus (TGEV) was first isolated by Doyle and Hutchings (1946). It is an enteropathogenic coronavirus which replicates in villus epithelial cells of the small intestine. Villus enterocytes become infected, degenerate and desquamate which results in atrophy of villi and in severe watery diarrhoea. TGEV can induce diarrhoea in swine of all ages with a mortality of near 100% in piglets until the age of 2 weeks (Hooper and Haelterman, 1969).

Between 12 and 24% of sows possessed seroneutralizing (SN) antibodies against TGEV in serological surveys carried out among the Belgian sow population between 1969 and 1984. In 1984, there was an unexpectedly high incidence of 68% animals with TGEV-SN antibodies. Since there had been no increased incidence of diarrhoea and since vaccination was not performed

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against TGEV in Belgium, it was concluded that a non-enteropathogenic virus, related to TGEV, had appeared. Subsequently, a virus was isolated in pigs and cell cultures. It was shown to be a coronavirus which replicates to high titres in the respiratory tract of piglets and is transmitted aerogenically. It was, therefore, called porcine respiratory coronavirus (PRCV) (Pensaert et al., 1986). PRCV has now spread to such an extent that nearly 100% of the swine farms in Belgium and surrounding countries have become infected.

The antigenic relationship of classical TGEV and the Belgian isolate of PRCV, designated TLM 83, has recently been studied (Callebaut et al., 1988). Both viruses show a complete cross-neutralization activity. Furthermore, they posses the same three structural proteins, namely a nucleoprotein (N), a glycoprotein (E1) associated with the envelope and a glycoprotein (E2) representing the surface projections, with similar molecular weights (N, Mw=48 000; E1, Mw=28 000; E2, Mw=200 000) and common antigenic determinants on each protein. Using monoclonal antibodies (MAb) against different TGEV-protein antigenic sites, it was shown that TGEV and PRCV have similar epitopes in the E1 and N proteins and in the neutralization-mediating antigenic site of the E2 protein. However, the E2 antigenic sites which stimulate non-neutralizing antibodies are different. Some E2 antigenic determinants of TGEV are, therefore, modified or absent on PRCV.

Pigs infected with PRCV or TGEV cannot be distinguished by the conventional SN-test since both viruses show a complete cross-neutralization activity. Nevertheless, differentiation is necessary for export to countries which require pigs to be free of infection with TGEV and also in research work on immunity and cross-protection. Since TGEV possesses antigenic sites in its E2 protein which are absent in PRCV, a differentiating ELISA test has been set up using one of the non-neutralizing MAb which was directed against this site in the E2 protein of TGEV. TGEV infected piglets have antibodies directed against this site which cannot be found in PRCV infected pigs. They are demonstrated by a competitive blocking ELISA (Callebaut et al., 1989).

Even though PRCV and TGEV are physicochemically and antigenically so closely related, they appeared to have a different cell tropism in pigs. Therefore, the virus-host interaction of PRCV was studied. One-week-old hysterectomy-derived and colostrum-deprived (HDCD) piglets were inoculated by aerosol with PRCV (Cox et al., 1990). Replication to high titre was observed in the respiratory tract, whereafter viraemia occurred. Subsequently, an intestinal infection began in the ileum and spread, within a few days, upwards to the duodenum. Replication, however, remained limited to a few unidentified cells located at villus- or crypt-sites. It was not clear if virus reached the intestine either by viraemia or by ingestion of infectious virus produced at the respiratory tract surface. Furthermore, we could not determine how the PRCV infection spread from caudal to cranial in the small intestine and if newly produced virus became released into the gut lumen.

The finding that PRCV can replicate in the intestine, may have important consequences with regard to the development of mucosal intestinal and lactogenic immunity in previously PRCV-infected pigs or sows when infected with the antigenically closely related TGEV. It was thus interesting to study the behaviour of PRCV in the intestine in more detail.

MATERIALS AND METHODS

This study was performed on 15 hysterectomy-derived and colostrum-deprived (HDCD) piglets, which were individually housed in Horsfall type units. They were inoculated with the Belgian isolate of PRCV, designated TLM 83, at the age of 1 week either using different inoculation routes or different inoculation dosages.

Three piglets were inoculated intravenously into the ear vein with 10^7 $TCID_{50}$ in 5 cc phosphate buffered saline (PBS) and were euthanatized at 1/2, 1 and 1 1/2 days post inoculation (PI). Twelve piglets were inoculated into the lumen of the gastrointestinal tract after laparotomy. One piglet was inoculated with 10^7 $TCID_{50}$ into the gastric lumen and was slaughtered at 3 days PI. Three piglets were inoculated with 10^7 $TCID_{50}$ into the lumen of the small intestine and killed 2, 3 and 3 days PI. The intestinal inoculation was performed at three sites, namely the cranial jejunum, mid jejunum and the ileum. Eight piglets were inoculated into the lumen of the cranial jejunum using different inoculation dosages. One piglet was inoculated with 10^7 $TCID_{50}$, two with 10^5, four with 10^3 and one with 10^1. Each dose was suspended in 5 ml PBS. One piglet inoculated with 10^5 $TCID_{50}$ and one with 10^3 were slaughtered 3 and 4 days PI respectively. The other piglets were followed for clinical signs and serological responses. Blood was sampled for the detection of TGEV-seroneutralization (SN) antibodies at 0, 10, 20 and 25 days PI. Rectal- and tonsillar swabs were collected daily for viral isolation (VI).

After euthanasia, the following tissues or samples were collected for viral isolation and/or immunofluorescence (IF): lung lobes, small intestine, mesenteric and bronchial lymph nodes, spleen, plasma and the contents of small intestine, large intestine and rectum. The small intestine was divided into seven segments equal in length and numbered from 1 (duodenum) to 7 (ileum). A piece of approximately 1.5 cm in length was collected for IF. VI was performed on the remaining parts. VI was performed according to standard procedures. The supernatant of a 20% suspension in PBS of each sample was inoculated on swine testicle (ST) cells in 10 tubes. Quantitative titrations of infectious virus were performed by inoculating 10-fold dilutions of the supernatants on ST cells in microtitre plates. The IF-test was performed only on tissues which were positive by VI. IF was performed using as hyperimmune serum a specific antiserum to TGEV. The SN-test was performed in SK6 cells using the Purdue-114 strain of TGEV.

TABLE 1

Results of virus titrations of tissues from piglets inoculated intravenously or into the lumen of the gastrointestinal tract

Inoculation		Piglet no.	Euthanasia (days PI)	Virus titre (log 10 $TCID_{50}$/g tissue)														
Route	$TCID_{50}$ (log 10)			Lung	Small intestinal segments							Content			Lymph nodes		Spleen	Plasma
					1	2	3	4	5	6	7	small intestine	large intestine	rectum	mesenteric	bronchial		
Intravenous	7	1688	1/2	1.3	–[1]	–	–	–	–	–	–	–	–	–	–	–	1.4	1.3
	7	1687	1	–	–	–	–	–	–	–	–	–	–	–	–	–	1.3	1.3
	7	1686	1 1/2	1.3	1.3	–	–	–	–	1.3	1.6	–	–	–	1.4	–	2.7	–
Lumen stomach	7	1587	3	–	2.1	1.4	–	–	–	2.0	2.7	ND[2]	–	–	1.9	–	–	–
Small	7	1762	2	–	ND	1.3	–	–	–	–	1.5	–	ND	–	1.3	–	ND	ND
Intestinal	7	1760	3	–	–	–	–	–	–	1.5	2.0	–	–	–	1.5	–	–	–
Lumen[3]	7	1588	3	–	1.3	2.7	3.0	3.2	3.0	3.2	3.0	ND	≥2.2	–	2.1	–	1.5	1.7
Lumen	3	1684	4	–	–	–	–	–	–	–	–	–	–	ND	–	–	–	–
Cranial jejunum	5	1685	3	–	–	1.3	–	–	–	–	2.1	–	–	–	1.5	–	–	–

[1]No virus isolated. [2]Not determined. [3]Inoculated in cranial and mid jejunum and ileum.

RESULTS

None of the inoculated piglets experienced respiratory distress or intestinal disorders.

Different inoculation routes

The results of the viral isolation and titration of piglets inoculated using different routes are given in Table 1. After intravenous inoculation, virus was isolated at low titers from the intestinal tract in the piglet killed at 36 h PI. Virus was also recovered from lungs, mesenteric lymph nodes and spleen in this piglet. Virus was not recovered from the intestinal tract, but in low titres from spleen, plasma or lungs at 12 and 24 h PI.

Three days after inoculation into the gastric lumen, virus was recovered from duodenum, cranial jejunum, caudal jejunum, ileum and mesenteric lymph nodes. The highest virus titres were found in the ileum. Upon inoculation of PRCV into the small intestinal lumen, virus could be recovered from ileum and from mesenteric lymph nodes in all three piglets killed 2 to 3 days PI. Virus was also isolated from cranial jejunum at day 2 PI, from caudal jejunum in one of both piglets killed at day 3 PI and from the entire small intestinal tract, large intestinal contents, spleen and blood in the other piglet.

After intravenous inoculation, few fluorescent cells were seen in the spleen of the piglet killed 36 h PI. These cells were large and oval and showed a weak IF. Fluorescent cells were also seen in lungs of this piglet. All other tissues were negative by IF. Upon inoculation with 10^7 $TCID_{50}$ TLM 83 into the gastrointestinal lumen viral antigen was detected by IF in the ileum of all three piglets killed at 3 days PI. More cranial intestinal segments were fluorescing in the piglets inoculated into the small intestinal lumen. Fluorescence, however, was always limited to a few unidentified cells which were located in or sometimes underneath the epithelial layer of villi and/or crypts.

Different inoculation dosages

The results of the viral isolation and titration of piglets inoculated with 10^5 or 10^3 $TCID_{50}$ TLM 83 and killed 3 and 4 days PI respectively are given in Table 1. No virus was isolated from tissues of the piglet inoculated with 10^3 $TCID_{50}$ TLM 83, whereas virus was recovered from caudal jejunum, ileum and mesenteric lymph nodes following inoculation with 10^5 $TCID_{50}$. Few fluorescing cells were observed in the ileum of this latter piglet. The other intestinal segments and tissues were negative by IF.

Seroconversion was observed in piglets inoculated in the cranial jejunum with 10^7 $TCID_{50}$, 10^5, and one of the piglets inoculated with 10^3. No serological response occurred in both other piglets inoculated with 10^3 $TCID_{50}$ and in the piglet inoculated with 10^1. The 50% infectious dose for the small intestinal lumen could be calculated from these results and is $10^{3.7}$ $TCID_{50}$. Virus

had been excreted for 4 to 10 days respectively in the faeces of the two piglets which became seropositive. Virus was isolated from tonsillar swabs in one of these piglets and this occurred 3 days after virus was recovered from faeces.

DISCUSSION

PRCV was observed to replicate in the small intestine upon inoculation by aerosol. Intestinal replication started in the ileum (Pensaert and Cox, 1989). The results of the present study indicate that PRCV can also reach the intestine via the lumen of the intestinal tract and via the blood. Indeed, virus was recovered from caudal small intestine and from mesenteric lymph nodes at slightly higher titres than from lung tissue at 1 1/2 days PI upon inoculation into the ear vein. However, viral replication in the small intestine, as evidenced by IF, was only observed after inoculation into the gastrointestinal tract and not after intravenous inoculation. The intravenously inoculated piglets were already killed at 1 1/2 days PI which may have been too early to observe intestinal fluorescence. Indeed, viral antigen was demonstrated by IF in the ileum only 2 days after aerosol inoculation (Cox et al., 1990) and from 3 days after gastrointestinal inoculation.

Results of the present study indicate that the small intestinal tract is not very susceptible to infection with PRCV, since inoculation of 10^3 $TCID_{50}$ TLM 83 into the cranial jejunum sometimes induced an infection and sometimes did not. We have been able to isolate virus at titers of 10^3 and $10^{4.3}$ from the stomach upon aerosol inoculation of piglets with TLM 83 in previously performed experiments. A sufficient amount of infectious virus appears, therefore, to be produced at the respiratory tract surface and to induce an intestinal infection if subsequently swallowed.

The intestinal replication of PRCV was totally different from that of TGEV. After TGEV is taken up, it comes in contact with the highly susceptible villus epithelial cells of the small intestine and causes a productive infection of these cells. All villus enterocytes become infected, degenerate and desquamate within a few days (Pensaert et al., 1970). PRCV infection, on the contrary, remains limited to a few unidentified cells, scattered over the mucosa. Small changes in molecular structure between PRCV and TGEV apparently have resulted in important changes in host cell tropism.

It was previously observed that the intestinal PRCV infection starts in the ileum and subsequently spreads to the duodenum. Since it is unlikely that the infection spreads via the gut lumen in the opposite direction of peristalsis, another route must be used. In the present study, inoculation of PRCV in the intestinal tract could induce an intestinal infection in the absence of a respiratory infection. This seriously indicates that PRCV virus is not spreading from the ileum to the duodenum via the blood as free virus, otherwise infection of the respiratory tract would be expected to occur consistently.

Virus was excreted in the faeces for several days without being isolated from tonsillar swabs. The intestinal infection with TLM 83, therefore results in production and release of infectious virus into the gut lumen.

It was shown that PRCV can replicate in the intestinal tract of 1-week-old HDCD piglets. However, pigs on farms usually become infected between 5 to 10 weeks of age, while having maternal antibodies. It still has to be determined if PRCV can replicate in the small intestine in piglets of this age and in the presence of maternal antibodies. Intestinal replication of PRCV could have important consequences regarding lactogenic immunity of pigs against TGEV infection if it induces anti-TGEV-IgA antibodies in the milk of sows by stimulating the gut-mammary link (Bourne et al., 1977).

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