

Key words: *TGE virus/porcine respiratory coronavirus/antigenic relationship*

Antigenic Differentiation between Transmissible Gastroenteritis Virus of Swine and a Related Porcine Respiratory Coronavirus

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(Accepted 25 March 1988)

SUMMARY

The antigenic relationship between a recently isolated porcine respiratory coronavirus (TLM 83) and transmissible gastroenteritis (TGE) virus of swine was studied by neutralization, immunoblotting and radioimmunoassay (RIA) using TGE virus-specific monoclonal antibodies (MAbs) and polyclonal antibodies specific for both viruses. A complete two-way neutralization activity between the two viruses was found. Immunoblotting revealed cross-reactions between TLM 83 and TGE virus antigens at the level of the envelope protein (E1), the nucleoprotein (N) and the peplomer protein (E2). By virus neutralization assays and RIA with TGE virus-specific MAbs, the presence of similar epitopes in the E1 and N proteins and in the neutralization-mediating antigenic site of the E2 protein were demonstrated. E2 protein-specific MAbs, without neutralizing activity and reacting with antigenic sites B, C and D (previously defined), failed to recognize TLM 83. These results indicated a close antigenic relationship and structural similarity between TLM 83 and TGE viruses and also suggested potential ways of differentiating between the two viruses.

Transmissible gastroenteritis (TGE) virus of swine is a member of the Coronaviridae family and was isolated for the first time in 1946 (Doyle & Hutchings, 1946). It is a specific enteropathogenic virus which replicates in the villous epithelial cells of the small intestine and causes degeneration, villous shortening and diarrhoea (Haelterman, 1972). Replication in the tonsils and in the respiratory tract has been reported, but appears to be secondary to the enteric infection (Furuuchi *et al.*, 1978/1979; Kemeny *et al.*, 1975). The virus spreads by the oral–faecal route; it is not known whether the aerogenic route constitutes a significant mode of transmission (Bohl, 1981). The viral particle is composed of a non-glycosylated nucleoprotein (N) and two glycoproteins, one of which (E2) is associated with the surface projections and the other (E1) with the lipid envelope (Garwes & Pocock, 1975).

A serological survey carried out in 1984 against TGE virus among the swine population in Belgium showed a sudden high incidence of antibodies in the absence of disease and there were indications that a virus related to TGE virus had appeared. Similar findings were reported in other European countries. A virus, designated TLM 83, was subsequently isolated in cell culture. Experimental pig inoculations with this isolate did not result in disease but the formation of antibodies which neutralized TGE virus (Pensaert *et al.*, 1986). Recent pathogenesis studies have shown that TLM 83 replicates to high titres in the respiratory tract but to a very low degree in the gut. It has been established that the virus is spread aerogenically (M. Pensaert & J. Hooyberghs, unpublished observation).

The present study was undertaken to determine whether the two viruses could be differentiated. To this end, their cross-neutralization was examined and the degree of relatedness was investigated, at the level of the individual structural polypeptides, by

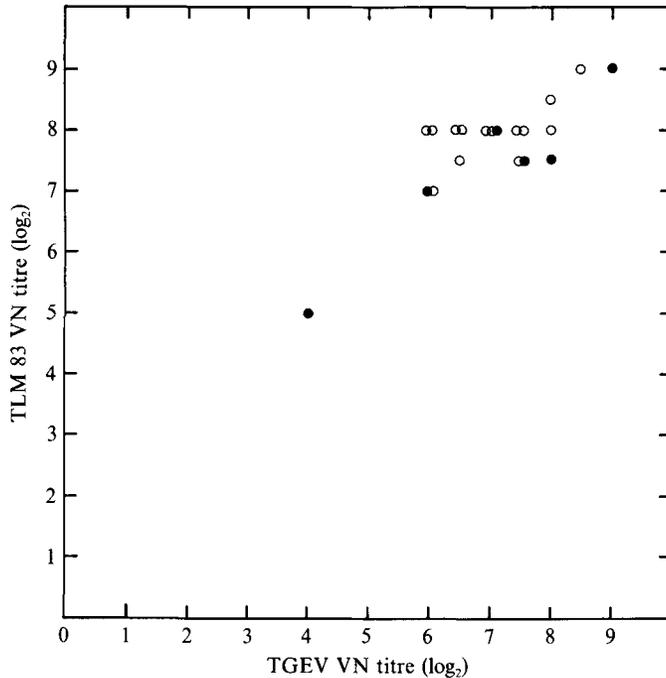


Fig. 1. Antigenic relationship between TLM 83 and TGE viruses examined by homologous and heterologous VN tests with sera from piglets experimentally infected with TLM 83 (○) and with TGE viruses (●). Titres are expressed as \log_2 of reciprocal endpoint dilution values.

immunoblotting analysis and by studying the reactivity with TGE virus-specific monoclonal antibodies (MAbs). The results may provide the basis for a differential serological diagnosis. The ability of virus neutralization (VN) tests to differentiate TGE and TLM 83 viruses was investigated by producing specific antisera to both viruses and comparing the homologous and heterologous antibody titres in cross-VN assays.

The TLM 83 virus isolate was used at the ninth passage in cell culture. It was cultivated in the swine kidney cell line PD5, grown as described by Horzinek *et al.* (1982). Following inoculation, cultures were incubated for 42 h at 37 °C; at that time the cells showed extensive cytopathic effect, characterized by clustering of rounded-up cells and initial loss of infected cells into the medium. The virus was harvested by two cycles of freezing and thawing. After clarifying, an infectivity titre of $10^{4.5}$ TCID₅₀/ml was obtained. The Purdue strain of TGE virus was the 114th passage in primary pig kidney (PPK) cells and, for the neutralization assays, was adapted to grow in PD5 cells by two passages. It reached an infectivity titre of $10^{5.5}$ TCID₅₀/ml. The porcine epidemic diarrhoea (PED) virus isolate CV 777 and the haemagglutinating encephalomyelitis (HE) virus isolate VW 572 were used as the third pig passage and the 15th passage in PPK cells, respectively, produced as described earlier (Debouck *et al.*, 1981; Pensaert & Callebaut, 1974).

Convalescent sera were raised in 1- to 6-week-old piglets, inoculated oronasally with the TLM 83 virus or orally with the Miller strain of TGE virus. Serum samples were collected 2 to 4 weeks after inoculation. VN tests with heat-inactivated (30 min at 56 °C) sera were performed in PD5 cells using the microtitre method described previously (Voets *et al.*, 1980).

The results of the neutralization tests with the convalescent sera are summarized in Fig. 1. Fourteen TLM 83 virus-specific antisera and six TGE virus-specific antisera had homologous VN titres between 7 and 9 and between 4 and 9 respectively. The titres of the sera prepared against each virus were similar in homologous and heterologous VN tests, indicating that there was a complete two-way neutralization activity between TLM 83 and TGE viruses.

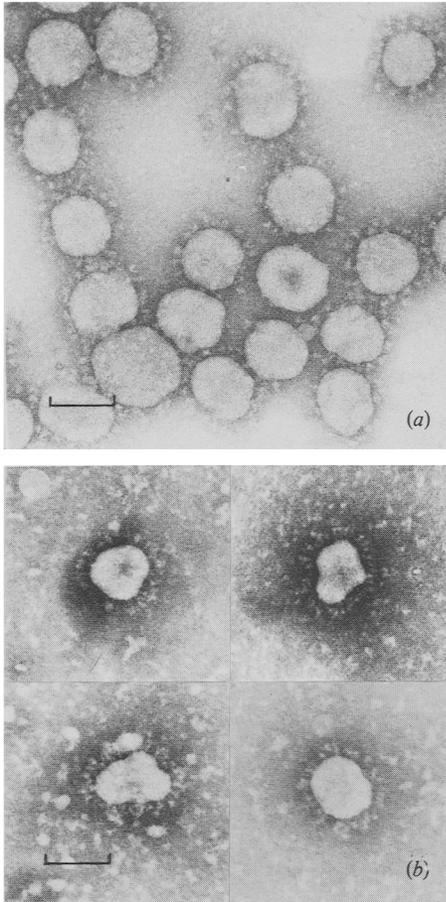


Fig. 2

Fig. 2. Electron microscopy of purified TGE (a) and TLM 83 (b) viruses, after negative staining with phosphotungstic acid. Bar markers represent 100 nm.

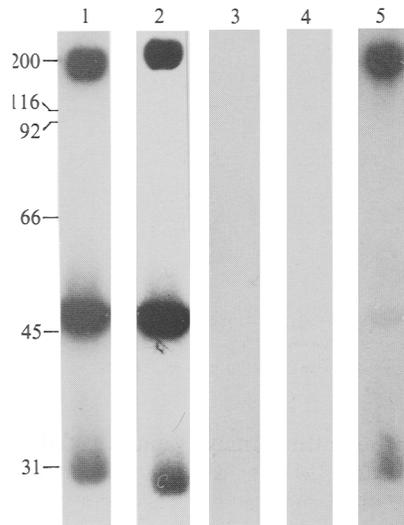


Fig. 3

Fig. 3. Immunoblot with TGE virus-specific and TLM 83-specific antisera with different porcine coronaviruses. Reactivity of TGE virus-specific antiserum with polypeptides of TGE, TLM 83, PED and HE viruses (lanes 1, 2, 3 and 4, respectively) and of TLM 83-specific antiserum with polypeptides of TGE virus (lane 5). M_r values ($\times 10^{-3}$) shown alongside.

In order to investigate the antigenic relatedness of TGE virus, TLM 83 virus, PED virus and HE virus at the level of each of the viral polypeptides E2, E1 and N, purified preparations of these viruses were analysed in parallel by immunoblotting. TLM 83, PED and HE viruses were purified by a procedure previously described (Callebaut & Pensaert, 1980) with some modifications. Briefly, crude virus suspensions were clarified at 15000 g for 20 min. Solid polyethylene glycol 6000 was added to a concentration of 15% and the suspension was stirred for 3 h at 4 °C. The precipitated virus was collected by centrifugation at 15000 g for 20 min and was resuspended in 0.02 M-Tris-HCl, 1 mM-EDTA, pH 7.4, to give a 100-fold concentration. This material was centrifuged through a linear 10 to 40% Urografin (Schering Corporation, Bloomfield, N.J., U.S.A.) density gradient at 76000 g in a Beckman SW41 Ti rotor for 60 min. After fractionation, the virus band was located by light scattering and was further purified by batch chromatography on hydroxyapatite (Bio-Rad) (Bernardi, 1971). The virus was absorbed to the gel for 30 min, washed three times with 0.02 M-phosphate buffer pH 6.5, and eluted by incubation with 0.3 M-phosphate buffer pH 6.5, for 30 min. The virus was pelleted at 30000 g in

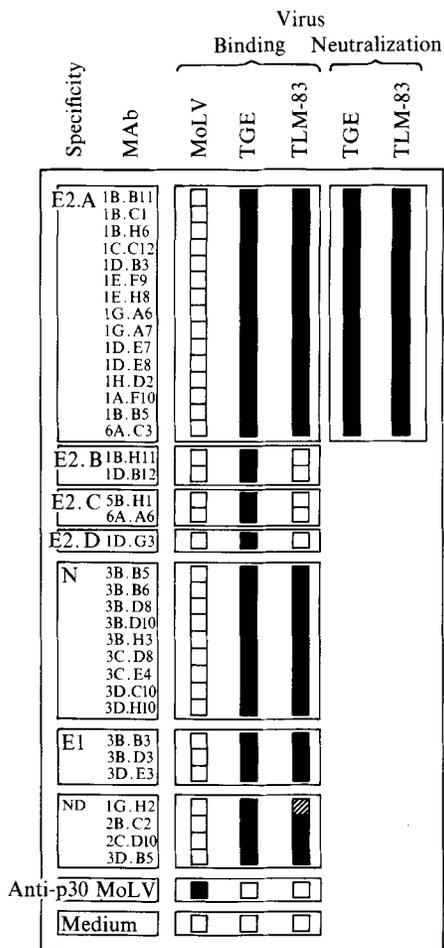


Fig. 4. Reactivity of TGE virus-specific MAbs with TLM 83 virus, determined by RIA and neutralization. MAb binding was determined by RIA. Empty, hatched and full squares represent binding of 0 to 19%, 20 to 40% and 41 to 100%, respectively. Moloney leukaemia virus (MoLV) and a hybridoma supernatant containing MoLV protein p30-specific MAb were used as a negative control. The neutralization index was determined by dividing the infectivity titre (expressed as the \log_{10} of the reciprocal endpoint dilution values) of virus mixed with normal medium by the infectivity titre of virus in the presence of MAb. Full squares represent neutralization indexes > 3.5 . ND indicates that the specificity of MAbs to TGE virus at the protein level was not determined.

a Beckman 42.1 rotor for 2 h and resuspended in a small volume of water. The Purdue strain of TGE virus was grown in the swine testicle ST cell line and purified according to the method described by Jiménez *et al.* (1986). Purified virus samples were processed for electron microscopy as earlier described (Debouck *et al.*, 1981). The preparations contained complete virions with a typical coronavirus morphology and were free of contaminating particulate material (Fig. 2).

The procedure for immunoblotting analysis has been described elsewhere (Jiménez *et al.*, 1986). Briefly, viral proteins were resolved by the method of Laemmli (1970) and transferred to nitrocellulose paper according to the method of Towbin *et al.* (1979). After saturating the non-specific binding sites of the nitrocellulose membrane, it was incubated with a 1:40 dilution of gnotobiotic porcine hyperimmune serum against TGE and TLM 83 viruses (Pensaert *et al.*,

1986). Detection of electrophoretically transferred proteins was performed by amplifying the reaction with a second antibody and ^{125}I -labelled Protein A (Correa *et al.*, 1988). A representative result of the immunoblot analysis is shown in Fig. 3. TGE virus-specific antiserum reacted to TGE and TLM 83 viral proteins, but not to those from HE or PED viruses. TLM 83-specific antiserum reacted to the three TGE viral proteins (Fig. 3), but did not react to those from HE or PED viruses (not shown); the homologous blot with TLM 83 virus was too weak to give conclusive data. The E2, N and E1 proteins of TGE virus had M_r values of 200 000, 48 000 and 28 000, respectively, in agreement with values previously published (Garwes & Pocock, 1975; Horzinek *et al.*, 1982; Laude *et al.*, 1986). TLM 83 proteins showed a pattern related to TGE virus proteins. E2 from TLM 83 was demonstrated to have an M_r in the same range as that from TGE virus, although some slight differences could not be excluded.

As common antigenic determinants appeared to be located on each of the structural polypeptides of TLM 83 and TGE viruses, the degree of the antigenic relatedness was assessed by comparing the individual epitopes of the two viruses, using a panel of TGE virus-specific MAbs. Both the binding assay and the MAbs which were used have been described previously (Jiménez *et al.*, 1986; Enjuanes *et al.*, 1987). Of these, 20, nine and three MAbs were specific for the E2, N and E1 proteins of TGE virus, respectively; we also used four MAbs to TGE virus, for which the specificity at the protein level was unknown. Fifteen E2 protein-specific MAbs, which were able to neutralize TGE virus and defined a single antigenic site, were designated anti-E2.A. The five remaining non-neutralizing E2 protein-specific MAbs recognized three separate antigenic sites and were designated anti-E2.B (two MAbs), anti-E2.C (two MAbs) and anti-E2.D (one MAb). The N and E1 protein-specific MAbs used did not neutralize TGE virus. In addition, the neutralization of TGE and TLM 83 viruses by the anti-E2.A MAbs was determined in PD5 cells as described above. The results of these assays are shown in Fig. 4. By radioimmunoassay (RIA) most MAbs recognized both TGE and TLM 83 viruses. No differences between the epitopes located on the E1 and N polypeptides could be detected, as all anti-N and anti-E1 MAbs bound to TLM 83 virus to a great extent. Furthermore, all E2.A-specific MAbs were fully reactive with TLM 83 virus both in the binding and the neutralization assays, indicating that the epitopes for the induction of neutralizing antibodies were indistinguishable. However, the non-neutralizing anti-E2.B, anti-E2.C and anti-E2.D MAbs failed to recognize TLM 83, indicating that the antigenic sites in the E2 protein of TGE virus, responsible for stimulating non-neutralizing antibodies, were modified or absent in the equivalent polypeptide of TLM 83 virus. All MAbs with unknown specificity extensively bound to TLM 83 virus.

These results demonstrated that TGE and TLM 83 viruses were closely related and structurally similar, as common antigenic determinants were found on E2, E1 and N proteins with porcine convalescent sera and with murine MAb preparations and it was found that these proteins were of similar M_r . Therefore, TLM 83 virus can be regarded as a TGE virus variant, which may have spontaneously emerged, rather than as a new porcine coronavirus species. The complete absence of serological cross-reactivity of TLM 83 virus with the porcine coronaviruses HE and PED, using immunofluorescent staining (Pensaert *et al.*, 1981) and immunoblotting, supports this conclusion.

Our results demonstrate that antigenic dissimilarities between TLM 83 and TGE viruses are associated with their respective peplomer proteins. The differences may be exploited for developing a competitive ELISA for the differential detection of antibodies against each virus. The lack of reactivity of site B-, C- and D-specific MAbs with TLM 83 is probably significant as these sites were present on three TGE virus clones of American (PUR 54), Japanese (SHI 56) and European (BRE 79) origin that were collected over a period of 25 years (Jiménez *et al.*, 1986). Other isolates of the two viruses are currently being analysed in order to confirm the similarities and differences between them. As a limited number of N and E1 protein-specific MAbs have been used, it cannot be ruled out that antigenic dissimilarities between TLM 83 and TGE viruses are also associated with these two proteins.

Further work is in progress to elucidate how the antigenic differences are related to the *in vivo* difference in host cell tropism between the two viruses.

We wish to thank Miss Lieve Sys for her able technical assistance. This study was supported by the Institute for Encouragement of Scientific Research in Industry and Agriculture (IWONL), Brussels, Belgium. This investigation was also funded by grants from the Consejo Superior de Investigaciones Científicas, Comisión Asesora para la Investigación Científica y Técnica, and Fondo de Investigaciones Sanitarias (Spain).

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(Received 21 September 1987)