MOLECULAR ASPECTS OF THE RELATIONSHIP OF TRANSMISSIBLE GASTROENTERITIS VIRUS (TGEV) WITH PORCINE RESPIRATORY CORONAVIRUS (PRCV)

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INTRODUCTION

Transmissible gastroenteritis, caused by a coronavirus (TGEV), has been recognised as a viral disease since 1946 when the virus was first isolated by Doyle and Hutchings (1). TGEV has been shown to cause diarrhoea in pigs of all ages but has a high mortality, often 100%, in neonatal piglets. The TGEV virion, like all coronaviruses, contains an envelope, whose lipids are derived from the host cell endoplasmic reticulum, a single-stranded RNA genome, of positive polarity, and three structural proteins. The virion proteins are: a surface glycoprotein (peplomer) of M_r 200000, a glycosylated integral membrane protein observed as a series of polypeptides of M_r 28000-31000 and a basic phosphorylated protein (nucleoprotein) of M_r 47000 associated with the viral genomic RNA (2). Like all the coronaviruses the TGEV proteins are expressed from a series of subgenomic mRNA species, six in the case of TGEV (3), which have common 3' ends but different 5' extensions. The region of each mRNA responsible for the expression of a protein appears to correspond to the 5'-terminal region, often referred to as the 'unique' region, that is absent from the preceding smaller species. The TGEV genome encompassing the structural protein genes has been cloned and sequenced from a virulent British isolate, FS772/70 (4, 5, 6, 7, 8 and unpublished results), and from the avirulent Purdue strain (9, 10, 11, 12, 13, 14). This has led to the identification of five other potential genes, one of which appears to be the polymerase gene (8), another that appears to be located in the host cell nucleus and not in TGEV virions (8, 15) and three others whose products have yet to be identified in TGEV infected cells.

A virus antigenically related to TGEV has recently appeared, and spread rapidly, throughout the pig population in several European countries between 1984 and 1986. The virus does not cause diarrhoea and has been shown to replicate in the respiratory tract with little or no clinical signs (16). The causative agent, isolated in Belgium (16) and in Britain (17), was identified as a coronavirus that produced a serological response that could not be distinguished from TGEV-infected pigs by available diagnostic tests. The respiratory form of TGEV has been named porcine respiratory coronavirus (PRCV) and shown not to replicate in the enteric tract upon oronasal inoculation. This is in contrast to TGEV which preferentially grows in the enterocytes covering the tips of the villi in the small intestine, causing diarrhoea and dehydration. TGEV has also been

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shown to be present in lung tissue by immune fluorescence and virus isolation (18). PRCV is fully neutralised by antisera prepared against TGEV and the majority of monoclonal antibodies (mAbs) raised against any of the TGEV virion proteins do cross react with PRCV. However, mAbs raised against antigenic determinants of the peplomer protein from either the virulent British isolate FS772/70 (19) or the avirulent Purdue strain (20) of TGEV, have been identified that do not recognise PRCV. None of the mAbs that reacted with TGEV but not PRCV had any neutralising effect on TGEV indicating that the difference between the peplomer molecules, recognised by these mAbs, is probably in a part of the molecule not involved in virus neutralisation. PRCV is not related to two other pig coronaviruses, porcine epidemic diarrhoea virus (PEDV) and porcine haem-agglutinating encephalomyelitis virus (HEV), neither of which belong to the TGEV subgroup (21).

Here we report the characterisation of polypeptides synthesised by a British isolate of PRCV in infected cells and describe differences in the subgenomic mRNA species of the two viruses.

METHODS

Virus production and RNA isolation

The 86/137004 isolate, Burkle strain, of PRCV and the FS772/70 strain of TGEV were grown and plaque assayed in secondary adult pig thyroid (APT/2) cell cultures (2). Viral subgenomic mRNA was isolated from infected LLC-PK1 cells as described previously (4). Monolayer cultures of APT/2 cells, 850 cm^2, in plastic roller bottles were infected with TGEV or PRCV (m.o.i. = 5 pfu/cell) for 2 h at 37°C, transferred to methionine-free medium for a further 2 h and metabolically labelled with [^{35}S]-methionine at 100 µCi/ml for a further 14 h. Cultures were frozen and thawed and the virions purified by sucrose gradient centrifugation (2).

Analysis of [^{35}S]-methionine labelled viral polypeptides

LLC-PK1 cells were infected with TGEV or PRCV, at a m.o.i. of 5-10 pfu/cell, and incubated at 37°C for 2 h. The inoculum was replaced with methionine free Eagles MEM medium and the cells incubated for a further 2 h as described previously (19). The infected cells were then incubated in the same medium containing 250 µCi of [^{35}S]-methionine and lysed (19). Protein samples were denatured by heating at 100°C for 2 mins in PAGE sample buffer and analysed on 12-20% polyacrylamide gels.

Messenger RNA analysis

Specific restriction fragments from TGEV cDNA clones were separated on agarose gels, purified by Geneclean™, and labelled with [α^{32}P-dATP] (5). TGEV and PRCV subgenomic mRNA species were denatured with 6M glyoxal, electrophoresed into 1% agarose gels, northern blotted onto Biodyne membrane and hybridised to ^{32}P-labelled TGEV cDNA fragments (5). The probes were hybridised and washed as described previously (7).

RESULTS

Viral polypeptides

TGEV and PRCV, grown in APT/2 cells in the presence of [^{35}S]-methionine, were purified by sucrose gradient centrifugation and Figure 1 shows that the two viruses have a similar overall structure, with the three major

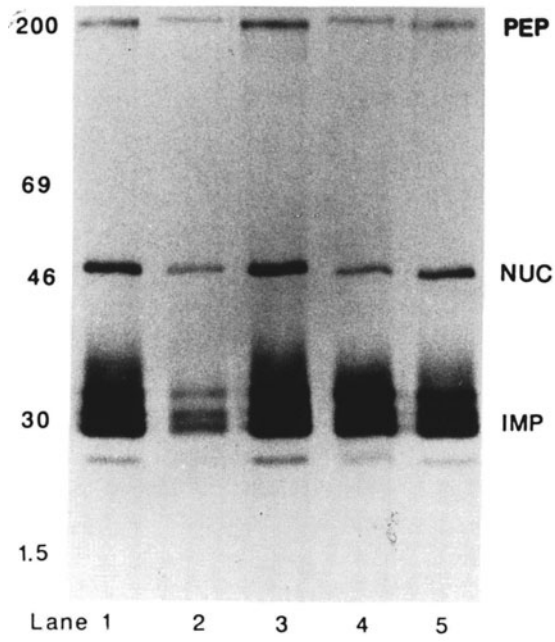

Fig. 1. Polypeptides purified from [^{35}S]-methionine labelled virions of TGEV (Tracks 2 and 4) and PRCV (Tracks 1, 3 and 5). PEP: peplomer; NUC: nucleoprotein; IMP: integral membrane protein; analysed by PAGE.

polypeptide species, the peplomer, nucleoprotein and integral membrane protein. LLC-PK1 cells, uninfected or infected with either TGEV or PRCV and incubated between 1-5 h, 5-10 h and 10-15 h post infection in the presence of [^{35}S]-methionine, were lysed and analysed by PAGE. Figure 2 shows that by 5 h after infection the TGEV nucleoprotein is detectable but the equivalent protein is not detectable in PRCV infected cells until between 5-10 h post infection. There is a marked suppression of host cell protein synthesis in the TGEV-infected cells between 5-10 h after infection bu this is not apparent in the PRCV cultures. The major polypeptide species discernible in the infected cel lysates with both viruses are the peplomer, nucleoprotein, the truncated form of the nucleoprotein (M_r 42000), the integral membrane protein and polypeptides of M_r 17000 and 14000. It can be seen from Figs. 1 and 2 that the PRCV peplomer protein (M_r 190000) is slightly smaller than the TGEV peplomer and that the PRCV nucleoprotein (M_r 48000) is slightly larger than the TGEV protein. The truncated form of the PRCV nucleoprotein (M_r 43000) is also slightly larger than the TGEV product. There is no detectable difference in the size of the PRCV integral membrane protein. The polypeptide of M_r 14000, TGEV ORF-4 gene product (5, 8, 15), is also present in PRCV infected cells and migrates identically to the TGEV product. A polypeptide of M_r 17000, observed in TGEV infected cells but not assigned to any TGEV gene (15), is also present in PRCV infected cells and migrates identically to the polypeptide observed in TGEV infected cells.

Viral mRNA analysis

Subgenomic mRNA from cells infected with either TGEV or two different isolates of PRCV were blotted onto membranes and probed with ^{32}P-labelled TGEV cDNA. This revealed the series of mRNA species shown in Figure 3.

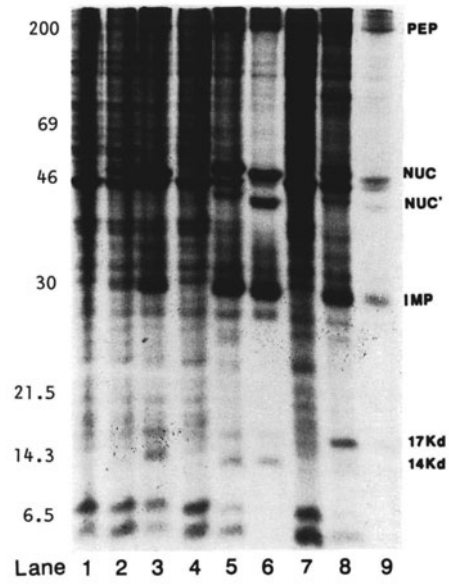

Fig. 2. Polypeptides from uninfected or virus infected cells labelled with [^{35}S]-methionine during 1-5 h (Tracks 1-3), 5-10 h (Tracks 4-6) and 10-15 h (Tracks 7-9) post infection. Uninfected cells (Tracks 1, 4 and 7); PRCV-infected (Tracks 2, 5 and 8); TGEV-infected (Tracks 3, 6 and 9).

The overall number of mRNA species was six for both viruses. The sizes of the TGEV species were 8.4, 3.9, 3.0, 2.6, 1.7 and 0.7 kb (3, 4, 5, 6, 7, 8). The pattern of mRNA from PRCV-infected cells was very similar, with

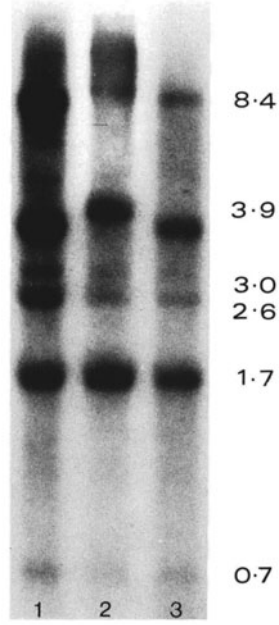

Fig. 3. Autoradiograph of northern blotted TGEV mRNA (Track 2) and from two strains of PRCV (Track 1; 86/137004, Track 3; 86/135308) probed with ^{32}P-labelled TGEV cDNA derived from several genes.

identical sizes for the 3.0, 2.6, 1.7 and 0.7 kb species but the PRCV equivalent to the TGEV 3.9 kb mRNA migrated faster than that of TGEV, relating to a size of 3.6-3.7 kb. The peplomer mRNA (8.4 kb TGEV) from PRCV also migrated faster than the TGEV equivalent.

DISCUSSION

Comparison of the virion proteins between British isolates of TGEV an PRCV shows very little difference in their molecular weights. The differences seen, a slightly smaller peplomer and slightly larger nucleoprotein in the case of PRCV, are of the same magnitude as those seen between TGEV and other members of the same serological group, namely feline infectious peritonitis virus (FIP) and canine coronavirus (CCV). The difference observed in the size of the peplomer polypeptides may be due to variations in the extent of glycosylation. the variations in the size of the nucleoprotein cannot be attributed to glycans as this protein is not glycosylated. It is interesting to note that the truncated form of nucleoprotein is also larger in the case of PRCV. The polypeptide of M_r 14000, product of the TGEV ORF-4 gene, is also present in PRCV infected cells.

TGEV has been shown to produce six subgenomic mRNA species and one, 3.9 kb, has two potential open reading frames at its 5' 'unique' region. The four smallest mRNA species from TGEV and PRCV appear to have the same size but the two larger species, 8.4 and 3.9 kb, appear to be smaller in PRCV. The mRNA equivalent to the TGEV 3.9 kb mRNA is 3.6-3.7 kb in PRCV infected cells. Comparison of the cDNA sequences, from TGEV and PRCV, corresponding to the 5' ends of this mRNA shows that there are deletions within this region of PRCV. Sequence data indicates that the smaller size of the mRNA in PRCV is probably due to a new consensus sequence, ACTAAAC, preceding the ORF-2 gene (unpublished result). This sequence appears to be involved in the synthesis of TGEV mRNA species and precedes the TGEV ORF-1 gene on the 3.9 kb mRNA species.

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