THE STRUCTURE OF THE CANINE CORONAVIRUS

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During a survey of canine sera in 1970, Norman and his associates detected antibodies to porcine transmissible gastroenteritis (TGEV). Since many of the dogs had never been in contact with pigs it was unlikely that this reflected infection with the porcine virus, suggesting the existence of a canine virus that was serologically related to TGEV. Further evidence to support this was provided by the study by Cartwright and Lucas (1972) of an outbreak of gastroenteritis in a kennel of 40 dogs in which rising antibody titres to TGEV were found. Although neither of these studies could find evidence of transmission of TGEV from pigs to dogs, this possibility could not be discounted since Haelterman (1962) had clearly demonstrated that dogs and foxes could be infected with the porcine virus. Dogs that were experimentally infected with TGEV showed no clinical signs but the virus could be reisolated from faeces and TGEV-neutralizing antibodies were produced in the serum.

The picture became clearer, however, when Binn and his associates (1975) reported their isolation of a canine enteric coronavirus, designated 1-71, from American military dogs suffering from diarrhoea. This virus was readily cultivated in puppies, in which it caused severe gastroenteritis, but would not infect piglets. It was antigenically related to TGEV but lacked the ability to infect porcine cells.

Our interest in the canine coronavirus (CCV) grew out of our studies on TGEV. For the past few years there has been very little TGEV in the U.K. although the pigs would seem to be fully susceptible. Since it is in the national interest to keep the number of TGEV outbreaks down to the minimum we became interested in the role of the dog and the cat (Reynolds and Garwes, 1979) as

potential carriers of the virus. In both of these instances, the epizootiology is confused by the presence of TGEV-related coronaviruses in the carrier, i.e. canine coronavirus and feline infectious peritonitis virus (Reynolds et al., 1977). We have studied the serological relationships of these viruses (Reynolds et al., 1980) and compared the structures of CCV and TGEV (Garwes and Reynolds, 1980). This paper summarizes our findings for the structural polypeptides of these two coronaviruses.

We used the 1-71 isolate of CCV for this study, grown in secondary dog kidney cells. To ensure that we were not working with a mixed population of virus we developed a plaque assay procedure and used this to clone the virus three times. Several clones were selected at each plaquing step and were subsequently tested to determine whether any obvious differences in their structural polypeptides could be detected but they were indistinguishable from each other and from the parent stock by this method.

Like many other coronaviruses, the growth of CCV in primary and secondary cell cultures does not result in very high titres. Yields of 10^6 - 10^7 TCID₅₀/ml were achieved but we did not consider that these were adequate to carry out chemical studies on the virus and we therefore resorted to the use of radiolabelled precursors, incorporated into the growth medium.

The virus was purified by the procedure that we had established for TGEV; removal of cell debris by low speed centrifugation, concentration of the virus by ammonium sulphate precipitation followed by 2 rounds of centrifugation in sucrose gradients (Garwes and Pocock, 1975). Following dissociation of the purified virus by sodium dodecyl sulphate and β -mercaptoethanol, the polypeptides were separated by electrophoresis on 5% acrylamide gels crosslinked with ethylene diacrylate using a phosphate buffer system.

The electropherograms shown in Fig. 1 were obtained from virus that had been grown in the presence of ^3H -leucine (a), ^{35}S -methionine (b) and ^3H -glucosamine hydrochloride (c). It can be seen that there are 4 major polypeptides of which 3 are glycosylated. The presence of minor components cannot be excluded but the small peaks and humps between the first and second major polypeptides tended to be inconsistent from preparation to preparation. By comparison with the rates of migration of proteins of known molecular weights, the sizes of the 4 major polypeptides of CCV were estimated from 18 determinations using 13 different batches of virus and this is summarised in Table 1. The molecular weights of gp 204, gp 32 and gp 22 are only approximate as the carbohydrate present on these glycopolypeptides will affect their migration rates.

We had previously shown that the high molecular weight glycopolypeptide of TGEV was associated with the peplomers that

Fig. 1. Polyacrylamide gel electropherograms of CCV polypeptides labelled with (a) ^3H -leucine, (b) ^{35}S -methionine and (c) ^3H -glucosamine. Migration was from the left.

Table 1. The Structural Polypeptides of Canine Coronavirus

Polypeptide Designation	Molecular Weight	Standard Deviation	Glycosylation
gp 204	203,800	6,400	+
p 50	49,800	900	-
gp 32	31,800	1,100	+
gp 22	21,600	1,500	+

Fig. 2. Polyacrylamide gel electropherograms of CCV polypeptides. Control, ●—● : NP40-treated, ●--●. Migration was from the left.

protrude from the surface of the virion and that these could be removed from the virus following solubilisation of the lipid envelope with detergent (Garwes et al., 1976). A similar procedure was attempted with CCV; purified virus was treated with 1% Nonidet P40 at room temperature for 15 minutes and then centrifuged through a sucrose gradient. The RNA-containing subviral particles were recovered from the gradient and compared with virus that had been treated with water and then centrifuged as a control. The resultant electropherograms of these 2 preparations are shown in

Fig. 2. The polypeptide profile of the detergent-treated virus resembles that of the control in most respects but it is obvious that gp 204 is lacking from the subviral particle. This is only circumstantial evidence for the role of gp 204 in the structure of the CCV peplomer, however, as electron microscopy of the subviral particles revealed structures that, although lacking the characteristic coronavirus projections, were so morphologically altered that there is no certainty that peplomers would be seen even if they were present (Fig. 3).

Fig. 3. Electron micrograph of CCV subviral particles. 2% sodium phosphotungstate, pH 7.2. The bar represents 100 nm.

The apparent molecular weights of the CCV polypeptides were strikingly similar to those found for TGEV (Garwes and Pocock, 1975). For an accurate comparison to be made between the two viruses, differences that might be caused by host-induced glycosylation patterns or by variation between polyacrylamide gels would need to be avoided. To this end, TGEV was grown in secondary dog kidney cells under identical conditions to those used for CCV. The titre of virus achieved was similar to that for CCV but TGEV produced much greater cytopathic changes in the cells, resulting in cell rounding and detachment within 18h after infection. We were unable to show

any replication of CCV in secondary pig thyroid cells, the culture system employed by us for TGEV (D. Reynolds, personal communication).

In order to analyse TGEV and CCV in the same polyacrylamide gel, we used ^3H -leucine and ^{35}S -methionine for double label experiments. When purified preparations of CCV that had been grown in ^3H -leucine and ^{35}S -methionine were pooled and subjected to gel electrophoresis,

Fig. 4. Polyacrylamide gel electropherogram of the structural polypeptides of ^{35}S -methionine-CCV (○) and ^3H -leucine-TGEV (●). Migration was from the left.

the resulting peaks coincided exactly, showing that the substitution of one label for another had no noticeable effect on the migration rates of the polypeptides. There was a marked difference in the relative proportions of ^3H -leucine and ^{35}S -methionine in gp 32, comparable with Sturman's observation of a relative richness of methionine in the membrane glycopolypeptide of the murine coronavirus A59 (Sturman, 1977).

Co-electrophoresis of CCV and TGEV that had been grown in different labels showed several differences. An example of ^3H -leucine-TGEV and ^{35}S -methionine-CCV is illustrated in Fig. 4 but a similar profile was obtained with ^{35}S -methionine-TGEV and ^3H -leucine-CCV (except for the height of the gp 32 peak of CCV, as just mentioned). Whereas p50 of both CCV and TGEV exactly coincided, gp 204 and gp 32 of CCV were slightly, but reproducibly, larger than their equivalents in TGEV. More noticeable, however, was the absence of a major polypeptide of 22,000 daltons in TGEV. We cannot rule out the possibility that TGEV may have a gp 22 (or equivalent) as a minor constituent but it is clearly not present in the quantities seen in CCV. It was possible that CCV gp 22 was a cleavage product of one of the other major viral polypeptides but this seems unlikely if one examines the candidates: gp 204 is removed by detergent but gp 22 is not; p50 is not glycosylated whereas gp 22 is; gp 32 is rich in methionine but gp 22 does not appear to be. It appears that CCV may have 2 membrane glycopolypeptides, a feature shown by several other coronaviruses (Garwes, 1980) including, as previously suggested (Garwes and Pocock, 1975), TGEV.

In conclusion, then, we can say that CCV and TGEV are related in their polypeptide structure as well as antigenically. Considering the lack of similarity between TGEV and other coronaviruses, including the other porcine member haemagglutinating encephalomyelitis virus, the close relationship between CCV and TGEV strongly suggests that they were derived either one from the other or both from a common parent during the evolution of the Coronaviridae. One might speculate that a mutation of CCV, resulting in a decrease in the size of gp 204 and gp 32 and in the loss of gp 22, allowed the modified CCV to grow in porcine cells yielding TGEV. Further, the new virus TGEV could grow in its old host (dog) as well as its new (pig) whereas the CCV stock could not grow in pig cells. This is, however, pure speculation but it is possible that more information on the evolution of this antigenic subgroup of coronaviruses will become available when the structure of the third member of the group, feline infectious peritonitis virus, is elucidated.

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