STRUCTURAL AND ANTIGENIC RELATIONSHIPS BETWEEN HUMAN, MURINE AND AVIAN CORONAVIRUSES

Malcolm R. Macnaughton

Division of Communicable Diseases
Clinical Research Centre
Harrow, Middlesex HA1 3UJ, UK

INTRODUCTION

There have been numerous reports in the literature describing the morphological and molecular structure of individual coronaviruses and comparing the structure of different strains of the same coronavirus species. However, there have been few attempts to compare the structure of different coronavirus species in the same laboratory. Some work has been done on the antigenic comparison of different coronaviruses, notably by Pederson et al. (17), but there is still considerable dispute about their antigenic relationships. This report is concerned with characterising the structural and antigenic relationships of a number of coronaviruses including various strains of human coronavirus (HCV), mouse hepatitis virus (MHV) and avian infectious bronchitis virus (IBV).

MORPHOLOGY

A previous study (4) has shown that these coronaviruses have similar morphologies. All the coronaviruses were pleomorphic, with a wide range in envelope diameters, although the three IBV strains examined, Massachusetts (Mass), Beaudette (Beau) and Connecticut (Conn) were significantly larger than MHV 3 and HCV 229E (Fig 1). Furthermore, two main types of surface projection were seen, both of about 20 nm in length. One type consisted of typical bulbous coronavirus projections and was seen on most IBV, MHV 3 and HCV 229E particles (Fig 1a). The other type of projection was in the form of a narrow cone and was seen mainly on MHV 3 particles (Fig 1b). We have previously suggested that both

Fig. 1. Purified coronavirus particles negatively stained with 2% potassium phosphotungstate, pH 6.5. a) MHV 3, b) IBV Conn. The bar represents 50 nm.

Fig. 2. Densitometer tracings of purified virus polypeptides on 7.5% polyacrylamide gels after staining with Coomassie brilliant blue. a) HCV 229E, b) IBV Conn. The numbers on the graphs represent the mol. wt. $\times 10^{-3}$, and are referred to in the text as VP (virus polypeptide) and VGP (virus glycopolypeptide) followed by the appropriate mol. wt. $\times 10^{-3}$.

projection types are manifestations of the same structures and that the bulbous projections are aggregates of thin cone shaped projections (4).

RNA AND RIBONUCLEOPROTEIN

It is generally accepted that the nucleic acid of coronaviruses consists of a large single stranded RNA molecule of positive polarity. Furthermore, we have shown that the genomes of IBV Beau, MHV 3 and HCV 229E all exist as similar single-stranded RNAs of mol. wt. about 5.8×10^6 with similar lengths of 3'-terminal poly(A) sequences (9).

We have shown that the ribonucleoproteins (RNP) of HCV 229E and MHV 3 can be readily visualised as helical structures by negative staining (12), although no IBV helices could be observed under similar circumstances. However, we have recently observed IBV RNP molecules by shadowing that were generally linear showing only a little helical structure (3). Thus, it appears that the stability of IBV RNP is different from that of HCV 229E and MHV 3, even though they have similar genomes and RNP polypeptides (see below).

PROTEIN COMPOSITION

The polypeptide composition of coronavirus particles is confusing, although most reports now tend to show a relatively simple polypeptide structure which may be common to many coronaviruses. Fig 2a shows a typical polypeptide profile of HCV 229E consisting of four major structural polypeptides. Similar structural polypeptides have been identified for three other HCV strains, HCV PR, HCV TO and HCV OC43, and MHV 3. Fig 2b shows the structural polypeptides of IBV, which are different from those of HCV or MHV. However, two recent reports (1,7) have shown IBV polypeptides more similar to those shown in Fig 2a, although other recent reports show more complex polypeptide patterns (2,16). The reported differences in the polypeptide composition of IBV may be due to contamination with host polypeptides (2) and/or be due to the aggregation of certain of the glycopolypeptides (10).

The position of the structural polypeptides in the virus particles has been studied, and those of HCV 229E (Fig 2a) are described as a typical example. The large mol. wt. glycopolypeptides, VGP 160 and VGP 105 (not resolved in Fig 2a), form the surface projections. This has been shown by digestion of the virus particles with bromelain or other proteolytic enzymes, or by treatment with dithiothreitol or nonidet P40 (10). In all cases VGP 160, and probably VGP 105, were removed from the virus particles along with the surface projections. RNP has been

isolated on sucrose density gradients from nonidet P40 treated virus particles and shown to consist of VP 50 and RNA. We have also suggested, by exclusion, that VGP 24 and VP 22 comprise the envelope polypeptides (10). Similar results were obtained with other HCV strains and MHV 3.

The polypeptide composition of the subcomponents of IBV has also been determined, and results broadly similar to those of HCV 229E have been obtained. Large mol. wt. polypeptides form the surface projections, VP 51 corresponding to VP 50 of HCV 229E comprises the RNP, and the small mol. wt. glycopolypeptide VP 33 forms the membrane polypeptide (3,11,13).

ANTIGENIC RELATIONSHIPS

Previous studies on the antigenic relationships of coronaviruses, using a variety of techniques, have produced a confusing pattern of interrelationships (14,19). A recent comprehensive study by Pederson et al (17) has shown that mammalian coronaviruses can be grouped into at least two main antigenic groups by fluorescent antibody studies. Many of these interrelationships have been confirmed and extended by other workers (summarised in 14, 17, 19). The viruses in these two antigenic groups are shown in Table 1. It is interesting to observe that this grouping of viruses cuts through virus groups by the species affected. IBV, the only avian coronavirus to be extensively studied, has been shown to be antigenically unrelated to the mammalian coronaviruses (14,19), and thus apparently forms a separate antigenic group.

The antigenic relationships between coronavirus species and strains within these species have been studied by enzyme-linked immunosorbent assay (ELISA). ELISA has been shown to be highly sensitive and to detect a broad range of antigenic specificity. The ELISA technique used has been described previously (5) using sucrose gradient purified antigens and immune sera to these antigens raised in rabbits (5). Detailed ELISA results for the IBV strains are shown below as examples, while results obtained for the HCV strains and MHV 3 are summarised for convenience.

Table 2 shows that the ELISA reaction is extremely sensitive for IBV Mass, detecting antibody at dilutions of rabbit anti-IBV Mass of 1:102400. Reactions were considered positive for ratios of immune to pre-immune sera absorbance values of 2 or more at the same dilution (5). Similar results were obtained for IBV Beau and IBV Conn against anti-IBV Beau and Conn, respectively. Table 3 shows that no antigenic differences could be detected by ELISA between IBV Mass, Beau and Conn, even though IBV Mass and Beau are not related to IBV Conn by neutralisation and apparently have different glycopolypeptides (2,16) from IBV Conn. Thus the ELISA is

Table 1 Antigenic relationships of selected
 mammalian coronaviruses^a

Group 1	Group 2
HCV OC43	HCV 229E
MHV	TGEV
CDCV	FIPV
HEV	CCV
RCV	
SDAV	

^a Adapted from Pederson et al (17) and incorporating other results summarised by Pederson et al. (17), McIntosh (14) and Tyrrell et al. (19). CDCV, calf diarrheal coronavirus; HEV, haemagglutinating encephalomyelitis virus of swine; RCV, rat coronavirus; SDAV, sialodacryoadenitis virus of rats; TGEV, transmissible gastroenteritis virus of swine; FIPV, feline infectious peritonitis virus; CCV, canine coronavirus.

Table 2 ELISA of IBV Mass against anti-IBV Mass^a

Serum Dilution	Anti-IBV Mass	
	Pre-immune	Immune
1 : 1600	0.04	0.69
1 : 6400	0.03	0.34
1 : 25600	0.03	0.17
1 : 102400	0.02	0.06
1 : 409600	0.02	0.03

^a Data are given as absorbance values at 405 nm after 30 minutes. IBV Mass dilution 1 : 400.

Table 3 ELISA of IBV strains Mass, Beau and Conn against anti-IBV Mass,
anti-IBV Beau and anti-IBV Conn^a

IBV strain ^b	Anti-IBV Mass ^c		Anti-IBV Beau ^c		Anti-IBV Conn ^c	
	Pre-immune	Immune	Pre-immune	Immune	Pre-immune	Immune
Mass	0.02	0.79	0.04	0.70	0.03	0.69
Beau	0.02	0.70	0.03	0.71	0.02	0.64
Conn	0.01	0.67	0.02	0.61	0.01	0.52

^a Data are given as absorbance values at 405 nm after 30 minutes

^b IBV dilutions 1:400

^c Anti-IBV dilutions 1:400

highly sensitive and detects no antigenic differences between distinct IBV strains.

Other studies have used sucrose gradient purified MHV 3 and several HCV strains in order to determine their antigenic relatedness by ELISA. Eight HCV isolates were obtained by Dr H.E. Larson at the Clinical Research Centre, Harrow and Dr S.E. Reed and Dr A.D. Macleod at the Common Cold Unit, Salisbury, from volunteers with colds (8, and unpublished data). Three of these isolates, KI, PR and TO, were readily adapted to growth in tissue culture, while the other isolates, AD, HO, GI, PA and RO, could only be grown in human foetal trachea and nasal mucosa organ cultures (8). Only the tissue culture adapted isolates were extensively analysed as only relatively small quantities of the organ culture adapted isolates were available. The morphology and polypeptide structures of PR and TO were shown to be the same as the prototype 229E and OC43 viruses, although PR and TO were shown to be distinct strains by neutralisation (Dr S.E. Reed, unpublished results).

The new isolates were compared with each other and strains 229E and OC43 using immune sera to these viruses prepared from rabbits, and sera from volunteers experimentally infected with HCVs. The HCV strains fell into at least two distinct antigenic groups, the HCV 229E group comprising strains 229E, KI, PR, TO and HCV OC43 (6). The other strains AD, HO, GI, PA and RO have not been completely characterised yet. However, preliminary results show that the strains are related to either the HCV 229E group viruses or HCV OC43. Experiments are in progress to resolve this problem and to identify any common HCV antigenic components. Finally, it is perhaps worth emphasising that the HCV 229E group viruses all readily grew in tissue culture; while HCV OC43 and HCV strains AD, HO, GI, PA and RO, were not readily adaptable to tissue culture, although recent reports have shown that HCV OC43 can be tissue culture adapted (15,18).

No antigenic relationship was observed between HCV 229E group viruses and MHV 3 by ELISA (5), although care had to be taken to remove contaminating serum components that can lead to spurious reactions. On the other hand, antigenic reactions were observed between HCV OC43, grown in suckling mouse brain (obtained from Dr S.E. Reed), and MHV 3 grown in mouse embryonic fibroblasts (12) using immune antisera obtained from rabbits. However, no such reactions have so far been obtained using AD, HO, GI, PA and RO strains grown in human organ culture against MHV 3 antisera from rabbits, or MHV 3 against human sera from volunteers inoculated with these HCVs. These results suggest that MHV 3 and HCV strains AD, HO, GI, PA and RO belong to different antigenic groups. However, the common antigenic components between HCV OC43 and MHV 3 (also described in 14, 17, 19) may be due to mouse antigens, as

Table 4 Antigenic groups of selected
 coronaviruses by ELISA

Group 1	IBV strains Mass, Beau and Conn
Group 2	HCV strains 229E, PR, KI, and TO
Group 3	HCV strain OC43 and MHV strain 3

The classification of HCV strains AD, HO, GI, PA and RO is unclear at present.

these studies all used HCV OC43 grown in suckling mouse brain. Thus, the coronaviruses studied can be grouped into at least three main antigenic groups by ELISA (Table 4), with the HCV strains AD, HO, GI, PA and RO probably being related to the HCV 229E group or HCV OC43.

CONCLUSIONS

There appear to be at least two main coronavirus groups. One group contains the IBV strains, which show significant morphological, structural and antigenic differences from the mammalian coronaviruses. Mammalian coronaviruses form the other main group, and these viruses are morphologically and structurally similar to one another, although they do seem to fall into different antigenic groups by ELISA. The HCV strains fall into at least two antigenic groups, the HCV 229E group and HCV OC43, with HCV OC43 being antigenically related to MHV 3. The classification of HCV strains AD, HO, GI, PA and RO is unclear at present.

ACKNOWLEDGEMENTS

Thanks are due to Miss M.H. Madge for technical assistance, Mrs H.A. Davies for electron microscopy, Dr C.A. Kraaijeveld for help with ELISA, Dr H.E. Larson and Dr S.E. Reed for HCV isolates and volunteer paired sera, and Dr S.E. Reed for HCV OC43 grown in suckling mouse brain. Dr C.A. Kraaijeveld, Dr S.E. Reed and Dr D.A.J. Tyrrell provided useful discussions during the course of this work.

REFERENCES

1. D. Cavanagh, Personal communication (1980)
2. M.S. Collins and D.J. Alexander, Avian infectious bronchitis virus structural polypeptides: effect of different conditions of disruption and comparison of different strains and isolates, Arch. Virol. 63: 239 (1980)
3. H.A. Davies, R.R. Dourmashkin and M.R. Macnaughton, Ribonucleoprotein of avian infectious bronchitis virus, J. gen. Virol. In press. (1981)
4. H.A. Davies and M.R. Macnaughton, Comparison of the morphology of three coronaviruses, Arch. Virol. 59: 25 (1979)
5. C.A. Kraaijeveld, M.H. Madge and M.R. Macnaughton, Enzyme-linked immunosorbent assay for coronaviruses HCV 229E and MHV 3, J. gen. Virol. 49: 83 (1980)
6. C.A. Kraaijeveld, S.E. Reed and M.R. Macnaughton, Enzyme-linked immunosorbent assay for detection of antibody in volunteers experimentally infected with human coronavirus 229E group viruses, J. clin. Microbiol. In press.
7. J.A. Lanser and C.R. Howard, The polypeptides of infectious bronchitis virus (IBV-41 strain), J. gen. Virol. 46: 349 (1980)
8. H.E. Larson, S.E. Reed and D.A.J. Tyrrell, Isolation of rhinoviruses and coronaviruses from 38 colds in adults, J. med. Virol. 5: 221 (1980)
9. M.R. Macnaughton, The genomes of three coronaviruses, FEBS Letts 94: 191 (1978)
10. M.R. Macnaughton, The polypeptides of human and mouse coronaviruses, Arch. Virol. 63: 75 (1980)
11. M.R. Macnaughton and H.A. Davies, Two particle types of avian infectious bronchitis virus, J. gen. Virol. 47: 365 (1980)
12. M.R. Macnaughton, H.A. Davies and M.V. Nermut, Ribonucleoprotein-like structures from coronavirus particles, J. gen. Virol. 39: 545 (1978)
13. M.R. Macnaughton, M.H. Madge, H.A. Davies and R.R. Dourmashkin, Polypeptides of the surface projections and the ribonucleoprotein of avian infectious bronchitis virus, J. Virol. 24: 821 (1977)
14. K. McIntosh, Coronaviruses: a comparative review, Curr. Tops. Microb. Immunol. 63: 85 (1974)
15. A.S. Monto and L.M. Rhodes, Detection of coronavirus infection of man by immunofluorescence, Proc. Soc. exp. Biol. Med. 155: 143 (1977)
16. E. Nagy and B. Lomniczi, Polypeptide patterns of infectious bronchitis virus serotypes fall into two categories, Arch. Virol. 61: 341 (1979)
17. N.C. Pederson, J. Ward and W.L. Mengeling, Antigenic relationship of the feline infectious peritonitis virus to coronaviruses of other species, Arch. Virol. 58: 45 (1978)

18. O.W. Schmidt, M.K. Cooney and G.E. Kenny, Plaque assay and improved yield of human coronaviruses in a human rhabdomyosarcoma cell line, J. clin. Microbiol. 9: 722 (1979)
19. D.A.J. Tyrrell, D.J. Alexander, J.D. Almeida, C.H. Cunningham, B.C. Easterday, D.J. Garwes, J.C. Hierholzer, A. Kapikian, M.R. Macnaughton and K. McIntosh, Coronaviridae: second report, Intervirology, 10: 321 (1978)