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Polypeptide Patterns of Infectious Bronchitis Virus Serotypes Fall Into Two Categories

Brief Report

By

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With 2 Figures

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Summary

Molecular weights of six major polypeptides of infectious bronchitis virus (IBV) are: 1. 75,000; 2. 50,000; 3. 45,000; 4. 35,000; 5. 28,000 or 24,000, and 6. 22,000 dalton. According to the mobility of protein 5 the polypeptide patterns of IBV serotypes fall into two main categories.

*

IBV is a member of coronaviridae (11). This family includes viruses with a large positive-stranded RNA genome (9, 14) and a lipid-containing envelope surrounded with characteristic projections. Studies on the size, chemical composition and localisation of virion polypeptides of IBV has not led to a uniform polypeptide pattern. The number of polypeptides found so far seems to vary between 7 and 16, and patterns of one and the same strain varied from study to study (1, 2, 10). In addition, no significant difference was found between two strains belonging to different serological groups (10). Although the present designation of an IBV serotype is somewhat arbitrary we have selected strains representing seven of the eight proposed serotypes (4). It is to be noted that probably many more serotypes exist (3) and newer ones emerge (7).

On studying the polypeptide pattern by polyacrylamide gel electrophoresis of different IBV strains two characteristic patterns were regularly observed. On examining 12 strains belonging to seven known serotypes it was found that all fell into one of two categories based on the characteristic polypeptide pattern.

Origin of the strains was as follows: strain Beaudette was kindly provided by Dr. V. VON BÜLOW, Bundesforschungsanstalt für Viruskrankheiten der Tiere, Tübingen; strains Connecticut, Iowa 97, Iowa 609, Australia T and SE 17 by Dr. D. J. ALEXANDER, Central Veterinary Laboratory, Weybridge; strains Massachusetts (M41), Lerida and Gray by Dr. E. NEUVONEN, State Veterinary

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Medical Institute, Helsinki; strain LED (Connecticut serotype) and strain PV (Massachusetts serotype) were isolated in Hungary (8).

Virus strains were propagated in 10-day-old embryonated eggs and purified at 4° C as follows. Virus was clarified at 10,000 × *g* for 30 minutes and the supernatant pelleted at 59,000 × *g* for 3 hours in a Type 19 rotor of Beckman L2-65B ultracentrifuge. The pellet was gently resuspended in NTE buffer (NaCl 100 mM, Tris-HCl pH 6.8 10 mM and EDTA 1 mM). After further clarification the virus suspension was pelleted through 30 per cent (w/v) sucrose made up in NTE-buffer in a SW-27 rotor with 100,000 × *g* for 1 hour. Resuspended and clarified virus was subjected to a velocity gradient centrifugation for 1 hour into 10 to 40 per cent (w/v) sucrose. The band (or double band) containing at least half of the virus applied was further purified on a 20 to 60 per cent (w/v) sucrose gradient at 80,000 × *g* for 14 hours. Equilibrium centrifugation alone resulted in virus preparations of insufficient purity as described (15). Virus peak was recovered by sedimentation and taken up in sample buffer (50 mM Tris-HCl pH 6.8, 10 per cent glycerol and 0.001 per cent bromphenol blue).

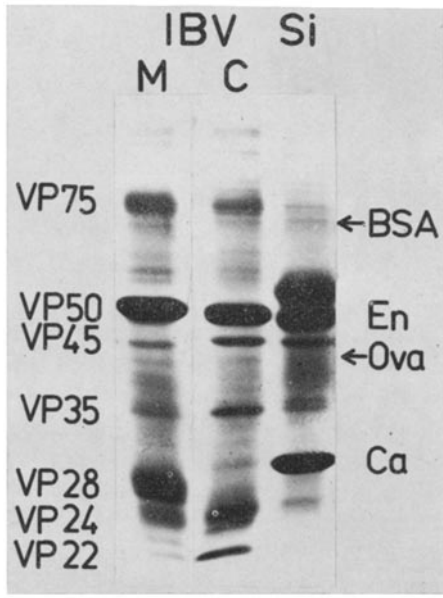

Fig. 1. Molecular weights of IBV proteins and the two main polypeptide patterns (lane M and C) of IBV serotypes. (Lane M = strain Beaudette; lane C = strain LED). A partially purified preparation of Sindbis virus (lane Si) is also shown to illustrate the presence of a cellular contaminant comigrating with VP45 of IBV. Arrows show the place of molecular weight markers bovin serum albumin (*BSA*, 68,000) and ovalbumin (*Ova*, 43,000), Sindbis virus envelope (*En*, 50,000) and capsid (*Ca*, 30,000)

Virus was dissolved in 2 per cent sodium dodecyl sulphate (SDS) and 0,5 per cent 2-mercaptoethanol in a boiling water bath for 3 minutes. Samples were run in 10 per cent slab gels (30:0.4 by weight acrylamide bis-acrylamide) using a discontinuous buffer system (5) for 14 hours at 50 volt. Gels were fixed in a mixture of methanol, acetic acid and water (5:1:5) and stained in 0.25 per cent Coomassie

blue. Molecular weights were calculated by using the following markers: myoglobin (17,200), ovalbumin (43,000), bovine serum albumin (68,000), Newcastle disease virus (12) and Sindbis virus proteins (13).

All IBV strains examined showed one of two protein patterns with polypeptides having apparent molecular weights in the range of 20,000 and 110,000 dalton (Fig. 1). Six major polypeptides were observed in all strains: VP75 (virion polypeptide of 75,000 dalton), VP50, VP45, VP35, VP28 or VP24 (depending on the serotype) and VP22. Minor polypeptides were also noted (VP110, VP48) but their viral origin is uncertain. The viral origin of VP45 is also doubtful for two reasons: a) there was a tendency of reduction of the relative amount of VP45 when velocity gradient centrifugation was combined with equilibrium gradient purification, b) this protein is equally present in preparations of egg-grown Sindbis virus which have been purified only by pelleting the virus under a 30 per cent sucrose layer, which also suggest that it may be a cellular contaminant (Fig. 1).

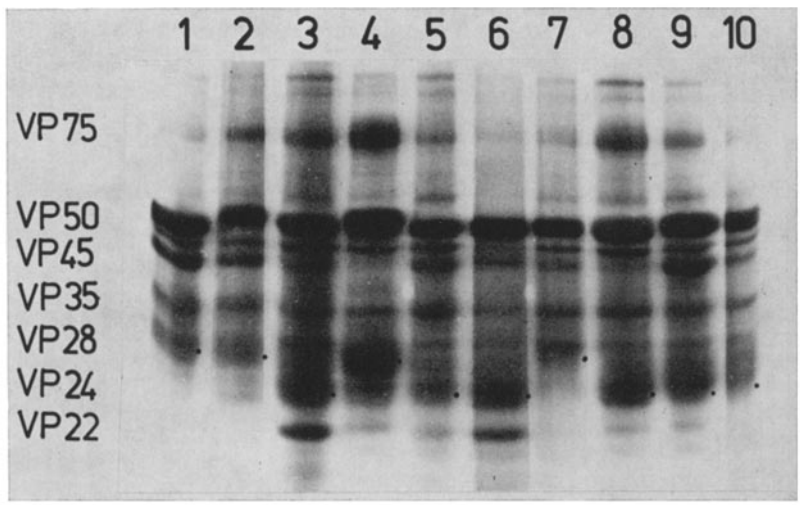

Fig. 2. Variation in the mobility of the "broad" polypeptide (marked with dots) in different serotypes of IBV. Lane 1 = strain SE17 of serotype Georgia; lane 2, 4 and 7 = strain M41, Beaudette and Lerida, respectively, of serotype Massachusetts; lane 3 = strain Gray of serotype Delaware; lane 5 and 6 = prototype strain and LED, respectively, of serotype Connecticut; lane 8 = prototype strain of Australia T; lane 9 = prototype strain of Iowa 609, lane 10 = prototype strain of Iowa 97

According to the variation in the mobility of a "broad" polypeptide at least two basic polypeptide patterns were recognized (Fig. 2). Pattern M (named after Massachusetts) includes strains from two serotypes, Massachusetts and Georgia (SE17), and here the "broad" protein has an apparent molecular weight of 28,000 dalton. Four strains (Beaudette, M41, PV, and Lerida) belonging to the Massachusetts serotype all showed identical M-pattern (PV is not shown). Pattern C (named after Connecticut) is characterized by a smaller "broad" protein of an apparent molecular weight of 24,000 dalton, and includes the five remaining serotypes studied: Connecticut, Delaware (Gray), Iowa 97, Iowa 609 and Australia T.

Three Connecticut strains (one prototype strain from Weybridge, another from Helsinki and LED) also exhibited identical C-pattern. Apart from variation in the mobility of the "broad" protein it was found that in strain Gray and LED VP22 was prominent relative to the other strains. Whether this suggests a closer relationship of the two strains, or an artifact, is presently unknown. It is to be noted, that there was no difference between the polypeptide patterns of heated and unheated samples. The polypeptide patterns presented here classify IBV serotypes into at least two main categories but no known biological properties common in serotypes belonging to a particular pattern can so far be identified.

Our results seem to be at variance with those of formerly published investigations (10) in two respects: a) molecular weights of the majority of the polypeptides in that study fall in the range of 33,000 to 130,000 dalton, although using harsher reducing conditions resulted in polypeptides smaller than 33,000 dalton; b) no significant difference was found between the protein pattern of strain Beaudette and Connecticut. The polypeptide patterns of IBV presented here, however, show a partial similarity to that published by COLLINS *et al.* (2), and a remarkable similarity to that obtained after a different purification method by LANCER and HOWARD (6). The similarity with pattern obtained in the latter works resides in the presence of smaller than 33,000 dalton proteins in the virions of IBV.

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