Monoclonal antibodies to three structural proteins of avian infectious bronchitis virus: characterization of epitopes and antigenic differentiation of Australian strains

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Ten monoclonal antibodies (MAbs) directed against three structural proteins of infectious bronchitis viruses (IBV), the peplomer (S), membrane (M) and nucleocapsid (N) proteins, were characterized and used to determine the antigenic relationship between Australian IBV strains. One MAb (MAb 5) was directed against an epitope on the S1 subunit of the peplomer, another (MAb 2) against an epitope on the M glycoprotein and eight MAbs (MAbs 1, 7, 9, 16, 24, 26, 27 and 51) were directed against seven non-overlapping epitopes on the N protein. None of the MAbs neutralized infectivity or inhibited haemagglutination of the virus. Conservation of the nine epitopes detected by these MAbs was determined in 13 serotypes of Australian IBV strains. Only epitope 5 on the S1 subunit of the peplomer was conserved in all strains. Epitope 2 on the M protein showed a high degree of conservation although this epitope was absent from four strains. None of the eight epitopes on the N proteins was conserved in all IBV strains but four epitopes (1, 16, 24 and 27) showed a high degree of conservation. Epitope 9 on the N protein was present only in IBV strains of one serotype whereas epitope 7 on the N protein distinguished vaccine viruses of serotype B from other IBV strains. The presence or absence of nine epitopes on three structural proteins differentiated IBV strains into five antigenic groups.

Introduction

Infectious bronchitis viruses (IBVs) are members of the Coronaviridae family of enveloped viruses with ssRNA of positive polarity. The virion contains three structural proteins: the peplomer (S) glycoprotein, located at the surface of the virion, consisting of two subunits, S1 and S2, with Mr values of 92K and 84K respectively; the membrane (M) glycoprotein, partially exposed at the surface of the virion, with an Mr ranging from 27K to 36K and the nucleocapsid (N) protein, internally located, with an Mr of 52K (Cavanagh, 1981, 1983; Wadey & Westaway, 1981). The S1 subunit of the peplomer contains determinants inducing neutralization and haemagglutination inhibiting antibodies (Cavanagh et al., 1984, 1986; Mockett et al., 1984) and is believed to be involved in protection (Cavanagh et al., 1986). The role of the M and N proteins in the immunobiology of IBV is not known.

IBVs induce a highly contagious respiratory infection or interstitial nephritis (Hofstad, 1984) in chickens of all ages and are endemic in chicken populations throughout the world. Although the disease is controlled by live attenuated vaccines, new variant strains of IBVs have been isolated frequently from outbreaks of the disease in vaccinated flocks (Cook, 1984). The antigenic changes occurring in strains of IBV have been identified by serum neutralization and haemagglutination inhibition assays which distinguished a large number of serotypes (Darbyshire et al., 1979; Wadey & Faragher, 1981; Cook, 1984; Lashgari & Newman, 1984). It is suspected that the mutations in the neutralization and haemagglutination sites are not the only antigenic changes occurring in IBV strains enabling variants to escape vaccinal immunity. There is no correlation between the serotype and degree of protection afforded by vaccine viruses (Arvidson et al., 1990). Nucleotide sequencing of the genes encoding the structural proteins of IBV has indicated that mutations occur also in the S2 subunit of the peplomer and the M glycoprotein and that variant strains might also arise by genetic recombination (Cavanagh & Davis, 1989; Kusters et al., 1989).

Apart from serum neutralization and haemagglutination assays, strains of IBV have been compared by gel electrophoresis (Nagy & Lomnicz, 1979; Collins & Alexander, 1980), limited proteolysis of viral glycoproteins (Cavanagh & Davis, 1987) and oligonucleotide fingerprinting (Clewley et al., 1981; Kusters et al., 1987).
These studies have also indicated that strains of different serotypes may be similar, whereas strains of the same serotype may differ in their structural proteins. In this paper we describe monoclonal antibodies (MAbs) directed against epitopes on the three structural proteins (S, M and N) of IBV and the use of the MAbs to differentiate IBV strains antigenically. The MAbs directed against the M and N proteins have revealed the antigenic changes in these proteins which were not identifiable previously and differentiated IBV strains into five distinct antigenic groups.

**Methods**

**Viruses.** IBV strains, obtained from Dr T. F. Faragher, National Biological Standards Laboratories, Parkville, Australia, have been described by Wadey & Faragher (1981). The strains designated Vac 3, Vac 4 and Vac 5 by Wadey & Faragher (1981) are synonymous with current infectious bronchitis vaccines Vic S Webster, Inghams and Steggleys, respectively. Other IBV strains used were: N3/62 synonym Gilvax and T1/82 synonym 2032. The N1/84, N1/87, N25/87, N1/88, N9/88, N1/89, N3/90 and Q3/88 viruses are field strains of IBV isolated from vaccinated broiler flocks and not previously described. That these strains were IBV was established by electron microscopy, the haemagglutination inhibition assay and passage in embryonated chicken eggs which resulted in the dwarfing and curling of embryos typical of IBV infection (J. Ignjatovic, unpublished results). Strain designation complied with the code of the reference collection of Australian IBVs (Geering & Bruce, 1970). The IBV strains, obtained as cloned viruses (Wadey & Faragher, 1981) or after cloning in tracheal organ cultures as described by Cook et al. (1976), were propagated in 10-day-old embryonated eggs. Titres, expressed as median ciliostatic doses (CD50), were determined in tracheal organ cultures.

**Preparation of purified virus and material for immunization.** Approximately 104 CD50 of IBV was injected into the allantoic cavity of 10-day-old embryonated specific-pathogen-free eggs and incubated for 48 h at 37 °C. The eggs were then chilled at 4 °C, allantoic fluid was collected, centrifuged at 3000 g for 30 min and virus was pelletted by centrifugation at 48000 g for 2 h at 25 °C in a Beckman ultracentrifuge. The virus pellet, resuspended in 100 mM-NaCl, 1 mM-EDTA, 10 mM-Tris–HCl pH 7-4 (NET) buffer to 1:100 of its original volume, is referred to as purified virus. For immunization, viruses were further purified by centrifugation at 95000 g for 3 h at 4 °C in a Beckman ultracentrifuge. The supernatant fluid above 25% sucrose was dialysed against PBS pH 7-2. Pelleted virus was resuspended in NET buffer containing 2% (w/v) n-octyl glucoside (Sigma) and 8 M guanidinium chloride (BDH). After incubation for 1 h at room temperature and centrifugation at 48000 g for 2 h at 25 °C in a Beckman ultracentrifuge, the supernatant was dialysed against PBS. The Newcastle disease virus (NDV) antigen was purified from allantoic fluid by the same method as used to prepare the purified IBV antigen. Protein concentration was determined by the method described by Lowry et al. (1951).

**Production of MAbs.** BALB/c mice, 6 to 12 weeks old, were primed by subcutaneous inoculation of 100 μg of either sucrose gradient-purified intact virus, dissociated virus or a fraction containing the S1 subunit of the peplomer, in complete Freund’s adjuvant. After 4 weeks, mice were boosted by intraperitoneal injection of the relevant antigen in incomplete Freund’s adjuvant. After a further 10 days a final boost of homologous sucrose gradient-purified intact virus was given intravenously. The mouse spleen was harvested for fusion with NS-1 myeloma cells 4 days after the final boost. Screening of culture fluid for hybridomas producing antibodies specific for IBV was performed by a direct ELISA and immunoblotting. Culture fluids, diluted 1:1 and 1:10 for ELISA and immunoblotting, respectively, were assayed simultaneously against homologous purified IBV and purified NDV which served as a control antigen. Clones that scored positive with the IBV antigen and did not react with control antigen were cloned by limiting dilution three times. Asicitic fluid was produced in the BALB/c mice pretreated with pristane (Aldrich). IgG was precipitated from ascitic fluid with 40% saturated (NH4)2SO4, pH 7-2 and dialysed against PBS. Eight milligrams of IgG (Mab IgG) was conjugated to horseradish peroxidase (HRP) (Sigma) (IgG–HRP) by the method of Wilson & Nakane (1978).

**Isotype determination.** The immunoglobulin isotypes and light chains of the MAbs were determined using a Misotest ELISA (Commonwealth Serum Laboratories).

**Direct ELISA.** Purified IBV antigen in 100 μl of 0-5 M-carbonate–bicarbonate buffer pH 9-6, was added to the wells of polystyrene microtitre plates (Disposable Products) and incubated overnight at 37 °C. The optimal antigen concentration was determined in a checkerboard titration for each IBV antigen and was in the range of 10 to 20 μg of protein/well. The wells were washed three times with PBS containing 0.05% Tween 20 and supernatant from hybridomas, or ascitic fluid, was added and incubated for 1 h at room temperature. Following washing, goat anti-mouse IgG–HRP (Tago) was added and incubated for 1 h at room temperature. After washing, a substrate solution containing 1 mg/ml 5-aminosalicylic acid (Merck) in phosphate buffer pH 5-95 supplemented just prior to use with H2O2 to give a final concentration of 0-005%, was added. After 1 h the absorbance at 450 nm (A450) was measured in a Titertek Multiskan (Flow Laboratories).

**Competition assay.** Microtitre plates were coated with 100 μl of purified Q1/76 antigen diluted in 0-5 M-carbonate–bicarbonate buffer, pH 9-5, overnight at 37 °C. The concentration of antigen for coating and dilution of MAb IgG–HRP were determined in advance by checkerboard titration and were those which yielded the maximum level of MAb IgG–HRP binding with the limiting amount of Q1/76 antigen. Plates were washed with PBS containing 0-05% Tween 20 after which IgG of each competing MAb, in concentrations of 10 to 10-5 μg/well, diluted in PBS containing 0-05%, Tween 20 and 5% bovine serum albumin, was added. After incubation for 1 h, the plates were washed, and the MAb IgG–HRP conjugate was added and incubated for 1 h at room temperature. Plates were washed, 5-aminosalicylic acid and H2O2 substrate added and the A450 was measured after 1 h. Binding of a MAb IgG–HRP conjugate to the Q1/76 antigen gave an A450 of between 0-60 and 0-98 and was taken to represent 100% binding. Each MAb was used as a competing antibody and as an IgG–HRP conjugate. Competing MAbs were considered to be directed against the same epitope on the N protein if reciprocal
competition for binding was greater than 50% and against overlapping epitopes if competition was between 25% and 50%. All competition pairings were assayed on the same day to minimize assay variations and were performed at least three times.

PAGE, SDS-PAGE was performed in a discontinuous slab gel as described by Laemmli (1970) with a 10% acrylamide concentration in the separating gel. Samples were prepared for electrophoresis in 0.125 M-Tris-HCl pH 6.8, 2.3% SDS, 5% 2-mercaptoethanol, 0.001% bromophenol blue and incubated at 100°C for 2 min. Proteins in the gel were detected by staining with Coomassie blue and glycoproteins were detected by periodate-Schiff's staining (Zacharius et al., 1969).

Immunoblotting. Proteins of purified IBV were separated by SDS-PAGE and transferred electrophoretically from the gel to a nitrocellulose membrane overnight at 200 mA (Burnette, 1981). After transfer, the nitrocellulose sheet was incubated in 10 mM-Tris-HCl, 150 mM-NaCl, pH 7.4 (TBS) containing 5% skim milk powder (blotto). The nitrocellulose sheet was cut into strips (0.4 cm) and incubated with MAb culture fluid or ascitic fluid, diluted 1:4 and 1:500, respectively, in blotto. The nitrocellulose strips were then washed with TBS containing 0.05% Tween 20 and incubated with rabbit-anti mouse IgG (Dako) diluted in blotto. After washing, the nitrocellulose strips were incubated with 2 μCi of 125I-labelled Protein A (Amersham) in blotto and exposed to X-ray film (Fuji).

Serotyping, virus neutralization and haemagglutination inhibition assays. The serotypes of IBV strains were determined by virus neutralization assay, which was performed in tracheal organ cultures with 100 CD50 of virus against the log2 dilution of chicken antisera or ascitic fluid (starting dilution 1:20 and 1:100, respectively). Antisera were obtained by intraocular inoculation of virus to 2-week-old specified pathogen-free chickens and bleeding them 8 weeks later. The neutralizing activity of ascitic fluid was assayed in the presence or absence of 2% guinea-pig complement (Gibco). The haemagglutination inhibition test was performed with 8 haemagglutinating units as described by Lashgari & Newman (1982) except that the haemagglutinating antigen was prepared by treatment of purified viruses with filtered culture supernatant of Clostridium welchii, strain ATCC 10543 (Collee, 1965), which was a kind gift from Dr R. Wilkinson, Microbiology Department, University of Melbourne, Australia.

Results

Properties of MAbs

Our purpose was to generate cross-reactive and vaccine virus-specific MAbs. For that reason mice were primed with either a virus fraction enriched in the S1 subunit of the peplomer, intact virus or disrupted virus. From 136 hybridomas producing antibodies directed against IBVs, the majority (133) were directed to the epitopes on the internal N protein, two to the epitopes on the S protein and one to an epitope on the M glycoprotein. From these hybridomas 10 were selected, on the basis of their overall superior reactivity in ELISA and, after cloning, further characterized (Table 1). Immunization of mice with a virus fraction enriched in the S1 subunit of the peplomer, or the intact virus, did not result in MAbs directed predominantly against the S1 and S2 subunits of the peplomer; instead MAbs directed against the N protein were preferentially produced. None of the MAbs neutralized the infectivity of homologous virus, either in the presence or absence of guinea-pig complement, and none inhibited the haemagglutinating activity of Vic S, Steggles, Q1/76 or N1/88 strains. All MAbs were of the IgG class and the predominant subclass was IgG1 (Table 1).

Table 1. Properties of monoclonal antibodies

<table>
<thead>
<tr>
<th>MAb</th>
<th>IBV strain</th>
<th>Inoculum*</th>
<th>Antigen specificity†</th>
<th>Isotype VN‡</th>
<th>HI§</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Q1/76</td>
<td>Peplomer</td>
<td>N</td>
<td>IgG1 (x)</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>Q1/76</td>
<td>Peplomer</td>
<td>N</td>
<td>IgG1 (x)</td>
<td>-</td>
</tr>
<tr>
<td>24</td>
<td>Q1/76</td>
<td>Peplomer</td>
<td>N</td>
<td>IgG1 (x)</td>
<td>-</td>
</tr>
<tr>
<td>26</td>
<td>Q1/76</td>
<td>Peplomer</td>
<td>N</td>
<td>IgG1 (x)</td>
<td>-</td>
</tr>
<tr>
<td>27</td>
<td>Q1/76</td>
<td>Peplomer</td>
<td>N</td>
<td>IgG1 (x)</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Q1/76</td>
<td>Peplomer</td>
<td>M</td>
<td>IgG1 (x)</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>Vic S</td>
<td>Disrupted virus</td>
<td>N</td>
<td>IgG2a (x)</td>
<td>-</td>
</tr>
<tr>
<td>51</td>
<td>Steggles</td>
<td>Disrupted virus</td>
<td>N</td>
<td>IgG1 (x)</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Q3/88</td>
<td>Intact virus</td>
<td>S</td>
<td>IgG2b (x)</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>N1/88</td>
<td>Intact virus</td>
<td>N</td>
<td>IgG2b (x)</td>
<td>-</td>
</tr>
</tbody>
</table>

* Used for primary immunization.
† N, M and S are the nucleocapsid, membrane and peplomer proteins, respectively.
‡ VN, virus neutralization assayed using 100 CD50 of homologous virus and ascitic fluid.
§ HI, haemagglutination inhibition with homologous and heterologous viruses.
∥ Virus fraction enriched in the S1 subunit of the peplomer.
¶- No neutralization or haemagglutination inhibition.

Protein specificity

The specificity of MAbs was determined by immunoblotting using ascitic fluid and sucrose gradient-purified viruses (Fig. 1). The eight MAbs 16, 24, 27, 7, 51, 1 (Fig. 1a) and 9 (Fig. 1b) reacted with a polypeptide with an approximate M, of 52K and were therefore directed against epitopes on the N protein. MAb 2 reacted with a polypeptide with an approximate M, of 26K, which corresponds to the M glycoprotein and MAb 5 reacted with a polypeptide of approximate M, 92K, indicating that it was directed against the S1 subunit of the peplomer. MAbs 1 and 51 obtained using Vic S and Steggles strains, respectively, cross-reacted in immunoblotting with the N protein of heterologous Q1/76 antigen (Fig. 1a, lanes 6 and 7) whereas MAbs 5 and 9 obtained by immunization with the Q3/88 and N1/88 strains, respectively, did not cross-react in immunoblotting with the S and N proteins of the heterologous Q1/76 antigen (Fig. 1 a, lanes 8 and 9). None of the MAbs 1, 7, 16, 24, 26, 27, 51 and 2, directed to epitopes on the N and M proteins, cross-reacted in immunoblotting with N1/88 and Q3/88 antigens (results not shown). None of the MAbs reacted with uninfected allantoic fluid or purified
NDV in immunoblotting (results not shown) and the control MAb, directed against infectious bursal disease virus, did not react with IBV antigens (Fig. 1).

Epitope specificity of MAbs

Since seven MAbs, 1, 7, 16, 24, 26, 27 and 51, reacted with the N protein of the Q1/76 virus in immunoblotting, the epitope specificity of these MAbs was determined in a competition assay (Fig. 2). MAbs 16 and 26 mutually competed for binding to the Q1/76 antigen in a manner that was similar to the homologous competition indicating that they are similar and directed against the same epitope on the N protein. MAb 1 competed partially with the MAbs 16 and 26 (30% and 20% competition respectively at 10 μg of IgG). The binding of MAb 1 IgG–HRP to Q1/76 antigen was, however, completely inhibited by MAbs 16 and 26, indicating that the epitope recognized by MAb 1 is within the epitope recognized by MAbs 16 and 26, although it is not identical to it. MAbs 24, 7 and 51 were each directed towards different epitopes on the N protein, as no other MAb competed for binding of their IgG–HRP conjugates to the Q1/76 antigen. MAb 27 was also shown to be directed to a unique epitope on the N protein as none of the MAbs 1, 7, 16, 24, 26 and 51 competed for the binding of MAb 27 IgG–HRP to the Q1/76 antigen (results not shown).

Relative avidity of MAbs

The avidity of MAbs 1, 7, 16, 24, 26 and 51 for binding to the Q1/76 antigen was similar (results not shown) although the Q1/76 antigen was the homologous antigen for MAbs 7, 16, 24 and 26 and a heterologous antigen for MAbs 1 and 51. All MAbs reached a plateau for binding to Q1/76 antigen at 1 μg/well of IgG (the range of IgG concentrations tested was between 0-0001 and 10 μg/well). The exception was MAb 27 which did not reach a plateau for binding to its homologous Q1/76 antigen within the range of IgG concentrations tested. The avidity of MAb 5 for binding to homologous Q3/88 and heterologous antigens V2/71, N1/62, V1/71, N1/88 and N1/75 was also determined (results not shown). MAb 5 showed variable avidity when binding to heterologous antigens and this avidity was generally higher than that for the homologous Q3/88 antigen. MAb 5 reached a plateau of binding to V2/71, N1/62 and N1/75 antigens at between 0-01 and 1 μg/well of IgG,
Antigenic differentiation of IBV

Fig. 2. Epitope specificity of Mabs directed against the N protein. Binding of a constant amount of MAb IgG-HRP conjugate, (a) 16 IgG-HRP, (b) 26 IgG-HRP, (c) 1 IgG-HRP, (d) 24 IgG-HRP, (e) 7 IgG-HRP and (f) 51 IgG-HRP, to Q1/76 antigen in microtitre plates was inhibited by prior addition of various concentration of MAb IgG: 16 (○), 26(■), 1 (▲), 24 (●), 27 (□), 7 (△) and 51 (▼). Binding of IgG-HRP conjugate was detected by addition of 5-aminoosalicylic acid-H₂O₂. Percentage competition was calculated in regard to the absorbance produced by binding of MAb IgG–HRP conjugate to the antigen alone, which was taken as 100%.

whereas the plateau of binding to Q3/88 and N2/75 antigens was not reached within the range of IgG concentrations tested (0-00001 to 10 µg/well). This differential reactivity of MAb 5 was independent of the antigen concentration.

Conservation of the epitopes in IBV strains

MAbs 1, 7, 9, 16, 24, 27 and 51, directed towards different epitopes on the N protein and MAbs 2 and 5 directed to the epitopes on the M and S glycoproteins respectively were used to determine the conservation of these epitopes in IBV strains of differing serotype (Fig. 3). The presence of an epitope was assayed by direct ELISA using purified viruses and MAbs in excess. An epitope was considered to be absent if the MAb did not bind to the antigen or binding was less than 10% of binding to the homologous antigen. Epitope 5 on the S1 subunit of the peplomer was the only epitope detected in all IBV strains. Epitope 2 on the M glycoprotein, recognized by MAb 2, was present in the majority of IBV strains. Of the seven epitopes on the N protein recognized by MAbs 1, 7, 9, 16, 24, 27 and 51, none was conserved in all IBV strains. Each MAb recognized a unique set of strains. Epitopes 1, 16, 24 and 27 on the N protein had the highest frequency of conservation but some strains (Vac-1, V1/71, N3/62, N9/74) lacked some or all (N1/88, Q3/88) of these epitopes. Epitope 1, which was found by competition tests to be within epitope 16, was absent from two strains in which epitope 16 was present, confirming that it was not identical with epitope 16. Epitopes 7 and 9 were each present in only Q1/76 and N1/88 strains, respectively. Epitope 51 was present in strains of serotypes B and E. The presence or absence of the epitopes (1, 7, 9, 16, 24, 27, 51 and 2) on the N and M proteins in 13 strains was confirmed by immunoblotting (results not shown).

From 13 IBV strains, six (Q1/76, Q1/73, T1/82, N1/62, N1/75 and N2/75) had four epitopes on the N protein (24, 1, 16 and 27) and one epitope on the M glycoprotein in common and thus could be distinguished as one antigenic group (antigenic group I) from the other IBV strains. The Vac-1, V1/71, V2/71 and N3/62 strains each had a unique set of conserved epitopes, lacking either epitopes 24, 1 or 16 on the N protein or epitope 2 on the M glycoprotein. These strains were considered to belong to antigenic group II. The N9/74 strain also differed antigenically from other IBV strains, lacking three epitopes in common on the N protein and so formed a separate antigenic group III. The N1/88 and Q3/88 strains differed significantly from other strains having no epitopes in common with other IBV strains either on the N or the M protein. The N1/88 strain also differed from Q3/88 as they did not share epitope 9 on the N protein and they therefore belonged to antigenic group IV and V respectively.

Specificity of the MAbs 7 and 9

Since MAbs 7 and 9 each reacted only with IBV of serotypes B and L respectively, the occurrence of these two epitopes on other strains belonging to these serotypes was determined. MAb 7 reacted with strains Vic S Webster, Inghams and Steggles, vaccines currently used in Australia, and with Vic S CSL, a discontinued vaccine, all of serotype B (Table 2). MAb 7 reacted also
Table 2. Specificity of MAb 7 for IBV strains of serotype B

<table>
<thead>
<tr>
<th>IBV strain</th>
<th>Serotype*</th>
<th>Titre†</th>
<th>MAb 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vic S Webster‡</td>
<td>B</td>
<td>2560§</td>
<td>+</td>
</tr>
<tr>
<td>Inghams‡</td>
<td>B</td>
<td>1280</td>
<td>+</td>
</tr>
<tr>
<td>Steggles‡</td>
<td>B</td>
<td>1280</td>
<td>+</td>
</tr>
<tr>
<td>Vic S CSL</td>
<td>B</td>
<td>1280</td>
<td>+</td>
</tr>
<tr>
<td>Q1/76</td>
<td>B</td>
<td>1280</td>
<td>+</td>
</tr>
<tr>
<td>N1/84¶</td>
<td>B</td>
<td>1280</td>
<td>+</td>
</tr>
<tr>
<td>N1/87¶</td>
<td>B</td>
<td>1280</td>
<td>+</td>
</tr>
<tr>
<td>V1/70</td>
<td>B</td>
<td>2560</td>
<td>-</td>
</tr>
<tr>
<td>N25/87¶</td>
<td>N</td>
<td>&gt;40</td>
<td>+</td>
</tr>
</tbody>
</table>

* Determined by virus neutralization assay in tracheal organ cultures with 100 CD50 of virus and log, dilution of chicken sera.
† Reciprocal of the last dilution of anti-Vic S Webster serum that neutralized 100 CD50 of corresponding virus.
‡ Strains currently used as vaccines.
§ Homologous reaction.
¶ †, Binding of MAb 7, assayed by ELISA, was equal to the binding with homologous Q1/76 strain; --, no binding.
¶ Strains isolated from commercial chickens vaccinated with Inghams vaccine.

Discussion

The majority of MAbs generated and characterized in this study were directed against epitopes on the internal nucleocapsid N protein and only two MAbs, 2 and 5, were directed against epitopes on the M and the S protein, respectively. The procedures for antigen preparations, immunization and selection of MAbs were similar to those used by others (Mockett et al., 1984; Koch et al., 1986; Niesters et al., 1987) to produce MAbs directed predominantly against the S1 and S2 subunits of the peplomer and M glycoprotein. The reasons as to why the production of MAbs directed against the N protein was favoured is not clear.

The eight MAbs directed against the N protein delineated seven epitopes. Epitopes 7, 51, 16, 24 and 27 were all non-overlapping whereas epitope 1 overlapped epitope 16 but was not identical to it. All six epitopes could be found on the same N protein in some strains of IBV. Epitope 9 was distinct from the other six epitopes in that it was found on the N protein of IBV strains of one serotype only.

Only one MAb (MAb 5) directed against an epitope on the S1 subunit of the peplomer was obtained and this epitope was conserved in all IBV strains isolated in Australia between 1962 and 1988. MAbs generated against the S1 subunit are usually directed against strain-specific neutralizing and haemagglutinating epitopes (Mockett et al., 1984; Koch et al., 1990) although a MAb against a conserved site on the S1 subunit of the peplomer has been obtained (Niesters et al., 1987).

Epitope 2 on the M glycoprotein was conserved in the majority of strains although four IBV strains lacked this epitope. Koch et al. (1986) obtained MAbs to two epitopes on the M glycoprotein that were conserved among all Dutch and U.S.A. strains and high conservation of the epitopes detected by MAbs directed against...
the M glycoprotein was also found in murine coronaviruses (Fleming et al., 1983).

The finding that none of the epitopes on the N protein was conserved among IBV strains was unexpected. Koch et al. (1986) generated MAbs to the epitopes on the N protein which were conserved in the majority of the Dutch strains although these epitopes were less conserved in the U.S.A. strains. MAbs have also been generated that are directed against non-conserved epitopes on the N protein of murine coronaviruses (Fleming et al., 1983). Another unexpected finding was that three epitopes, 7, 9 and 51, on the N protein were present in only some strains of IBV. Epitope 9 was serotype L-specific and distinguished these strains from other strains. Epitope 7 was specific for vaccine viruses Vic S Webster, Inghams and Steggles, whereas epitope 51 was specific for serotypes B and E only. Such strain-specific MAbs, directed against epitopes on the N protein, have not been reported before. From the strain-specific MAbs directed against the S1 subunit of the peplomer (Mockett et al., 1984; Niesters et al., 1987), the majority, but not all, were directed against the neutralizing and haemagglutinating epitopes indicating that strain-specific epitopes might exist outside neutralizing and haemagglutinating epitopes.

Conservation of the four common epitopes 1, 16, 24 and 27 on the N and epitope 2 on the M protein allowed grouping of IBV strains that belonged to 13 serotypes into five antigenic groups. The strains within each of the four antigenic groups I, III, IV and V had the same set of epitopes on the M and N proteins and therefore could be considered antigenically similar. Two antigenic groups, I and II, were unrelated to the serotype and three, III, IV and V, were. The antigenic grouping, although not taking into consideration the changes that might have had occurred in the peplomer protein, nevertheless revealed the striking degree of changes that have occurred in some IBV strains. The N1/88 and Q3/88 strains had no epitope in common with strains from other antigenic groups either on the M or N proteins. This indicates that profound mutations have occurred, not only in the S1 subunit involving the neutralization site, but also in the other two structural proteins of these strains.

The MAbs generated in this study were useful for differentiation of vaccine viruses of serotype B from other strains of IBV and also in ascertaining that strains isolated from vaccinated flocks were variant viruses. Three currently used infectious bronchitis vaccine viruses, Vic S Webster, Inghams and Steggles, were antigenically similar if not identical by MAb analysis and could be differentiated from other IBV strains by MAb 7. Two strains, N1/84 and N1/87, that were isolated from flocks vaccinated with the Inghams vaccine were identical to the vaccine virus in both its serotype and MAb antigenic profile and were therefore considered as arising by re-isolation of vaccine viruses. The strains N25/87 and V1/70, however, could be distinguished by MAbs and serotyping as variants. Fingerprinting with T1 ribonuclease has given some insight into strain differences that are unrelated to the serotype (Clewley et al., 1981; Kusters et al., 1987) but this method is not well suited for the rapid characterization of viruses.

The panel of MAbs generated in this study has enabled the assessment of the degree of antigenic similarity between viruses, the identification of mutation in variant viruses and the differentiation between strains of viruses of the same or different serotypes, which was not previously possible. As such, these MAbs are useful tools for epidemiological studies, vaccine development and studies aimed at determining the role of individual viral antigens in protection and immunity.

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References


