The Characterization of Monoclonal Antibodies to Newcastle Disease Virus

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SUMMARY

Monoclonal antibodies to the haemagglutinin–neuraminidase (HN), fusion (F), polymerase and nucleocapsid polypeptides of Newcastle disease virus were prepared. Two epitopes were recognized on the HN polypeptide: one was associated with inhibition of haemagglutination and poor neutralization and the other with good neutralization and no inhibition of haemagglutination. The most effective neutralizing antibody was that produced against the F polypeptide. The poorer neutralization associated with the antibody against the HN epitope was augmented by antiglobulin or complement. The monoclonal antibodies that inhibited haemagglutination also inhibited neuraminidase activity when fetuin but not neuraminyl lactose was the substrate.

Newcastle disease virus (NDV) isolates vary in pathogenicity but cannot be subtyped by conventional haemagglutination inhibition (HI) tests (Kendal & Allan, 1970). Monoclonal antibodies to NDV (Ulster 2C), a non-pathogenic field isolate, have recently been used to classify 40 NDV isolates into eight groups by an indirect immunoperoxidase (IIP) test and viruses within each group appeared to share a number of biological and epizootiological properties (Russell & Alexander, 1983). In this paper the binding and biological activities of the same monoclonal antibodies are characterized by immunoprecipitation, 'Western' blotting, blocking radioimmunoassay, IIP, HI, neuraminidase inhibition (NI), and virus neutralization.

The nine hybridomas are designated by their original clone numbers, 14, 32, 38, 86, 424, 445, 479, 481 and 688. Each hybridoma had been subcloned twice by limiting dilution and produced antibody of a single isotype (Table 1) and was therefore considered to be secreting monoclonal antibody. Six of the monoclonal antibodies bound in an ELISA test to virus purified by a sucrose gradient and they also immunoprecipitated single polypeptides from infected chick embryo cells (Table 1; Fig. 1a). Thus, monoclonal antibodies 14, 32, 86 and 445 bound to the haemagglutinin–neuraminidase (HN) glycopolyptide, 481 to the fusion (F) glycopolyptide and 38 bound to the nucleoprotein (NP). In a 'Western' blotting test, four of the monoclonal antibodies, 14, 32, 86 and 38, bound to the same polypeptides and a fifth, 688, bound to the viral polymerase (P) polypeptide (Fig. 1b). Monoclonal antibody 688 also bound to two polypeptide bands under non-reducing conditions (Fig. 1b) and this result was repeated with six tertiary subclones of 688. On the other hand, monoclonal antibodies 445 and 481 bound to HN and F by immunoprecipitation but not by 'Western' blotting, suggesting that their epitopes had probably been irreversibly denatured during SDS–polyacrylamide gel electrophoresis. Monoclonal antibodies 424 and 479 failed to bind polypeptides either by immunoprecipitation or by 'Western' blotting.

The topography of the epitopes bound by the monoclonal antibodies was analysed by blocking assays based on competition assays developed for Sendai virus by Yewdell & Gerhard (1982). The seven monoclonal antibodies that bound in the ELISA test also bound to purified virus after
Fig. 1. (a) Analysis of antibody binding by immunoprecipitation. Primary chicken embryo cells were infected overnight with NDV, labelled with $^{35}$S-methionine for 1 h and then extracts were made. The lysates were mixed with a 1:100 dilution of ascitic fluid and then pelleted with undiluted rabbit anti-mouse Ig and analysed by SDS-polyacrylamide gel electrophoresis and fluorography (Russell et al., 1981). Lane 1, antibody 14; lane 2, 32; lane 3, 38; lane 4, 86; lane 5, 424; lane 6, 445; lane 7, 479; lane 8, 481; lane 9, 688; lane 10, monoclonal antibody A12 to simian virus 5; lane 11, $^{35}$S-labelled adenovirus-infected cell extract as molecular weight ($\times 10^{-3}$) markers. (b) Analysis of antibody binding by 'Western' blotting. Polypeptides were separated from 50 μg of sucrose-banded allantoic NDV on 8% SDS-polyacrylamide gels and then transferred to nitrocellulose sheets. The lanes were stained by Coomassie Brilliant Blue (CBB) or blocked with 3% bovine serum albumin overnight and stained by IIP using hybridoma 688 tissue culture fluid. The NDV polypeptides were labelled by reference to Smith & Hightower (1981). Lane 1, reduced purified adenovirus mol. wt. markers + CBB; lane 2, non-reduced NDV + CBB; lane 3, non-reduced NDV + antibody 688; lane 4, reduced NDV + CBB; lane 5, reduced NDV + antibody 688.

radioiodination (Table 1) and the binding ratios were at least sixfold higher when NDV was used, compared to those with Sendai virus (data not shown). In the blocking assay, a 1 in 10 dilution of ascitic fluid was allowed to bind for 3 h before labelled IgG was added in duplicate, at concentrations of 1 μg and 0.1 μg/ml. In homologous blocking assays, each of the seven antibodies which bound to virus caused 90 to 100% inhibition of binding by either concentration of IgG, whereas antibodies 424 and 688 (which failed to bind to virus by ELISA) caused less than 5% inhibition. Homologous blocking was therefore saturating and specific. Three types of heterologous blocking occurred: 95 to 99% in the nine combinations of unlabelled and radiolabelled antibodies 14, 32 and 86, 50 to 70% by unlabelled antibodies 14, 32 and 86 against radiolabelled antibody 445 in a non-reciprocal manner and 60 to 65% by unlabelled antibody 38 against radiolabelled 479 also in a non-reciprocal manner. The four monoclonal antibodies to HN had similar avidities (Table 1) and it was therefore considered that monoclonal antibodies 14, 32 and 86 bound to the same epitope, which was termed HN-1, whilst antibody 445 appeared to bind to a second epitope (HN-2). Monoclonal antibody 479 was assumed to bind to NP because it was blocked by antibody 38 (which immunoprecipitated the NP polypeptide) although the avidity of antibody 38 exceeded that of antibody 479, which may explain why the block appeared to be non-reciprocal.

When antibody binding was assessed by IIP on infected Madin-Darby bovine kidney (MDBK) cells, monoclonal antibodies to HN or F stained the cell membrane whereas those to
Short communication

Table 1. Summary of the properties of monoclonal antibodies to NDV

<table>
<thead>
<tr>
<th>Monoclonal antibody no.</th>
<th>Epitope</th>
<th>Isotype*</th>
<th>Distribution§</th>
<th>log₁₀ Titre</th>
<th>log₁₀ Titre</th>
<th>HI† at IgG concn. of 1 µg/ml</th>
<th>log₁₀Titre</th>
<th>log₁₀Titre</th>
<th>VN†</th>
</tr>
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<tbody>
<tr>
<td>14</td>
<td>HN-1</td>
<td>2a m</td>
<td>6</td>
<td>5</td>
<td>1276</td>
<td>327</td>
<td>2.7</td>
<td>3.3</td>
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<tr>
<td>32</td>
<td>HN-1</td>
<td>2a m</td>
<td>6</td>
<td>5</td>
<td>1239</td>
<td>190</td>
<td>2.9</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>NP</td>
<td>2a i</td>
<td>6</td>
<td>&gt;5</td>
<td>4021</td>
<td>1084</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>86</td>
<td>HN-1</td>
<td>2a m</td>
<td>6</td>
<td>&gt;5</td>
<td>1119</td>
<td>173</td>
<td>3.2</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>424</td>
<td>?</td>
<td>2b c</td>
<td>4</td>
<td>&lt;2</td>
<td>25</td>
<td>3</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>445</td>
<td>HN-2</td>
<td>2b m</td>
<td>6</td>
<td>5</td>
<td>1414</td>
<td>307</td>
<td>&lt;1</td>
<td>3.8</td>
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<tr>
<td>479</td>
<td>NP</td>
<td>2a i</td>
<td>4</td>
<td>3</td>
<td>329</td>
<td>32</td>
<td>&lt;1</td>
<td>&lt;1</td>
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</tr>
<tr>
<td>481</td>
<td>F</td>
<td>1 m</td>
<td>5</td>
<td>5</td>
<td>196</td>
<td>67</td>
<td>&lt;1</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
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<td>P</td>
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<td>NR</td>
<td>NR</td>
<td>&lt;1</td>
<td>&lt;1</td>
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</tr>
</tbody>
</table>

* Determined by immunodiffusion against standard anti-mouse sera.
† IIP, ELISA, HI and virus neutralization (VN) tests were performed in microwells as described by Russell et al. (1983) and Russell & Alexander (1983), except that HI tests utilized heat-inactivated ascitic fluid and both chick and bovine red blood cells.
‡ RIA microwells were treated overnight with 2-5 µg of virus in 50 µl of phosphate-buffered saline A (PBSA), fixed with 50 µl 10% formalin for 10 min, washed and then immersed in newborn calf serum for 2 h at 37 °C. Monoclonal IgG was ¹²⁵I-labelled using Iodogen to give specific activities of about 10⁶ cts/min/µg, was diluted in 50 µl PBSA + 10% foetal bovine serum and incubated in duplicate wells for 1 h at 37 °C and then overnight at 4 °C. Wells were washed five times, counted for 5 min in a Packard 5260 Autogamma counter and the machine background of 27 cts/min was subtracted.
§ Distribution: m, cell membrane; i, intracytoplasmic inclusion bodies; c, cytoplasm.
|| NR, No result.

NP or P polypeptide stained intracytoplasmic inclusions (Table 1). The one uncharacterized monoclonal antibody, 424, stained the cytoplasm in a diffuse manner; when the concentration of formalin used for fixation was reduced from 10% to less than 2%, the antibody additionally stained the nucleus more intensely. Monoclonal antibody 424 was also of interest because it distinguished the 18 viscerotropic velogenic isolates of NDV from 22 less pathogenic isolates (Russell & Alexander, 1983).

The results of the monoclonal antibody binding detected by IIP staining with different isolates of NDV confirmed that HN-1 and HN-2 were different epitopes, since HN-1 was present in 36 of 40 isolates and HN-2 co-existed on only 7 of these isolates (Russell & Alexander, 1983).

Other biological activities of the monoclonal antibodies were also related to their epitopes. Thus, three monoclonal antibodies to HN-1 had both HI and NA activity whereas those to HN-2 and F did not. Neutralization was most effective using monoclonal antibody to F, weaker using monoclonal antibody to HN-2 and weakest with the three monoclonal antibodies to HN-1 (Table 1 and see below). If virus neutralization were assessed by plaque reduction using 100 µg/ml of separated IgG, then the three monoclonal antibodies to HN-1 neutralized 50% of the virus whereas those to HN-2 and F neutralized formation of 99% and 99.99% of plaques respectively. If complement (1 in 100 dilution of fresh guinea-pig serum) or antiglobulin (1 in 100 dilution of heat-inactivated goat anti-mouse globulin) was added to the three monoclonal IgGs to HN-1, at least 99.8% of virus infectivity was then neutralized whereas the antiglobulin and complement controls caused 1% and 40% plaque reduction respectively. Such augmentation was not apparent if the monoclonal IgG was increased in concentration from 10 µg/ml to 500 µg/ml. (At 500 µg/ml the monoclonal IgGs were known to be anticomplementary and they would have contained more antibody molecules than the 1 in 100 dilution of antiglobulin serum.)

Monoclonal antibodies to the neuraminidase of influenza virus have been described which inhibit the action of the enzyme on fetuin but not on neuraminyl lactose (Jackson & Webster, 1982). A similar result was obtained by using monoclonal antibodies to the HN-1 epitope since a 1 in 100 dilution of ascitic fluid caused 85 to 95% inhibition with fetuin as a substrate, compared to only 22 to 38% inhibition with neuraminyl lactase.
These studies have demonstrated that there are at least two epitopes on the HN polypeptide of NDV and it is significant that there appeared to be a separation of those concerned with haemagglutination and virus neutralization. This parallels work with reovirus type 3 and seal influenza virus (Burstin et al., 1982; Kida et al., 1982). Antibodies to the HN-1 epitope, associated with HI, appear to overlap functionally with the HN-2 epitope in blocking assays and this might explain their ability to neutralize in a weak manner. Virus neutralization by monoclonal antibodies to HN-1, HN-2 or F can also occur after virus has attached to cells which may explain the dissociation of virus neutralization and HI (P. H. Russell, unpublished results). The strongest neutralization is associated with the one monoclonal antibody to F and this F epitope does not overlap the HN-1 or HN-2 epitope, as judged by blocking assays. NDV can therefore be separately neutralized by monoclonal antibodies to HN and F as has been described for conventional antisera to the purified HN and F proteins (Avery & Niven, 1979).

Previous evidence for antigenic variation between NDV isolates has come from virus neutralization studies and not from HI tests (see Avery & Niven, 1979) and it is relevant, therefore, that the HN-1 epitope was conserved on 36 of 40 virus isolates whereas HN-2 and F, which are better epitopes for virus neutralization, were on only 7 and 22 of the 40 isolates respectively (Russell & Alexander, 1983).

The importance of antibodies to F for vaccine protection against paramyxoviruses has recently been emphasized (McClelland, 1980). Indeed, if the F polypeptide is the most important target for neutralization of NDV, as suggested by the small number of monoclonal antibodies used in the present work, this may explain why in some circumstances vaccine protection against NDV fails even when HI titres exceed 1 in 20 (Partadiredja et al., 1979). It should also be borne in mind that although neutralization by monoclonal antibodies to HN-1 was weak in vitro this may not completely reflect in vivo results because complement could increase their neutralizing ability.

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REFERENCES


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