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Short Communication

MONOCLONAL ANTIBODIES AGAINST THE HAEMAGGLUTININ-NEURAMINIDASE GLYCOPROTEIN OF NEWCASTLE DISEASE VIRUS STRAIN AF2240

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SUMMARY

The Malaysian velogenic-viscerotropic Newcastle disease virus (NDV) strain AF2240 is the reference strain that has often been used for vaccine development. Eight hybridoma clones producing monoclonal antibodies (mAbs) against NDV strain AF2240 were established as a result of fusion between myeloma Sp2/0-Ag14 and Balb/c B-lymphocytes. Isotyping of mAbs showed that clones 1B9, 2D6 and 9D7 produced IgG1, clones 1D5, 5A10 and 5F10 produced IgG2a and clones 2G3 and 5E10 produced IgG3. All the mAbs possessed kappa (κ) light chain. The mAbs were reacted towards the haemagglutinin-neuraminidase (HN) glycoprotein of the virus and able to inhibit in the haemagglutination with chicken red blood cells but not the haemolysis-inhibition test which confirmed that they were specific to the HN glycoprotein and not to the fusion protein.

Keywords: Glycoprotein, haemagglutinin-neuraminidase, monoclonal antibodies, NDV

INTRODUCTION

Newcastle disease virus (NDV), like the other members of the *Paramyxoviridae*, contains a lipid bilayer envelope that is derived from the plasma membrane of the host cell in which the virus is grown. The genome encodes six major viral proteins: haemagglutinin-neuraminidase (HN), fusion (F), matrix (M), nucleoprotein (NP), phosphoprotein (P) and large (L) (Peeters and Koch, 2002). An excellent review on NDV structural proteins and functions is available in Yusoff and Tan (2001). Lai and Ibrahim (1987) had reported that NDV strain AF2240 was responsible for very high morbidity and mortality in Malaysia. It was recovered during an outbreak in the 1960s. AF2240 has been found to have an intravenous pathogenicity index (IVPI) of 2.56 and intracerebral pathogenicity index (ICPI) of 1.9 that can be classified as viscerostropic velogenic NDV.

Kohler and Milstein (1975) had successfully developed a hybridoma technique that allows the growth of cells secreting antibodies with a defined specificity. In this technique the B-lymphocytes isolated from immunised animals were fused with myeloma cells. The resulting hybrid cells known as hybridomas were able to be cultured *in vitro*. Antibodies secreted from a hybridoma clone are known as monoclonal antibody. MAbs had been raised from a variety of NDV strains regardless of the pathotypes with three main objectives: (i) to study the antigenic regions and functional epitopes; (ii) to study the mAbs neutralising properties toward viral glycoproteins and (iii) to develop as a tool for diagnostic and epizootic studies (Russell, 1988; Aldous and Alexander, 2001). Numerous overlapping and non-overlapping antigenic regions had been identified by mAbs against the HN glycoprotein (Iorio and Bratt, 1983; Nishikawa *et al.*, 1983; Russell and Alexander, 1983; Iorio *et al.*, 1986, 1989; Yusoff *et al.*, 1988; Panshin *et al.*, 1999).

MATERIALS AND METHODS

Hybridoma clones were generated by fusion of myeloma Sp2/0-Ag14 and sensitised Balb/c B-lymphocytes at proportion of 1:10 using polyethylene glycol with the molecular weight of 1450 (Harlow and Lane, 1988). Limiting dilutions were performed on positive clones for four times before determining the isotypes. Indirect-ELISA technique using NDV-coated plate was employed on all screening assays. Subsequently, the highest mAbs producing clones were expanded into tissue culture flask and the hybridomas were injected into the pristine-primed female Balb/c to raise ascites fluid. Protein G was used to purify the mAbs in ascites fluid

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and the concentration was determined by the absorbance value at 280 nm (Delves, 1995). The Laemmli (1970) discontinuous buffer system was employed in 12% SDS-PAGE and transfered onto nitrocellular membrane as described by supplier. A series of two-fold dilutions haemagglutination-inhibition (HI) tests were carried out to investigate the ability of mAbs in neutralising the haemagglutination (HA) activity of HN glycoprotein. Haemolysis-inhibition (HLI) test was employed to examine the neutralising ability of mAbs towards viral F glycoprotein that cause fusion and eventually lysis of red blood cell (RBC). Cross-reactivity test among the NDV pathotypes was carried out using ELISA based viral coated plate.

RESULTS

Eight hybridoma clones were established by selecting colonies that produced the highest absorbance values by ELISA (data not shown). All the clones (1B9, 1D5, 2D6, 2G3, 5A10, 5E10, 5F10 and 9D7) were established after four times of limiting dilutions. Three clones (1B9, 2D6 and 9D7) produced IgG1, three clones (1D5, 5A10 and 5F10) were produced IgG2a and 2 clones (2G3 and 5E10) produced IgG3. All mAbs were found to possess kappa (κ) light chain. Two μg of NDV which consists of six major proteins were subjected to 12% SDS-PAGE (Figure 1a) and transferred to nitrocellulose membrane for immunoblot analysis. From the membrane, two groups of mAbs can be characterised: (1) mAbs that recognised HN linearised epitopes at 75 kDa and (2) mAbs that did not recognise any linearised epitopes.

The first group (mAbs 1D5, 5A10 and 5E10) showed positive results toward both the ELISA test and immunoblot indicating that it recognised conformational and linearised epitopes, respectively. On the other hand, the second group (mAbs 1B9, 2D6 and 9D7) showed positive results in ELISA test of whole virus but not on the immunoblot indicating that it only recognised conformational epitopes (Figure 1b). MAb 1B9 exhibited the highest titering HIU with 2^8. The remaining mAbs showed either 2^4 or 2^5 HIU. Thus, the overall HIU was considered low compared to these as the polyclonal antibodies (pAbs) which had the titering 2^{11} HIU. None of the mAbs inhibited the F protein from lysing the RBC in HLI test (Table 1) which indicates that all of the mAbs raised were against the HN glycoprotein and not the F protein. A mixture of responses could be observed from the cross-reactivity test among the NDV pathotypes (Table 2).

The hybridoma clones established in this study can be divided into three groups; those that produce mAbs of IgG1, IgG2a and IgG3. All three groups produced kappa (κ) chain. Harlow and Lane (1988) discovered that murine B-lymphocytes carries 800 potential κ chain and five lambda (λ) chains. As a result, chances of hybridoma producing κ chains are higher than λ chains. A major factor which determines the success of immunoblotting is the nature of the epitope which is recognised by the antibodies. The electrophoresis techniques involve denaturation of the antigen, thus only mAbs which recognise denaturing-resistant (linear) epitope will bind. Most pAbs typically contain at least some antibodies of this type but many mAbs will not react with linearised antigens (Harlow and Lane, 1988). Nevertheless, it showed agreement with our immunoblotting results. Only three (1D5, 5A10 and 5E10) out of the six selected mAbs recognised linear epitopes. A single band of 75 kDa which corresponded to the HN glycoprotein was observed (Figure 1b, lane 1, 2 and 3). A second band of

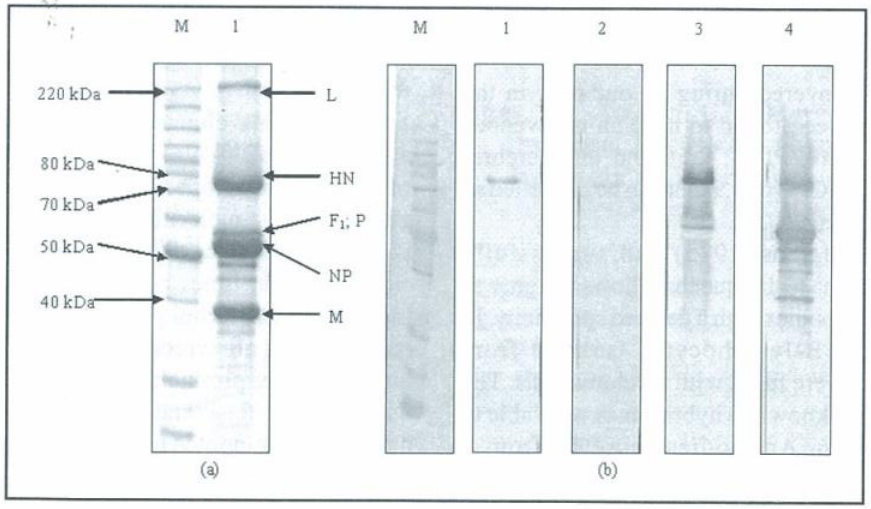

Figure 1: (a) NDV protein profile. Lane M: Protein ladder marker, lane 1: NDV proteins: L at 220 kDa, HN at 75 kDa, F_1 and P at 55 kDa, NP at 53 kDa and M at 38 kDa. (b) Immunoblotting against selected mAbs. Lane M: Protein ladder marker, lane 1: mAb 1D5, lane 2: 5A10, lane 3: mAb 5E10, lane 4: murine pAbs.

Table 1: Haemagglutination-inhibition and haemolysis-inhibition tests

MAb	HI units	Haemolysis-inhibition test OD (540 nm) ± SD
1B9	2^8	0.50 ± 0.02
1D5	2^4	0.35 ± 0.01
2D6	2^5	0.53 ± 0.01
5A10	2^4	0.49 ± 0.01
5E10	2^5	0.63 ± 0.02
9D7	2^4	0.61 ± 0.01
Chicken pAbs	2^{11}	0.02 ± 0.00
Negative control	*	0.43 ± 0.00

* No inhibition was observed using the Sp2 myeloma supernatant.

Table 2: Cross-reactivity test among NDV pathotypes

Virus strain	Pathotype	mAb				
		1B9	1D5	5A10	5E10	9D7
AF2240	Velogenic	+++	+++	+++	+++	+++
00/IKS	Velogenic	++	+	+	++	++
01/C	Velogenic	++	+	+	+	+
01/GNS	Velogenic	++	+	+	++	++
01/TM	Velogenic	++	++	+	+	++
Mukteswar	Mesogenic	+	+	+	+	+
B1	Lentogenic	++	+	+	+	++
F	Lentogenic	+++	++	++	++	++
La Sota	Lentogenic	++	+	+	+	+
V4 (QUE)	Lentogenic	+++	++	+	++	+++
V4 (UPM)	Lentogenic	+++	++	++	++	++

ELISA was employed to measure the cross-reactivity of different NDV strains. (+) indicated weak reactivity; (++) indicated moderate reactivity and (+++) indicated strong reactivity. Weak reactivity indicated by absorbance value of sample was 1 - 5 times higher than absorbance value of negative control. Moderate and strong reactivity indicated by absorbance values of sample 6 - 10 times and more than 10 times higher than absorbance value of negative control, respectively. Absorbance value of negative control for 1B9 was 0.04, 1D5 (0.04), 5A10 (0.03), 5E10 (0.04) and 9D7 (0.04).

approximately 53 kDa was detected by mAb 5E10 (lane 3) which might be the degraded HN. Samson (1986) postulated that the HN glycoprotein may have been fragmented at some stage and the epitopes were trapped by the other viral proteins. However, all the mAbs were confirmed to recognise the HN glycoprotein based on ELISA.

The HI test is still the most widely used conventional serological method for detecting antibodies to NDV and is considered to be the laboratory standard test for this disease. It has been established that the HI titre correlates very well with protection against NDV (Reynolds and Maraqa, 2000). However, most of the HI tests were carried out using mAbs harbour low titre (Yusoff *et al.*, 1988; Jestin *et al.*, 1989). In our study, mAb 1B9 produced the highest HI titre (2^8 HIU) but this was still lower than the titres obtained by the pAbs (2^{11} HIU). It is most likely that all the mAbs, except mAb 1B9, recognised an epitope that did not block or neutralise the HN glycoprotein from binding to RBC receptors. Our results are further supported by Iorio and Bratt (1985) who showed that a large percentage of mAb-resistant mutants retained infectivity despite binding to neutralsing antibody. Reynolds and Maraqa (2000) reported that chickens passively immunised with pAbs against a combination of both HN/F and UV-NDV were protected from further introduction of NDV. This showed that pAbs have higher protection ability than mAbs in general but specific mAbs against the HN and F glycoproteins do play a significant role in blocking HA and haemolysis activities.

A cross-reactivity test was carried out for NDV inter-strains to detect any overlapping epitopes of HN protein which might provide cross-protection. As for the velogenic NDV group, each of the mAbs exhibited different binding properties. The results obtained would suggest that mAb 5A10 bound to a unique epitope that

distinguished strain AF2240 from the other velogenic strains. Tan *et al.* (1995) have reported that HN protein of AF2240 strain is unique because three nucleotides (at positions 1298 – 1300) which encode an Arginine residue (at position 403) were absent compared to the other published NDV strains (Sakaguchi *et al.*, 1989). However, it also bound moderately to lentogenic strains F and V4 (UPM) suggesting the presence of a partially overlapping epitope. As mAb 1B9 reacted with all of the velogenic strains in this study, it is very likely that it recognised a conserved epitope among these velogenic stains. However, antigenic variation does exist among the same pathotype group and many mAbs have been used to define antigenic variation at the level of the HN subunit (Rusell and Alexander, 1983). All mAbs bound weakly to Mukteswar strain (mesogenic NDV). Ali *et al.* (1996) reported that all their mAbs against AF2240 also cross-reacted strongly with strains V4 (QUE) and V4 (UPM). Their results further substantiated ours except for mAb 5A10 which had a weak reactivity.

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