Protective effect of monoclonal antibodies to Newcastle disease virus in passive immunization

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A series of monoclonal antibodies (MAbs) against the haemagglutinin-neuraminidase (HN) and fusion (F) glycoproteins and the matrix (M) protein of Newcastle disease virus (NDV) were tested for protective effects in passive immunization of newborn chickens against challenge with a virulent heterologous strain of NDV (Italien). MAbs with high virus-neutralizing activity directed to one antigenic site of the HN protein delayed virus growth and significantly prolonged survival time, but all chickens eventually succumbed to infection. MAbs directed to two antigenic sites of the F protein completely suppressed virus growth and prevented death of chickens, although the neutralizing activities of these anti-F MAbs were lower than those of the above anti-HN MAbs. Combined administration of the anti-HN and anti-F MAbs had a synergistic protective effect, but no protective effects were shown by MAbs against the M protein.

Newcastle disease virus (NDV) is a member of the paramyxovirus group, containing two surface glycoproteins and at least four internal proteins (Nagai et al., 1976; Sato et al., 1987; McGinnes et al., 1988). It is generally agreed that the protective effect of antibodies against the haemagglutinin-neuraminidase (HN) glycoproteins of paramyxoviruses upon viral infection parallels their virus-neutralizing activity (Giraudon & Wild, 1985; Wolinsky et al., 1985). On the other hand, the role of antibodies against the fusion (F) glycoprotein in protection has not been definitively established.

To elucidate the relative roles of antibodies to the HN and F glycoproteins of NDV on protection we previously studied the effect of passively administered chicken polyclonal antibodies to either proteins upon lethal infection of chickens (Umino et al., 1987). Both anti-HN serum with virus-neutralizing activity and anti-F serum with only a slight virus-neutralizing activity markedly prolonged the survival of infected birds and the protection by both sera administered together was significantly greater than by either serum given alone.

In the present study we assessed the protective effect of murine monoclonal antibodies (MAbs) against different epitopes of the HN, F and matrix (M) proteins of NDV and the protective effect of various combinations of MAbs.

Embryonated eggs laid by unvaccinated specific pathogen-free (SPF) White Leghorn hens were used for preparation of chicken embryo cell cultures and for raising chickens. Chickens were used for passive immunization at the age of 10 days and were kept in flexible plastic film poultry isolators throughout the experiment. Primary chick embryo (CE) cells from 11-day-old embryonated eggs were used for the virus titration by plaque assay. The Italien strain of NDV was used throughout the study. MAbs prepared from mice immunized with the NDV Ulster strain have been described (Umino et al., 1990) and mouse ascitic fluids containing MAbs were used throughout the study.

For characterization of MAbs, methods for haemagglutination inhibition (HI), virus neutralization (NT) and haemolysis inhibition (HLI) tests previously described were followed (Umino et al., 1984), except that the NDV Italien strain was used as an antigen in the present study. For the plaque-inhibition (PI) test CE cell monolayers in 12-well cluster plates were infected with 0.1 ml of the NDV Italien strain containing about 30 p.f.u. After a 60 min adsorption at room temperature the inoculum was removed. Cultures received 1 ml of agar overlay medium consisting of Eagle's minimal essential medium (EMEM), 0.5% agarose, 2% calf serum and 10-fold serially diluted MAbs and, 3 days later, 1 ml of EMEM containing 0.03% neutral red. Plaques were counted on the following day and the PI titre was expressed as the highest dilution of ascitic fluid that reduced the number of plaques by 50%.

The method of passive immunization has been described previously (Umino et al., 1987). Briefly, four or five 10-day-old chickens received intravenous injections of 0.1 ml of MAb-containing ascitic fluid. Twenty-four h
later the chickens were challenged intramuscularly with 0.1 ml of the NDV Italien strain containing about 20 p.f.u., a dose sufficient to kill all birds (Umino et al., 1987). Birds were kept under observation until day 10 and the mean death time (MDT) was determined. NDV-infected chickens were sacrificed at intervals for determination of infective virus in different organs. The signs of the disease at the time of sacrificing were graded from 1 to 4 according to severity, where 4 points correspond to death. For titration of virus in organs, chickens were bled by cardiac puncture under anaesthesia with ether. Organs were carefully removed to minimize contamination with blood or other tissues and were washed three times with phosphate-buffered saline (PBS), homogenized in a glass homogenizer with 2 ml of PBS and centrifuged at 3000 r.p.m, for 20 rain at 4 °C. The supernatant was assayed for infectivity by plaque assay in CE cells (Umino et al., 1984). Lungs from passively immunized chickens were assayed by the following procedures. The thorax of anaesthetized chickens was opened and a 20-gauge needle equipped with a three-way valve and two 20 ml syringes was inserted into the right ventricle of the heart. Alternate aspiration of blood and injection of 5 ml of cold PBS were repeated 10 times and the lung was excised. Lungs were processed for virus titration as described above. The blood was washed out to 1/50 of the original content by the treatment.

The preparation and characterization of various MAbs are described in the accompanying report, using the Ulster strain as an antigen (Umino et al., 1990). Since the Italien strain is highly virulent we used this virus to study the protective effect of MAbs and the results of serological tests of MAbs using the Italien strain as an antigen are summarized in Table 1. In general they were similar to those obtained with the Ulster strain as antigen (Umino et al., 1990). Anti-HN Mabs were classified into three groups by competitive binding assays (Umino et al., 1990). MAbs 142 (HN-I), 298 (HN-I) and 284 (HN-II) had high HI and neutralizing activity, but low PI activity. MAb 190 (HN-II) had lower HI and neutralizing activity but higher HLI and PI activity. MAbs 110 (HN-II) weakly inhibited the haemolysing activity of the virus. This antibody also reduced plaque size without affecting plaque number (data not shown). Anti-F MAbs were similarly classified into three groups (Umino et al., 1990). MAbs 36 (F-I) and 313 (F-II), directed to each of two non-overlapping antigenic sites, had high neutralizing, HLI and PI activity. Anti-M MAb 45 had neither HI, neutralizing, HLI nor PI activity.

Newborn chickens were passively immunized with 0.1 ml of each of the MAbs listed in Table 1 and then challenged by lethal NDV infection. The protective effect of passive immunization with MAbs to the F protein is shown in Fig. 1. MAb 142 (HN-I) markedly prolonged the survival period (MDT 244 h as opposed to 98 h in control chickens, $P < 0.01$), although protection was not complete. A similar degree of protection was provided by two other MAbs, 51 and 298, both belonging to the same group, HN-I (data not shown). MAb 284 (HN-II), 190 (HN-II) and 110 (HN-II) were less protective (MDT 142 h, 128 h and 138 h, respectively). MAbs 223 (HN-II), 265 (HN-III) and 129 (HN-III) without neutralizing activity showed no protective effect (MDT 93 h, 98 h and 112 h, respectively). The results of the passive immunization with MAbs against the F protein are shown in Fig. 2. Two MAbs, 36 (F-I) and 313 (F-II), when administered undiluted, provided complete protection and all chickens survived without showing

### Table 1. Activities of MAbs

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Class and subclass</th>
<th>Antigenic site</th>
<th>Antibody concentration (mg/ml)</th>
<th>HI</th>
<th>NT</th>
<th>HLI</th>
<th>PI</th>
<th>Protective effect (MDT)*</th>
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<tr>
<td>142</td>
<td>G3</td>
<td>HN-I</td>
<td>4.5</td>
<td>1024</td>
<td>39800</td>
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<td>25</td>
<td>244</td>
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<td>HN-I</td>
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<td>2048</td>
<td>63000</td>
<td>600</td>
<td>25</td>
<td>218</td>
</tr>
<tr>
<td>284</td>
<td>G3</td>
<td>HN-II</td>
<td>0.8</td>
<td>1024</td>
<td>2000</td>
<td>540</td>
<td>63</td>
<td>142</td>
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<tr>
<td>223</td>
<td>G1</td>
<td>HN-II</td>
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<td>&lt;8</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>93</td>
</tr>
<tr>
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<td>HN-II</td>
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<td>16</td>
<td>200</td>
<td>1400</td>
<td>5010</td>
<td>128</td>
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<tr>
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<td>2000</td>
<td>4500</td>
<td>12590</td>
<td>275†</td>
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<td>3200</td>
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<td>&lt;8</td>
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<tr>
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* MDT on administration of 0.1 ml of each of the undiluted MAbs, using four or five birds.
† MDT on administration of 1:10 diluted MAb.
Fig. 1. Effect of passive immunization with anti-HN MAbs on death time. Groups of four or five 10-day-old newborn SPF chickens received intravenous injections of 0.1 ml of undiluted mouse ascitic fluid containing the indicated anti-HN MAb or PBS. On the following day the chickens were infected intramuscularly with 20 p.f.u. of the NDV Italian strain. Columns show death times of individual birds. The survival time was censored by negative-exponential transformation according to Liddell (1978), using 0 and 240 h for 0 and T, respectively. Statistical significance of the difference in death time between each test group and the control group assessed by Student’s t-test is given at the right-hand side.

Fig. 2. Effect of passive immunization with anti-F MAbs on death time. Procedures were similar to those in Fig. 1, except that MAbs 36 and 313 were given at a dilution of 1:10. Statistical significance of the difference in death time between each test group and the control group assessed by Student’s t-test is given at the right-hand side.

Anti-HN MAb 298 (HN-I) and anti-F MAb 36 (F-I) were marginally effective at dilutions of 1:10 and 1:50 (MDT 140 h and 145 h, respectively). Combined administration of the two antibodies resulted in significantly prolonged death time (MDT 199 h) in comparison with the single administration of either antibody, indicating a synergistic effect of the two antibodies (Fig. 4).

There have been a number of studies dealing with the protective effect of MAbs to individual envelope glycoproteins of paramyxoviruses, i.e. mumps virus (Wolinsky et al., 1985; Løve et al., 1986), parainfluenza virus type 3 (Rydbeck et al., 1988), respiratory syncytial virus (Taylor et al., 1984), measles virus (Rammohan et al., 1981; Giraudon & Wild, 1985; Varsanyi et al., 1987), Sendai virus (Örvell et al., 1982) and NDV (Meulemans et al., 1986; Lana et al., 1988). It is generally agreed that anti-HN MAbs with neutralizing activity have a protective effect upon virus infection. On the other hand, the role of anti-F MAbs in protection has been less well documented. Recently, Meulemans et al. (1986) have presented data that MAbs directed against the HN and F day. The kidneys and lungs contained more virus than any other organs (data not shown). The kinetics of virus growth in the lungs of control chickens and those passively immunized with MAbs 142 (HN-I) and 36 (F-I) are shown in Fig. 3. No virus was detected in the lungs of chickens passively immunized with undiluted anti-F MAb 36 and no clinical signs developed. On the other hand, after a delay of 3 to 4 days, virus grew in the lungs of chickens that received anti-HN MAb 142 and eventually killed all birds.

Fig. 3. Virus growth in chicken lungs. Chickens were passively immunized with MAbs 142 (HN-I) (●), 36 (F-I) (▲), or PBS as a control (○) and infected with 20 p.f.u. of NDV 24 h later. Points represent geometric mean virus titres of lung homogenates from two birds. (−) No clinical signs; (+) listlessness; (++) increased respiration rate and inactivity; (+++) paralysis and respiratory difficulty.

any symptoms. Administration of 1:10 diluted MAbs provided incomplete protection, with a MDT of 275 h each. MAb U63 (F-III), prepared by immunization with the purified F protein (Umino et al., 1990), prolonged the survival period to 147 h. MAbs 207 and 262 (F-III), whose epitopes are located on the same site of the F protein as that of U63, gave no protection at all (MDT 86 h and 105 h, respectively), nor did anti-M MAb 451). All results are summarized in Table 1 as the MDTs.

Virus growth in various organs of infected chickens was tested. Virus started to increase on day 2 in the kidneys, lungs, intestines, muscle, liver and blood, and reached a plateau on day 3, when chickens began to die. Virus growth in the brain and spleen lagged behind by 1
proteins with neutralization activities provided passive protection to susceptible chickens. Moreover, protection afforded by anti-F MAbs has been higher than that observed with anti-HN MAbs. The data demonstrate the importance of the F protein in the immune response against NDV. In another study anti-F MAbs without neutralizing activity were protective against mumps virus infection in the central nervous system (Löve et al., 1986).

We have previously studied the protective effect of polyclonal chicken antisera specific to the HN and F proteins of NDV and found that anti-F serum with a marginal neutralizing activity had a protective effect comparable to that of anti-HN serum, with high neutralizing activity. We speculated that the protective effect of the anti-F serum may result from a possible PI activity limiting cell-to-cell spread of infection through cell fusion in the presence of antibodies in agar overlay throughout the plaque assay (Umino et al., 1984), but the PI activity of the antisera had not been determined (Umino et al., 1987).

The following findings in the present study are thought to be particularly noteworthy. First, some MAbs to the F protein not only have high PI activity, but also neutralizing activity and are more effective in protection than those to the HN protein, which have an even higher neutralizing activity as Meulemans et al. (1986) pointed out. PI activity appears to be more relevant to the protective effect than to neutralizing activity. Secondly, supposedly hyperimmune anti-F sera from rabbits (Umino et al., 1984) and chickens (Umino et al., 1987) were devoid of sufficient neutralizing activity, whereas some MAbs to the F protein prepared in this study had neutralizing activity in addition to PI activity. The finding reported in the accompanying paper (Umino et al., 1990) that some epitopes of the F protein are labile to detergents and acetone may be relevant to the question. The rabbit and chicken antisera were prepared by immunizing animals with the F protein isolated from detergent-solubilized virions. Immunogenicity of the F protein could have been impaired by the detergent treatment. The antibody-binding activity of the F protein of measles virus was reported to be abolished by acetone (Armstrong et al., 1979) and Tween--ether (Norrbuy & Gollmar, 1975) and the anti-F antibody reacting with acetone-labile epitopes had neutralizing as well as HLI and fusion-inhibiting activity (Sato et al., 1989). Thirdly, the finding that the combined administration of two MAbs made the survival time significantly longer than that due to either of the two MAbs administered singly, but in a concentration twice as high as in the combined administration, indicates a synergistic effect. This suggests that the antibodies to the two proteins have different mechanisms of protection.

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**References**


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