Monoclonal antibodies to three structural proteins of Newcastle disease virus: biological characterization with particular reference to the conformational change of envelope glycoproteins associated with proteolytic cleavage

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Monoclonal antibodies (MAbs) to the haemagglutinin–neuraminidase (HN), fusion (F) and matrix (M) proteins of Newcastle disease virus were prepared and characterized. At least three non-overlapping or partially overlapping antigenic sites were delineated on the HN, three on the F and three on the M proteins by competitive binding assays. Antigenic sites on the HN and F proteins roughly represented functional domains defined by serological tests. Two antigenic sites on the F protein were involved in virus neutralizing and haemolysis-inhibiting activity. These antigenic determinants were readily affected by treatment with certain surfactants and acetone. Proteolytic cleavage of the HN and F proteins was associated with conformational change, revealed by altered reactivity with MAbs and by altered topological arrangements of some epitopes. None of the anti-M MAbs inhibited any biological activities of the virus.

Introduction

The unique ability of enveloped viruses to fuse with cellular membranes is an important mechanism of host cell entry for these viruses and is effected by virus-encoded envelope glycoproteins. Envelope glycoproteins are also the targets of the host immune response.

Post-translational proteolytic cleavage is involved in the activation of the fusion (F) glycoproteins of paramyxoviruses. The precursor of the fusion protein (F₀) is cleaved into fragments F₁ and F₂ (F₁₂), which are present as a disulphide-bonded molecule. Cleavage of F has been observed for different paramyxoviruses (for reviews see Ishida & Homma, 1978; Compans & Klenk, 1979). In the case of Newcastle disease virus (NDV) haemagglutinin–neuraminidase (HN) glycoprotein, the precursor HN₀ has been reported for certain strains of NDV (Nagai et al., 1976). HN₀ is converted to HN by cleavage, which releases a carboxy-terminal glycopeptide into the culture medium (Schuy et al., 1984). These proteolytic cleavages mediated by specific proteases of host cells are not indispensable for the formation of virus particles, but are essential for the biological activities of glycoproteins and hence for virus infectivity.

NDV consists of a wide variety of strains that differ in their virulence for chickens. Strain-specific differences in the susceptibility of the envelope glycoproteins to proteolytic cleavage account for differences in biological properties, such as host range, organ tropism and pathogenicity of the virus (Nagai et al., 1976; Nagai & Klenk, 1977). Cleavage reactions are therefore important for the biological behaviour of the virus.

We showed in an earlier study that cleavage is associated with a conformational change of the glycoproteins of NDV, demonstrable by physicochemical and spectroscopic analyses (Kohama et al., 1981). In the present study we have prepared various monoclonal antibodies (MAbs) to Newcastle disease viral proteins, some of which recognize the cleavage-associated conformational change based upon their reactivity with uncleaved or cleaved forms of the glycoproteins. The results confirm the previous conclusion that changes in conformation indeed parallel the proteolytic cleavage and that reactivity of the F protein with some MAbs is abolished by detergents and acetone.

Methods

Cells and viruses. Madin-Darby bovine kidney (MDBK) cells were grown in Eagle's minimal essential medium (MEM) containing 5% foetal calf serum and 10% tryptose phosphate broth. Eleven-day-old embryonated eggs were used for preparation of monolayer cultures of chick embryo (CE) cells. The Ulster strain of NDV was used throughout the study. Virus was grown in MDBK cells in the absence
(designated non-trypsin-grown virus) or in the presence of 10 μg/ml trypsin in the medium (Difco; trypsin-grown virus) (Nagai et al., 1976).

The octylglucoside-solubilized, isoelectric focusing-purified F and HN glycoproteins from trypsin-grown, purified virus were prepared as described previously (Kohama et al., 1981). Infectivity titration and neutralization tests were done by plaque assay in monolayer cultures of CE cells maintained under the agar medium containing 10 μg/ml of trypsin (Nagai et al., 1976; Umino et al., 1984).

Radiolabelling and purification of virus. Confluent monolayers of MDBK cells on square plastic tissue culture plates (Nunclon, 245 × 245 × 20 mm; Nunc) were inoculated with the virus at an m.o.i. of 5 p.f.u./cell. After 60 min of adsorption the inoculum was removed, the cells were washed once with MEM and overlaid with 50 ml of glucose-free MEM containing 5 μCi/ml of [3H]glucosamine. After 36 to 48 h of incubation the medium was harvested and virions were purified as previously described (Kohama et al., 1981).

Preparation of MAbs. Two 8-week-old BALB/c mice were immunized by an intraperitoneal injection of 200 μg of purified non-trypsin-grown live virus without Freund's adjuvant. Three additional booster injections with 80 μg each of the same antigen were given intraperitoneally.

Another mouse was subcutaneously immunized with 150 μg of the F glycoprotein purified from the trypsin-grown virus emulsified with Freund's complete adjuvant. The same dose of the antigen without adjuvant was administered intraperitoneally 6 weeks later.

Immune spleen cells from each group of mice were fused with SP2/O Ag 14 myeloma cells at a ratio of 7:1. Fusion using polyethylene glycol (M, 1549; Wako Chemical) and selection and subsequent culture of hybridomas were done by the method described by Umino et al. (1985). Culture fluids of hybridomas were screened for anti-NDV antibody by ELISA using the non-trypsin-grown virus as an antigen (Table 1, ELISA-A). Antibody-positive cultures were subjected to further cloning by colony formation in soft agar. Hybridoma clones secreting MAbs were finally inoculated into pristane-primed BALB/c mice. Ascitic fluid was collected 1 to 2 weeks later. The isotype of the antibodies was determined by ELISA using anti-mouse immunoglobulins (Nordic Immunological Laboratories, Tilburg, The Netherlands). The concentration of antibody in a given ascitic fluid was expressed as the protein concentration after purification of the antibody by the method described by Ey et al. (1978).

ELISA. The method was as previously described (Umino et al., 1985). Purified non-trypsin-grown virus at a protein concentration of 4 μg/ml, and the HN and the F protein separated from trypsin-grown virus were used as antigens.

Competitive binding ELISA. Peroxidase was conjugated to immunoglobulin prepared by ammonium sulphate precipitation from 0.25 ml of ascitic fluid by the method described by Wilson & Nakane (1978). The method was as previously described (Umino et al., 1985), except for simultaneous reaction of non-conjugated and peroxidase-conjugated antibody with a solid-phase antigen in the present study.

Immunoprecipitation and PAGE. Radiolabelled, purified virus was lysed in 50 to 100 μl of either radioimmunoprecipitation (RIPA) buffer (0:15 M-NaCl, 1% sodium deoxycholate, 1% Triton X-100, 0.1% SDS, 0.01 M-Tris-HCl pH 7.4, 1 mM-PMSF, 1 mM-methionine and 50000 K units/500 ml Trasylol aprotonin) (Lamb et al., 1978) or phosphate-buffered saline (PBS) containing 1.5% Tween 20 in a microcentrifuge tube (Beckman P/N 348349 polyallomer, 1.5 ml) and centrifuged for 15 min at 35000 r.p.m. in a TLA-100.3 rotor using a Beckman TL-100 ultracentrifuge. The supernatant was mixed with 20 to 30 μl of ascites fluid. Radioimmunoprecipitation, gel electrophoresis in 10% polyacrylamide slab gels and fluorography were carried out essentially as described by Kohama et al. (1985).

Immunofluorescence of infected cells. MDBK cells grown on glass coverslips were infected with NDV strain Ulster and incubated for 9 h. Half of the cultures were treated with 10 μg/ml of trypsin for 2 h at 37°C. Cells were then subjected to immunofluorescence staining either without fixation or after fixation with acetone for 10 min (Kohama et al., 1986). MAbs and fluorescein-conjugated goat anti-mouse IgG (Cappel Laboratories) were used at a dilution of 1:20 and 1:50, respectively.

Haemagglutination inhibition (HI) test. The HI test was performed on plastic microtitre trays using 0.5% chicken erythrocytes and 4 haemagglutinating units (HAU) of the virus grown in eggs as the antigen.

Virus neutralization (NT) test. The method described previously (Umino et al., 1984) was followed, except that the Ulster strain was used and trypsin (60 μg/ml) was added to the agar overlay. The neutralization titre was expressed as the highest dilution of serum that reduced the number of plaques by 50%.

Neuraminidase inhibition (NI) test. The standard procedure described by Seto & Rott (1966) was used. Fettuin was used as the substrate for viral neuraminidase assays and the antibody titre was expressed as the highest dilution that inhibits 50% of the enzyme activity.

Haemolysis inhibition (HLI) test. The procedure described previously (Umino et al., 1984) was followed, except that 200 HAU of virus was used and the A545 was measured using a Titertek Multiskan Type 310 C photometer.

Results

Preparation and characterization of the MAbs

Two mice were immunized with virus grown in the absence of trypsin in MDBK cells and one mouse was immunized with F protein isolated from trypsin-grown virus. Twenty-seven hybridomas were derived from the former mice and two (U63 and U21) from the latter (Table 1). For the ELISA antigen, non-trypsin-grown virus was used (Table 1, ELISA-A), as well as the octylglucoside-solubilized, isoelectric focusing-purified HN and F glycoproteins (isolated from trypsin-grown virus; Table 1, ELISA-B and -C, respectively) (Kohama et al., 1981). Five clones (numbers 313, 262, U63, U21 and 207) were found to secrete antibodies reacting with the purified F protein (Table 1, ELISA-C and 14 clones (numbers 51, 53, 142, 271, 298, 284, 223, 224, 190, 192, 193, 278, 110 and 263) to secrete those reacting with the HN protein (Table 1, ELISA-B). The hybridomas were inoculated into the peritoneal cavity of syngeneic mice and ascitic fluids containing MAbs were obtained. The specificity of these antibodies was then determined by RIPA and SDS–PAGE. Seven clones of the hybridomas (the aforementioned five clones and two additional clones, 36 and 83) produced antibodies specific to the F protein. It should be noted that in particular antibody 83,
Table 1. Immunological properties of the MAbs to NDV strain Ulster

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Subclass</th>
<th>Antigenic site</th>
<th>Antigenic concentration (mg/ml)</th>
<th>ELISA*</th>
<th>Antibody titre</th>
<th>Reactivity</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
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<tr>
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<td>G2b</td>
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<td>+1</td>
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</tr>
<tr>
<td>313 (F)</td>
<td>G2a</td>
<td>II</td>
<td>2.2</td>
<td>+2</td>
<td>-1</td>
<td>&lt;8</td>
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<tr>
<td>U63 (F)</td>
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<tr>
<td>U21 (F)</td>
<td>M</td>
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<td>ND</td>
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<tr>
<td>262 (F)</td>
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<td>+3</td>
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<tr>
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<td>G2a</td>
<td>III</td>
<td>0.8</td>
<td>+2</td>
<td>-3</td>
<td>&lt;8</td>
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<tr>
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<td>+3</td>
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<td>I</td>
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<td>+3</td>
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<td>+3</td>
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<tr>
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<td>-</td>
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<tr>
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<td>I</td>
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<td>37 (M)</td>
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<td>+2</td>
<td>-</td>
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<tr>
<td>179 (M)</td>
<td>G1</td>
<td>II</td>
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<td>+2</td>
<td>-</td>
<td>&lt;8</td>
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<tr>
<td>126 (M)</td>
<td>G2a</td>
<td>III</td>
<td>1.0</td>
<td>+2</td>
<td>-</td>
<td>&lt;8</td>
</tr>
</tbody>
</table>

* ELISA A, NDV Ulster (MDBK, non-trypsin-grown); B, purified HN from trypsin-grown virus; C, purified F from trypsin-grown virus were used as antigens.

† RIPA-PAGE, using RIPA buffer for solubilization of antigens and immunoprecipitation.

§ ND, Not done.

which did not react with the purified F protein in ELISA, precipitated the F₁,₂ protein when radiolabelled, trypsin-grown virus was used as an antigen (Fig. 1). Sixteen clones (the former 14 clones and two additional clones, 129 and 130) produced MAbs specific to the HN protein and six clones to the M protein.

Effect of proteolytic cleavage of the envelope glycoproteins on the reactivity with MAbs

The MAbs specific to F and HN were tested for reactivity with uncleaved and cleaved forms of the glycoproteins by RIPA (Fig. 1 and 2). Out of six anti-F antibodies of the IgG class, three (207, 262 and U63) precipitated predominantly the F₁,₂ protein, whereas three others (36, 83 and 313) precipitated more F₀ protein than F₁,₂ protein (Fig. 1a). We previously speculated that some epitopes on the F protein are susceptible to detergent (Umino et al., 1984). In order to avoid a possible interference of detergents contained in RIPA buffer with the reactivity of the F protein, immunoprecipitation was carried out using virus solubilized by 1:5% Tween-20. As shown in Fig. 1(b), antibodies 83 and 313 precipitated predominantly the F₁,₂ protein under the milder solubilizing condition (Fig. 1b, lanes 9 and 11) in contrast to the condition using RIPA buffer (Fig. 1b, lanes 10 and 12). After the milder solubilization, HN protein was coprecipitated with the F₁,₂ protein, presumably because of incomplete dissociation of the two proteins (Fig. 1b, lanes 9, 11 and 13). Antibody U63 precipitated more F₁,₂ than F₀ protein after disruption both with Tween-20 and RIPA buffer (Fig. 1b, lanes 13 and 14). These observations suggest that the epitopes 83 and 313 are present both on the F₀ and the F₁,₂ proteins and that the epitopes on F₀ are masked in mild detergent, but recognized in RIPA, whereas on F₁,₂ the epitopes are accessible under both conditions. In contrast, epitope U63 is detected only in
F₁,₂. Thus there are two types of anti-F MAbs with differential affinity to the uncleaved and cleaved forms of the F protein. Antibodies 36, 83 and 313 reacted with both the uncleaved and cleaved forms, whereas U63, 262 and 207 reacted predominantly with the cleaved form of the F protein. In addition, epitopes 313 and 83 on the cleaved form of the F protein are labile to RIPA buffer.

Of 10 anti-HN antibodies, nine precipitated both the HN₀ and the HN glycoprotein equally well (Fig. 2). In contrast, antibodies 129 (Fig. 2) and 130 (data not shown) reacted predominantly with the HN₀ protein.

**Delineation of non-overlapping antigenic sites by competitive antibody binding**

Antigenic sites to which the MAbs bound were analysed by competitive binding assays. Three non-overlapping or partially overlapping antigenic sites were demonstrated by the assays using the two viral glycoproteins. Competition among anti-F antibodies U63, 262 and 207 is shown in Fig. 3. When trypsin-grown virus was used as the antigen, labelled antibody 262 was competed against by U63 and 207 as well as by homologous 262, whereas labelled U63 was strongly competed against by U63 and 207, but to a lesser extent by 207 (Fig. 3e, d). Labelled antibody 207 was competed against only by homologous antibody (Fig. 3f) and there was a non-reciprocal competition between antibodies 207 and 262. These results indicate that epitopes U63 and 262 are close to each other, whereas epitope 207 is separated from them.

Interaction among the three antibodies was altered when the ELISA antigen was replaced by non-trypsin-grown virus (Fig. 3a to c). In contrast to the low reactivity of these MAbs with the uncleaved form of the F protein in RIPA, as described above, they reacted well with the same antigen in the competitive ELISA. Competition of antibodies U63 and 207 with each other was higher with the uncleaved rather than with the cleaved form of the F protein used as an antigen. Epitopes U63, 207 and 262 were no longer distinguishable from each other on the uncleaved F protein. Based
MAbs to NDV proteins

Fig. 2. SDS–PAGE fluorogram of the HN proteins precipitated by anti-HN MAbs from the purified virus. Radioactively labelled virus mixture was solubilized with RIPA buffer. Lane 2, 51 (HN-I); lane 3, 110 (HN-II); lane 4, 129 (HN-III); lane 5, 142 (HN-I); lane 6, 190 (HN-II); lane 7, 224 (HN-II); lane 8, 265 (HN-III); lane 9, 271 (HN-I); lane 10, 284 (HN-II); lane 11, 298 (HN-I); lane 1, supernatant without immunoprecipitation.

on such competitive binding assays MAbs 36, 83, 313, U63, 262 and 207 could be assigned to three distinct antigenic sites (Fig. 4). Fig. 4 also shows that cleavage of the F protein has a substantial influence on the topological relationships of the binding sites.

Three non-overlapping and partially overlapping antigenic sites of the HN glycoprotein have been defined using non-trypsin-grown virus as antigens as summarized in Fig. 5. Binding of labelled antibody 51 (group I) was inhibited only by 51 itself and by those of the same group. In contrast, the labelled antibodies belonging to group II were competed against not only by antibodies of their own group, but also by those of other groups. Thus, there was a unidirectional competition among anti-HN antibodies. A similar result was obtained when a competitive ELISA was carried out using trypsin-grown virus as the antigen, with the exception of the three antibodies belonging to group III (129, 130 and 265), which did not react in competitive binding assays with trypsin-treated virus (data not shown). These results suggested that antigenic site III is probably located at some distance from antigenic sites I and II, whereas the latter sites are located in close proximity to each other, forming a continuous cluster of epitopes. Partial overlapping within these epitopes might explain the observation that antibody 129 precipitated only the HN₀ protein, whereas antibody 265 reacted with both the HN₀ and the HN protein, as demonstrated by the RIPA-assay (Fig. 2).

The MAbs specific to the M protein were classified as recognizing three partially overlapping antigenic sites and our results with anti-M antibodies appear to resemble those reported by Nishikawa et al. (1987).

Immunofluorescence study of the F glycoprotein in NDV-infected cells

It is known that the cleavage of the F protein of the Ulster strain occurs only at the cell surface and not in the cytoplasm when trypsin is added to the culture medium (Nagai et al., 1976). When MDBK cells infected with NDV strain Ulster and grown in the absence or presence of trypsin were examined by immunofluorescence with antibody 83 (F-I), both uncleaved and cleaved forms of the F protein were demonstrable on the cell surface (Fig. 6a and c). The intensity of fluorescence appeared to be greater for the cleaved form than the uncleaved form of the F protein. When the cells were
fixed and permeabilized with acetone the presence of the uncleaved form of the F protein was demonstrable both on the cell surface and in the cytoplasm (Fig. 6b). Acetone treatment completely abolished the cell surface fluorescence of the cleaved form of the F protein (compare Fig. 6c and d), but did not markedly affect the cytoplasmic fluorescence of its uncleaved form (compare Fig. 6a and b). This shows that epitope 83 on the cleaved form of the F protein is highly sensitive to acetone.

Fig. 3. Competitive binding ELISA of three anti-F MAbs U63, 262 and 207. Absorbance values given by a peroxidase-conjugated anti-F antibody in the presence of non-conjugated antibodies at the indicated concentrations were expressed as a percentage of the absorbance value in the absence of non-conjugated antibody. (a to c) Non-trypsin-grown virus and (d to f) trypsin-grown virus as solid-phase antigens. Peroxidase-conjugated antibody: (a) and (d), U63; (b) and (e), 262; (c) and (f), 207. Non-conjugated antibody: (●) U63; (○) 262; (△) 207.

Biological activities of MAbs

As expected none of the anti-M MAbs had HI, NT, NI or HLI activity. On the other hand, anti-HN MAbs were quite diverse, reflecting various functional domains of the HN molecule (Table 1). Antibodies directed to antigenic site I possessed a high level of HI, NT, NI and HLI activities. Antibodies directed to antigenic site II possessed either low or no HI activity, but possessed NT, NI and HLI activities to a considerable level, except for antibody 284, which showed high HI activity. Antibody 278, the only anti-HN antibody of the IgM class so far obtained, possessed high NI, low HI and HLI, but no NT activity. The relatively high NI activity of this antibody may have resulted from a steric effect due to its being IgM. A moderate level of HLI activity was detected with antibody 110 (HN-II), with the absence of HI, NT and
MAbs to NDV proteins

Fig. 5. Antigenic site assignment of anti-HN MAbs using non-trypsin-grown virus as the antigen. The procedure and symbols are the same as those described in the legend to Fig. 4.

Fig. 6. Indirect immunofluorescence staining of NDV-infected MDBK cells cultured in the absence (a and b) and presence of 10 μg/ml of trypsin in the medium (c and d) with MA b 83 (F-I) at 1:20 dilution. Cells were processed for surface staining without fixing (a and c) or for cytoplasmic as well as surface staining after fixing with acetone (b and d).

Discussion

MAbs have previously been made against NDV proteins F, HN, phosphorylated (P), nucleoprotein (NP) and matrix (M) aiming at comparison, antigenic mapping, and functional analyses (Russell & Alexander, 1983; Russell et al., 1983; Russell, 1984, 1986; Iorio & Bratt, 1983, 1984a, b; Iorio et al., 1986; Nishikawa et al., 1983, 1986; Long et al., 1986; Abenes et al., 1986; Erdei et al., 1987; Toyoda et al., 1988). In the present study we show that various MAbs specific to the F and the HN protein of NDV Ulster can serve as tools for investigating the conformation of these proteins in relation to their biological functions and to analyse the chemical stability of their functional domains.

We have previously shown, by use of physicochemical techniques, that the F protein of NDV undergoes a change in its secondary structure during proteolytic cleavage (Kohama et al., 1981). The findings that antibodies 83 (complementation group F-I) and 313 (complementation group F-II) precipitate both uncleaved and cleaved forms of the F protein in RIPA, whereas those belonging to complementation group F-III react only with the cleaved F₁₂ protein, reflect the
rearrangement of antigenic sites on the F protein molecule during cleavage. Additional evidence for a conformational change of the F protein resulting from cleavage is provided by the antigen-dependent variations in the reactivity of the antibodies belonging to complementation group F-III. As indicated by competitive binding assays epitopes U63, 262 and 207 are located close to each other on the uncleaved F protein. However, epitope 207 was separated from epitopes U63 and 262 on cleaved F protein. This suggests that steric relationships among the epitopes are altered by cleavage. F₁ and F₂ might be rearranged through the connecting disulphide bond in such a way that regions distant from each other before cleavage come closer (Scheid & Choppin, 1977). On the other hand, using MAbs specific for different epitopes on F it could be shown with NDV strain Australia-Victoria that this protein undergoes a conformational change during intracellular transport prior to cleavage (Morrison et al., 1987). Thus, the existence of two different types of changes in the secondary structure of the F protein during maturation processes have been established by use of MAbs. As indicated by RIPA and immunofluorescence assay some epitopes on sites F-I and F-II of the cleaved F protein molecule are highly sensitive to certain detergents or organic solvents. This finding strongly suggests that the fusogenic activity residing in these epitopes is also susceptible to those reagents. Since the F protein is known to be hydrophobic (Chambers et al., 1986), even mild procedures used for the isolation of the F protein, such as octylglucoside solubilization and isoelectric focusing (Kohama et al., 1981), might have affected its biological activity. The inability of antibodies 36 and 83 to react with the purified F protein in ELISA supports this concept. Attempts to demonstrate the F antigen by the Western blot method were unsuccessful with any of the anti-F MAbs. All the antigenic determinants on the F protein were irreversibly inactivated by treatment with SDS before electrophoresis (data not shown). In contrast some epitopes of other viral proteins, such as the HN, M and NP proteins, could react with specific antibodies in Western blotting (data not shown). Thus the F protein appears to be more susceptible to detergents. A similar observation was reported by Long et al. (1986). The antigenic sites F-I and F-II observed with NDV strain Ulster in this study may also be present with strains Italien and La Sota. With these viruses it was found that several anti-F MAbs, which lost their reactivity in the Western blotting assay but not in RIPA, also had high neutralizing activity. Out of several anti-HN MAbs only two (129 and 130) reacted predominantly with the HN₀ protein in RIPA. It is known that HN₀ is converted into the HN protein by removal of a glycopeptide fragment from the carboxyterminal end (Schuy et al., 1984; Millar et al., 1988). It is therefore likely that the loss of reactivity in RIPA observed after cleavage reflects the elimination of an antigenic site on the glycopeptide. On the other hand it is possible that cleavage of HN₀ causes a change in the steric structure of the HN protein, resulting in the loss of the epitope. Despite these two HN₀-specific MAbs, the original intention of this study to obtain a large panel of MAbs specific to either the uncleaved or the cleaved forms of HN and F, was not attained. The pathway between injection of antigens and their presentation to antibody-producing cells is so complex that it may be irrelevant whether they are offered in the uncleaved or the cleaved form. Furthermore, the antigenic instability of the F protein to detergents had not been anticipated. Nevertheless MAbs of sufficient variety were obtained to allow us to monitor the conformational change associated with the cleavage of the HN and F proteins. Two types of neutralization activity appear to exist with respect to anti-HN antibodies in the present study; the neutralization accompanied by high HI activity and that with either low or no HI activity (MAbs to sites HN-I and HN-II, respectively). With the former type virus adsorption to the host cell might be blocked. This type of neutralization was observed before, when we used polyclonal anti-HN antibodies (Umino et al., 1984). With the latter type an unknown function of the HN protein, different from adsorption, may be inhibited. This type of neutralization activity has also been observed with anti-HN MAbs to Sendai virus (Tozawa et al., 1986).

Recently we have presented data showing that passive administration of antisera raised against the HN and F proteins provided protection to susceptible chickens (Umino et al., 1987). The protective effect of MAbs is presented in a separate paper (Umino et al., 1990).

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