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Identification of Amino Acids Relevant to Three Antigenic Determinants on the Fusion Protein of Newcastle Disease Virus That Are Involved in Fusion Inhibition and Neutralization

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Nucleotide sequence analysis of F protein antigenic variants of Newcastle disease virus mapped three distinct antigenic determinants to positions 343, 72, and 161 on the protein. The high fusion-inhibiting and neutralizing capacities of all of the monoclonal antibodies used for selection suggested close functional and structural relationships of the three positions with the fusion-inducing N-terminal region of the F_1 subunit. The former two positions were located at the cysteine cluster domain near the C terminus of the F_1 subunit and at the major hydrophilic domain in the F_2 subunit, respectively, and both domains appeared to represent the major antigenic determinants of paramyxovirus F protein.

Paramyxovirus infection is initiated by the action of two envelope glycoproteins. One of these mediates attachment of the virus to host cell receptor and is designated HN (hemagglutinin-neuraminidase) for the genus *Paramyxovirus*. The other glycoprotein, designated F, is responsible for virus penetration into the host cell and virus-induced cell fusion and hemolysis. These two surface glycoproteins are important as the targets of host immune response. One characteristic feature in host response to paramyxovirus infection is that although antibodies to either glycoprotein can neutralize infectivity in in vitro tests, antibodies to F protein appear to be predominantly necessary and important for preventing infection and spreading of virus in vivo (16). Despite this prime importance of F protein for the immune response, our knowledge of its antigenic structure has been limited. So far, only one epitope has been localized on Sendai virus F protein (22). This contrasts with the situation of HN protein, in which the amino acids important for a number of determinants have been identified and the relationships between the determinants and the biologically active sites are now under fairly extensive investigation (6, 11, 22, 32).

The biologically active F protein consists of two disulfide-linked subunits F_1 and F_2, which are derived from the inactive precursor F_0 protein by proteolytic cleavage at a specific site (12, 18, 26). The hydrophobic N-terminal region of F_1 generated by the proteolysis is thought to drive membrane fusion by a hydrophobic interaction with cellular membrane (10, 24).

Abenes et al. (1) prepared a panel of monoclonal antibodies to the F protein of Newcastle disease virus (NDV), an avian paramyxovirus, and showed that the F protein had four antigenic determinants, I, II, III and IV. However, we found that the antibodies to the fourth site were not directed to the F protein but to another viral glycoprotein, HN. These antibodies were, therefore, ignored in the present study. The other three sites were confirmed to be on the F protein. Also confirmed was that all of them were highly potent at neutralizing the infectivity and inhibiting both hemolysis and fusion activities (1) (Table 1).

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Antigenic variants were isolated by growing the parental Sato strain in chick embryo or by plaquing on the monolayers of baby hamster kidney cells in the presence of each antibody (1, 11, 19). Only one variant clone was obtained in each experiment with an antibody. This was repeated three times with the use of separate parental virus stocks, each being plaque purified, and thus three independent variants were obtained for each antibody. The nucleotide sequencing of the F genes of variants and parental strain were done by the dideoxy method with the virion RNAs as templates (11). There was no sequence variability in F genes among the three stocks of parental virus. The coding region of the parental virus exhibited only 1.1 and 2.4% differences in the nucleotide and deduced amino acid sequences, respectively, compared with the previously published F gene sequence of Miyadera strain (30). The deduced amino acid substitutions in the variants are summarized in Fig. 1 and Table 1, and the positions of substitutions are illustrated in the hydropathicity profile and diagram of the F protein in Fig. 2. All three isolates selected with one antibody displayed an identical single point mutation, which resulted in an amino acid substitution of nonconservative nature. In addition, two independent antibodies to a single site (site I) induced an identical amino acid change (Table 1).

The leucine-to-proline change at position 343 was responsible for the loss of site I, the position being 226 residues downstream from the fusion-inducing F_1 N terminus. This change could introduce a new bend to the protein and result in a conformational change, thereby affecting antibody binding at some distant site. However, the fact that the position was located to the cysteine cluster domain near the C terminus (Fig. 2) raises another possibility that the domain involving Leu-343 is important antigenically as well as structurally and functionally, in view of the following observations. First, most cysteine residues near the C terminus are highly preserved for all the paramyxovirus F proteins so far sequenced (3–5, 7–9, 15, 17, 20, 23, 25, 27–31, 33), possibly contributing to the formation of a bunching structure characteristic of these proteins. Second, of a panel of 16 monoclonal antibodies to Sendai virus F protein, only a single antibody inhibited fusion and infectivity, and the corresponding determinant was also located to the cysteine

TABLE 1. Characterization of antigenic variants and antibodies used for selection

Antigenic site	Antibody	Antibody characterization: Assay result[a]							Variant characterization		
		Isotype	ELISA (10^3)	HI	NI	HLI	FI	N	Variant	Nucleotide change (position)	Amino acid change (position)
I	320/1	G2bk	1,245	<16	<40	3,584	9,088	13,312	V320/1	T(1074)→C	L(343)→P
									V320/2	T(1074)→C	L(343)→P
									V320/3	T(1074)→C	L(343)→P
	743/1	G2ak	360	<16	<40	1,064	4,480	3,789	V743/1	T(1074)→C	L(343)→P
									V743/2	T(1074)→C	L(343)→P
									V743/3	T(1074)→C	L(343)→P
II	70/1	G2ak	606	<16	<40	1,136	11,776	16,384	V70/1	A (261)→G	D (72)→G
									V70/2	A (261)→G	D (72)→G
									V70/3	A (261)→G	D (72)→G
III	59/1	G1k	311	<16	<40	656	6,400	4,692	V59/1	C (528)→T	T(161)→I
									V59/2	C (528)→T	T(161)→I
									V59/3	C (528)→T	T(161)→I

[a] ELISA, Enzyme-linked immunosorbent assay; HI, hemagglutinin inhibition assay; NI, neuraminidase inhibition assay; HLI, hemolysis inhibition assay; FI, fusion inhibition assay; N, neutralization assay. Each number indicates reciprocal of the highest antibody dilution in each test. For details, see Abenes et al. (1).

cluster domain (22). Third, an F protein mutant of measles virus, whose replication can no longer be inhibited by a fusion-inhibiting peptide, had three amino acid changes, and two of them were also located to the cysteine cluster domain (14).

The amino acid substitutions of glycine for aspartic acid 72 and isoleucine for threonine 161 resulted in the loss of recognition by antibodies to sites II and III, respectively. By analogy to influenza virus antigenic variations (2), these positions could be involved in the sites recognized by the antibodies.

The Asp-72 was located to the major hydrophilic domain in the F_2 subunit (Fig. 2). Close to this position, there is one cysteine residue (position 76), which is involved in disulfide linkage between the F_1 and F_2 subunits. This cysteine residue probably facilitates formation of site II and its access to the F_1 subunit or to the F_1 N terminus, thereby conferring a high steric fusion-inhibitory capacity to the corresponding antibodies. Sequence and hydropathicity comparison of paramyxovirus F proteins by our program (13) (Genetyx, SDC, Tokyo, Japan) indicated that the cysteine in the middle of F_2 sequence is highly conserved and that there is at least one major hydrophilic domain near this cysteine residue (data not shown). Thus, it can be predicted that this domain serves as another major antigenic determinant of paramyxovirus F protein. The amino acid relevant to site III was located relatively close to the F_1 N terminus. Compared with the other two sites, this site was located at a less hydrophilic domain (Fig. 2).

None of the antibodies could recognize either the nascent polypeptide chain or the denatured F protein and its fragments for Western blot (immunoblot) analysis (not shown), indicating that all the determinants are highly dependent on protein folding and conformation. Thus, we did not carry out further analysis involving antibody reactivity with synthetic peptides representing the presumable determinants.

In summary, we identified amino acids relevant to three antigenic determinants of NDV F protein, two of these being located at the F_1 subunit and one at the F_2 subunit. The antibody to each of the sites displayed high inhibitory capacity for hemolysis, membrane fusion, and infectivity. Thus, if one assumes that these inhibitions are due to steric hindrance rather than to conformational change induced by antibody binding and that the identified amino acids are

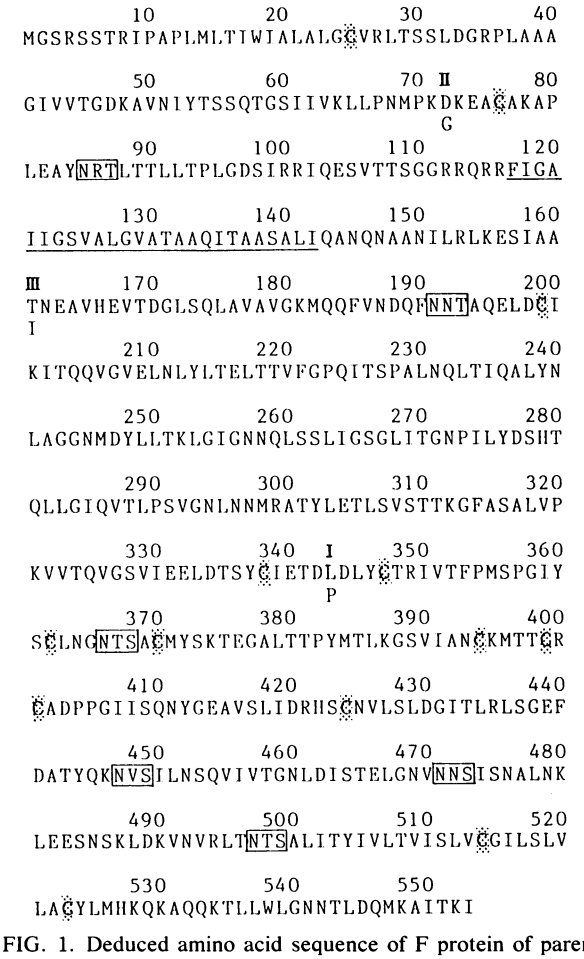

FIG. 1. Deduced amino acid sequence of F protein of parental NDV Sato strain and amino acid substitutions relevant to site I, II, and III. The fusion-inducing F_1 N terminus is underlined. The potential N-glycosylation sites and cysteines are boxed or shadowed.

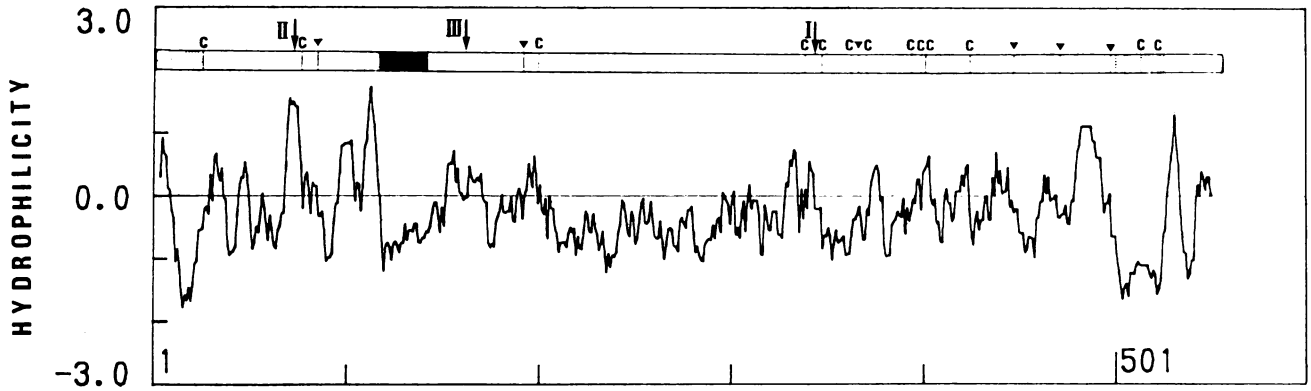

FIG. 2. Location of the amino acid substitutions in variants relevant to sites I, II, and III on the diagram and hydropathicity profile of NDV F protein. A window of 7 amino acids was used to calculate the hydropathicity profile. ■, Fusion-inducing F_1 N-terminal region; ▩, N-terminal signal sequence and C-terminal transmembrane domain; C, cysteine residues; ▼, potential N-glycosylation sites.

involved in the site for antibody recognition, the three amino acid positions could be located close to the fusion-inducing domain at the F_1 N terminus in the three-dimensional structure. It is significant that sites II and III are in close enough proximity to be partially inhibited by reciprocal competitive antibody-binding tests (1). These two sites, together with site I, may surround the fusion-inducing domain. Alternatively, besides the F_1 N terminus, some other sites, such as the cysteine cluster domain, may be involved in or necessary for the membrane fusion.

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