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Characterization of a Natural Mutation in an Antigenic Site on the Fusion Protein of Measles Virus That Is Involved in Neutralization

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Although measles virus is an antigenically monotypic virus, nucleotide sequence analysis of the hemagglutinin and nucleoprotein genes has permitted the differentiation of a number of genotypes. In contrast, the fusion (F) protein is highly conserved; only three amino acid changes have been reported over a 40-year period. We have isolated a measles virus strain which did not react with an anti-F monoclonal antibody (MAb) which we had previously shown to be directed against a dominant antigenic site. This virus strain, Lys-1, had seven amino acid changes compared with the Edmonston strain. We have shown that a single amino acid at position 73 is responsible for its nonreactivity with the anti-F MAb. With the same MAb, antibody-resistant mutants were prepared from the vaccine strain. A single amino acid change at position 73 (R→W) was observed. The possibility of selecting measles virus variants in vaccinated populations is discussed.

In vivo, mutation rates for RNA viruses are on the order of 10^{-3} to 10^{-4} (15). Certain viruses such as measles are antigenically stable, whereas others readily give rise to antigenic variants. Serologically, measles virus (MV) is a monotypic virus, as a single infection gives lifelong immunity. Sequence studies on its two most variable proteins, the hemagglutinin (H) and the nucleoprotein (NP), have enabled measles virus strains to be classified into at least eight genotypes (1, 12, 13, 16). Sequence data obtained by analyzing strains isolated over the last 40 years have shown that there is an accumulation of mutations in the circulating viruses (13). As measles vaccination was initiated during the 1960s, this accumulation may correspond to immune selection pressure by the vaccine virus, or it may simply reflect a natural phenomenon. Despite these noted differences, the wild-type strains isolated over this period are all neutralized in vitro with a polyclonal serum to the vaccine virus strain, although less efficiently (2).

MV contains two glycoproteins, the H and the fusion (F) protein. The former is responsible for the attachment of the virus to the host-cell receptor, and the F protein brings about the fusion of the host cell and viral membranes (19, 20). Murine monoclonal antibodies (MAbs) to either of these antigens can neutralize virus infectivity in vitro and passively protect in vivo (7, 8). Similarly, serum from convalescent patients has activities directed against both antigens which are neutralizing (14). Thus, to escape immunological elimination, the virus would have to mutate in both antigens. We were therefore surprised when we found that a wild-type isolate did not react with an anti-F MAb which had been previously shown to be a dominant epitope, inducing neutralizing antibodies. In the present study, we have cloned and analyzed the sequence of the cDNA coding for the F protein of this strain of MV and shown that among the seven amino acids which differ from the vaccine strain, a single one (amino acid 73) is responsible for the loss of the antigenic activity. Further, mutation of this amino acid to that found in the vaccine strain reconstituted the vaccine antigenic site.

Characterization of the Lys-1 MV strain. The Lys-1 strain of MV was isolated from a measles patient in France who had recently returned from West Africa. Peripheral blood lymphocytes from this patient were stimulated with phytohemagglutinin and cocultured with B95-8 cells. The virus was subsequently passaged on B95a cells. The antigenic epitopes of this isolate were compared with other MV strains with a bank of MAbs specific for the H and F proteins (Table 1). Although positive for all the other MAbs in the panel, the Lys-1 strain was negative for the anti-MV F MAb F 186. This result was surprising, as our previous studies showed that this MAb identified an epitope defining a major dominant antigenic site inducing neutralizing antibodies (8).

To identify the changes in the F protein responsible for the altered antigenicity of Lys-1, the F gene was cloned from mRNA, and the cDNA was subsequently sequenced. In the predicted amino acid sequence (Table 2), seven differences (four in the F2 and three in the F1) were observed compared to the amino acid sequence of the vaccine strain Edmonston.

TABLE 1. Reactivity of anti-MV H and F MAbs with MV isolates[a]

Isolate (location and yr of isolation)	Reactivity of MAb			
	Virus genotype	H 55	F 263	F 186
Hallé (laboratory strain)	A	+	+	+
Y14 (Cameroon, 1983)	B1	+	+	+
R 96 (Gabon, 1984)	B2	+	+	+
R113 (Gabon, 1984)	B2	+	+	+
Joint (France, 1974)	?[b]	+	+	+
Loss (Russia, 1988)	?	+	+	+
MVO (England, 1974)	D1	+	+	+
Mad93a (Spain, 1993)	D6	+	+	+
Lys-1 (France, 1996)	?	+	+	−

[a] Vero or B95a (Mad93a, Lys-1) cells were infected (0.1 to 0.01 PFU/cell) with the different MV isolates. When 20 to 30% of the cells were involved in syncytia, they were fixed with acetone and examined by immunofluorescence with MAbs anti-H (6) and anti-F 263 and 186 (8) as primary antibody and a fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin (Dako).

[b] ?, not determined.

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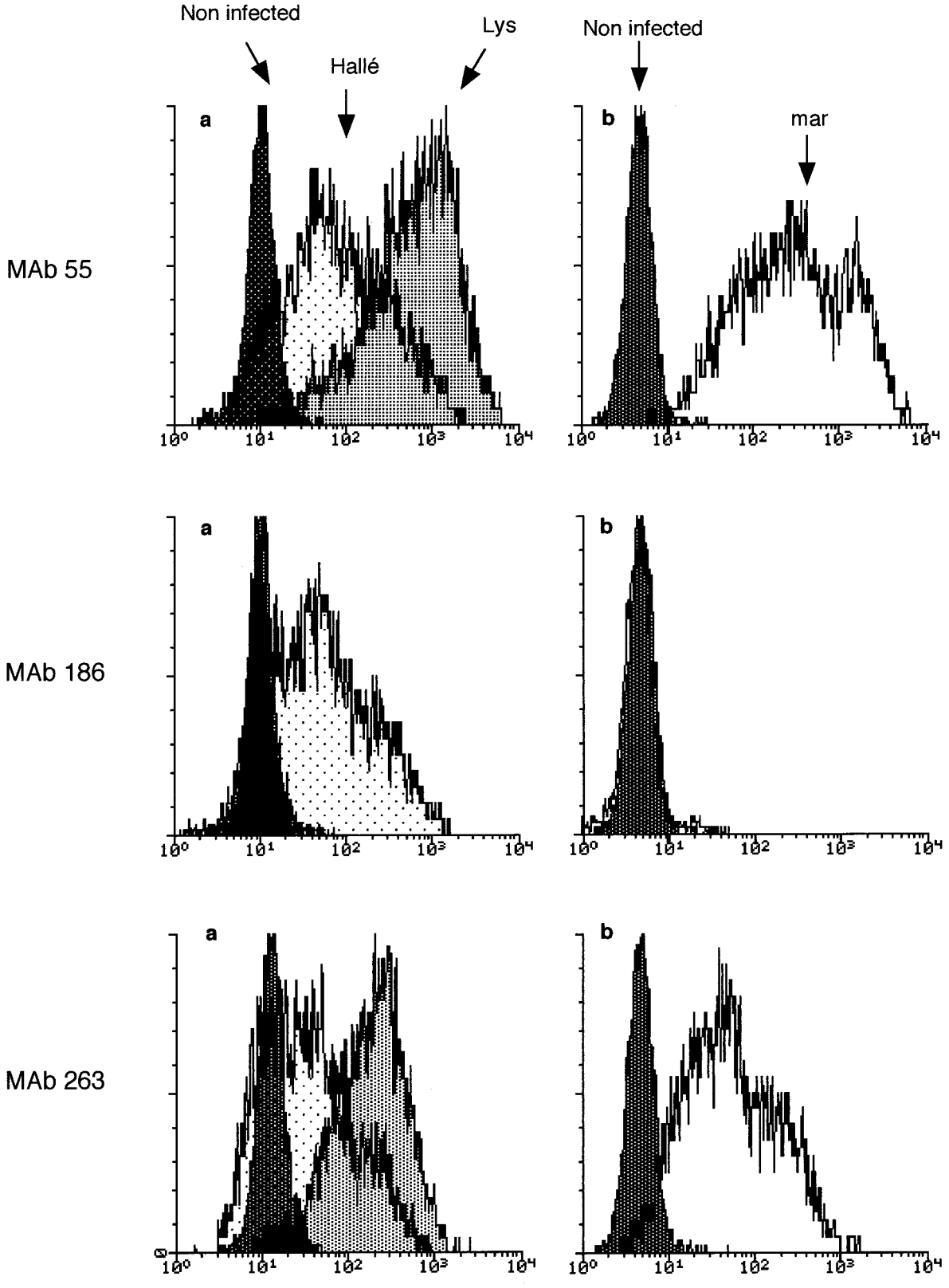

FIG. 1. FACScan analysis of MV-infected BJAB/P3 cells. Cells were infected with the MV strain Hallé or Lys-1 (a) or mar (b) (multiplicity of infection = 0.1 PFU/cell), and 3 days later the surface expression of the H and F MV glycoproteins was examined by FACScan analysis. The MAbs used were 55 (H), 186 (F antigenic site group 1), and 263 (F antigenic site group 2).

TABLE 2. Nucleotide sequence analysis and predicted amino acid sequences of Edmonston, Lys-1, and mar mutant[a]

Nucleotide/amino acid	Nucleotide (amino acid)		
	Edmonston	Lys-1	mar[b]
73/25	A (thr)*	G (ala)	A
186/62	G (met)	T (ile)	G
217/73			T (trp)
218/73	G (arg)	A (lys)	G
332/111	G (arg)	T (met)	G
425/142	A (gln)	G (arg)	A
1265/422	A (his)	G (arg)	A
1543/515	G (ala)	A (thr)	G

[a] The predicted amino acid sequences of the F genes of the Edmonston and Hallé MV strains are identical (3). The nucleotide differences leading to amino acid changes are shown above. Other differences were found at nucleotides 114, 234, 249, 300, 306, 318, 525, 540, 585, 606, 670, 675, 708, 720, 732, 771, 810, 900, 1014, 1188, 1242, 1254, 1284, 1335, 1413, 1416, 1528, 1629, and 1644. The sequence is deposited in EMBL/GenBank under accession no. MVY17840.

[b] Hallé strain of MV selected for its resistance to neutralization by MAb F 186.

mar. To locate the amino acid(s) responsible for the loss of reactivity with the anti-F 186 MAb, we prepared MAb-resistant (mar) escape mutants from our laboratory strain, Hallé (amino acid sequence identical to that of Edmonston) (3), by growing the virus in the presence of MAb F 186. The selection of virus mutants which resist neutralization by MAbs has been used to map epitopes in a number of viral systems. The Hallé mar mutants produced in this way were negative by immunofluorescence and FACScan analysis with F 186 but retained their reactivity with an anti-F MAb of group 2 (MAb F 263), which reacts with a second epitope implicated in neutralization (8) (Fig. 1). In order to identify mutations in the Hallé F responsible for the loss of activity with MAb F 186, we extracted RNA from cells infected with one of the mar mutants, made cDNA copies, and then sequenced the one corresponding to the MV F (Table 2). Compared to the parental strain, there was a single amino acid substitution at position 73. Arginine had been replaced by tryptophan. In the Lys-1 F primary sequence, there is a lysine at this position rather than an arginine.

Amino acid 73 plays a role in the dominant antigenic site of F. As reactivity to MAb 186 was lost when the arginine mutated to a tryptophan in the mar mutant, we investigated whether the lysine in the Lys-1 strain at this position was solely responsible for its loss of reactivity with F 186. Site-directed mutagenesis was used to mutate lysine-73 of Lys-1 to arginine (the amino acid found in the Hallé strain). The corresponding cDNA was then expressed in HeLa cells. The K73R Lys-1 F protein mutant was recognized by MAb F 186 (Table 3). To confirm the importance of the amino acid at position 73, arginine-73 of the Hallé strain was mutated to lysine (the amino acid found in Lys-1), and the cDNA was expressed and examined by immunofluorescence with MAb F 186 activity. This mutation completely destroyed recognition by this antibody. The MV F was also coexpressed with the MV H gene so we could monitor for fusion activity. None of the mutations which played a positive or negative role in the constitution of this antigenic site had an effect on the functional role of the molecule (fusion) (Table 3).

Arginine-73 (R73) is located in the F2 subunit of the MV F just two amino acids C-terminal to the subunit's single cysteine residue (C71), which forms a disulfide bond with the F1 subunit. Interestingly, a single substitution, R73K, of the F protein of human parainfluenza virus type 3 (PIV-3), which is also adjacent to the F2 cysteine, confers resistance to anti-PIV-3 neutralizing MAbs (17). We suggest that antigenically different strains may arise by immune selection during replication in partially immune children. Immune selection of neutralization-resistant variants is the mechanism that drives antigenic drift of influenza A virus in the human population, and this can be mimicked in vitro by the selection of antigenic variants with neutralizing anti-H MAbs (18).

TABLE 3. Antigenicity of MV F protein mutated at amino acid 73[a]

F protein	Anti-FMab			
	Ost 3	F 186	F 263	Fusion
Hallé	+	+	+	+
Hallé-mar	+	−	+	+
Lys-1	+	−	+	+
Hallé R73K	+	−	+	+
Lys K73R	+	+	+	+

[a] HeLa cells were infected with 0.1 PFU/cell of a vaccinia recombinant virus coding for the MV H (4) and then transfected by the Lipofectin method (Gibco-BRL) with plasmids coding for the F protein under the control of the vaccinia H6 promoter (10). The cells were observed for fusion and antigen expression by immunofluorescence 20 h later. Site-directed mutagenesis was carried out with M13 phage as previously described (20).

Analyses of the MV genes of a large number of strains have established that the F gene is among the most stable; only three amino acid changes were noted between the Edmonston strain and viruses isolated up to 1989 (10a, 11). In contrast, evolutionary changes in the H and NP genes have been reported, although the rate of change is of a lower order of magnitude than for influenza virus (11). However, it was reported that the mutation rate for the period from 1977 to 1989 was twice that for the period from 1954 to 1989 (11). It has been speculated that this might be due to the influence of vaccination, especially as it has been reported that MV can circulate in vaccinated populations (5, 9). The MV H is responsible for host cell attachment and so will be the primary antigen to be subjected to immunomodulation; however, there could also be immunoselective pressure to favor the emergence of viruses mutated in F which permit virus entry in the presence of vaccine-induced antibody. The wild-type virus we have studied, Lys-1, was isolated from a patient in France who developed measles upon his return from West Africa. It will be interesting to examine other isolates from this and other regions to determine whether such strains are widespread and whether they represent antigenic drift induced by vaccine antibodies.

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