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Contribution of Measles Virus Fusion Protein in Protective Immunity: Anti-F Monoclonal Antibodies Neutralize Virus Infectivity and Protect Mice against Challenge

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To study the contribution of the measles virus fusion (F) protein in the immune response, anti-F monoclonal antibodies were prepared by using a vaccinia-measles virus F recombinant. In contrast to previously described anti-F monoclonal antibodies, these antibodies not only neutralized virus infectivity and inhibited fusion but also passively protected mice. Since these monoclonal antibodies recognize a configurational epitope, presentation of the antigen during infection may play an important role in the immune response. These factors are discussed in relation to vaccination.

Measles virus, a member of the paramyxovirus group, is one of the main causes of infant mortality in the Third World. Although vaccination with a live attenuated virus has been successful in a number of countries, inactivated and subunit vaccines have met with less success (7, 20). The two membrane proteins of measles virus, the hemagglutinin (H) and fusion (F) proteins, play an important role in the induction of protective immunity. During infection, the H protein is responsible for virus attachment to the cell receptor and the F protein aids penetration of the virus through the cell membrane. The latter protein is also responsible for cell-to-cell fusion (syncytia) characteristic of this group of viruses. Immunization of children with either inactivated or subunit vaccines, although eliciting good virus-neutralizing responses, led to clinical complications when the vaccinees became infected (7, 16, 20). It was proposed that the lack of protection is due to poor antibody response to the F protein (14).

Studies to elucidate the role of the individual antigens in the immune response have shown that monoclonal antibodies (MAbs) against the H protein both neutralize virus in vitro and protect animals when administered passively (9, 27). In contrast, anti-F mono- or polyclonal antibodies did not neutralize virus and failed to protect animals from infection (27). This finding has led to the suggestion that the F protein may play a role at the T-cell level. To support this idea, it was shown that F-specific T-helper clones protected animals from a lethal infection (5; P. De Vries, Doctorat thesis, Rijksuniversiteit te Utrecht, Utrecht, The Netherlands, 1988). However, studies on a number of paramyxoviruses have shown that the F antigen elicits neutralizing antibodies and protects animals against infection (1, 2, 11, 13, 17–19, 21, 23, 24, 28). Recently, we showed that a vaccinia-measles virus F recombinant induced low levels of neutralizing antibody in mice (6). This finding has led us to reinvestigate the role of the humoral response to the F protein in immunity against measles infections. Since immunization with vaccinia virus recombinants is particularly efficient for stimulating biologically active murine MAbs against paramyxovirus fusion proteins, we have used the vaccinia-measles virus F recombinants to make anti-F MAbs. In this report, we show that these MAbs, in contrast to those previously described, neutralize virus infectivity in vitro and protect mice against measles virus infection.

BALB/c mice were vaccinated by tail scarification with the vaccinia-measles virus F recombinant (3×10^7 PFU per mouse). A booster inoculation was given intraperitoneally at 6 months, and 4 days later the spleen cells were fused with SP 2/0-Ag 14 myeloma cells to produce hybrid cell lines as previously described (8). The tissue culture supernatants were screened by immunofluorescence on measles virus-infected Vero cells, and cells from positive wells were cloned by limiting dilution. The clones were then checked by immunofluorescence, using cells infected with either the wild-type or F-recombinant vaccinia virus. Positive clones were subsequently expanded, and ascites was produced in BALB/c mice. By this method, more than 25 anti-F-specific hybridoma cell lines were obtained.

The characteristics of nine of the MAbs are shown in Table 1. All were of the immunoglobulin G1 (IgG1) isotype and neutralized measles virus in a plaque reduction assay. Canine distemper virus, which is immunologically closely related (22), was not neutralized by the measles virus anti-F MAbs (titer of <20 in all cases). It has been previously reported that nonneutralizing measles virus anti-F MAbs react with canine distemper virus (22). To define the number of epitope sites identified by our MAbs, we isolated virus mutants (mar) that were resistant to neutralization with a given MAb. Measles virus (Hallé strain) was incubated with undiluted ascites fluid at 37°C for 1 h, and the virus resistant to antibody neutralization was isolated on Vero cells, using an agarose overlay medium containing 0.2% MAb (ascites fluid). The number of mutants isolated by this procedure was on the order of 2×10^{-5} to 10×10^{-5}. After two successive plaque purifications in the presence of the homologous MAb, the virus clone was tested for reactivity with the other MAbs. Using neutralization as a basis for distinguishing the MAbs, two epitope sites were determined.

The ability of the MAbs to inhibit fusion was studied in HeLa cells infected with a vaccinia virus recombinant containing both the H and F measles virus proteins. In this test, the anti-F MAbs efficiently inhibited syncytium formation. The MAbs also inhibited the measles virus-induced lysis of

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TABLE 1. Biological properties of the anti-F measles virus MAbs[a]

MAb	Epitope[a]	Neutralization[b]	Fusion inhibition[c]	Hemolysis inhibition[d]
M186B19	I	12,800	ND	ND
186A	I	25,600	4,590	38,000
319	I	25,600	≥13,770	13,000
M186B22(28)	I	51,200	≥13,770	60,000
M186B22	I	51,200	4,590	35,000
M263-5	II	400	510	7,000
M263-18	II	200	510	4,800
M77-4	II	800	4,590	25,000
27	II	100	510	4,800

[a] Defined by preparing MAb-resistant mutants with the Hallé strain of measles and testing their neutralization against the other MAbs as defined in the text. MAbs attributed to the same group did not neutralize the appropriate mar mutant, whereas MAbs attributed to a different group reduced the titer (20 μl of ascites fluid) by at least 4 log units of infectivity.

[b] Measured by incubating dilutions of the MAb with 50 PFU of measles virus in 96-well plates for 1 h at 37°C. Vero cells (20,000 per well) were added and incubated for 5 days. The highest antibody dilution neutralizing the virus is given as the titer.

[c] HeLa cells were infected with a vaccinia recombinant (0.01 PFU per cell) expressing both the H and F genes. Antibody dilutions were added, and the cells were examined for syncytia 18 h later. ND, Not determined.

[d] Determined as described by Norrby and Gollmar (15) except the final volumes were reduced to 200 μl and the optical density was read in a Bio-Rad plate reader. ND, Not determined.

TABLE 2. Effect of passive administration of anti-F MAbs to 5- to 7-day-old mice upon challenge with the Yamagata-1 strain of measles virus[a]

MAb	Epitope	PFU/brain[b]
M186B19	I	10^3
None		1.5×10^5–2×10^5
186A	I	<50
None		$\geq 10^6$
319	I	≤100
None		5×10^5–$\geq 10^6$
M186B22	I	<50
None		$>10^5$
M77-4	II	2×10^3–6×10^3
None		1.5×10^5–2×10^5

[a] Mice were inoculated intracerebrally with the Yamagata-1 strain of measles virus. Half of the mice were then inoculated intraperitoneally with 50 μl of ascites fluid anti-F MAb except for MAb M77-4 (5 μl). The brains were removed 7 days later, homogenized, and titrated on Vero cells. The quantity of IgG in the ascites fluids inoculated into the mice was determined by an enzyme-linked immunosorbent assay, using mouse IgG (Sigma Chemical Co.) as a standard and an anti-mouse IgG-peroxidase conjugate. The quantities of IgG injected corresponded to 200 to 400 μg per mouse except for M77-4 (50 μg). When mice were given measles virus anti-NP MAbs, no reduction in virus titer was observed.

[b] Expressed as the range obtained from individual animals (at least five mice per group).

monkey (vervet) erythrocytes. None of the MAbs inhibited the attachment of monkey erythrocytes to measles virus-infected Vero cells (values of <10 in all cases).

Although the anti-F MAbs reacted with measles virus in an immunodot assay, prior treatment of the virus with different concentrations of sodium dodecyl sulfate before adsorption onto nitrocellulose led to a loss of immunological activity when the sodium dodecyl sulfate concentration exceeded 0.05%. It is assumed that the MAbs recognize conformational epitopes. Immunofluorescence studies on acetone-fixed measles virus-infected cells revealed the F protein to be stained specifically at the membrane (Fig. 1), suggesting that the MAbs recognize a fully processed form of the antigen.

To investigate the in vivo properties of the MAbs, we developed a mouse model. BALB/c mice (5 to 7 days old) inoculated intracerebrally with 10^3 PFU of the Yamagata-1 strain of measles virus (10) developed a nonlethal neurological infection. Virus could be isolated from the brains of the mice and titrated on Vero cells. Seven days after inoculation, approximately 10^6 PFU of virus could be recovered from a brain. The fusion protein of the Yamagata-1 strain of measles virus differs by nine amino acids in the ectodomain from that of the Hallé strain used for establishing the MAbs (3, 29). Comparative studies (immunofluorescence) showed that the MAbs reacted equally well with both strains of virus. To study the effect of passively administered MAbs on the infection, 5 to 50 μl of MAb (ascites fluid) was injected intraperitoneally immediately after infection. Seven days later, the brains were removed and the virus was titrated (Table 2). All of the MAbs examined reduced the virus titer in the brain, normally to an undetectable level. Thus, the measles virus anti-F MAbs were active both in vitro and in vivo.

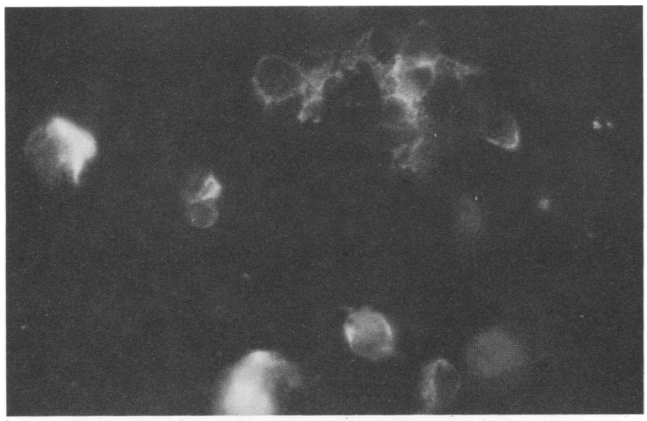

FIG. 1. Immunofluorescence with anti-F MAb of measles virus-infected, acetone-fixed Vero cells.

Our results underline the importance of the humoral response against the F protein. Although the anti-H antibodies inhibit virus-cell attachment, it is the anti-F antibodies that play a role in inhibiting cell-to-cell transmission (12). Studies on human parainfluenza virus type 3 suggest that the functions of neutralization and fusion inhibition may be at different sites (26). However, the MAbs used in our study inhibited both functions. Studies with MAb escape mutants of several paramyxoviruses to map the epitope sites have shown the involvement of three regions (19, 24, 25, 30). In respiratory syncytial virus, two of these sites have been shown to interact with the same MAb (21). The interrelationship of these sites, if any, remains to be clarified. Our successful preparation of biologically active anti-F MAbs may be due to the method of immunization used. Our previous studies using measles virus failed to produce anti-F MAbs with the described biological activities. In contrast, immunization with the vaccinia-measles F recombinant virus induced antibodies that neutralized virus both in vitro and in vivo. In view of this finding, subunit or inactivated vaccines should be carefully analyzed for the nature of the immunological response. A similar opinion regarding live and inactivated viruses has recently been discussed for human immunodeficiency virus vaccination (4).

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