

Passive Protection against Rotavirus-Induced Diarrhea by Monoclonal Antibodies to the Heterotypic Neutralization Domain of VP7 and the VP8 Fragment of VP4

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A murine model was used to determine whether neutralizing monoclonal antibodies (MAbs) with heterotypic specificity directed to VP7 (MAB 57-8) or to the VP8 fragment of VP4 (MAB M14) passively protect mice against challenge with various strains of rotavirus. (The gene 4 product, an outer capsid protein, has traditionally been called VP3. It has been proposed, however, that the rotavirus gene 4 product be named VP4. The gene 3 product, a core protein, has been identified recently and named VP3 [M. Liu, P. A. Offit, and M. K. Estes, *Virology* 163:28-32, 1988].) Suckling mice orally inoculated with MAB 57-8 did not develop diarrhea when challenged with virulent serotype 3, 4, or 6 rotaviruses, while those inoculated with MAB M14 were passively protected from challenge with serotype 3 or 6 rotaviruses, as predicted by *in vitro* neutralization tests. These MAbs, however, did not protect mice from infection when the mice were challenged with rotaviruses of other serotypes. We conclude that specific neutralization epitopes on each surface protein are capable of mediating protection against one or several rotavirus serotypes.

Rotaviruses are the leading cause of severe diarrhea in infants throughout the world (4, 9, 17). They are associated with a high rate of infant mortality in developing countries and significant morbidity in developed countries. Rotaviruses have also been found to cause sporadic outbreaks of diarrhea among elderly (8, 22), acutely ill (10), and immunocompromised patients (33). Virulent strains of atypical rotaviruses have recently been implicated in epidemics of diarrheal illness in normal adults in China (14).

Rotavirus particles consist of 11 segments of double-stranded RNA enclosed in a double-layered viral protein capsid. The outer capsid is composed of two viral proteins, VP7 and VP4. VP7 is a 37-kilodalton glycoprotein encoded by gene segment 8 or 9 (depending on the viral strain) and is the serotype-specific rotavirus neutralization antigen (1, 6, 7, 23, 25, 26). Recent studies indicate that this protein mediates the attachment of rotaviruses to cells (5, 23, 31, 32). VP4 (19), an 86.5-kilodalton protein that is encoded by gene segment 4, functions as the viral hemagglutinin (15, 16). Viral infectivity is enhanced when VP4 is cleaved by trypsin into two fragments, VP8 (28 kilodaltons) at the amino terminus and VP5 (60 kilodaltons) at the carboxy terminus (3, 15).

Each of the outer capsid proteins independently induces the production of antibodies that neutralize the virus *in vitro* (6, 7, 13, 18, 23, 26) and protect against infection *in vivo* (12, 25, 27). Monoclonal antibodies (MAbs) to these rotavirus surface proteins have been useful in defining the epitopes that mediate viral neutralization (34, 35). The locations of the amino acids on VP7 and VP4 that are involved in neutralizing rotaviruses of the same serotype (homotypic) or different serotypes (heterotypic) have recently been determined by

sequencing rotavirus variants selected by homotypic or heterotypic neutralizing MAbs (2, 20, 21, 36, 37).

In a previous study (25), we demonstrated that neutralizing MAbs directed to two distinct epitopes of rhesus rotavirus (RRV) VP7 passively protected mice from challenge with RRV. Mice orally inoculated with neutralizing MAB 2G4 directed to an epitope of VP4 were passively protected from challenge with three distinct rotavirus serotypes. Two other VP4-specific, RRV-neutralizing MAbs directed at nonconserved epitopes failed to protect mice from RRV challenge *in vivo*. Nonneutralizing MAbs directed to VP7 and to the inner capsid protein VP6 did not protect mice from challenge.

Since the time of the study by Offit et al. (25), a heterotypic VP7-directed MAB has been isolated and characterized, and the neutralizing domains defined by this MAB and others have been mapped. Recent variant analyses by Dyll-Smith et al. (2), Mackow et al. (20, 21), and Taniguchi et al. (36, 37) have identified the sites involved in VP7- and VP4-specific neutralization. The homotypic VP7-specific MAbs that protected suckling mice from challenge with RRV (25) are directed to nonconserved neutralization epitopes (RRV VP7 amino acid 94 or 96 in the A region or amino acid 211 in the C region) (20). Similar analyses have shown that the heterotypic VP4-specific MAB that protected mice from challenge with three serotypes of rotavirus (25) is directed to a conserved neutralization domain on the VP5 portion of VP4 (RRV VP4 amino acid 393) (21). At least one of the two MAbs that neutralized RRV alone and failed to protect mice from challenge with RRV or any of the rotaviruses tested (25) is directed to an epitope on the VP8 fragment of VP4 (RRV VP4 amino acid 188) (21).

In light of these recent advances, the study by Offit et al. (25) leaves two important questions unanswered. (i) Do

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TABLE 1. Protection against rotavirus-induced diarrhea by monoclonal antibodies directed to specific epitopes on VP7 and VP4

MAb	Amino acid (AA) mutation site selected by MAb	In vitro PRN titer ^a against virus (serotype):				In vivo protective titer ^b against virus (serotype):			
		RRV(3)	ST3xRRV(4)	OSU(5)	NCDV(6)	DxRRV(1)	RRV(3)	R13-3(4)	NCDV(6)
57-8	VP7 AA 94	409,600	409,600	<100	409,600	<200	2,500	3,200	>12,500
M14	VP4 AA188 (in VP8)	51,200	1,600	<200	6,400	ND ^c	7,500	<100	2,500

^a Plaque reduction neutralization (PRN) assays were done as previously described (29).

^b The reciprocal of the dilution of ascites at which 50% of suckling mice were protected against the indicated rotavirus challenge.

^c ND, Not determined.

heterotypic VP7 MAbs passively protect suckling mice from rotavirus challenge? (ii) Are MAbs to the VP8 portion of VP4 capable of passively protecting mice from challenge with virulent rotaviruses? We undertook the current study to address these issues.

The in vivo mouse protection model used in this study has been described previously (28). Three- to four-day-old CD-1 mice were given a 100- μ l oral inoculum of serial fourfold dilutions (1:100 to 1:6,400) of either neutralizing MAb 57-8 or M14. MAb 57-8 was originally recovered from a mouse immunized with a serotype 4 porcine rotavirus (Gottfried strain) (D. A. Benfield, E. A. Nelson, and Y. Hoshino, *Abstr. 7th Int. Congr. Virol.* 1987, p. 111). This MAb neutralizes serotype 3 simian rotaviruses RRV and SA11, serotype 4 porcine rotavirus Gottfried, and serotype 6 bovine rotaviruses NCDV and UK in vitro (Benfield et al., *Abstr. 7th Int. Congr. Virol.* 1987). Sequence analysis by Mackow et al. (20) has shown that MAb 57-8 consistently selects variants of RRV that have a mutation at amino acid 94 in the A region of VP7, the same site selected by other MAbs with homotypic specificity. Competition inhibition studies, however, have shown that MAb 57-8 also competes for viral binding with MAbs that select viral variants with mutations in the C region (20). This has led to the hypothesis that MAb 57-8 is directed to a conformationally determined epitope of VP7 composed of the A and C regions. MAb M14 was derived from a mouse immunized with serotype 3 rotavirus RRV and efficiently neutralizes this rotavirus strain and serotype 6 rotavirus NCDV in vitro (21). Variants of RRV selected by MAb M14 have a mutation at amino acid 148 in the VP8 portion of VP4.

Thirty minutes after the specified dilution of MAb was administered, the mice were orally challenged with serotype 1 reassortant rotavirus DxRRV 6-1-1 (24) (1.6×10^7 PFU), serotype 3 rotavirus RRV (2×10^7 PFU), serotype 4 reassortant rotavirus R13-3 (1.8×10^6 PFU), or serotype 6 rotavirus NCDV (1×10^7 PFU). R13-3 is a reassortant rotavirus that was kindly provided by I. Holmes and B. Fu. The gene segment 9 alone (hence VP7) of R13-3 was derived from a serotype 4 parent rotavirus ST-3 (Saint Thomas-3), while the remaining gene segments were from simian rotavirus SA11 or bovine rotavirus UK parents. The other strains of rotavirus used in this study have been characterized previously (11).

Control animals were given 100 μ l of medium instead of MAb and were challenged with the various rotaviruses. The dose of rotavirus specified above caused diarrhea in approximately 95% of mice in the control group.

Our results are summarized in Table 1. We found that both neutralizing MAbs, 57-8 and M14, protected mice from challenge with virulent rotaviruses to the extent predicted by plaque reduction neutralization studies in vitro. MAb 57-8 neutralized serotype 3, 4, and 6 rotaviruses in vitro and protected mice against challenge with these rotaviruses but did not protect against challenge with a serotype 1 reassor-

tant rotavirus DxRRV. MAb M14 neutralized serotype 3 and 6 rotaviruses in vitro and protected against challenge with these viruses but did not protect against challenge with a serotype 4 reassortant rotavirus R13-3.

These results and those of Offit et al. (25) collectively demonstrate that neutralizing MAbs directed to either of the rotavirus outer capsid proteins (VP7 or VP4) or to either of the trypsin cleavage products of VP4 (VP8 or VP5) are capable of passively protecting suckling mice from rotavirus challenge. Specific epitopes on each of these surface proteins, such as those defined by VP7-specific MAb 57-8, VP8-specific MAb M14, and VP5-specific MAb 2G4, mediate protection against at least two rotavirus serotypes. Other epitopes identified on these proteins by homotypic neutralizing MAbs mediate protection against only one rotavirus serotype or one member of that serotype. The nature of the relationship, if any, between the determinants of serotype and the stimulation of homotypic or heterotypic protection or neutralization remains to be elucidated.

In vitro neutralization studies appear to be able to predict the specificity and efficacy of the MAbs used in in vivo protection studies thus far, with the exception of two VP4 MAbs with homotypic specificity (8B3 and 7A12) tested previously (25). There are no examples at this time of rotavirus MAbs that protect in vivo but do not neutralize in vitro. The extent to which homotypic or heterotypic antibodies (or both) directed to specific epitopes of VP4 and VP7 are responsible for protection after natural infection or experimental vaccination remains to be determined. Each surface protein clearly has epitopes that are capable of inducing heterotypic immunity, at least in the experimental situation. Cytotoxic T lymphocytes may also play a significant role in determining heterotypic protection (30). Future studies designed to elucidate the factors which regulate the relative immunogenicity of epitopes on both VP4 and VP7 during infection or immunization and the factors which stimulate the cellular immune response will be important in the ongoing efforts to prevent rotavirus-induced diarrheal disease and in understanding the molecular basis of heterotypic immunity.

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