Immunological Properties of Monoclonal Antibodies Specific for Meningococcal Polysaccharides: the Protective Capacity of IgM Antibodies Specific for Polysaccharide Group B

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Two IgM monoclonal antibodies, MB32 and MB34 specific for meningococcal polysaccharide group B have been raised. Both were detectable by radioimmunoassay and agglutination, but only MB34 was effective in counter immunoelectrophoresis and complement fixation. MB34 was also far more potent than MB32 when tested for passive protection of mice infected with either *Neisseria meningitidis* group B or *Escherichia coli* K1. These data demonstrate that group B-specific antibodies do play a protective role in mice infected with these bacteria.

INTRODUCTION

Antibodies to the capsular polysaccharide are known to play a major protective role in human immunity to meningococci (Goldschneider *et al.*, 1969; Gotschlich *et al.*, 1978). However, the *in vivo* biological and protective properties of antibodies specific for group B meningococcal polysaccharide are not very well understood, due in part to the poor immunogenicity of this particular antigen (Kasper *et al.*, 1973*a*; Wyle *et al.*, 1972). Even though the existence of B-specific antibodies with demonstrable bactericidal (Kasper *et al.*, 1973*a*) and protective (Frasch *et al.*, 1976) activity has been reported, their low titres make characterization difficult, particularly in the presence of antibodies with specificities other than group B.

It was considered that monoclonal antibodies, specific for group B polysaccharide could help in clarifying some of these issues, and this report describes the preparation of such antibodies, their immunological characterization and ability to protect mice challenged with lethal doses of *Neisseria meningitidis* B and also *Escherichia coli* K1 whose colominic acid antigen is chemically and antigenically undistinguishable from group B (Bhattacharjee *et al.*, 1975, Counts & Turck 1977; Kasper *et al.*, 1973*b*).

METHODS

Bacterial strains. *Neisseria meningitidis* strains CN7619, CN7622 (both group B) were obtained from Dr C. Frasch, Bureau of Biologics, Food and Drug Administration, Bethesda, Maryland 20014, U.S.A., and correspond to his designates B16B6 and M931, respectively. *Neisseria meningitidis* CN7783 (group B) was obtained from the Public Health Laboratory, Manchester, and is equivalent to their Branham M993 strain. *Neisseria meningitidis* CN7038 (group C) was obtained from Dr E. Gotschlich, The Rockefeller University, New York, U.S.A., and corresponds to his C11 strain. *Escherichia coli* CN6158 came from the American Type Culture Collection and corresponds to NCTC 9007. *Neisseria meningitidis* strains were freeze dried and recovered as required on either blood agar or Mueller Hinton agar (Oxoid). They were then grown overnight in Tryptone Soya broth (Oxoid). Formalinized suspensions were prepared by adding formalin to 0.5% (w/v). After 24 h the cultures were spun down, washed and resuspended in phosphate-buffered saline, pH 7.2 [PBS containing 50% (v/v) glycerol]. Suspensions prepared in

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this way, containing about 10^{11} organisms ml^{-1} , were maintained at -20°C ; under these conditions they were found to remain stable for at least 6 months.

Preparation of meningococcal polysaccharide group B. MPS(B) was prepared from strain CN7783 according to the procedure described by Gotschlich *et al.* (1972). Chemical analysis indicated 1.3% protein and 0.8% nucleic acid contamination. Lipopolysaccharide was estimated to be less than 0.25%. Identification was confirmed by chemical analysis of *N*-acetylneuraminic acid, sensitivity to neuraminidase and nuclear magnetic resonance. Immunological reactivity was estimated by counter immunoelectrophoresis with rabbit anti-B serum (Wellcome Diagnostics, Dartford). The content of MPS(B) in the sample was estimated to be at least 95%.

Immunization procedure for obtaining monoclonal antibodies. Female CBAT⁶T⁶ mice, 8 weeks old, were given 0.7 mg *Corynebacterium parvum* (Coparvax, Wellcome) on day 0 for non-specific stimulation of the immune response and splenomegaly (Adlam & Scott, 1972). This was followed by 10^8 *N. meningitidis* B (CN7783) given intravenously on days 4 and 11. Spleens from mice having the highest anti-B titre by solid-phase radioimmunoassay were pooled and used for fusion.

The procedure used for fusion followed the general method of (Kohler & Milstein, 1975) using a procedure described previously (Coates *et al.*, 1981). Supernatants of primary wells were screened for specific (anti-B) antibodies by solid-phase radioimmunoassay, positive wells were cloned by limiting dilution and finally grown as ascitic tumours in BALB/c $\times$ CBAT⁶ F1 hybrid mice primed 3 d earlier with 0.5 ml pristane (Aldrich). Ascitic supernatants were pooled and tested for activity either directly or after precipitation of the immunoglobulin at 18% (w/v) Na_2SO_4 , followed by dialysis and freeze drying. MB32 and MB34 were both IgM antibodies.

Immunological assays. Solid-phase radioimmunoassay was performed on round bottomed soft titration plates (Dynatech) pretreated with $100\text{ }\mu\text{g}$ poly-L-lysine ml^{-1} (Sigma, mol. wt 50000) for 1 h followed by $10\text{ }\mu\text{g}$ polysaccharide ml^{-1} and blocked with 3% (w/v) bovine serum albumin (BSA). The sera were diluted in 3% BSA (twofold dilutions), plates were incubated with ^{125}I -labelled, immunopurified rabbit anti-mouse immunoglobulin ($1-3 \times 10^4$ c.p.m. per well; $7-8 \times 10^6$ c.p.m. μg^{-1}) and counted. Plates were washed five times with PBS after every incubation. Antisera were diluted in 3% BSA/PBS.

Counter immunoelectrophoresis was performed as described previously (Lifely *et al.*, 1981) using a range of antigen concentrations from 0.1 to $100\text{ }\mu\text{g ml}^{-1}$.

Complement fixation was done in microtitre plates (Linbro, flat bottomed microelisa). Serial twofold dilutions of monoclonal antibody (25 μl) were mixed with a range of 0.1 to $100\text{ }\mu\text{g}$ antigen ml^{-1} (25 μl) followed by 1.8 haemolytic units of baby rabbit serum as a source of complement (50 μl). After incubation for 1 h at 37°C , a 2% (v/v) suspension of (50 μl) sheep red blood cells previously sensitized with rabbit anti-sheep red blood cell haemolysin (Wellcome) was added and reactions were incubated for a further 2 h. Plates were centrifuged at 300 g for 15 min at an angle of 30° and haemolysis read at 405 nm in a Titertek Multiskan.

Bacterial agglutination was performed on a slide with formalin-fixed and washed bacteria ($2 \times 10^{10}\text{ ml}^{-1}$).

Mouse protection model. The method used was similar to the procedures described by Calver *et al.*, (1976), Holbein (1980) and Holbein *et al.* (1979). CBAT⁶ female mice (8 weeks old) were injected intraperitoneally with 10 mg iron dextran (Imferon, Fisons) followed within 30 min by an intraperitoneal injection of 32 LD₅₀ of *N. meningitidis* suspended in PBS. Mortality was recorded for a 3 d period. The LD₅₀ for the different strains used were as follows: CN7619, 260 c.f.u.; CN7620, 380 c.f.u.; CN7038, 7.8×10^4 c.f.u.; CN6158, 5 c.f.u.

RESULTS

Two IgM monoclonal antibodies (MB32 and MB34) with specificity for meningococcal polysaccharide B were used in these studies. Their potency by solid-phase radioimmunoassay is illustrated in Fig. 1. The linear segment of the curve was used to obtain the titre for each monoclonal antibody by extrapolating to zero binding, and gave values of 250 and 100 ng immunoglobulin ml^{-1} for MB32 and MB34, respectively. These titres are considerably lower than those obtained for other monoclonal antibodies specific for A and C meningococcal polysaccharides (data not shown). It is not clear whether this reflects differences in the assay system, poor avidity of anti-B monoclonals or other factors. Both monoclonals gave negative results when tested with meningococcal polysaccharides groups A and C, BSA and poly-L-lysine which served as controls for specificity.

Other immunological assays performed with MB32 and MB34 are listed in Table 1. MB34 gave positive results in all the assays, counter immunoelectrophoresis was positive with MPS(B) but was negative with other meningococcal polysaccharides (A and C) and after treatment of MPS(B) with neuraminidase. MB32 reacted weakly in agglutination tests, was non-precipitating by counter immunoelectrophoresis and did not fix complement.

Fig. 1. Solid-phase radioimmunoassay performed with serial dilutions of MB34A1 (a) and MB32A1 (b). The plate was coated with meningococcal polysaccharide B.

Table 1. *Immunological assays performed with monoclonal antibodies specific for meningococcal group B polysaccharide*

Assay	Monoclonal antibody:	
	MB32	MB34
Radioimmunoassay	+	+
Counter immunoelectrophoresis	-*	+
Agglutination:		
<i>N. meningitidis</i> (B)	+	+
<i>E. coli</i> K1	+	+
Complement fixation	-†	+

* Negative at a concentration of 2.5 mg immunoglobulin ml⁻¹.

† Range tested: 500–0.5 µg immunoglobulin ml⁻¹.

The protective capacity of MB32 and MB34 was assayed in a mouse test (Fig. 2). This experiment indicated a clear passive protection bestowed by both MB32 and MB34 monoclonal antibodies, as seen in Fig. 2. From these results it was clear that the minimally protective dose of MB34 demonstrable when mice were challenged with 32 LD₅₀ of strain CN7619 was between 1 to 2 × 10⁻⁵ mg per mouse. In contrast, MB32 monoclonal antibody, although it was also an IgM antibody was about 500 times less potent and no further protection studies done with it are reported here. The protective effect was also demonstrable when MB34 monoclonal antibody was given 4 h after (Table 2) or 24 h before challenge (Table 3). In both cases, however, the protective capacity was clearly less than when antibody was given within 1 h of challenge. Since *E. coli* K1 polysaccharide antigen is very similar, if not identical, to MPS(B) (Bhattacharjee *et al.*, 1975), the capacity of MB34 to protect mice from lethal challenge with this organism was also explored (Table 4). Although these experiments showed only partial protection by MB34 monoclonal antibody, the results however are consistent with a concept of cross-protection between *N. meningitidis* B and *E. coli* K1. The observed difference in susceptibility is probably due to the large difference in virulence between the challenge organisms. The LD₅₀ for *E. coli* K1 was found to be less than 5 c.f.u. mouse, i.e. 100 times more virulent than strain *N. meningitidis* CN7619 (group B). To verify the specificity MB34 was also tested in mice subsequently challenged with 32 LD₅₀ of a group C strain (CN7038) without any positive effect. Conversely, mice challenged with group B (CN7619) strain were not protected either by normal mouse serum, or unrelated monoclonal antibodies. These results are not shown.

Fig. 2. Protective capacity of monoclonal antibodies MB32 (○) and MB34 (●). CBAT⁶ female mice received dilutions of a stock solution of antibody (2.2 mg antibody ml⁻¹) intraperitoneally 1 h prior to challenge with 32 LD₅₀ of *N. meningitidis* CN7619, group B, in combination with 10 mg iron dextran.

Table 2. *Passive protection with MB34 monoclonal antibody given 4 h after challenge with N. meningitidis*

CBA/T⁶T⁶ female mice (five per group) were injected with 32 LD₅₀ of strain CN7619 and 10 mg iron dextran followed 4 h later by 0.2 ml of antibody MB34. All injections were given intraperitoneally.

Amount of MB34 injected (µg per mouse)	Mortality at:		
	24 h	48 h	72 h
8.8	0	0	0*
4.4	0	0	1*
2.2	0	0	1*
1.1	0	0	0*
0	2	5	5

* A χ^2 test indicated that these values are significantly different from the control, with a value of $P < 0.01$.

Table 3. *Effect of MB34 monoclonal antibody given 24 h before challenge with N. meningitidis*

Conditions were the same as for Table 2, except that antibody MB34 was given 24 h before challenge with 32 LD₅₀ *N. meningitidis* CN7619.

Amount of MB34 injected (µg per mouse)	Mortality at:		
	24 h	48 h	72 h
4.5	0	1	1*
0.45	0	5	5
0.045	0	3	4
0	0	4	5

* Significantly different from the control, with $P < 0.01$.

Table 4. *Protective effect of MB34 monoclonal antibody on mice challenged with E. coli K1*

CBA/T⁶T⁶ female mice (15 per group) received MB34 intraperitoneally 1 h prior to challenge with 40 c.f.u. of *E. coli* K1 and 10 mg iron dextran (both injected intraperitoneally).

Amount of MB34 injected (µg per mouse)	Mortality at 72 h
4.5	12
0.45	7*
0.045	12
0	15

* Significantly different from the control, with $P < 0.01$.

DISCUSSION

The data present demonstrate the protective role of anti-group B meningococcal antibodies in mice. Some protection was obtained against *E. coli* K1; this is in agreement with previous observations that passive antibody can protect rats injected with *E. coli* K1 (Bortolussi & Ferrieri, 1980) and confirms the specificity of the effect. The difference in protective capacity obtained with monoclonal antibody MB34 when tested with *N. meningitidis* CN7619 and *E. coli* K1 (CN6158) probably reflects the 100-fold difference in virulence observed for these strains, although other explanations are possible. MB34 is an IgM, complement-fixing antibody, and the complement dependence of protection against *N. meningitidis* (Nicholson & Lepow, 1979) and *E. coli* K1 (Stevens *et al.*, 1978) has been reported although it has not established unequivocally whether protection is due to bactericidal activity, to C3b-dependent opsonization or both. A relationship between protection against *E. coli* K1 and complement activity restricted to the classical pathway has been established (Stevens *et al.*, 1980), but this is not necessarily the case for *N. meningitidis*.

Poor protection was observed with MB32 and complement fixation could not be demonstrated with this antibody. These findings tend to strengthen the association between complement fixation and protection. However, other factors could play a role: MB34 seems to have greater avidity for antigen than MB32 and rough estimates with the crude systems available to us would indicate approximately a 10-fold difference in avidity between the two. Moreover, Mandrell & Zollinger (1982), have recently published a comparative study for avidity of human and mouse antibodies specific for meningococcal B and C antigens, and found the former to be low. It is difficult to interpret data of this kind; but it might very well be the case that MB32 did not protect for two reasons: lack of complement fixation and poor avidity.

The protective capacity of IgM monoclonal antibodies specific for *Streptococcus pneumoniae* type 3 (Briles *et al.*, 1981*a*) and phosphorylcholine (Briles *et al.*, 1981*b*) has been established. According to these reports, as little as 20 µg antibody (or possibly less) confers complete protection on CD mice. This compares well with the protection obtained with MB34 for *N. meningitidis*, as seen in Fig. 2. Here, complete protection was obtained with 1 µg antibody, and a minimally protective dose can be put as low as 10 ng antibody per mouse. In terms of antibody molecules these data correspond to about 0.5×10^6 molecules per bacterium, a very large quantity by comparison with IgM anti-*Salmonella adelaide* antibody where only eight molecules per bacterium promoted phagocytosis *in vivo* (Rowley & Turner, 1966). However in these experiments the bacteria and antibodies were premixed before injection, thus maximizing the efficiency of the antibody.

Although a simpler murine model based on mortality has been published (Huet & Suire, 1981), *N. meningitidis* is considered as poorly pathogenic for laboratory animals (Goldschneider *et al.*, 1969) unless it is treated with mucin or administered along with iron (Holbein *et al.*, 1979; Jones *et al.*, 1980) which has been described as essential for bacterial multiplication *in vivo* (Holbein, 1980). The fact that iron-promoted infection by *N. meningitidis* can be controlled with anti-B antibodies suggests that such a model could be used to study not only passive protection but also active immunity in vaccinated mice and can be considered as a useful complement to the mouse bacteraemia model as described by Craven & Frasch (1979).

The central issue in this communication is the fact that IgM antibodies specific for polysaccharide B are sufficient to confer protection. A similar protective role of murine IgG₃ specific for phosphorylcholine and anti-SIII pneumococcal polysaccharide has been reported recently (Briles *et al.*, 1981*a*). We now intend to clarify whether B-monoclonal antibodies belonging to other classes and subclasses are also effective.

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