Detection of IgG and IgM to meningococcal outer membrane proteins in relation to carriage of Neisseria meningitidis or Neisseria lactamica

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Abstract

Carriage of non-serogroupable Neisseria meningitidis or Neisseria lactamica induces antibodies protective against meningococcal disease. Antibodies directed against outer membrane proteins are bactericidal and the serotype and subtype outer membrane protein antigens are being examined for their value as vaccine candidates for serogroup B disease. The aim of this study was to examine the effect of carriage of these two Neisseria species among children and young adults on induction of antibodies to outer membrane components from strains causing disease in Greece. Among 53 patients with meningococcal disease, IgG or IgM antibodies were detected by ELISA in 9 of 13 (69%) from whom the bacteria were isolated and 27 of 40 (67%) who were culture-negative. For military recruits (n = 604), the proportion of carriers of meningococci with IgM or IgG to outer membrane proteins was higher than non-carriers, P < 0.05 and P = 0.000000, respectively. Among school children (n = 319), the proportion with IgM or IgG to outer membrane proteins for carriers of meningococci was higher compared with non-carriers, P = 0.000000 and P = 0000043, respectively. Carriage of N. lactamica was not associated with the presence of either IgM or IgG to the outer membrane proteins in the children. The higher proportion of children (50%) with IgM to outer membrane proteins compared with recruits (10%) might reflect more recent exposure and primary immune responses to the bacteria. The lack of association between antibodies to outer membrane proteins and carriage of N. lactamica could reflect observations that the majority of N. lactamica isolates from Greece and other countries do not react with monoclonal typing reagents. Bactericidal antibodies to meningococci associated with high levels of IgG to N. lactamica were found in a previous study; these are thought to be directed to antigens other than outer membrane proteins or capsules and imply antigens such as lipo-oligosaccharide are involved in induction of antibodies cross-reactive with meningococci. © 1999 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

Adults and older children who become colonized
with *Neisseria meningitidis* develop bactericidal antibodies against strains of homologous and heterologous serogroups, suggesting the carriage plays a role in induction and maintenance of antibodies to meningococci [1,2]. Carriage of *Neisseria lactamica* is also suggested to induce antibodies protective against meningococcal disease. Gold et al. [3] found that 40% of children who carried *N. lactamica* developed increased titers of bactericidal antibodies reactive with meningococcal isolates of serogroups A, B and C. We found that adsorption with *N. lactamica* of sera containing bactericidal antibodies to serogroupable strains of meningococci significantly reduced the level of bactericidal activity [4]. The same study found that carriers of meningococci whose sera had bactericidal activity for capsulate strains had significantly higher levels of IgG to *N. lactamica* (but not to a non-groupable strain of meningococci). These were serogroup B and C isolates, including the B:4:P1.15 strain which caused an outbreak in the school population from which the sera were obtained [5].

Outer membrane proteins (OMP) have been shown to be immunogenic and are being assessed for their potential as vaccine candidates. IgG antibody levels to serogroup B OMP antigens were significantly higher for carriers before and after immunization with the meningococcal vaccine under investigation in Norway [6]. The objective of the current study was to use ELISA to detect IgM and IgG antibodies specific for OMP from isolates expressing phenotypes associated with meningococcal disease in Greece to determine if carriage of meningococci or *N. lactamica* was associated with induction of antibodies to these antigens.

2. Materials and methods

2.1. Sources of sera

There were 53 sera from patients with meningococcal disease, 13 were culture-positive for *N. meningitidis* and 40 were culture-negative. The diagnosis of disease was made on the basis of clinical symptoms and identification of *N. meningitidis* by culture and/or microscopy. Sera obtained from 604 military recruits (age range 16–35 years) from an earlier study were used [7]. Among these, the proportion of sera from carriers was 163/604 (27%). Sera from 319 children obtained in our study of ethnic Greeks who had recently immigrated from Russia were tested as there was a high level of carriage of meningococci (13.1%) and *N. lactamica* (17.3%) in this group [8].

2.2. Detection of IgG and IgM specific for OMP

2.2.1. Preparation of OMP

OMP were prepared from the following three isolates obtained from Greek patients with meningococcal disease: strain 342 (B:4:P1.15); strain AK74 (C:2a:P1.2); strain BM30 (B:15:P1.7). While these phenotypes are identified among strains isolated from patients, they are not common among isolates obtained from either recruits or school children who are carriers of meningococci [8,9].

Preparation of the OMP was based on a method supplied by Dr. Andrew Fox (Public Health Laboratory Service, Manchester, UK). The bacteria were cultured on chocolate blood agar (20 plates per strain) at 37°C in a humidified atmosphere containing 5% CO₂. The growth was harvested by scraping the surface with a sterile glass rod and the bacteria were suspended in 30 ml deoxycholate buffer (0.5% sodium deoxycholate, 0.1 M EDTA, pH 8.5). The suspension was transferred to a 250-ml conical flask and shaken with polyester beads (5 mm diameter) at 66°C for 30 min at 300 rpm in an orbital incubator. The suspension was transferred to 10 ml polypropylene tubes and centrifuged at 12 500 rpm at 4°C in a Sorval (RC5B) centrifuge. The pellet was discarded and the supernatant was recentrifuged at 28 000 rpm for 90 min. The supernatant fluid was discarded, the pellet resuspended in 0.5 ml double-distilled water, and the material treated with a MSE Soniprep 150 for three 10-s intervals. The protein content of each preparation was standardized with the BCA protein assay reagent (Biochem) to a concentration of 400 μg ml⁻¹; the OMP from the three preparations were mixed in ratios of 1:1:1, aliquoted and stored at −20°C.

2.2.2. ELISA for detection of IgM and IgG to OMP

The OMP antigens were thawed and diluted 1 in 1000 in bicarbonate buffer (pH 9.6) and 100 μl added to the wells of 96-well ELISA plates (Sarstead). The
plates were incubated overnight at 4°C and washed three times with washing buffer composed of phosphate buffered saline (PBS) containing 0.5% (v/v) Tween 20 (pH 7.2). Non-specific binding was blocked by incubating the wells for 1 h at 37°C with 100 μl PBS containing bovine serum albumin (BSA) (3% w/v). The plates were washed three times with the washing buffer and 100 μl of the test serum added. The serum samples were diluted 1 in 1000 in the serum diluent containing the following per 100 ml PBS: Tween 20 (0.05 ml); powdered milk (1 g) (Carnation); and 5 ml normal goat serum (Sigma). The plates were incubated at 37°C for 90 min and washed three times with washing buffer.

Controls included positive and negative sera kindly provided by Dr. Fox. Horseradish peroxidase labeled anti-human IgG or IgM (Sigma) were diluted 1 in 1000 in the serum diluent and 100 μl added to the plates to detect specific IgG or IgM bound to the OMP antigens. The plates were incubated for 60 min at 37°C then washed three times. The substrate (100 μl), 3,3′,5,5′-tetramethylbenzidine (TMB) (Pierce, lot no. 1854050) (0.4 g l⁻¹), was added and the plates incubated at room temperature for 30 min. The reaction was stopped by adding 50 μl 5 N H₂SO₄ and the absorbance measured at 492 nm (A₄₉₂) with a Titretek Multiscan plate reader.

All samples were tested in duplicate and the A₄₉₂ values for the pairs averaged. For each experiment, the cut-off levels for IgM and IgG were calculated separately by the following formula:

\[ \frac{A_{492} \text{ negative control}}{(A_{492} \text{ positive control} - A_{492} \text{ negative control}) \times 100} \]

Values ≥ that of the cut-off value for the positive control were considered positive.

2.3. Statistical methods

The results were stored in a data base and analyzed with the EpiInfo package.

3. Results

3.1. Detection of IgG and IgM to OMP in sera from patients

Among the 13 culture-positive patients, four (31%) had no detectable IgG or IgM to the OMP; seven (54%) had IgM and two (15%) had both IgG and IgM antibodies specific for the OMP. In the sera from the 40 culture-negative patients, 13 (33%) had no detectable antibodies, 21 of 40 (52.5%) had IgM to the OMP, five (12%) had IgG and IgM and one had IgG only (2.5%) (Fig. 1). Matched acute and convalescent sera were available from two patients. In the sera from a culture-positive patient, the first

![Fig. 1. Proportion of sera with IgG (G⁺) and/or IgM (M⁺) to the OMP preparation from patients with meningococcal disease from whom the bacteria were isolated (culture +) or from whom no organism were obtained (culture -).](image-url)
sample contained IgM only, but the second contained both IgG and IgM. In the culture-negative patient, both acute and convalescent sera were negative for both isotypes.

3.2. Detection of IgG and IgM to OMP in sera from military recruits

Among military recruits (n = 604), the proportion of carriers (17 of 163, 10.4%) with IgM to OMP was higher than non-carriers (24 of 441, 5.4%) ($\chi^2 = 3.92, P < 0.05$). The proportion of carriers (64 of 163, 39%) with IgG to OMP was significantly higher than that among non-carriers (68 of 441, 15%) ($\chi^2 = 38.24, P = 0.000000$) (Fig. 2).

3.3. Detection of IgG and IgM to OMP in sera from school children

Sera were obtained from 319 school children, 34 (10.6%) of whom were carriers of meningococci. The proportion of IgM positive sera among the carriers was 17 of 34 (50%) compared with 29 of 285 (10%) non-carriers ($\chi^2 = 35.88, P = 0.000000$). Among carriers, the proportion of IgG positive sera was higher (8 of 34, 23%) compared with non-carriers (9 of 285, 3%) ($\chi^2 = 21.1, P = 0.000043$) (Fig. 3). Carriage of N. lactamica was not associated with presence of either IgM or IgG to the OMP.

4. Discussion

The method for obtaining the OMP preparations did not have a specific step to remove lipo-oligosaccharide (LOS) or capsular polysaccharides; therefore, it could be suggested that some of the antibodies were directed against endotoxin or capsular antigens present in the preparation. The lack of association between carriage of N. lactamica indicates that much of the antibody response detected is directed against the OMP because N. lactamica lacks capsular antigens and the majority do not react with serotype or subtype reagents.

There were no significant differences in detection of IgG or IgM antibodies in sera from patients from whom meningococci were isolated compared with those who were culture-negative. The ELISA successfully detected IgG or IgM in sera of 69% of patients from whom meningococci were isolated and 67% of those who were culture-negative. There was a higher proportion of IgM positive sera than sera with IgG only or sera with both IgG and IgM (Fig. 1). This is the pattern expected if the assay is detecting primary antibody responses during the initial acute stage of infection.

In both the school children and the recruits, carriers had significantly higher levels of IgG and IgM to the OMP antigens. The patterns of isotype detected in the recruits differed significantly from that...
observed in children (Figs. 2 and 3). The higher proportion of children (50%) with IgM to OMP compared with recruits (10%) might reflect primary responses among the children elicited by recent or concurrent exposure to the bacteria. The predominance of IgG responses to the OMP among recruits suggests these are secondary responses to the OMP antigens.

In our previous study, bactericidal antibodies to capsulate strains of meningococci were associated with high levels of IgG to *N. lactamica*, but not with high levels of IgG antibodies to a non-groupable strain of *N. meningitidis* [4]. The lack of association between antibodies to OMP and carriage of *N. lactamica* in this study could reflect observations that the majority of *N. lactamica* isolates from Greece and other countries do not react with monoclonal typing reagents directed against proteins 1, 2, and 3.

We conclude that the results presented here complement our previous suggestion that OMP antigens of *N. lactamica* are not involved in induction of bactericidal antibodies to meningococci. We are currently investigating the role of endotoxin from *N. lactamica* isolates for immunogenicity and cross reactivity with meningococci expressing different lipo-oligosaccharide immunotypes.

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References


