

Incidence of haemagglutinating antibodies to meningococci in north-west England

D. M. JONES AND BARBARA M. TOBIN

From the Public Health Laboratory and Department of Bacteriology, University Hospital of South Manchester

SYNOPSIS Using an indirect haemagglutination technique the incidence of meningococcal antibodies in adults was found to be: group A 21%, group B 3%, and group C 20%. The reasons for some differences between these results and those obtained in America are discussed. A low incidence of antibody was detected in preschool children but the incidence in school children was found to approach that of adults. Comparison of the antibody incidence in the adult population of an area where clinical infections were occurring with that in a similar population elsewhere failed to detect any significant difference.

Antibodies to meningococci may be demonstrated by a variety of serological techniques. Indirect haemagglutination detects group specific antibody, is very sensitive, and is adaptable for use in large-scale surveys. Sanborn and Vedros (1966) demonstrated that haemagglutinating antibody developed in relation to clinical infection and also to carriage of the organisms. The method has been developed (Edwards and Driscoll, 1967) and also used to investigate the antibody response in a community outbreak (Huntley and Reed, 1966). Using purified polysaccharide to sensitize erythrocytes the method has been extensively used, together with other serological techniques, to investigate the antibody response to meningococcal polysaccharide vaccines (Artenstein, Brandt, Tramont, Branche, Fleet, and Cohen, 1971a; Artenstein, Schneider, and Tingley, 1971b; Artenstein, 1971). The occurrence of an increased frequency of group B meningococcal infections in Bolton where approximately 40 cases occurred during late 1970 and 1971 prompted a survey of meningococcal antibodies in the population of Bolton and also of the surrounding areas where clinical infection was not occurring.

Methods

ANTIGENS

The meningococci used for the preparation of sensitizing antigens were a group B strain isolated from a case of meningitis that occurred in the Bolton outbreak, and strains group A M1027, group B

M993, group C M1628 (Sara Branham), and group Y (K. W. Slaterus). The organisms were grown on Difco Mueller Hinton agar with 1% Difco supplement B overnight (groups A, C, and Y) or for six hours (group B) before harvesting into saline with a cottonwool swab. After 24 hours at 4°C merthiolate was added to the suspension which was then centrifuged to remove most of the organisms. These crude saline extracts are rich in group-specific polysaccharide but also contain other antigens.

ANTISERA

The antisera used to establish the haemagglutination technique were prepared as previously described (Abbott and Graves, 1972).

PREPARATION OF FOWL RED CELLS

A 7% suspension of freshly collected fowl cells in phosphate-buffered saline, pH 7.2, was added to an equal volume of 7% formaldehyde. The mixture was incubated at 37°C overnight, the cells were then thoroughly washed and stored in saline containing 1/1000 sodium azide.

INDIRECT HAEMAGGLUTINATION

A 3.75% suspension of formalinized fowl cells was tanned with 1:20 000 tannic acid, washed and resuspended with equal volumes of tenfold dilutions of meningococcal antigen in phosphate-buffered saline pH 6.4. The mixtures were incubated for 30 minutes at 37°C, washed and resuspended in saline containing 1.5% normal rabbit serum to give a 1.5% suspension of sensitized cells. The optimum

Received for publication 18 August 1972.

sensitizing dilution of antigen was determined by chess board titrations. Antisera were diluted in barbitone buffer pH 7.2 and to each 0.1 ml of serum dilution was added 0.1 ml cell suspension. The plates were shaken and the results read after standing one hour at room temperature.

The specificity and sensitivity of the test systems were demonstrated using purified polysaccharide prepared from groups A, B, and C meningococci (kindly supplied by Dr M. S. Artenstein) in haemagglutination-inhibition tests. The serological response in a small number of cases of meningitis has been studied with this haemagglutination technique and the titres observed have been of the same order as those reported by Artenstein *et al* (1971).

Sera for testing were first inactivated then diluted with buffer and 0.1 ml was put into each of four wells in a plastic plate. An equal volume of sensitized cells (group A, B, and C) was added to each of three wells and unsensitized cells in the fourth. Sera reacting with unsensitized cells (about 2% of those tested) were absorbed with fowl cells and retested. Sera that reacted in the screening test were titrated with relevant sensitized cells. In this investigation cells were freshly tanned and sensitized each day that tests were performed and antisera were titrated with each batch of tests to detect any variations in sensitivity. Some sera from children were tested in addition with group Y sensitized cells.

PATIENTS' SERA

The sera from adult patients were selected from specimens submitted to the Central Serology Laboratory, Withington Hospital. These came mainly from antenatal clinic patients and also hospital inpatients over the whole of the Manchester region. Sera collected from children on admission to hospitals in N. Lancashire were kindly supplied by Dr D. N. Hutchinson, Preston.

Results

The indirect haemagglutination technique was found to be very sensitive, highly group specific, and gave results that were readily reproducible. The comparison of tests by three techniques using rabbit antisera are shown on Table I. The antigen prepared

from the 'epidemic' strain of group B meningococcus was used to examine 2250 sera collected from adults over the whole Manchester Region including the Bolton area (Table II). The low incidence of antibody in this survey lead us to examine more sera using antigens prepared from stock strains of group A, B, and C meningococci. One thousand sera were selected so that approximately half came from the Bolton area, and the results are shown in Table III. In these studies the range of titres was found to extend up to 1/128 but most positive sera reacted at dilutions of 1/8 or 1/16. There was no detectable difference in the distribution of antibody between males and females.

Antiserum Group	Agglutination	Immunofluorescence	Indirect Haemagglutination
A	1/10	1/160	1/3200
B	1/40	1/320	1/1600
C	1/20	1/160	1/3200

Table I *Comparative titres by three techniques of meningococcal group specific rabbit antisera*

Origin	Number of Sera Examined	Percentage of Sera with Antibody \geq 1/8
Manchester region	1 457	3.5
Bolton area	793	3.1

Table II *Comparison of the incidence of haemagglutinating antibody to group B meningococcus ('epidemic strain') in the Manchester region and Bolton area*

Sera collected from children on admission to hospitals in various parts of the region were also examined and some of these were examined in addition for antibody to group Y meningococci (Table IV).

Discussion

For the performance of the indirect haemagglutination test a ruptured cell antigen, chemical extracts of cultures, purified polysaccharide and simple saline extracts have all been previously used by other workers. We adopted simple saline extraction of cultures and obtained satisfactory sensitization of

Origin	Number of Sera Examined	Sera with Antibody \geq 1/4	Sera with Antibody (%) to		
			Group A	Group B	Group C
Manchester region	472	176 (37%)	21	2.7	23
Bolton area	518	167 (32%)	21	1.7	17

Table III *Comparison of the incidence of haemagglutinating antibodies to meningococci in adults from north-west England*

Origin	No. of Sera Examined	Sera with Antibody	Sera with Antibody to			
			Group A	Group B	Group C	Group Y
N Lancashire preschool age	170 ¹	10 (6%)	4	3	3	0
N Lancashire school age	41 ¹	11 (27%)	2	0	6	3
Bolton (neonates)	23 ²	1 (4.3%)	0	1	0	NT
S Manchester (cord sera) ⁴	40 ³	1 (2.5%)	0	0	0	1 ³

Table IV Comparison of the incidence of haemagglutinating antibodies to meningococci in children from NW England

¹Sera examined at a dilution of 1/4.

²Sera examined at a dilution of 1/8.

³Titre unchanged after treatment with 2 mercaptoethanol.

⁴Cord sera found to contain raised levels of IgM and/or IgA.

NT = Not tested

tanned formalinized fowl red cells. These red cells were chosen because it has been shown (Dr P. J. L. Sequeira, personal communication) that only a small proportion of human sera agglutinate unsensitized fowl cells and we were able to avoid absorbing all but a very small proportion of the sera that we examined. Antigens other than polysaccharides may be important in immunity to the meningococcus and a tanned cell system was adopted so that protein antigens would also adhere to the cells. The indirect haemagglutination technique has been shown to be very sensitive and group specific in demonstrating the antibody response to infection, carriage, and vaccination with purified bacterial polysaccharide (Artenstein *et al.*, 1971). The haemagglutinating antibody is predominantly but not exclusively found in the IgM class of immunoglobulin (Gotschlich, Goldschneider, and Artenstein, 1969). The haemagglutinating antibody that develops following vaccination has been shown to persist for many months (Artenstein, 1971) but there is little information on the persistence of naturally acquired antibody either following infection or associated with carriage. The haemagglutinating antibody is indicative of group specific (polysaccharide) antigenic experience but it has been inferred that it is the bactericidal antibodies that are truly protective although these too are generated by vaccination with pure polysaccharide (Goldschneider, Gotschlich, and Artenstein, 1969). The techniques for demonstrating bactericidal antibody are not suitable for examining large numbers of sera, so that probably the haemagglutinating antibody is at present the best index of population immunity. It is reasonable to conclude that the incidence of haemagglutinating antibody will reflect what groups of meningococci are or have been prevalent in a population, whether clinical infections are occurring or not.

In our study the incidence of group A antibody (21%) and group C antibody (20%) may be compared with that in a small group of normal young adults examined in America where the incidence was

group A (51%) and group C (19%) (Artenstein *et al.*, 1971). The most striking difference between our results is with group B antibody: in the American study 72% of 40 adults had titres greater than 1:8 whereas in over 3000 adults in England antibody was found in only 3%. In a recent survey of meningococcal carriage in families in the USA group B strains accounted for half of the meningococci isolated (Greenfield, Sheeche, and Feldman, 1971). It is also worth noting that group B meningococcal infections are now uncommon in the United States where group C causes most of the clinical infections (Artenstein *et al.*, 1971b). In the United Kingdom group B strains are still the most common cause of clinical infection (Abbott and Graves, 1972). The difference in the populations may therefore be a real one and reflect a greater population susceptibility to group B strains existing in the United Kingdom.

It has been shown that the introduction of a carrier into a household is followed by establishment of the carrier state in adult females of that household (Greenfield *et al.*, 1971). Although the meningococcal infections that occurred in the Bolton area during the study were mainly in preschool children, the haemagglutinating antibodies of antenatal patients (who were the bulk of our sample), many of whom have children of this age, probably reflects current antigenic experience of the children. We failed to demonstrate either a deficiency or an increase of group B antibody in the Bolton population compared with control areas. There was also no detectable difference in incidence of antibody to the other serogroups and we conclude that the localization of the outbreak of clinical infection cannot be explained on the basis of these results.

Our studies on the sera from children confirm that preschool children have little antibody but that in older children the incidence of antibody approaches that of adults. This parallels the higher incidence of disease in the preschool age group. There was no evidence of antibody formation by neonates, and, as most haemagglutinating activity is associated with

macroglobulin, little maternal haemagglutinating antibody passes the placenta.

We are indebted to Dr P. J. L. Sequeira for access to specimens submitted to the Central Serology Laboratory, Withington Hospital.

This work was supported by a grant from the Manchester Regional Hospital Board.

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