DEVELOPMENT OF ANTIBODIES TO MENINGOCOCCAL PROTEIN AND LIPOPOLYSACCHARIDE SEROTYPE ANTIGENS IN HEALTHY CARRIERS

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The role of bactericidal antibodies directed against meningococcal serogroup (polysaccharide) antigens in protection against disease is well established, and there are effective polysaccharide vaccines for meningococcal groups A and C. The group-B polysaccharide is poorly immunogenic (Wyle et al., 1972) and in disease caused by group-B meningococci the serotype protein antigen and the lipopolysaccharide antigen may be relatively more important in the development of protective antibody. Antibodies to these protein serotype antigens and lipopolysaccharide serotype antigens of meningococci develop in the course of meningococcal carriage and in convalescence after infection, but information on the occurrence of these antibodies is still limited (Zollinger, Pennington and Artenstein, 1974; Frasch, 1977). Recent work (Frasch and Robbins, 1978) has shown that a vaccine made from serotype-2 protein is protective in the experimental animal. The serotype-2 protein was chosen for study because this antigen is common on strains of meningococci of all groups that produce either sporadic or epidemic infection (Jones and Tobin, 1976; Frasch and Friedman, 1977). The present investigation was undertaken as a study of the antibody response to the protein and lipopolysaccharide antigens which are induced by the carriage of meningococci by healthy subjects. We paid special attention to the development of bactericidal antibody to serotype-2 protein in carriers because of the potential importance of this phenomenon in natural immunity to meningococcal infection.

MATERIALS AND METHODS

Eighty Royal Air Force recruits were studied during their first six weeks of basic training. During the period of study there was no meningococcal disease amongst these recruits. Nasopharyngeal swabs were taken weekly to establish patterns of carriage and acquisition of meningococci. All the meningococcal isolates were serogrouped and they were also serotyped by the bactericidal method of Frasch and Chapman (1972). Some strains were lipopolysaccharide serotyped by the haemagglutination inhibition test (Zollinger and Mandrell, 1977). Serum samples were collected from each recruit on the day of entry and again six weeks later. These were examined for meningococcal antibodies to the group polysaccharide by haemagglutination (Jones and Tobin, 1972) and to a whole bacterial-cell extract by complement fixation. The sera were examined for bactericidal antibodies by the method of Frasch and Chapman (1972) and for antibodies to serotype lipopolysaccharide by haemagglutination of fowl red cells sensitised with phenol-water extracts (Zollinger et al., 1974).

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RESULTS

Meningococcal carriage and acquisition

Carrier rates amongst the recruits did not vary much during the period of study (table I). The principal acquisitions were of group-W135 strains, most of which carried the serotype-2 antigen but some were "untypable". The strains of other assorted groups isolated were all "untypable" in the context of this investigation, i.e. they did not carry any of the protein serotype antigens 1–12 (Frasch, 1977).

Serological findings

The distribution of haemagglutinating antibody to the group-specific polysaccharides is shown in table II. Approximately half of the recruits reacted with at least one polysaccharide antigen and 12 recruits showed a significant rise in antibody titre, all associated with the acquisition of meningococci during the training period. The complement-fixation test gave similar results; 48% of the recruits were positive on entry and 63% after training. A rise in titre was detected in the same 12 recruits who showed a rise in haemagglutinating antibody titre.

TABLE I

Carriage of meningococci in 80 recruits during six weeks' basic training

<table>
<thead>
<tr>
<th>Week no.</th>
<th>Number of recruits carrying meningococci of group</th>
<th>Total number of carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A  B  C  X  Y  Z  29E  W135 not groupable</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0  9  0  0  3  0  2  0  6  20</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0  9  0  0  3  0  2  2  6  22</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0  7  0  0  2  0  2  5  5  21</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0  4  1  0  2  0  2  8  2  19</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0  4  1  0  3  0  3  6  4  21</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0  5  1  0  4  0  3  13 10 36</td>
<td></td>
</tr>
</tbody>
</table>

TABLE II

Meningococcal antibody in paired sera from 78 recruits

<table>
<thead>
<tr>
<th>Serum specimen</th>
<th>Number of recruits with haemagglutinating antibody to group-specific polysaccharide</th>
<th>complement-fixing antibody to group-B/P2/L2 strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A  B  C  X  Y  Z  29E  W135</td>
<td></td>
</tr>
<tr>
<td>First</td>
<td>16  0  4  1  10  9  7  4</td>
<td>38 (49%)</td>
</tr>
<tr>
<td>Second</td>
<td>14  0  5  0  11  8  4  15</td>
<td>48 (62%)*</td>
</tr>
</tbody>
</table>

* 12 recruits showed a rise in titre of complement-fixing antibody.
† 10 recruits showed a rise in titre of bactericidal antibody.
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All sera were tested for bactericidal antibody to the protein serotype 2 against a group-B/P2/L2 strain that had caused infection in the same camp a year previously, but was not cultured from any of the current recruits. On entry 11 men had anti-P2 antibody and six weeks later this was present in 19 (24%); 10 men showed a rise in titre. Details of the group and protein-serotype antigens of the meningococci isolated from the 10 recruits who showed a rise in titre are shown in table III. Acquisition of a group-W135 protein serotype-2 meningococcus was the predominant finding. The lipopolysaccharide antigens on these strains were shown to be different from that on the test strain (B/P2/L2) by haemagglutination inhibition, so that it is reasonable to conclude that the bactericidal activity of these sera was directed against the P2 antigen. Two recruits appeared to be established carriers of non-typable meningococci, one non-groupable and one group B. We cannot be certain that these recruits did not also acquire the W135/P2 strain because we did not make a particular effort to detect the concurrent carriage of more than one serogroup, although this phenomenon was observed in several recruits.

Two other recruits acquired an untypable W135 strain and these produced homologous bactericidal antibody to W135 polysaccharide and to the homologous lipopolysaccharide but, as one would expect, no anti-P2 antibody.

DISCUSSION

In group-B meningococcal infection the antibody response is directed principally against the protein and lipopolysaccharide antigens and there is a poor or undetectable response to the group polysaccharide. Immunity may
depend on naturally developed antibody to the protein and polysaccharide antigens, although there is probably synergy between the systems (Frasch et al., 1976). In the present investigation, we have studied the natural development of antibody to serotype-2 protein in carriers among a military recruit population. Acquisition and carriage of avirulent strains of meningococci carrying the serotype-2 protein resulted in the development of antibody bactericidal to a virulent group-B serotype-2 strain that had been associated with meningitis in the recruit camp one year previously. In that outbreak we found that the carriage rate of the group-B/P2 strain amongst contacts of the cases was very low but that the rates for other meningococci were quite high (Tettmar, Abbott and Jones, 1977). The occurrence of low carriage rates of virulent strains that carry the serotype-2 protein in other outbreaks has been commented upon (Jacobson and Frasch, 1978). Our experience with healthy recruits reported here and in other studies has been that strains of meningococci that are not associated with disease but carry the serotype-2 protein, may be acquired readily and that protective antibody results from carriage. We conclude that such strains play an important part in the development of naturally acquired immunity, particularly relevant to group-B meningococcal infection.

**Summary**

The nasopharyngeal acquisition of meningococci was followed in healthy military recruits during their primary training. The production of antibodies to meningococcal serotype protein antigens and serotype lipopolysaccharide antigens accompanied the carrier state. Bactericidal antibody to protein serotype 2 was generated in response to the carriage of meningococci of low virulence that carried this antigen.

It is a pleasure to acknowledge the co-operation and help of our colleagues Dr J. D. Abbott and Mrs E. M. Sutcliffe, and Dr R. E. Tettmar, Royal Air Force, Nocton Hall.

**REFERENCES**


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