Lack of Immunity in University Students before an Outbreak of Serogroup C Meningococcal Infection

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Immunity to meningococci was determined in infected and uninfected students before and during an outbreak of serogroup C meningococcal infection at a university in the United Kingdom. No immunity against the outbreak strain was detected in serum taken from infected students prior to the outbreak or at the time of admission; bactericidal activity developed during convalescence. Carriage of all strains of serogroup C meningococci in asymptomatic students was low (0.9%), and no carriage of the outbreak strain could be detected. Immunity in the at-risk student population before the outbreak was low: 90% of students had no significant bactericidal activity against the outbreak strain. A low prevalence of carriage of the outbreak strain, together with a low prevalence of protective immunity within the student population, was associated with a high incidence of invasive disease in those who acquired the outbreak strain.

The proportion of invasive meningococcal infection due to serogroup C meningococci has increased in several countries in recent years, including the United Kingdom (UK), United States, and Canada. This has often been associated with clusters of infection, particularly in institutions [1±4]. For the majority of persons, however, acquisition of meningococci does not cause invasive disease and merely results in colonization of the nasopharynx. Carriage of meningococci occurs in ~10% of the population but is considerably higher in institutions, particularly military camps or other closed or semiclosed communities [5]. In several recent clusters of serogroup C infection in institutions, studies of susceptible persons failed to detect or detected only a very low prevalence of colonization by disease-causing strains, compared with strains not specifically associated with that cluster of infections [6, 7].

Factors that predispose some persons to invasive disease, while the majority of colonized persons remain unaffected, are poorly understood. However, it is generally accepted that there is a strong correlation between the presence in serum of complement-dependent bactericidal activity against meningococci and immunity of the individual to subsequent infection. Most of our knowledge of immunity prior to an outbreak of meningococcal infection is based on the classic studies by Goldschneider and colleagues [8±10]. They demonstrated that most soldiers entering a training camp possessed bactericidal antibodies to the epidemic strain, largely directed against capsular polysaccharide, and did not develop infection. Soldiers who lacked such bactericidal antibodies had a high incidence of invasive disease [8].

In recent years, there have been a number of outbreaks of infection caused by serogroup C meningococci in educational establishments within the UK. In October 1997 an outbreak of serogroup C infection occurred at a UK university, with 6 cases among first-year students and 3 deaths [11]. Serum samples taken 1 month prior to the outbreak were available from 1 infected student and from classmates of that student who lived in the same residential complex. This provided a unique opportunity to investigate levels of immunity to meningococcal infection, both in infected and in uninfected students, before and during the outbreak.

Methods

Samples. Acute serum samples were obtained from 5 of 6 patients, and convalescent serum samples were available from 2 of 3 surviving patients. Serum samples taken from patient 2, 1 month before the outbreak, were also available, together with serum samples taken for routine health screening from 75 other members of this cohort of first-year health care students who lived in the same residential complex as 5 of the infected students. Throat swabs

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Table 1. Characterization of strains isolated from infected students during a group C meningococcal outbreak.

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Day of presentation</th>
<th>Serologic characterization of strain</th>
<th>Serosubtype determined by porA sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>C:2a:NST*</td>
<td>P1.5,2‡</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>C‡</td>
<td>P1.5a,10d</td>
</tr>
<tr>
<td>3</td>
<td>16</td>
<td>C:NT:P1.5‡</td>
<td>P1.5a,10d</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>18</td>
<td>C‡</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>C:NT:P1.5‡</td>
<td>P1.5a,10d</td>
</tr>
</tbody>
</table>

**NOTE.** Dashes indicate assays negative on available samples from culture-negative cases.

* In order of notification to health authorities.

† Serogroup:serotype:subtype. NST, nonsubtypeable; NT, nontypeable.

‡ Isolated from blood cultures.

§ Class 1 protein not expressed.

* Culture negative: serogroup determined by SiaD polymerase chain reaction of plasma or cerebrospinal fluid.

were taken to determine meningococcal carriage in 587 students prior to chemoprophylaxis as part of outbreak control measures.

**Molecular characterization of isolates by sequencing of por genes.** Meningococci isolated from infected students were cultured by standard techniques. The porB gene and the variable regions VR1 and VR2 of the meningococcal porA gene were amplified by polymerase chain reaction (PCR) and sequenced as described elsewhere [12].

**Bactericidal assay.** The bactericidal activity of test serum was determined against meningococcal strains by use of 25% baby rabbit serum as a complement source, as described elsewhere [12, 13].

**Results**

**Meningococci isolated during the outbreak.** During the 20-day outbreak, 6 cases were identified (table 1). Patients 1, 2, and 6 died as a result of their infection. Meningococci were isolated from blood of patients 1, 3, and 6. Meningococci isolated from patient 1 were serologically identified as serogroup C, serotype 2a, nonsubtypeable (C:2a:NST). This case occurred 15 days before the first of 5 additional cases that manifested over 5 days. Isolates from patients 3 and 6 were serologically identified as serogroup C, nontypeable, subtype P1.5 (C:NT: P1.5). Sequencing of the porA gene, which encodes for the subtype-specific class 1 protein, demonstrated that the isolate from patient 1 was nonsubtypeable due to nonexpression of the P1.5,2 class 1 protein, whereas the isolates from patients 3 and 6 expressed subtype P1.5a,10d. Sequencing of the porB gene, which encodes the serotype-specific class 2/3 protein, confirmed that case 1 was serotype 2a and was clearly distinct from cases 3 and 6. PCR amplification and sequencing of DNA extracted from the blood or cerebrospinal fluid of culture-negative patients 2, 4, and 5 demonstrated that case 2 was also caused by a serogroup C, P1.5a,10d strain. Patient 5 could be characterized only as having serogroup C infection, and case 4 was not confirmed; however, both were linked epidemiologically to patient 2. All 3 students had visited the same night-club on the same evening the previous week. This link was not noted for the remaining cases.

**Meningococcal carriage at the time of the outbreak.** Between days 18 and 27 of the outbreak, throat swabs were taken from 587 students, and meningococci were isolated from 25% (n = 147). Nongroupable meningococci were most common (11.1%), followed by serogroups B (6.1%) and Y (2.6%). Serogroup C meningococci were isolated from only 0.9% of the sample population, and none of these isolates was consistent with the antigenic profile (C:NT:P1.5a,10d) of the outbreak strain. None of the nongroupable strains possessed the group C SiaD gene, as determined by PCR [14].

**Serum bactericidal activity in serum samples from infected students.** Paired serum samples were available from 3 patients. Serum samples taken 28 days before the outbreak and acute serum samples taken at the time of admission were available for patient 2. Acute and convalescent serum samples were available from patients 4 and 5. All serum samples were tested with rabbit complement for bactericidal activity against both the outbreak and sporadic strains. Serum samples from patient 2 taken before the outbreak showed no demonstrable bactericidal activity against either strain (<1 : 2), and the bactericidal activity did not increase at admission. Similarly, serum samples from patients 4 and 5 showed no detectable activity at the time of admission, but during convalescence significant bactericidal activity developed against the outbreak strain (titers of 1 : 256 and 1 : 512, respectively) and against the sporadic strain (titers of 1 : 512 and 1 : 8192, respectively). Similar results were obtained with human serum as the complement source.

**Serum bactericidal activity in the student population before the outbreak.** Serum samples taken 28 days before the outbreak were available for 76 students and were tested for bactericidal activity against the outbreak strain (figure 1). Only 8 students (10.5%) had significant bactericidal activity, defined as a bactericidal titer ≥ 1 : 4 [8]; the others had no demonstrable bactericidal activity. Of these 8 students, 4 showed significant bactericidal activity against the isolate from the sporadic case.

![Figure 1. Bactericidal activity in student serum samples before meningococcal outbreak. No significant bactericidal activity against the outbreak strain was detected in 68 of 76 students. Of 8 students with a bactericidal titer ≥ 1 : 4, 4 also showed significant activity against the sporadic strain.](image-url)
No student had bactericidal activity against only the sporadic strain.

Discussion

Natural immunity to meningococcal infection prior to an outbreak can only be studied in unusual circumstances, since outbreaks are rare and unpredictable and since it is unusual that substantial numbers of serum specimens immediately predating an outbreak are available from the at-risk population. The availability of serum samples obtained prior to this outbreak presented a unique opportunity to study protective immunity in a modern institutional setting.

The isolate from patient 1 was clearly distinguishable, by DNA sequence analysis of the \textit{porA} and \textit{porB} genes, from the isolates from patients 3 and 6 and from amplified DNA from culture-negative patient 2. Although complete strain information could not be obtained for patients 4 and 5, they were closely related to case 2 by temporal and epidemiologic factors. These data suggest that 2 distinct organisms were responsible for the cases of meningococcal infection: one caused a single sporadic case, and the other caused a cluster of 5 outbreak cases. These strains were differentiated only by DNA sequence analysis. Recently, multiple-locus sequence typing of \textit{porA} and \textit{porB} genes and of stable housekeeping genes has been used to establish the clonal lineage of meningococcal isolates [15]. Such molecular techniques are powerful tools to determine the association of strains from loosely related clusters of meningococcal infection.

In the asymptomatic at-risk students, the point prevalence of carriage of all strains of serogroup C meningococci was very low, and no carriage of either the sporadic or outbreak strains could be detected. This is in accordance with recent studies of clusters of serogroup C meningococcal infection that reported a very low prevalence of asymptomatic carriage of serogroup C strains and even lower carriage of the outbreak strain [6, 7]. This is in contrast with the classic studies of Goldschneider and colleagues [8–10], who followed an epidemic of serogroup C meningococcal infection in a military training camp at Fort Dix, New Jersey, during 1967–1968. They found throat carriage of serogroup C meningococci in 6%–10% of recruits on entry into the camp, which rose to 50% in week 3 and 75% in week 8.

After the studies of Goldschneider and colleagues [8–10], it was generally accepted that the most important correlate of protection against meningococcal infection is the presence of serum bactericidal activity against the invasive strain [8, 9]. During the Fort Dix outbreak >80% of new recruits possessed serum bactericidal activity, at a titer \(\geq 1:4\), against the epidemic meningococcal strain on entry into the camp. None of these persons developed meningococcal infection. In contrast, invasive disease developed on acquiring the epidemic strain in nearly 40% of recruits who had serum bactericidal titers <1 : 4.

In our study, no significant bactericidal activity against the outbreak strain was detected in serum samples from patient 2 that were obtained 1 month before the outbreak or at the time of admission. Serum samples from patients 4 and 5 also showed no activity on admission and a marked increase during convalescence. This bactericidal activity was directed against both the outbreak and sporadic strains, indicating the development of a substantial amount of anticapsular antibody during infection.

Immunity in the student population before the outbreak was determined in serum samples obtained 1 month earlier from classmates of patient 2 who lived in the same residential complex. The prevalence of serum bactericidal activity against both the sporadic strain and the outbreak strain in preoutbreak serum samples was low. Only 8 (10.5%) of 76 students had bactericidal titers of \(\geq 1:4\) against the outbreak strain, and, of these, 4 (5.2%) also had bactericidal activity against the sporadic strain, indicating the presence of anti-group C antibodies. These data, in combination with observations that the outbreak strain was isolated only from infected students and not carriers, indicate a low prevalence of carriage of the outbreak strain together with a low prevalence of protective immunity within the student population. This was associated with a high incidence of invasive disease in those acquiring the outbreak strain.

These conclusions contrast with the earlier studies on military recruits, which found both a high prevalence of carriage of serogroup C meningococci within the training camp and a high prevalence of protective immunity. The differences between these findings may be explained by the significant epidemiologic differences between military recruits entering a US training camp in 1967 and students entering a UK university in 1997. Smoking, overcrowding, lower socioeconomic status, and male sex are recognized as factors that predict higher rates of meningococcal carriage, particularly in institutions [5]. The effects of changes in these epidemiologic factors on the development of natural immunity to meningococcal infection over 30 years are unknown. However, any tendency toward a reduction in the frequency of carriage may result in a lesser development of natural immunity to infection. Certainly, the lower level of carriage of serogroup C meningococci in the student population is consistent with more recent studies [6, 7] and also with the lower levels of immunity observed in our study.

In the context of the low prevalence of natural immunity to the outbreak strain found before the outbreak, the initiation of prompt chemoprophylaxis and mass vaccination of the students with serogroup C meningococcal polysaccharide is likely to have prevented more cases from occurring. The conjugate serogroup C vaccine was recently introduced into the UK and is likely to prevent further serogroup C clusters in UK educational institutions. If such low prevalence of natural immunity is reproduced in similar student populations elsewhere,
then the introduction of conjugate serogroup C vaccines should become a priority.

Acknowledgments

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References