Complement Deficiency Predisposes for Meningitis Due to Nongroupable Meningococci and Neisseria-Related Bacteria


Nongroupable meningococci or bacteria related to the genus Neisseria rarely cause meningitis. Complement deficiency has been identified as a major predisposing factor for meningococcal disease. To assess whether patients with meningitis due to such strains have a complement deficiency, we studied 12 persons. Six patients had meningitis due to nongroupable strains of meningococci, and six patients had meningitis due to Moraxella species or Acinetobacter species. Inherited complement component C7 or C8 deficiency was found in two persons who had had meningitis due to nongroupable meningococci, and one C8-deficient person had had meningitis caused by Moraxella osloensis. Hypocomplementemia resulting from CSF drain–associated shunt nephritis was found in one person with meningitis due to Moraxella nonliquefaciens and in one person with meningitis due to Acinetobacter lwoffi. This rather high frequency of inherited or acquired complement deficiencies among patients with meningitis due to nongroupable meningococci, Moraxella species, and Acinetobacter species justifies the recommendation that such patients must be studied for complement deficiency.

The complement system has an important function in host defense against bacteria. C3 promotes the phagocytosis of bacteria, and the membrane attack complex (C5–C9) effects serum bactericidal activity [1]. So, individuals with a deficiency of a component (C1, C2, or C4) of the classic pathway of complement activation or with a deficiency of C3 have an increased risk for acquiring infections due to various bacterial pathogens, including Neisseria meningitidis [2]. Patients who have acquired complement deficiencies due to autoimmune antibodies to complement components or a chronic disease leading to an increased turnover of complement components [3] are also at increased risk for bacterial infections [4, 5]. Deficiencies in the alternative pathway (factor D, factor H, or properdin) and in the terminal pathway (C5, C6, C7, C8, or C9) of the complement system are especially associated with infections (meningitis and sepsis) due to N. meningitidis [1, 2].

N. meningitidis is the most frequently isolated pathogen from patients with bacterial meningitis [6]. Meningococcal serogroups are defined by the capsular polysaccharides. Serogroups B and C represented 96% of the meningococcal strains isolated from patients with meningitis in the Netherlands in 1990 [7]. Meningitis caused by nongroupable meningococci or the Neisseria-related Moraxella species and Acinetobacter species is a relatively rare event [7–10]. Nongroupable meningococci do not produce capsular polysaccharides or produce low amounts [11, 12]. Individuals with a deficiency of a complement (C5, C6, C7, C8, or C9) who have no capacity for complement-mediated serum bactericidal activity lack a basic defense against the so-called serum-sensitive bacteria such as nongroupable meningococci [10, 11]. Whether the Neisseria-related Moraxella species and Acinetobacter species, common inhabitants of the human skin and mucosal surfaces and most often unencapsulated [6, 10], are sensitive to the complement-mediated bacterial activity is unknown. To our knowledge, no study has been performed in which the prevalence of complement deficiency among persons with meningitis due to these pathogens has been assessed. Therefore, we investigated the prevalence of complement deficiencies among patients who had had meningitis caused by nongroupable meningococci, Moraxella species, and Acinetobacter species.

Patients and Methods

The Netherlands Reference Laboratory for Bacterial Meningitis (Department of Medical Microbiology, University of Amsterdam, Amsterdam, and National Institute for Public Health and Environmental Protection, Bilthoven, the Netherlands) collects bacterial pathogens causing meningitis in the Netherlands. Strains are identified, grouped, and typed in this laboratory. Meningococci are serogrouped by means of
Ouchterlony tests with use of rabbit antisera (produced at the Reference Laboratory) to the capsular polysaccharides of serogroups A, B, C, 29E, H, I, K, L, W135, X, Y, and Z [13]. Nongroupable strains included in this study were retested after enhancement of encapsulation by growth to an early stationary phase under iron limitation (in the presence of 30 μM of deferoxamine; Ciba-Geigy, Basel, Switzerland) [12].

Serotyping and subtyping of meningococci by means of ELISA were performed as described elsewhere [7]. Other bacterial isolates of meningitis were identified according to standard bacteriologic methods [14]. Isolates of Moraxella species and Acinetobacter species were sent to the Laboratory for Bacteriology of the National Institute for Public Health and Environmental Protection for confirmation.

Fourteen nongroupable meningococcal isolates (1959–1990), four Moraxella isolates (1976–1990), and nine Acinetobacter isolates (1976–1990) had been cultured from the CSF of 27 patients. Patients with infections due to these isolates were traced according to the ethical codes for protection of privacy. Clinical data for the patients were provided by the general practitioners and medical specialists in the hospitals to which the patients had been admitted. One of the authors (C.A.P.F. or H.G.T.) visited the patients to collect blood samples for complement screening, to interview the patients for various predisposing factors for meningitis, and to determine whether meningitis had recurred after the first episode.

Screening for complement deficiency. Serum samples were flash-frozen and stored at −70°C. Hemolytic screening tests for the alternative, classic, and terminal pathways of complement activation were performed in duplo both in gel [15] and in free solution [16]. Defects in each of these pathways were determined by quantitative measurement of all components in the affected pathway. Quantitative assays for single complement components were ELISA (properdin, C8, and C9), double radial immunodiffusion in agarose (C3, factor H, and factor B), and hemolytic assays (C3, C5, C6, C7, factor D, and C3NeF) [17, 18]. C8β deficiency was identified by western blotting. The C1q binding test measuring immune complexes was performed by standard methods [19].

Results

Since 1959, 6,559 meningococcal isolates from patients have been collected at the Netherlands Reference Laboratory. Nongroupable meningococci represent 0.36% of the meningococcal isolates received. Other bacteria causing meningitis have been collected since 1976. Among the 12,766 isolates collected by the Reference Laboratory in the period 1976–1990, 31% were N. meningitidis, 0.05% were Moraxella species, and 0.2% were Acinetobacter species.

Nongroupable meningococci, Moraxella species, and Acinetobacter species were isolated from CSF samples from a total of 27 patients. Of the 14 patients with meningitis due to nongroupable meningococci, eight were not included in the study because of the following reasons: five could not be traced, two had emigrated, and one had died of multiple myeloma. One of the six persons included in the study developed meningitis shortly after a traffic accident that had resulted in a skull fracture. Gram staining of CSF from this patient showed many granulocytes and few gram-negative diplococci. This patient recovered completely. Her complement system was intact.

Two of the remaining five patients had a complement deficiency (table 1). One patient was an otherwise healthy female who developed meningitis at the age of 16 years; she had a C7 deficiency. Her only sibling, a brother, was heterozygous for this deficiency (his C7 level was 53% of the normal reference value) and had not suffered from bacterial meningitis. The other patient was a male who had had meningitis and sepsis at the age of 17 years; he had a C8β deficiency. This patient was a member of a family in which meningococcal diseases frequently occurred. His mother had had meningitis due to N. meningitidis serogroup W135 at the age of 30 years and had also been found to be C8β deficient [5]. Six more relatives appeared to be C8β deficient, and three of them had had a total of seven episodes of presumed meningococcal disease. It is unfortunate that only one isolate was serogrouped; it belonged to serogroup C.

None of the six patients with meningitis due to nongroupable meningococci who were included in our study had a recurrence of meningococcal disease after antibiotic treatment. Serotyping and subtyping of the six nongroupable meningococcal strains studied revealed the following results: 2b:P1.2,5; nt:P1.6; 2b:P1.2,5; ntnt; 4:P1.4; and 2b:P1.10. All strains were susceptible to penicillin.

A Moraxella species was isolated from CSF samples from four patients. One patient could not be traced. Two of the three persons with moraxella meningitis had a complement deficiency (table 1). One patient with a C8β deficiency developed meningitis at the age of 15 years; he presented with fever, vomiting, petechiae, meningeal irritability, and disturbance of consciousness. Analysis of CSF showed a slightly elevated leukocyte count (82/mm³), and culture of CSF yielded Moraxella osloensis that was susceptible to penicillin, sulfonamide, and cephaloridine. Blood cultures were negative. He recovered completely after receiving penicillin treatment and was discharged 11 days after admission. At the age of 24 years, he developed meningitis due to meningococcal serogroup A [23]. One sister among his six siblings studied also had a C8β deficiency, but she did not contract bacterial meningitis.

The second patient had meningitis at the age of 19 years. 10 years after the implantation of a ventriculoatrial shunt for progressive congenital hydrocephalus. She presented with otitis media, but after 4 days she developed nuchal rigidity and high fever. Gram staining of the CSF showed a few leukocytes and gram-negative bacteria. Culture of CSF yielded
Table 1. Summary of characteristics of complement-deficient patients (including five patients in the present report) with infections due to nongroupable meningococci, *Moraxella* species, and *Acinetobacter* species.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Sex</th>
<th>Clinical syndrome</th>
<th>Age at clinical syndrome (year)</th>
<th>Isolate</th>
<th>Complement deficiency</th>
<th>Associated disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR</td>
<td>Female</td>
<td>Meningitis</td>
<td>16 y (1977)</td>
<td>NGM</td>
<td>C7</td>
<td></td>
</tr>
<tr>
<td>[PR]</td>
<td>Male</td>
<td>Meningitis and sepsis</td>
<td>17 y (1990)</td>
<td>NGM</td>
<td>C8β</td>
<td></td>
</tr>
<tr>
<td>PR</td>
<td>Female</td>
<td>Meningitis</td>
<td>19 y (1984)</td>
<td><em>Moraxella nonliquefaciens</em></td>
<td>C1q, C4, C2, C3*</td>
<td>CSF drain</td>
</tr>
<tr>
<td>[22]</td>
<td>Male</td>
<td>Gastroenteritis, sepsis, pneumonia</td>
<td>4.5 y (1975), 6 y (1977)</td>
<td><em>Salmonella group B, M. nonliquefaciens; HSV and CMV</em></td>
<td>C4</td>
<td>SLE with nephritis</td>
</tr>
</tbody>
</table>

NOTE. PR = present report; NGM = nongroupable meningococcus; HSV = herpes simplex virus; CMV = cytomegalovirus; SLE = systemic lupus erythematosus.

* Acquired deficiencies.

Moraxella nonliquefaciens. She recovered after treatment with cefotaxime that was followed by treatment with tobramycin and amoxicillin. At the time of this infection, this female patient had signs of shunt nephritis (persistent macroscopic hematuria), but complement studies were not performed. In our study, 8 years later, we found low serum hemolytic activity in the classic pathway. Low levels of C1q (1 mg/dL; reference range, 10-14 mg/dL), C4 (7%; reference range, 68%-104%), C2 (14%; reference range, 68%-104%), and C3 (37 mg/dL; reference range, 68-104 mg/dL) were found repeatedly in association with a slightly elevated level of C3d (19 μg/mL; reference range, 4.7-13.9 μg/mL) and a normal level of C1 inhibitor (41 mg/dL; reference range, 29-41 mg/dL). The C1q binding test was strongly positive (92 μgEq; reference range, <10 mgEq), thus suggesting the presence of circulating immune complexes, possibly due to shunt-associated nephritis [24].

Of nine *Acinetobacter* isolates, three were considered as contaminants since neither the CSF profile nor the clinical symptoms of the patients supported the diagnosis of meningitis. One patient could not be traced. *Acinetobacter* species were isolated from CSF from the remaining five patients; these patients had either pleocytosis (20-516 cells/mm³) or an elevated CSF level of protein (0.38-4.1 g/L) associated with fever, nuchal rigidity, and/or vomiting, thus fulfilling the criteria of Odio et al. [25] for bacterial meningitis. Two of these patients were not included in the study: one was treated for non-Hodgkin’s lymphoma at the time of the study, and one died of metastasized bronchus carcinoma at the time of meningitis.

Complement levels were normal in two of the remaining three patients. The third patient had a ventriculoatrial drain implanted at the age of 1 year because of hydrocephalus that occurred after meningitis, for which no cause was identified (table 1). At the age of 6 years, the drainage system had to be replaced. Twelve years later this patient developed meningitis; he presented clinically with drain malfunction, disturbed consciousness, a slightly elevated temperature, and vomiting. Examination of CSF showed sporadic leukocytes, and culture of CSF yielded *Acinetobacter lwoffi*. The strain was susceptible to amoxicillin, chloramphenicol, and sulfamethizole. At the time of this infection, the patient had membranoproliferative glomerulonephritis with macroscopic hematuria and proteinuria. The serum had low levels of C1q (12%; reference range, 69%-128%), C4 (8%; reference range, 37%-161%), and C3 (28%; reference range, 66%-124%) and a positive C1q binding test. After temporary removal of the drain and treatment with chloramphenicol, the complement levels and the results of the C1q binding test returned to the normal ranges. Thirteen years after this episode, the results of screening tests for the classic and alternative pathways of complement activation were normal.

Discussion

Analysis of the complement systems among six patients with meningitis due to nongroupable meningococci showed the presence of a complement deficiency in two. To our knowledge, meningococcal disease caused by nongroupable meningococci has been previously described in only three complement (C6 and C7)-deficient patients (table 1). Nongroupable meningococci that are unencapsulated or that produce only very small amounts of polysaccharides are generally considered to be vulnerable to the bactericidal activity...
of the complement system [11, 12]. The serotype-subtype combinations in all nongroupable meningococci studied were quite common in the Netherlands [7]. Among patients with meningococcal disease due to the uncommon meningococcal serogroups (X, Y, Z, W135, and 29E), the prevalence of complement deficiency was 31% [5, 23]. In isolates recently recovered in South Africa, one apparently nongroupable meningococcal strain was found in conjunction with other uncommon meningococcal serogroups (W135 and Y) to be a cause of meningitis in complement-deficient individuals (A. Orren, personal communication). This finding supports our observation and suggests that such strains are invasive and that the complement system is the most important system for warding off infections due to these uncommon meningococcal strains, including the nongroupable strains.

Both complement-deficient patients had had meningococcal disease in their late teens, whereas the other four had had meningococcal disease at the mean age of 6 years (range, 1–11 years). This age is in accordance with the still unexplained observation that complement-deficient individuals have their first episode of meningococcal disease at a mean age of 16 years [1, 2], whereas in the general Dutch population, meningococcal disease occurs at the mean age of 3 years [7].

*Moraxella* species and *Acinetobacter* species are included in the family of *Neisseriaceae* [10]. *Moraxella* species are frequently unencapsulated [14]. It has been questioned whether *Acinetobacter* species should be classified within this family [10]. Previous reports indicate that infections due to *Moraxella* species and *Acinetobacter* species generally occur in patients with defective immunity [8, 9], and these organisms are generally considered as opportunistic pathogens [10]. None of the six patients with such infections who we studied had nosocomial infections. To our knowledge, only one case of a moraxella infection has been previously described in a complement (C4)-deficient patient (table 1). Our patient had had a ventriculoatrial shunt implanted 10 years before the infection.

Infection associated with ventriculoatrial shunts occurs in 0 to 38% of cases [26]. Most shunt infections (70%) are associated with the surgical implantation of drainage systems. *Staphylococcus aureus* and *Staphylococcus epidermidis* are predominantly isolated in such infections [25–27]. Infections not associated with surgical procedures are caused relatively often by gram-negative strains [28]. The frequency of glomerulonephritis in association with a ventriculoatrial shunt is rather low (0.9%) [24]. The most common clinical features of shunt nephritis are fever and hematuria in association with moderate proteinuria, anemia, and splenomegaly. Circulating immune complexes, as measured by the C1q binding test, are nearly always present [24], and typically C3 and C4 levels are decreased.

In our study, two patients with a ventriculoatrial shunt infection due to *Moraxella* species or *Acinetobacter* species had hematuria and other signs suggestive of shunt nephritis at the time of the onset of meningitis. Immunologically, the low C1, C4, C2, and C3 levels and the positive C1q binding test support the diagnosis of shunt nephritis and represent an acquired complement deficiency. Whether the low complement levels increased the susceptibility for infection due to these uncommon strains or whether the levels were due to meningitis cannot be answered. However, the occurrence of *M. osloensis* meningitis in an otherwise healthy C8β-deficient patient favors the hypothesis that low complement levels, either acquired or congenital, increase the risk for meningitis due to such microorganisms.

Our findings suggest that inherited or acquired complement deficiency is frequently associated with meningitis due to nongroupable meningococci, *Moraxella* species, and *Acinetobacter* species. The recommendation that such patients should be tested for complement deficiency seems justified.

**Acknowledgments**

The authors thank all patients and medical doctors for their cooperation in the study.

**References**


