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# Characterization of Strains of *Neisseria meningitidis* Recovered from Complement-Sufficient and Complement-Deficient Patients in the Western Cape Province, South Africa

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**Complement deficiency has been associated with increased susceptibility to meningococcal disease. In order to determine whether special meningococcal strains caused disease in complement-deficient (CD) patients, 17 *Neisseria meningitidis* strains recovered from patients in the Western Cape Province, South Africa, known to be CD were compared with 124 routine isolates obtained from patients living in the same area. Serogrouping of the strains from the CD subjects revealed that the common serogroups, particularly serogroup B, predominated. However, the prevalence of rare serogroups among isolates from CD subjects was significantly higher than that found among isolates from the control group. Sero- and subtyping of the class 1 and class 2 or 3 outer membrane proteins showed no significant difference between isolates from CD subjects and the routine clinical isolates. Multilocus enzyme electrophoresis of the 141 isolates revealed six clusters of electrophoretic types (ETs) and two unrelated ETs. The same degree of genetic diversity existed in ETs of isolates from CD subjects and the control group. However, the ET-5 complex, which is known to be associated with epidemic disease, was found in 22 (18%) of the routine clinical isolates but in none of the isolates from the CD subjects. This difference was marginally significant. What was highly significant was the finding that 8 of the 17 isolates from CD subjects were in one ET cluster, cluster F, which comprised a total of 20 isolates. Thus, our results show a difference in the clonal compositions of the strains that infect CD subjects in comparison with the clonal compositions of those that cause clinical infections in the population at large.**

Deficiencies in certain complement components in humans have been recognized for some time as being responsible for increased susceptibility to *Neisseria meningitidis* infections (17). The deficiencies particularly associated with these infections are (i) deficiencies of terminal complement components (30), (ii) deficiencies associated with markedly reduced levels of the third component of complement (C3) such as factor I deficiencies or the presence of nephritic factor (28), and (iii) deficiencies of the alternative complement pathway components, especially properdin (24). Terminal component deficiencies are responsible for by far the largest number of reported cases (9), although the frequencies of different deficiencies are subject to regional variations (14, 30). The terminal components comprise the components C5 through C9, which, following activation of C5, combine together to form the multimolecular complex that is capable of penetrating cell membranes and producing cellular lysis. Infections in deficient individuals in Europe and the United States have been reported sporadically. Epidemiological data on organisms recovered from complement-deficient (CD) individuals are now accumulating. However, because the studies have been performed in Western countries, the data have frequently been drawn from areas where meningococcal disease is relatively rare.

A number of epidemiological tools have been used to investigate the distribution and spread of meningococcal disease. Serogrouping on the basis of the antigens of the polysaccharide capsule is the most frequently used test in routine clinical investigations and has often been the only epidemiological test applied to isolates from CD subjects. The reports from the United States (21), The Netherlands (10, 27), and Denmark (14) have demonstrated that the infections in CD individuals have frequently been caused by organisms with unusual polysaccharide capsules; in addition, infections with nonencapsulated bacteria have also been reported (12).

Classification of *N. meningitidis* according to the antigenic reactivities of the outer membrane proteins is a powerful epidemiological tool. Within this system, class 2 or 3 proteins are responsible for serotype determination (11) and the class 1 proteins are responsible for subtype determination (2). Sero- and subtyping assays have led to the identification of strains associated with clinical disease and virulence (18), while a variety of sero- and subtypes have been found in carrier strains (3).

Multilocus electrophoresis of meningococcal metabolic enzymes provides a system for characterizing the chromosomal genomes and a means of estimating the genetic relatedness of strains. It therefore allows the study of the clonal relationships of isolates (6) and the clonal spread of disease (4). It has been used to show differences between strains from patients and

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carriers (5) and thus is potentially very useful for investigating the relative virulences of organisms that infect CD hosts.

In Western Cape Province, South Africa, deficiency of the sixth component of complement (denoted as C6D) is relatively frequent (16). Moreover, *N. meningitidis* infections are still endemic to the region, with an average incidence of 20 cases per 100,000 population per year, rising to more than 100 cases per 100,000 population per year in epidemic periods (19). In the present study serogrouping, sero- and subtyping, and multilocus enzyme electrophoresis were used to examine the epidemiological relationship between isolates obtained from the population at large and those from CD individuals in the Cape Town, South Africa, area.

## MATERIALS AND METHODS

**Routine clinical isolates.** Specimens for routine clinical investigations of meningococcal disease were initially cultured in the Pathology Laboratories serving the relevant hospitals in the Western Cape Province, including the greater Cape Town area as well as rural hospitals. Positive cultures were sent to the South African Institute of Medical Research for serogrouping. From these, 124 isolates collected from 1985 to 1990 (approximately 20 per year) were randomly selected and sent to the World Health Organization Collaborating Centre for Reference and Research on Meningococci, National Institute of Public Health, Oslo, Norway, for multilocus enzyme electrophoresis analysis and to The Netherlands Reference Laboratory for Bacterial Meningitis, University of Amsterdam, to confirm the serogroups and for sero- and subtyping.

Limited personal data were available for most ($n$ = 106) patients. Fifty-six percent of the patients were male, the median age of the infected patients was 2 years, but the age range of the patients was 0.1 to 58 years. Twenty-nine isolates had been obtained during prospective studies of complement function of all patients with meningococcal disease admitted to the City of Cape Town Hospital for Infectious Diseases during two 3-month periods in 1985 and 1989. Complement function was found to be normal in these 29 subjects, who made up the subset of complement-sufficient patients. In this subset 39% were male, the median age of the infected patients was 3 years, and the age range was 0.1 to 58 years.

**CD patients.** Data for 17 episodes of infection in 13 CD subjects were included in the study. Eight of these subjects were male. All except one of the subjects had C6D; the remaining one had a subtotal C3 deficiency (less than 5% of the control level of native C3) secondary to the presence of nephritic factor, and she had suffered recurrent meningococcal infections.

Clinical details of the CD patients have been reported elsewhere (16, 19). Briefly, the complement deficiencies were ascertained because of meningococcal infections. The episodes of infection were bacteriologically confirmed meningitis, sometimes with septicemia (19), as in the complement-sufficient patients. Three of the infections caused by the isolates described here were primary, 13 were recurrent infections (second to fifth episodes), and one was unknown. The patients lived in the greater Cape Town area or the rural Western Cape; there were no marked economic, racial, or social differences between these patients and the 29 subjects in the complement-sufficient group for whom we had data, and we have no reason to believe that these 29 patients differed in this respect from the larger group from whom the remaining routine clinical isolates were obtained. All 141 isolates were obtained from patients as part of the routine investigations of suspected meningococcal disease. The CD patients and the 29 complement-sufficient subjects all recovered from their diseases. The one marked difference between the CD subjects and those who provided the routine clinical isolates (including the complement-sufficient subset) was that those in the former group were older; the median age of the subjects with infections described here was 19 years (range, 9 to 36 years), and the median age of those with primary infections was 11 years (9, 11, and 16 years). This older age of the infected subjects is in keeping with what has been reported for CD patients with infections in the United States (9) and Europe (10) as well as Cape Town (16). Three different isolates were obtained from one subject with C6D and two each were obtained from two other subjects with C6D. All but one of the isolates were obtained in the same period as the routine clinical isolates (1985 to 1990); one isolate was obtained from a patient with C6D late (October) in 1984. We attempted to investigate all possible isolates from CD patients, and all such isolates that were available were sent to the laboratories in Oslo and Amsterdam for the same studies that the routine clinical isolates underwent. However, we were limited by the number of isolates that had originally been sent to the South African Institute of Medical Research. Thus, the total number of strains available was less than the number of episodes of infection in CD patients that have been reported for this period (16).

**Serogroup, serotype, and subtype determinations.** The initial routine grouping was carried out in Cape Town by using meningococcal agglutination sera (Wellcome, Beckenham, United Kingdom). These recognized polysaccharide antigens A, B, C, X, Y, Z, and W135. Serogrouping results were confirmed at the Reference Laboratory for Bacterial Meningitis, Amsterdam, The Netherlands, by using double-diffusion Ouchterlony and rabbit antisera produced at the Meningococcal Reference Laboratory (25). Sero- and subtyping were done by using the whole-cell enzyme-linked immunosorbent assay method (1) and monoclonal antibodies. The anti-class 2 or 3 antibodies recognized serotypes 1, 2a, 2b, 4, 14, 15, and 16, and the anti-class 1 antibodies recognized subtypes P1.1, P1.2, P1.4, P1.6, P1.7, P1.9, P1.10, P1.14, P1.15, and P1.16. In addition, some isolates were tested with an anti-P1.5 antibody.

**Electrophoresis of enzymes.** Methods for starch gel electrophoresis and selective enzyme staining were similar to those described by Selander et al. (23). The 14 enzymes assayed were malic enzyme, glucose 6-phosphate dehydrogenase, peptidase, isocitrate dehydrogenase, aconitase, NADP-linked glutamate dehydrogenase, NAD-linked glutamate dehydrogenase, alcohol dehydrogenase, fumarase, alkaline phosphatase, indophenol oxidases 1 and 2, adenylate kinase, and an unknown dehydrogenase.

Electromorphs of each enzyme, numbered in order of decreasing anodal mobility, were equated with alleles at the corresponding structural gene locus. Numerical allele designations were cognate with those previously recorded in *N. meningitidis* (6). Each isolate was characterized by its combination of alleles at the 14 enzyme loci, and distinctive multilocus genotypes were designated electrophoretic types (ETs). ETs were numbered sequentially according to the position in the dendrogram (Fig. 1). With the exception of ET-5, ET numbers were not cognate with those assigned previously (6).

**Statistical analyses.** Genetic diversity ($h$) at an enzyme locus among ETs was calculated as $h = (1 - \Sigma x_i^2)\,[n/(n-1)]$, where $x_i$ is the frequency of the $i$th allele and $n$ is the number of ETs. Mean genetic diversity ($H$) is the arithmetic average of $h$ values over all loci. The genetic distance between pairs of ETs was expressed as the proportion of enzyme loci at which dissimilar alleles occurred (mismatches), and clustering was performed

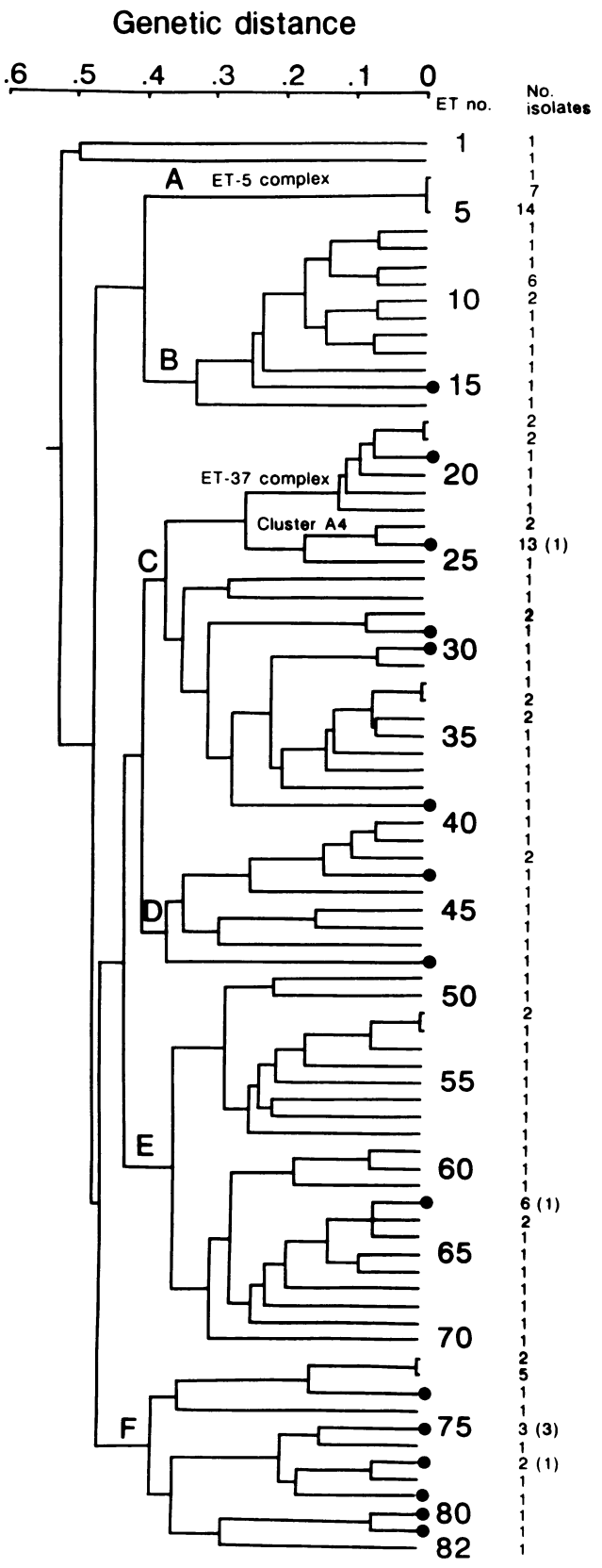


FIG. 1. Genetic relationships among 82 ETs of *N. meningitidis* isolates recovered from patients with or without complement deficiencies in Cape Town. The dendrogram was generated by the average-linkage method of clustering from a matrix of coefficients of genetic distance on the basis of 14 enzyme loci. ETs are numbered sequentially from top to bottom. ETs of isolates from individuals with complement deficiencies are marked by a solid circle. Letters A through F indicate clusters of ETs that diverge at genetic distances of less than 0.40. Three clusters of ETs of epidemiological importance that have been described previously (5, 6) are also indicated. The number of isolates of each ET is indicated, with the number of isolates from CD patients if the ET was represented by more than one isolate given in parentheses.

from a matrix of genetic distances by the average-linkage method (26).

## RESULTS

Table 1 presents the ET, serogroup, and sero- and subtypes of all 141 strains. Examination of the serogrouping results showed that of 17 infections in CD subjects, 10 (59%) were infections with common serogroups (8 serogroup B and 2 serogroup C), 6 (35%) were with rare serogroups Y or W135, and one was nonserogroupable (NG). Serogroup B isolates predominated, although the rare serogroups (W135, Y, and NG) together were responsible for 41% of the infections in CD individuals. Among the routine clinical isolates, rare serogroups were indeed seldom seen prior to 1989 and accounted for only three (two serogroup W135 and one serogroup X) of 75 strains. However, during 1989 and 1990 rare serogroups accounted for 6 of 49 routine clinical isolates and 6 of the 8 isolates from CD patients. The difference between the frequency of W135, Y, and NG serogroups in isolates from CD patients and routine clinical isolates was highly significant ($P < 0.001$ by chi-square analysis) for the whole study as well as for the period 1989 and 1990 ($P < 0.001$ by chi-square analysis) but was not significant for the period from 1985 to 1988.

Serogrouping together with sero- and subtyping showed the most common antigenic combinations among the routine isolates to B:4:P1.15 and C:2b:P1.2, and these accounted, respectively, for 17 and 8 of the 121 routine isolates that were sero- and subtyped. Among the isolates from CD patients, these antigenic combinations were represented by one isolate each.

Analysis of the sero- and subtyping results for the routine isolates and the isolates from CD patients showed a slightly higher frequency of nonserotypeable strains among those recovered from CD individuals (76%) in comparison with the frequency among routine isolates (54%; $P < 0.1$ by chi-square analysis). The frequency of nonsubtypeable strains was also higher among the isolates from CD patients (35%) in comparison with the frequency among routine clinical isolates (17%; $P < 0.1$ by chi-square analysis). When the sero- and subtyping results could be ascribed, there were no statistically significant differences between the results for the isolates from the two patient groups.

In the collection of 141 isolates, multilocus enzyme electrophoresis showed that 1 (indophenol oxidase 1) of the 14 enzyme loci was monomorphic and that 13 enzyme loci were polymorphic for 2 to 10 alleles. The average number of alleles per locus was 4.7 (Table 2). A total of 82 distinctive multilocus genotypes were identified. Because of the amount of data, the actual allelic combinations are not tabulated but are available from the corresponding author upon request. Among the 82 ETs the mean genetic diversity per locus was 0.446. The 124 routine clinical isolates belonged to 70 ETs, while the 17 isolates from CD patients represented 15 ETs (Table 1 and Fig. 1). The dendrogram in Fig. 1 consists of six major clusters

TABLE 1. Characteristics of 141 isolates of 82 ETs of *N. meningitidis* recovered from complement-sufficient and CD patients in the Western Cape Province, South Africa

| ET | Serogroup | Serotype:subtype[a] | Yr of isolation |
|---|---|---|---|
| 1 | B | 2b:P1.2 | 1988 |
| 2 | B | NT:– | 1986 |
| 3 | B | 4:P1.15 | 1988 |
| 4 | B | 4:P1.15 (3) | 1986–1989 |
| 4 | B | ND | 1987 |
| 4 | B | 4:P1.12 (2) | 1989 |
| 4 | B | 4:P1.4 | 1990 |
| 5 | B | 4:P1.15 (10) | 1986–1989 |
| 5 | B | 4:P1.12 | 1988 |
| 5 | B | NT:P1.15 (2) | 1985 |
| 5 | B | 4:P1.14, 15 | 1986 |
| 6 | B | NT:P1.2 | 1989 |
| 7 | B | 15:P1.2 | 1987 |
| 8 | B | NT:P1.9 | 1985 |
| 9 | B | NT:– (3) | 1985–1987 |
| 9 | B | NT:P1.2 | 1987 |
| 9 | B | NT:P1.12 | 1989 |
| 9 | B | NT:P1.5, 2 | 1989 |
| 10 | B | NT:P1.2 | 1986 |
| 10 | B | NT:P1.5, 2 | 1990 |
| 11 | B | NT:P1.2 | 1986 |
| 12 | B | 4:P1.14 | 1987 |
| 13 | B | NT:P1.5, 2 | 1989 |
| 14 | B | NT:P1.1 | 1986 |
| **15**[b] | **B** | **NT:–** | **1987** |
| 16 | B | 14:P1.14 | 1988 |
| 17 | W | 2a:P1.2 | 1986 |
| 17 | W | 2a:P1.5, 2 | 1990 |
| 18 | B | 2a:P1.2 (2) | 1985 |
| **19** | **W** | **2a:P1.2** | **1989** |
| 20 | B | 2a:P1.2 | 1986 |
| 21 | B | 2a:P1.2 | 1988 |
| 22 | B | 2a:P1.2 | 1986 |
| 23 | C | 2b:P1.2 | 1986 |
| 23 | C | 2b:P1.5, 2 | 1990 |
| **24** | **C** | **2b:P1.2** | **1988** |
| 24 | C | 2b:P1.2 (2) | 1987–1989 |
| 24 | C | 2b:P1.7, 1 | 1989 |
| 24 | C | 2b:– | 1989 |
| 24 | C | 2b:P1.5, 2 (4) | 1990 |
| 24 | C | 2b:P1.6 | 1990 |
| 24 | B | 2b:P1.5, 2 (2) | 1990 |
| 24 | NG | ND | 1989 |
| 25 | B | 2b:P1.2 | 1989 |
| 26 | B | 4:P1.15 | 1986 |
| 27 | B | NT:P1.1 | 1986 |
| 28 | W | NT:– | 1990 |
| 28 | W | ND | 1990 |
| **29** | **W** | **NT:P1.6** | **1990** |
| **30** | **B** | **NT:P1.12** | **1984** |
| 31 | B | NT:P1.6 | 1986 |
| 32 | B | 4:P1.15 | 1989 |
| 33 | C | NT:P1.9 (2) | 1989 |
| 34 | B | 4:– | 1985 |
| 34 | B | NT:– | 1986 |
| 35 | W | NT:P1.6 | 1986 |
| 36 | B | NT:P1.7 | 1987 |
| 37 | B | 15:– | 1986 |
| 38 | B | 1:– | 1987 |
| **39** | **B** | **4:P1.15** | **1986** |
| 40 | B | 16:P1.5, 2 | 1989 |
| 41 | B | NT:P1.2 | 1986 |
| 42 | B | NT:P1.2 | 1987 |
| 42 | B | NT:P1.5, 2 | 1989 |
| **43** | **B** | **NT:–** | **1990** |
| 44 | B | NT:P1.2 | 1985 |
| 45 | B | 2a:P1.14 | 1988 |
| 46 | B | NT:P1.2 | 1988 |
| 47 | C | 15:P1.15 | 1988 |
| **48** | **B** | **NT:P1.2** | **1986** |
| 49 | B | NT:P1.2 | 1986 |
| 50 | B | NT:– | 1985 |
| 51 | B | NT:P1.7, 14 | 1985 |
| 51 | B | NT:– | 1986 |
| 52 | B | NT:P1.6 | 1987 |
| 53 | B | NT:– | 1987 |
| 54 | B | NT:– | 1986 |
| 55 | B | NT:P1.6 | 1987 |
| 56 | B | NT:P1.5 | 1990 |
| 57 | B | NT:P1.6 | 1989 |
| 58 | B | NT:– | 1985 |
| 59 | B | NT:P1.2 | 1988 |
| 60 | B | 2b:– | 1988 |
| 61 | B | NT:P1.7, 1 | 1989 |
| **62** | **B** | **NT:P1.2** | **1987** |
| 62 | B | NT:P1.7 | 1985 |
| 62 | B | NT:– (4) | 1985–1989 |
| 63 | B | NT:P1.2 | 1985 |
| 63 | B | NT:– | 1986 |
| 64 | B | NT:– | 1989 |
| 65 | B | NT:– | 1988 |
| 66 | B | NT:– | 1989 |
| 67 | B | NT:P1.2 | 1988 |
| 68 | A | NT:P1.3 | 1990 |
| 69 | B | 2a:– | 1987 |
| 70 | B | NT:P1.2 | 1989 |
| 71 | A | 4:P1.10 | 1988 |
| 71 | A | 21:P1.10 | 1989 |
| 72 | A | NT:P1.10 (5) | 1985–1990 |
| **73** | **B** | **4:P1.7, 1** | **1990** |
| 74 | X | NT:P1.2 | 1985 |
| **75** | **Y** | **NT:– (2)** | **1985–1989** |
| **75** | **Y** | **NT:P1.5, 2** | **1990** |
| 76 | B | NT:P1.16 | 1986 |
| **77** | **Y** | **NT:P1.2** | **1990** |
| 77 | Y | NT:P1.5, 2 | 1990 |
| 78 | Y | NT:P1.5 | 1990 |
| **79** | **NG** | **NT:P1.5** | **1990** |
| **80** | **C** | **NT:–** | **1988** |
| **81** | **B** | **NT:–** | **1985** |
| 82 | B | NT:P1.5, 2 | 1990 |

[a] ND, not done; NT, nonserotypeable; –, nonsubtypeable. The number of isolates, if more than one, with the same characteristics are indicated in parentheses.

[b] Information on strains from CD patients is indicated in boldface type.

of two or more closely related ETs that diverged at a genetic distance of less than 0.40 (designated by the letters A through F) and two ETs (ET-1 and ET-2) that individually had no close relationship to the other ETs. The genetic diversity among the ETs of isolates recovered from patients with complement deficiencies ($H = 0.444$) was very similar to that among the ETs of isolates from other patients ($H = 0.439$). Of the 17 ETs identified more than once, 3 ETs (ET-24, ET-62, and ET-73) were found among both routine strains and strains from CD subjects, and three isolates, all from CD subjects, were ET-75. ETs 3 through 5, represented by 22 isolates, were clones of the ET-5 complex. Remarkably, none of the strains from patients with complement deficiencies belonged to the ET-5 complex ($P < 0.05$ by the Fischer exact probability test). ETs 17 through

TABLE 2. Genetic diversity in 82 ETs of 141 isolates of *N. meningitidis* from patients with or without known complement deficiencies

| Enzyme locus[a] | No. of alleles | Genetic diversity in ETs of isolates from: | | |
|---|---|---|---|---|
| | | CD patients ($n$ = 15) | Complement-sufficient patients ($n$ = 70) | All patients ($n$ = 82) |
| ME | 6 | 0.810 | 0.658 | 0.687 |
| G6P | 5 | 0.552 | 0.571 | 0.567 |
| PEP | 8 | 0.790 | 0.771 | 0.775 |
| IDH | 10 | 0.895 | 0.866 | 0.870 |
| ACO | 3 | 0.362 | 0.559 | 0.536 |
| GD1 | 2 | 0.133 | 0.325 | 0.303 |
| GD2 | 4 | 0.457 | 0.306 | 0.341 |
| ADH | 4 | 0.248 | 0.350 | 0.341 |
| FUM | 3 | 0.000 | 0.188 | 0.162 |
| ALK | 9 | 0.762 | 0.804 | 0.810 |
| IP1 | 1 | 0.000 | 0.000 | 0.000 |
| IP2 | 4 | 0.514 | 0.404 | 0.429 |
| ADK | 3 | 0.000 | 0.084 | 0.072 |
| UDH | 4 | 0.695 | 0.261 | 0.359 |
| Mean | 4.7 | 0.444 | 0.439 | 0.446 |

[a] Enzyme abbreviations: ME, malic enzyme; G6P, glucose 6-phosphate dehydrogenase; PEP, peptidase; IDH, isocitrate dehydrogenase; ACO, aconitase; GD1, NADP-linked glutamate dehydrogenase; GD2, NAD-linked glutamate dehydrogenase; ADH, alcohol dehydrogenase; FUM, fumarase; ALK, alkaline phosphatase; IP1 and IP2, indophenol oxidases 1 and 2; ADK, adenylate kinase; UDH, an unknown dehydrogenase.

22 represented clones of the ET-37 complex (5), while ETs 23 through 26 belonged to cluster A4 (6). These two clone complexes have been shown to be responsible for significant numbers of infections in South Africa in the late 1970s to early 1980s (7). Among the 20 isolates belonging to cluster F (ET-71 through ET-82), 8 were from among the 17 isolated from CD patients (odd ratio, 8.3; statistical significance, $P < 0.0001$). The results for isolates of the ET-5 complex and cluster F have been discussed selectively because they are the two most significant results; however, this type of selection could result in an overestimate of the significance of our findings (13). To compensate for multiple tests of significance, the significance ($P$) can be multiplied by the number of groups tested (i.e., six ET clusters). This procedure abolishes the significance of the negative association between ET-5 strains and isolates from CD individuals, but the positive association of isolates from CD individuals with ET cluster F remains highly significant.

## DISCUSSION

Limited data were available for most of the subjects from whom the routine clinical isolates were obtained. In order to establish whether the subjects from whom the routine clinical isolates were obtained were representative of the complement-sufficient population as a whole, we tested the subset of isolates from 29 subjects who had been shown to be complement sufficient. The CD subjects were markedly older at the time of infection than either the 29 known complement-sufficient patients or the remainder of the group of subjects from whom the routine isolates were obtained. The distribution of serogroups among the isolates from the 29 complement-sufficient subjects was not significantly different from that of the serogroups among the remainder of the routine isolates (data not shown). Fijen and coworkers (10) found that a high proportion of individuals clinically infected with organisms of serogroups W135 or Y when they were 10 years old or older were CD. However, in the present study only two of the subjects from whom seven routine serogroup Y or W135 isolates were obtained were over 10 years of age (four subjects were less than 1 year old and no information was available for the other subject). In studies of complement deficiency in patients from the Cape with primary meningococcal infection, only one case of complete complement deficiency was found among 123 patients studied (16). Thus, these factors taken together indicate that if there were any CD individuals among those from whom the routine clinical isolates were obtained, they would make up a low proportion of the whole group of 124 subjects.

Workers in the United States (9, 21) and The Netherlands (10, 27) have reported that CD patients tend to suffer clinical *N. meningitidis* infections with organisms bearing unusual serogroups. Our study confirms these observations in part, in that rare serogroups were significantly more common among isolates from CD individuals than among the routine clinical isolates. However, examination of Table 1 shows that of 17 infections in CD subjects, 10 (59%) were infections caused by isolates with common serogroups and that the most prevalent single serogroup was B. Meningococcal infections remain endemic in the Cape at about 20 cases per 100,000 population per year, whereas the incidence in The Netherlands reported by Fijen and coworkers (10) at the time of the study was 2.7/100,000 population per year. In the United States in 1986 there were approximately 1.8 meningococcal infections per 100,000 individuals (29). The higher background rate of infection in the Cape indicates that members of the Cape population are at higher risk of infection. The high exposure level to serogroup B organisms in the Cape might counterbalance the factors that operate to favor serogroup Y or W135 infections in CD individuals. A recent report from Italy (8) also found that meningococcal disease in subjects with deficiencies of the late-acting complement components was caused by strains of the serogroup that was responsible for the majority (61%) of cases of disease in complement-sufficient subjects. In this instance serogroup C predominated, and it was the only serogroup identified among organisms that caused infections in the CD subjects, even though serogroup Y organisms were most commonly found among healthy carriers in Italy.

Another factor leading to differences in the serogroups isolated from CD patients in different countries may lie in differences in the nature of the deficiencies themselves. Properdin deficiency has been found relatively frequently in

northern European countries (9, 14, 25) and has also been found to be associated with serogroup W135 and Y infections (11); this implicates the importance of the alternative complement pathway in the host defense against infections with organisms with these rare serogroups. However, properdin deficiency has not been found in the Cape population. Another observation, made by Ross and coworkers (22), was that serogroup B organisms are susceptible to opsonophagocytosis, whereas the serogroup Y polysaccharide (and probably the related W135 polysaccharide) renders organisms relatively resistant to opsonophagocytosis. This may at least partly explain the higher prevalence of serogroup Y and W135 infections in our patients.

With regard to the NG isolates, it is not possible to ascertain whether these strains were nonencapsulated in vivo because the assays were done after the strains had been freeze-dried, and freeze-drying could be associated with capsule loss (15). One of these isolates was from a complement-sufficient subject and was ET-24 (belonging to the A4 cluster). We thus believe that our failure to serogroup this strain may well have been because of technical factors. However, the other was from a CD subject and was found to be NG both in The Netherlands and Norway, and a nonencapsulated strain is known to have caused clinical disease in twin infants with C6D (12). Thus, this strain could also well have been nonencapsulated in vivo.

It has previously been suggested that primary infection in CD individuals may predispose those individuals to further meningococcal infection (16). We therefore considered the possibility that the properties of the isolates responsible for primary infection might differ from those that cause recurrent infections. Unfortunately, of the 17 isolates listed in Table 1, only 3 were known to be associated with primary infection. Two were serogroup B and one was NG, and there are insufficient data from which to draw any conclusions. The other, even more important, question is whether CD patients tend to be infected more than once with organisms of the same serogroup and that carry the same potentially protective subcapsular antigens. If this is true, the immune system has only a very limited protective role in these patients. However, it may be that what appears to be recurrent infections are really new infections with organisms of different serogroups and different outer membrane proteins (22), and these infections are a manifestation of profoundly increased susceptibilities of CD subjects to clinical meningococcal infections. If this is the case the immune system does protect against reinfection with the same organisms. It is very difficult to obtain sufficient data to answer this question conclusively, although the answer might have a profound influence on the management of these patients.

There were higher proportions of nonserotypeable and nonsubtypeable strains among the isolates from CD individuals than among the routine clinical isolates. However, the prevalence of infections with nonserotypeable strains was also high among the routine clinical isolates (54%). Thus, the class 2 or 3 antigens of a number of pathogenic strains in South Africa have not yet been identified. It is therefore difficult to analyze the differences between the isolates from the two patient groups, but no significant differences in the identifiable class 1 and class 2 or 3 outer membrane proteins were observed. Little information has yet been reported on the sero- or subtypes of meningococcal isolates from U.S. or European CD subjects. Ross and coworkers (20) demonstrated that there was no difference in susceptibility to bactericidal killing between isolates from CD and complement-sufficient individuals; they concluded that suggestions that strains that would normally only be associated with carrier status tend to cause disseminated disease in CD individuals are probably incorrect. Unfortunately, sero- and subtyping are not particularly efficient in indicating isolates that would be nonvirulent in complement-sufficient individuals.

Multilocus enzyme electrophoresis, on the other hand, has been shown to be a highly sensitive method that can be used for tracing clones and differentiating between epidemic strains and carrier strains (5, 6). The studies of the case strains presented here failed to show any difference in the genetic variabilities of strains from complement-sufficient and CD patients. Isolates of the ET-5 complex have been found to be associated with epidemic disease (4). In the present study, ETs 3 through 5, represented by 22 routine isolates, were clones of the ET-5 complex, but none of the strains from patients with complement deficiencies belonged to the ET-5 complex. This difference is marginally statistically significant, and the results do suggest a difference in the susceptibility pattern. CD subjects may develop clinical disease because of exposure to the organism they are most likely to encounter, even if it has low virulence potential. In a situation in which there is a high rate of carriage of virulent strains, there will be little difference in the strain composition infecting complement-sufficient patients and those with complement deficiencies, while if the virulent strains have a low carriage rate, it might explain why they seldom cause disease in patients with complement deficiencies. Although relatively little is known concerning the carriage rate of virulent clones, several studies have shown that ET-5 has a low carriage rate, low transmissibility, but a high level of virulence (3, 5). This might explain why ET-5 was not found among the isolates obtained from patients with complement deficiencies.

The increased frequency of isolates from CD individuals belonging to the cluster F was highly significant. The eight relevant isolates were of serogroups B ($n = 2$), Y ($n = 4$), NG ($n = 1$), and C ($n = 1$); thus, it is not possible to ascribe the association of cluster F with strains from CD individuals only to the properties of a particular polysaccharide capsule. The fact that only 12 of 124 (<10%) routine isolates belonged to this F cluster may reflect the fact that cluster F strains are less virulent in complement-sufficient individuals. Further work on the properties and epidemiology of organisms belonging to the F cluster needs to be done. Unfortunately, no strains were taken from South Africans who were carriers at the time of our study. In any event, the data presented here suggest that the CD patients develop overt disease with less common meningococci, whether determined by serogrouping or multilocus enzyme electrophoresis. Whether there are differences in virulence and the reasons for such differences remain to be elucidated.

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