Recent emergence of serogroup C meningococcal disease in Greece

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Abstract

The number of cases of meningococcal disease reported to the Meningitis Reference Laboratory in Athens rose dramatically in 1996–1997. The aims were (1) to determine if the increase was due to introduction of new strains, (2) to assess the geographic and age distribution of the cases, (3) to compare antibiotic sensitivity patterns of the current isolates with strains from the early 1990s. In 1993–1994, 151/19 (74%) of the cases for which information on age was available were in children ≤5 years; in 1995–1997, 80/179 (45%) of cases were in children ≤5 years and 99 (55%) in the older age range (P < 0.02). From 593 cases in 1993–1997, 214 (36%) isolates were available for characterisation. Serogroup B was predominant in the early 1990s but by 1997, serogroup C accounted for 46/72 (64%) of isolates and serogroup B for 25/72 (35%). Serogroup B was predominant in children ≤5 years (44/78, 56%) but only 19/99 (18%) of older children and adults (P = 0.0000005). Sulfonamide resistance decreased from 10/22 (45%) in 1993–1994 to 27/92 (14%) in 1995–1997 (P < 0.01). Multilocus enzyme electrophoresis of 70 strains obtained during this period identified the epidemic ET-15 clone in 24 (34.3)%. The profiles of the Greek ET-15 isolates were identical to C:2a:P1.2(P1.5) strains responsible for the epidemic in the Czech Republic which began in 1993. This genotype was not found in Greek strains isolated prior to 1993. We conclude that the increase in meningococcal disease is due to introduction of the epidemic serogroup C:2a:P1.2(P1.5) strain responsible for disease in the Czech Republic and Canada. © 1999 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

During the past 2 years, the number of cases of meningococcal disease due to Neisseria meningitidis reported to the National Meningitis Reference Laboratory has risen dramatically in Greece. This prompted an assessment of the phenotypic and genotypic characteristics of the strains isolated from patients to determine if
there was a parallel with the rapid increase in numbers of meningococcal disease due to serogroup C reported in the Czech Republic [1].

Previous studies on meningococcal disease and carriage in Greece (1989–1992) found only sporadic cases, primarily due to serogroup B. Most isolates did not react with the current serotype and subtype reagents [2], and among the minority that were typeable, the predominant serosubtype combination was 2b:Pl.10. There was also a high proportion of isolates with reduced susceptibility to penicillin (48%) as well as resistance to sulfonamides (16%) in the same period [3].

The aims of the present study were: (1) to determine if the increased numbers of cases were associated with introduction of new strains of meningococci; (2) to assess the distribution of the isolates among different age groups and geographic regions in Greece; (3) to compare antibiotic sensitivities of strains isolated in the early 1990s with those obtained during the past 3 years.

2. Materials and methods

2.1. Source and identification of isolates

In Greece, cases of meningococcal diseases are required by law to be reported to the Ministry of Health; however, the information regarding age and classification of the disease (e.g., meningitis with or without septicaemia) is often incomplete. The diagnosis of meningococcal disease was made on the basis of symptoms and identification of the bacteria in blood or cerebrospinal fluid by microscopy and/or culture. Collation of this information and analysis of the isolates is carried out by the Meningitis Reference Laboratory, National School of Public Health in Athens. The material examined in this study included cases from 1993 to 1997 from all areas of Greece and patients ranged in age from 1 month to 65 years. There were 214 isolates from 593 cases reported (36%); 20 were from isolates obtained during 1993–1994 and 194 from cases occurring in 1995–1997.

The results were compared with data from studies published previously on carriage among military recruits (1990–1991), school children in Athens (1993–1994) and immigrant groups (1995) [2–5]. To obtain more recent information on strains of meningococci in the population, in June 1998 250 military recruits from all regions of the country were screened for carriage of meningococci; 67 isolates obtained from this group were characterised. All isolates were grown on chocolate blood agar and characterised by Gram stain, oxidase test and by the rapid carbohydrate utilisation test (Gallerie Pasteur).

2.2. Determination of phenotype

Serogroups were determined by slide agglutination with polyclonal antisera to serogroups A, B, C, W-135, X, Y, and Z (Wellcome Diagnostics). Serotype and subtype were determined by whole-cell enzyme-linked immunosassay (WCE) with monoclonal antibody reagents supplied by Dr. J.T. Poolman (RIVM, Bilthoven, The Netherlands) [2]. The monoclonal antibody designated ‘22’ was also included in the present study [4,6]. Serotype and subtype of 70 isolates sent for genotypic characterisation were assessed in the reference laboratory in Prague by WCE with a second set of monoclonal antibody reagents supplied by NIBSC, Potters Bar, UK.

2.3. Antibiotic sensitivities

Sensitivities to penicillin (PG), rifampicin (RF), ciprofloxacin (CI), cefazidime (TZ), cefaclor (CF), erythromycin (EM), chloramphenicol (CL), ceftriaxone (TX), sulfadiazine (SU), tetracycline (TC) and cefotaxime (CT) were determined by the E-test (AB Biodisc, Solna, Sweden). Classification of resistance was based as before on the recommendations of the working party for the British Society for Antimicrobial Chemotherapy [7]. The break point of ≥10 mg l⁻¹ for sulfamethoxazole was that used in our previous study [3] recommended by the meningococcal reference laboratory in Scotland (the late Dr. R.J. Fallon, personal communication).

2.4. Enzyme electrotypes (ET) determination

Genotypic analysis of 70 isolates was carried out by multilocus enzyme electrophoresis (MLEE) at the meningococcal reference laboratory in Prague. Enzyme electrotypes (ET) were determined by MLEE.
as described previously [8,9]. The following enzymes were assayed: malic enzyme (ME; EC 1.1.1.40), glucose 6-phosphate dehydrogenase (G6P; EC 1.1.1.49), leucine aminopeptidase (PEP; EC 3.4.11.1), isocitrate dehydrogenase (IDH; EC 1.1.1.42), aconitase (ACO; EC 4.2.1.3), glutamate dehydrogenase (NADP-dependent) (GD1; EC 1.4.1.4), glutamate dehydrogenase (NAD-dependent) (GD2; EC 1.4.1.4), alcohol dehydrogenase (ADH; EC 1.1.1.1), fumarase (FUM; EC 4.2.1.2), alkaline phosphatase (ALP; EC 3.1.3.1), and adenylate kinase (ADK; EC 2.7.4.3).

3. Results

3.1. Meningococcal disease in Greece 1993–1997

Between 1993 and 1997, 593 cases of meningococcal disease were reported to the National Meningitis Reference Laboratory in Athens: 55 in 1993, 70 in 1994, 111 in 1995, 133 in 1996 and 224 in 1997 (Fig. 1). This reflected an increase in incidence per 100 000 of the population: 0.57 in 1993; 0.83 in 1994; 1.31 in 1995; 1.57 in 1996; and 2.65 in 1997. The numbers of deaths reported to the reference laboratory also rose sharply from 1996 to 1997: 3 in 1993; 2 in 1994; 2 in 1995; 3 in 1996; 16 in 1997. During 1997 when the greatest rises in cases and deaths were noted, there were 27 cases of septicaemia and localised outbreaks of meningococcal disease: 4 cases in a recruit camp in Thrace; 2 cases in a recruit camp in the Peloponnese; 4 cases within 10 days in a kindergarten in Thessaly.

3.2. Age ranges affected by the increase in meningococcal disease

Information on age was not available for all cases; however, for those cases for which data were available, there was a significant shift in the age groups affected. In 1993–1994, 14 of the 19 cases (74%) were in children aged 5 years or younger. Among the 179 cases reported in 1995–1997, 80 (45%) were in the younger age range with 99 (55%) older than 5 years of age ($\chi^2 = 5.46, P < 0.02$) (Fig. 2).

3.3. Serogroups of strains

From the 593 cases reported, 214 (36%) isolates were sent to the reference laboratory for characterisation, 22 from the period 1993–1994 and 192 from the period 1995–1997. The serogroups of the meningococcal strains isolated during this period are illustrated in Fig. 3. Serogroup B had been the predominant serogroup from 1990 [2]. From 1994, there was an increase in the proportion of serogroup C and a reduction in the proportion of serogroup B isolates. When the numbers of cases started to increase in 1995, the proportions of group B (16/32, 50%) and
group C (15/32, 47%) strains were similar. In 1996, the proportion of group C isolates remained constant (44/88, 50%), but the proportion of B isolates was reduced (31/88, 35%). In this year there were also 4 cases due to serogroup W135, 2 to serogroup Y; isolates from 6 cases were non-groupable. A further increase in serogroup C was observed among the 1997 isolates, accounting for 46/72 (63.9%) with a slight reduction of serogroup B isolates 25/72 (34.7%) (Fig. 3).

Analysis of the serogroup distribution by age found that serogroup B was the predominant isolate among the age group 1 month to 5 years: 44/78 (56%) compared with 19/99 (18%) in older children and adults ($\chi^2 = 25.25$, $P = 0.0000005$). In the older age ranges, particularly the 16-20-year group, serogroup C was the most frequent cause of disease (Fig. 4).

### 3.4. Serotypes of the isolates

Until 1996, there was no predominant serotype: 40% of the strains were not typable with the available monoclonal antibody reagents. The serotypable isolates were distributed mainly among the serotypes 2b, 2a, 15 and 22. During 1996, 2a became the most common serotype accounting for 36.5% (32/88) of the strains. There was a further increase in the 1997 isolates, to 59.7% (43/72). During 1996 and 1997 there was a decrease of the non-typable strains to 31.8% and 20.8% respectively. There were only six 2a isolates among the 81 (7%) obtained from 1993-1995 compared with 48 of the 133 (36%) obtained between 1996-1997 ($\chi^2 = 20.14$, $P < 0.0001$); of the six 2a isolates from the earlier period, four were isolated in 1995.

### 3.5. Serosubtypes of the isolates

Before 1995, there were only 3/22 (13.6%) isolates that were subtype P1.2 or P1.5. This rose to 81/192 (42%) in the 1995-1997 period ($\chi^2 = 5.60$, $P < 0.02$). During 1996 subtype P1.2 increased to 22.1% and subtype P1.5 to 16.3%. By 1997 these two serotypes were predominant, with 30.5% of the isolates typable with the monoclonal against P1.2 and 25% with P1.5.

The predominant serogroup:serotype:subtype combination from 1996 was C:2a:P1.2(P1.5), and this phenotype was isolated from 20/27 (74%) of the 1997 cases in which septicaemia was reported.

### 3.6. Geographic distribution of the C:2a:P1.2,5 isolates

The C:2a:P1.2,5 phenotype was found in all but one area of the country (Corfu) and comprised 43-50% of all group C isolates. The highest proportions
of the new phenotype were found in northern areas, Macedonia (11/24, 46%), Thrace (3/6, 50%), Thessaly (3/6, 50%) and Epirus (9/19, 47%). In Athens and the Peloponnese there were slightly lower proportions of the new phenotype, 37/85 (43.5%) and 11/25 (44%) respectively. Among the six group C isolates from Crete, only 2 (33%) were C:2a:P1.2,5. The survey of military recruits in 1998 found only 2/67 (3%) of the strains with this phenotype: one from Macedonia and the other from the Aegean Islands.

3.7. Electotypes of the isolates

Electrotypes were determined for 70 strains, isolated in 1993–1997. MLEE analysis revealed the epidemic ET-15 clone (a member of the ET-37 clonal complex) in 24/70 (34.3%) of the strains. The enzyme profiles of the Greek ET-15 isolates were identical to those obtained for strains responsible for the epidemic in the Czech Republic which began in 1993. The most frequent phenotype among the ET-15 strains was C:2a:P1.2 (9 strains) and C:2a:P1.2,P1.5 (8 strains), followed by C:2a:NST (4 strains), C:NT:P1.2 (2 strains), and Y:2a:P1.2, P1.5 (1 strain) (Table 1).

3.8. Antibiotic sensitivities

All strains were sensitive to cefaclor, ceftriaxone, chloramphenicol, cefotaxime and ciprofloxacin, erythromycin and tetracycline. The proportion of penicillin-insensitive strains isolated in the 1993–1994 period was 1/22 (4%) compared with 13/192 (6.7%) for the 1995–1997 period. Only one rifampicin-resistant isolate was detected among the 214 strains tested. There was, however, a significant decrease in sulfonamide resistance from 10/22 (45%) in the 1993–1994 period to 27/192 (14%) in 1995–1997 ($\chi^2 = 11.5$, $P < 0.001$).

4. Discussion

Since there has been no major change in the meth-
ods for collecting information or isolates, the increase in the numbers of cases of meningococcal disease in Greece noted since 1995 appears to be due to introduction of the ET-15 clone with the predominant phenotype C:2a:P1.2,P1.5. The situation is similar to that reported for the Czech Republic and Canada [1,10].

In 1993 a previously unrecognised meningococcal clone appeared in the Czech Republic. The new strain was identified as C:2a:P1.2,5. There was a high age-specific morbidity in 15–19-year age range and a high case fatality (20%) with an atypical course with a high incidence of Waterhouse-Friderichsen syndrome and meningococcal sepsis [1,11]. In Greece there was also a sharp increase in numbers of deaths reported and in cases of septicaemia during 1997. Three quarters of the septicaemia isolates were infected with the C:2a:P1.2,5 strain.

In the Czech Republic, the incidence of meningococcal disease increased from 1.3/100 000 in 1993 to 1.9/100 000 in 1994 and 2.2/100 000 by 1995 [12]. An even greater increase in incidence was observed in the Greek population between 1996 (1.57/100 000) and 1997 (2.65/100 000). While targeted immunisation in the 15–19-year age group was used to control serogroup C disease in the Czech Republic [11], in Greece immunisation was carried out only in the kindergarten affected and vaccination trials are being carried out among recruits.

The Czech Republic, there was a change in the age group most affected to that of the 15–19-year age range and the emergence of the serogroup C strain. In Greece, there has been a significant increase in the age range >5 years, but no specific peak in the 15–19-year range.

Resistance to sulfonamide among meningococci was observed during the epidemics of World War II [13] and later with sporadic outbreaks in the United States [14]. In northern Europe, increased mortality rates were associated with sulfonamide-resistant strains [15,16] and this pattern was observed with all major serogroups [17]. In Greece, there has been a significant reduction in the proportion of strains resistant to sulfonamide. This could be due to the high proportion of group C strains and also adoption by the reference laboratory of the more accurate E-test method in 1995. In the Czech Republic, the proportion of strains resistant to sulfonamide was low even before the emergence of the new meningococcal clone [18], and no significant change in the proportion of sulfonamide-resistant strains was observed.

In our studies of meningococcal carriage, we did not identify the C:2a:P1.2(5) phenotype among recruits examined in 1990–1991 [2], school children in Athens during 1992–1994 [2], Russian immigrants to Greece in 1995 [5] or Kurdish immigrants (unpublished observations). The low carriage rate of the C:2a:P1.2(5) phenotype among the recruits in 1998 (0.8%) indicates that a virulent strain causing many cases of disease can be found in a low proportion of carriers.

Further evidence for recent introduction of the C:2a:P1.2(5) strain into Greece comes from the genetic analyses of the strains. In both Greece and the Czech Republic the new strain belonged to the ET-15 clone and the predominant phenotype was C:2a:P1.2,P1.5. Antigenic variations of ET-15 strains found in Greece are similar to those found in the Czech Republic [12]. The clone was absent in a collection of Greek strains isolated in 1989–1992 [19]. Three recent 1998 isolates expressed the phenotype B:2a.P1.2; however, these have not yet been examined for genotype to determine if they belong to the same clone.

We conclude that the increase in meningococcal disease reported to the Ministry of Health in Greece is due to the introduction of the virulent new clone C:2a:P1.2,P1.5 first noted in Canada and more recently in the Czech Republic.

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