EPIDEMIOLOGY

Meningococcal disease in The Netherlands, 1959–1981: the occurrence of serogroups and serotypes 2a and 2b of Neisseria meningitidis

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Summary

By means of a filter radioimmunoassay and the use of monoclonal anti-2a and anti-2b antibodies, we have serotyped 3164 of 3688 strains of Neisseria meningitidis isolated from patients in The Netherlands between 1959 and 1981. Serotypes 2a and 2b were distributed differently among the major serogroups A, B, C, and W-135. Neither of the types was found among group A strains. Type 2b strains of serogroup B emerged in 1965, causing a country-wide epidemic which reached a peak incidence in March and April of 1966 and continued to predominate within group B until 1979. Type 2a strains of serogroup C were responsible for a substantial number of sporadic cases over a long period without any association with outbreaks or with a shift in the pattern of the serogroup. After the appearance of group W-135 in 1971, W-135 strains caused a small non-focal epidemic wave. The upsurge of disease due to virulent sub-populations of strains B:2b and C:2a appeared to be closely related to a basic pattern of regular cyclical waves with peak intervals which differed for serogroups A, B, and C. In recent years both serotype 2a and 2b strains within the different serogroups fell to insignificant numbers. Our results show that retrospective large-scale serotyping of collected strains provides insight into the epidemiological patterns of endemic meningococcal disease.

Introduction

The epidemiology of endemic meningococcal disease is characterised by a low incidence (< four cases per 100,000 population) with recurrent rises every 6–10 years as was shown in Western Germany and the U.S.A. Changes in the distribution of the major serogroups (A, B, or C) may occur over the years, new serogroups being introduced (Y, W-135); geographical differences are found also. Furthermore, the various serogroups show differences in case-to-carrier ratio, in the age distribution of cases and possibly in fatality rate.

Sub-division of meningococci by serotyping on the basis of immunologically distinct outer membrane proteins (OMPs) and lipopolysaccharides gives more
insight into the epidemiology of meningococcal disease and may contribute to the understanding of serogroup-related differences.\textsuperscript{12–14} Frasch described 15 different serotypes,\textsuperscript{15} of which serotypes 2 and 15 (both OMPs) are associated with outbreaks of meningococcal disease, whereas other types were found only occasionally.\textsuperscript{14–18} In sporadic disease, many strains are not typable, although in various studies a predominance of serotype 2 has been reported.\textsuperscript{14, 18–20} A high case-to-carrier ratio has been noted for serotype 2 which may indicate a high degree of virulence.\textsuperscript{15}

Serotype 2 has recently been subdivided into three subtypes 2a, 2b, and 2c by Poolman and colleagues.\textsuperscript{14} Serotype 2a was found predominantly in group C, W-135, and Y strains, serotype 2b in group B strains and serotype 2c in group Y strains.\textsuperscript{14, 18}

As part of a longstanding survey of meningococcal disease in The Netherlands we present data about the occurrence of serotypes 2a and 2b during the period 1959–1981. More than 3000 isolates from cases were serotyped by the filter radioimmunoassay (FRIA), a method recently developed for large-scale serotyping and for which monoclonal antibodies are used.\textsuperscript{21}

Materials and methods

Strains

Isolates of \textit{Neisseria meningitidis} from 3688 cases with systemic meningococcal disease were submitted to our Reference Laboratory by the hospital and public health laboratories in The Netherlands during the period 1959–1981. During this period the population grew from 11.4 to 14.2 million inhabitants with an average density of about 380/km\(^2\) of land-area (Netherlands Central Bureau of Statistics). The strains were isolated from cerebrospinal fluid or blood, or from both of these. In addition, 432 strains randomly isolated from the nasopharynx, sputum, or genital tract of healthy non-case-contact carriers were collected during 1966–1975. Unless stated otherwise, these carrier strains were excluded from the data. All strains were subcultured from the various transport media on to Mueller Hinton agar supplemented with 1% (v/v) yeast extract (Difco), and incubated at 37 °C in a ‘candle jar’. Identification as \textit{N. meningitidis} was made on the basis of colony morphology, Gram stain, production of oxidase and catalase and acid production from glucose and maltose. The strains were stored at −70 °C in peptone broth containing 8% glycerol.

Antisera

Antisera against serogroups A, B, C, W-135, X, Y, Z, and Z‘ (29E) were prepared according to the method described by Slaterus\textsuperscript{22} by means of the reference strains previously described.\textsuperscript{23} Monoclonal antibodies to serotypes 2a and 2b were kindly provided by Dr W. D. Zollinger (Walter Reed Army Institute of Research, Washington, DC, USA).\textsuperscript{21}

Serogrouping and serotyping

The strains were serogrouped by agglutination and microprecipitation techniques.\textsuperscript{22} Serotyping was performed by the filter radioimmunoassay
(FRIA) simultaneously for the entire collection and included 201 controls (20 group A, 97 group B, 42 group C, 24 group W-135, and 18 group Y strains) scattered over the 3164 strains, as described elsewhere.21

Results

Occurrence of serogroups during 1959–1981

From 1959 to 1981 we collected 3688 isolates of N. meningitidis. These amounted to 83% of the cases notified during that period (Fig. 1). Since 1972, with the single exception of 1976, the yearly total of isolates exceeded the number of cases notified, thereby showing incompleteness of official notification. The incidence of meningococcal disease displayed a characteristic cyclical pattern with peaks in 1966, 1972, and 1978. In 1966 the incidence of notified cases reached 4.1/100000.24 Most cases occurred in the months of March and April (attack rate: 10.6/100000 extrapolated for the year), constituting an epidemic peak (Fig. 2).

Serogrouping revealed an overall predominance of serogroup B (mean 66.1%), with serogroups A and C each accounting for about 15% of the strains (Table I). Since 1971 group A strains have been on the increase with a concomitant decline of group B strains. The numbers of serogroup C rose during the last years studied. In addition to these trends, all three serogroups appeared to demonstrate regular cyclical waves with intervals between the peaks differing among the groups (Fig. 3). In this study, group B showed peaks every 6 years: 1966, 1972, and 1978, coinciding with the overall peaks (Fig. 1 and 3). Increases of group A strains were found every 7 years (1966,
Table I  Strains isolated from CSF, blood, or both, 1959–1981, according to serogroup

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>No. of strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>537 (14.6)</td>
</tr>
<tr>
<td>B</td>
<td>2437 (66.1)</td>
</tr>
<tr>
<td>C</td>
<td>564 (15.3)</td>
</tr>
<tr>
<td>W-135</td>
<td>98 (2.7)</td>
</tr>
<tr>
<td>X</td>
<td>7 (0.2)</td>
</tr>
<tr>
<td>Y</td>
<td>32 (0.9)</td>
</tr>
<tr>
<td>Non-groupable</td>
<td>13 (0.4)</td>
</tr>
<tr>
<td>Total</td>
<td>3688 (100.0)</td>
</tr>
</tbody>
</table>

Figures in parentheses denote percentages.

Fig. 2. Seasonal distribution of strains isolated (a) 1965–1967 and (b) general pattern 1959–1981. ■, Serotype B:2b; □, group B, other types; □, other groups.

1973, 1980), and group C peaked at 9-year intervals (1963, 1972, 1981). It should be noted that the comparison with absolute numbers of strains before 1966 is limited because of the incomplete numbers of strains submitted (Fig. 1). Serogroup W-135, first isolated in 1971, showed a peak in 1974 (21 cases) followed by a decline to only a few cases in recent years. Strains of serogroups X and Y and non-groupable strains were found very infrequently
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(altogether 1.5%). No strain belonging to serogroup Z or Z' (29E) was encountered.

Distribution of serotypes during 1959–1981

The results of screening 3164 of 3688 strains for the presence of serotypes 2a and 2b by FRIA are summarized in Table II. Serotype 2a predominated in serogroup W-135 (60%), and made up 21% of group C. It was less frequent in serogroup B (4%). Serotype 2b occurred to a substantial extent only within group B (44%). Taking into account the fact that the FRIA yielded a few false positive reactions, the total absence of both serotypes 2a and 2b among group A strains is notable.

The occurrence of serotypes 2a and 2b in each serogroup during the period studied is depicted in Fig. 3. Among serogroup B strains, serotype 2b (B:2b) showed a sudden rise in 1966 (1959–1964: <2.5%; 1965: 12.5%; 1966: 65%). This indicates that the appearance of this serotype was responsible for the epidemic in 1966 [Figs. 1, 2, 3(a)]. From the beginning of the epidemic, B:2b strains were isolated simultaneously in all parts of the country. In the early months of the epidemic neither focal outbreaks nor traceable evidence of spread could be detected. Figure 2(a) showing the seasonal incidence during 1965–1967 reveals that the majority (56%) of cases in March and April 1966 were due to B:2b strains. Other strains belonging to both serogroup B and other serogroups also increased during these months. The annual seasonal pattern for the period 1959–1981 is depicted in Fig. 2(b).

After 1966, serotype 2b gradually declined with lower peaks in 1972 and 1978 coinciding with the cyclical waves of other types within group B [Fig. 3(a)]. In 1981 only 14 serotype 2b strains (13%) were found within group B.
Table II  Strains isolated from CSF, blood, or both, 1959–1981, according to serotypes 2a and 2b within serogroups

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>Serotype 2a</th>
<th>Serotype 2b</th>
<th>Other</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3 (1)*</td>
<td>5 (1*)</td>
<td>473 (98)</td>
<td>481 (100)</td>
</tr>
<tr>
<td>B</td>
<td>78 (4)</td>
<td>916 (44)</td>
<td>1082 (52)</td>
<td>2076 (100)</td>
</tr>
<tr>
<td>C</td>
<td>100 (21)</td>
<td>25 (5)</td>
<td>344 (73)</td>
<td>469 (100)</td>
</tr>
<tr>
<td>W-135</td>
<td>56 (60)</td>
<td>3 (3)</td>
<td>34 (37)</td>
<td>93 (100)</td>
</tr>
<tr>
<td>Other</td>
<td>—</td>
<td>4 (9)</td>
<td>41 (91)</td>
<td>45 (100)</td>
</tr>
<tr>
<td>Total</td>
<td>237</td>
<td>953</td>
<td>1974</td>
<td>3164</td>
</tr>
</tbody>
</table>

Figures in parentheses denote percentages.
* When retested by immunoprecipitation, eight group A strains, which were positive by FRIA with anti-2a antiserum (three strains) and anti-2b antiserum (five strains) yielded negative results for both serotypes.

Table III  Distribution of strains isolated from cases and carriers, 1966–1975, in relation to serogroup and serotypes 2a and 2b

<table>
<thead>
<tr>
<th>Serogroup</th>
<th>Source</th>
<th>Serotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2a</td>
</tr>
<tr>
<td>A</td>
<td>Cases</td>
<td>2*</td>
</tr>
<tr>
<td></td>
<td>Carriers</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>Cases</td>
<td>49 (4)</td>
</tr>
<tr>
<td></td>
<td>Carriers</td>
<td>4 (4)</td>
</tr>
<tr>
<td>C</td>
<td>Cases</td>
<td>68 (30)</td>
</tr>
<tr>
<td></td>
<td>Carriers</td>
<td>3 (9)</td>
</tr>
<tr>
<td>W-135</td>
<td>Cases</td>
<td>47 (71)</td>
</tr>
<tr>
<td></td>
<td>Carriers</td>
<td>15 (56)</td>
</tr>
</tbody>
</table>

Figures in parentheses denote percentages.
* When retested by immunoprecipitation, four group A strains, which were positive by filter radioimmunoassay with anti-2a antiserum (two strains) and anti-2b antiserum (two strains) yielded negative results for both serotypes.

From the early 1960s serotype 2a was encountered in about 32% of serogroup C strains until 1975, falling to very low percentages in the period 1976–1981 [Fig. 3(d)]. Increases in the number of serotype 2a strains were observed during the peak years of group C.

Serotype 2a appeared to be highly associated with the greater numbers of serogroup W-135 strains during the 3 years after the first appearance of this serogroup [Fig. 3(b)].
Serotypes among carrier and patient strains during 1966–1975

Between 1966 and 1975, 432 non-case-contact carrier strains were serogrouped: 1·9 % were of serogroup A, 36·8 % of group B, 12·5 % of group C, 6·7 % of group W-135, 2·5 % of group X, 9·3 % of group Y, 3·2 % of group Z, 3·2 % of group Z' and 23·8 % were non-groupable.

Out of the 250 carrier strains belonging to serogroups A, B, C, and W-135, 177 strains were available for serotyping. Table III displays the distribution of serotypes 2a and 2b among these carrier strains. Comparison of serotyping results of these strains with patient strains collected during the same years, indicates that serotypes B:2b and C:2a were found significantly more often among patient strains (55 and 31 %, respectively) than among carrier strains (B:2b, 9 %; C:2a, 9 %). In serogroup W-135, during this period, serotype 2a was more equally distributed among patient and carrier strains.

Discussion

In this study, we used filter radioimmunoassay, a method very suitable for large-scale serotyping, in order to detect serotypes 2a and 2b in our collection of approximately 3500 strains of meningococci gathered in The Netherlands between 1959 and 1981.

The results of serotyping demonstrate different distributions for types 2a and 2b among the various major serogroups (A, B, C, and W-135). Type 2a and 2b antigens were not found among group A strains. This is consistent with other serotyping studies.

In group B, which predominated during the period of study, serotype 2b played a major role, whereas serotype 2a appeared infrequently. After a period in which few if any serotype 2b strains were detected, the emergence of B:2b strains in 1965 gave rise to an epidemic in The Netherlands in the following year. The appearance of B:2b strains coincided with a peak in the cyclical pattern of sporadic disease (Fig. 1). The spectrum of meningococcal strains in 1966 shows that serotype B:2b caused 56 % of all cases during the months of March and April, although other types and groups increased as well (Fig. 2). Regular cyclical waves with a different periodicity for serogroups A, B, and C were apparent (in this study, every 7, 6, and 9 years, respectively), resulting in 1966 in simultaneous peak incidences for groups A and B in addition to a minor elevation of group C strains. The concomitant rise of these strains hampered the recognition of an epidemic strain in a previous study of the epidemic. Moreover, serotyping methods were not then available. The pattern of cyclical waves, which may be observed for the various serogroups and serotypes within groups B and C [Fig. 3], strongly suggests a cyclical increase in susceptibility within the community. The sudden upsurge of meningococcal disease due to B:2b strains was probably enhanced by this overall susceptibility in 1966 as well as by a lack of type-specific immunity to this epidemic strain. The seasonal epidemic caused by B:2b strains related to the general annual seasonal incidence, a feature also reported for a group C epidemic in Brazil.

From the very beginning of the epidemic, B:2b strains were isolated from
cases in all parts of the country without substantial civilian or military clusters, and with no traceable evidence of spread of the epidemic. This feature suggests a high rate of transmission through healthy carriers. Case-to-carrier ratio was found in this study to be relatively low, as in other surveys of B:2b disease.\textsuperscript{17, 29, 30} Since the overall carrier rate in endemic areas varies between 5\% and 20\%, however, and since group B is often found among carrier strains (ranging from 25 to 50\%\textsuperscript{31, 32}) the calculated proportion of healthy carriers of B:2b strains in the community seems adequate for country-wide spread in The Netherlands within a short time. The rapid spread of B:2b strains described in this study does not seem to agree with the hypothetical model of Griffis,\textsuperscript{33} which is based on the assumption of slow spread due to faecal–oral transmission of cross-reacting enteric bacteria. These bacteria were postulated to increase susceptibility to meningococci by the induction of type-specific blocking serum IgA antibodies. The absence of any special recrudescence in the densely populated parts of the country argues against this theory of faecal–oral transmission. In addition, there was no evidence for the presence of a common (cross-reactive) serotype among the various serogroups of meningococci on the increase in 1966.

Another important finding is the fact that although the B:2b epidemic in 1966 affected the whole country in a short time, it did not spread from The Netherlands to the neighbouring countries of Belgium and Western Germany. In Belgium, a serotype B:2b outbreak began later in 1969;\textsuperscript{30} in Western Germany, any real epidemic has not yet been recognised.\textsuperscript{4} Similar observations were made for the Finnish group A epidemic in relation to other Scandinavian countries.\textsuperscript{34} Possibly, contacts might not be sufficiently close for such spread. In addition, cyclical waves of disease in The Netherlands and in Western Germany have not appeared at the same time,\textsuperscript{4} probably reflecting the different states of susceptibility of these communities.

The origin of B:2b strains is not clear. For the period up to 1965, the year of their appearance in The Netherlands, a serotype B:2b epidemic has not been reported in other countries. Because early serotyping studies\textsuperscript{5, 35} did not differentiate between serotypes 2b and 2a, of which the latter accounted for about 13\% of group B strains isolated in The Netherlands during 1959–1965 [see Fig. 3(a)], precise data concerning type B:2b strains in other parts of the world before 1965 do not exist. Besides the possibility that this type was introduced into The Netherlands from abroad, its emergence could be explained by mutation in a class 2 outer membrane protein.\textsuperscript{25, 36} Once mutated, the protein may have been stably expressed by the meningococcus, thereby allowing the serotype to persist within the community for a long time as found in this study for B:2b strains [Fig. 3(a)]. Whether serotype 2b originated from serotype 2a is an intriguing question, since the two relevant proteins were found by peptide mapping to be closely related.\textsuperscript{37} The question of an evolutionary change within classes of stably expressed OMPs may also be raised in the case of the epidemic serotype B:15, first described in northern Norway.\textsuperscript{16} After 1966, the number of B:2b strains (following the cyclical changes of group B) gradually fell to become very few during the last years of the study. Serotype B:15 did not appear to a substantial extent in The Netherlands until 1981 (unpubl. data).
Serotype 2a was mainly found within group C and group W-135. A small wave of group W-135 with a peak in 1974 was highly associated with type 2a strains. After the first 3 years the proportion of W-135:2a strains decreased. During the last years of this investigation group W-135 was encountered infrequently [see Fig. 3(b)].

Until 1975, serotype 2a accounted for about 30% of the group C patient strains, and was encountered significantly more often among patients than non-case-contact carriers. In contrast to the situation in the USA,7 there was not any shift in serogroup distribution in favour of serogroup C and outbreaks due to serotype C:2a strains were not observed. Although there was a rise in numbers of group C after 1977, as part of its cyclical pattern, the proportion of serotype 2a became unimportant.

This study shows that in sporadic meningococcal disease new serotypes may arise associated with certain serogroups (e.g. B:2b, W-135:2a). Changing incidences of these serotypes influence the epidemiological picture, particularly when they are associated with virulence (as B:2b). Epidemiological characteristics of sporadic meningococcal disease such as changes in serogroup prevalence, minor outbreaks, and geographical differences may be due largely to varying patterns of serotypes. The upsurge of disease due to virulent subpopulations of strains (B:2b, C:2a) appears to be closely related to the serogroup-specific cyclical patterns which characterise the overall picture of endemic meningococcal disease. The emergence and subsequent disappearance of virulent serotypes necessitates an epidemiological survey on a large scale in order to predict which OMP-vaccines38 may be efficacious, especially with respect to group B, because of the poor immunogenicity of the group B capsular polysaccharide.15

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References