Serogroup B, Electrophoretic Type 15 *Neisseria meningitidis* in Canada

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Invasive meningococcal disease is nationally reportable in Canada. In recent years, a serogroup C genotype, designated electrophoretic type 15 (ET15), has been the most frequently isolated meningococcal genotype in Canada and has caused epidemics across the country. Between August 1993 and September 1995, there were 9 cases of invasive meningococcal disease caused by a variant of this genotype, expressing group B capsular polysaccharide. The appearance of serogroup B:ET15 was related temporally and geographically to mass immunization campaigns designed to control serogroup C meningococcal disease in Canada. Since there is no vaccine available to control serogroup B meningococcal disease, the appearance of this variant may have public-health significance if it demonstrates the same epidemic potential as its serogroup C counterpart.

In Canada, invasive meningococcal disease (IMD) has been a major public-health issue during the last decade. From 1985 to 1993, there was an annual increase in the national incidence of IMD that was associated with the emergence of a new serogroup C clone. Multilocus enzyme electrophoresis placed this clone in the electrophoretic type (ET) 37 complex [1] but distinguished it by identification of a unique allelic variant for the enzyme fumarase [2]. Isolates belonging to this clone, designated ET15, were predominantly serotype 2a, subtypes P1.2,5. From 1988 to 1993, the percentage of all laboratory-confirmed invasive meningococcal disease in Canada attributable to serogroup C:ET15 increased from 2.0% to 51.8%. Epidemiologically, ET15 was associated with a significant increase in IMD incidence among persons 5–19 years old and a significant increase in the case fatality rate for all ages [3]. In response to this increase in serogroup C disease, there were 9 mass immunization campaigns (defined as >20,000 people immunized in a campaign) against meningococcal disease in Canada between 1992 and 1994. Serogroup C:ET15 has also been increasingly responsible for epidemic and endemic disease in the United States [4], England and Wales [5], and the Czech Republic [6].

In contrast to serogroup C:ET15, which has caused several community and institutional outbreaks [7], serogroup B *Neisseria meningitidis* has been responsible primarily for endemic disease in Canada. From 1987 to the present, most typeable serogroup B meningococcal isolates have been serotype 15 or 4 [8]. Here, we describe the epidemiologic and microbiologic characteristics of 9 cases of invasive serogroup B, serotype 2a, ET15 *N. meningitidis* found in Canada since 1993. This genotype may be especially interesting to public-health officials, because of its emergence following mass immunization to control serogroup C:ET15 disease, its potential for epidemic disease (given the behavior of its serogroup C counterpart), and the lack of an effective vaccine for serogroup B *N. meningitidis*.

**Methods**

In Canada, IMD is nationally notifiable according to standardized case definitions [9]. The Laboratory Centre for Disease Control (LCDC) maintains a national database that correlates case-by-case epidemiologic reports with results from laboratory evaluation. The epidemiologic reports include the date of disease onset and the patient’s age, sex, residence (health unit), clinical syndrome, and outcome.

All sterile-site meningococcal isolates are forwarded to the LCDC for serogrouping, serotyping, and serosubtyping at the National Laboratory for Bacterial Diseases [10]. Multilocus enzyme electrophoresis and pulsed-field gel electrophoresis, as described previously [2, 7], are used routinely to characterize all serogroup C meningococcal isolates as well as serogroup B isolates that are suspected of belonging to the ET37 complex.

**Results**

From 1985 to the present, there have been 9 cases of invasive infection with serogroup B:ET15 *N. meningitidis* in Canada (table 1). All cases occurred between August 1993 and September 1995. Eight (89%) of the isolates came from the provinces of Quebec (6 isolates) and Saskatchewan (2 isolates). None of the cases were epidemiologically linked. Two of the isolates were from patients in the 5–19 years age group. Three isolates were recovered from blood and 5 from cerebrospinal fluid. There was one fatality, a 3-month-old child. Two patients in Quebec (cases 5 and 6) had been previously immunized with an A/C bivalent meningococcal vaccine as part of a mass immunization campaign. In cases 3 and 9, the patients had never been immunized. The immunization status of the remaining 5
Table 1. Cases of invasive disease caused by serogroup B, electrophoretic type 15 \textit{N. meningitidis} in Canada.

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Province</th>
<th>Date of onset (d/m/y)</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Vaccine status</th>
<th>Outcome</th>
<th>Site of isolate</th>
<th>Serogroup: subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Québec</td>
<td>24/8/93</td>
<td>20</td>
<td>M</td>
<td>U</td>
<td>Survived</td>
<td>CSF</td>
<td>B:2a:P1.±</td>
</tr>
<tr>
<td>2</td>
<td>Québec</td>
<td>13/9/93</td>
<td>48</td>
<td>F</td>
<td>U</td>
<td>Survived</td>
<td>CSF</td>
<td>B:2a:P1.2,5</td>
</tr>
<tr>
<td>3</td>
<td>Saskatchewan</td>
<td>2/11/93</td>
<td>3 m</td>
<td>M</td>
<td>N</td>
<td>Died</td>
<td>Blood</td>
<td>B:2a:P1.2,5</td>
</tr>
<tr>
<td>5</td>
<td>Québec</td>
<td>14/12/94</td>
<td>4</td>
<td>M</td>
<td>Y</td>
<td>Survived</td>
<td>CSF</td>
<td>B:2a:P1.2,5</td>
</tr>
<tr>
<td>6</td>
<td>Québec</td>
<td>11/1/95</td>
<td>2</td>
<td>F</td>
<td>Y</td>
<td>Survived</td>
<td>CSF</td>
<td>B:2a:P1.2,5</td>
</tr>
<tr>
<td>7</td>
<td>Québec</td>
<td>15/2/95</td>
<td>56</td>
<td>F</td>
<td>U</td>
<td>Survived</td>
<td>Blood</td>
<td>B:2a:P1.2,5</td>
</tr>
<tr>
<td>8</td>
<td>Saskatchewan</td>
<td>23/2/95</td>
<td>19</td>
<td>F</td>
<td>U</td>
<td>Survived</td>
<td>Blood</td>
<td>B:2a:P1.2,5</td>
</tr>
<tr>
<td>9</td>
<td>Québec</td>
<td>30/9/95</td>
<td>2</td>
<td>M</td>
<td>N</td>
<td>Survived</td>
<td>CSF</td>
<td>B:2a:P1.2,5</td>
</tr>
</tbody>
</table>


patients was unknown, although 2 would not have been eligible to receive publicly funded meningococcal vaccine as part of any immunization campaign because of age (case 3, 48 years, exceeding maximum age of 20 years) or location (case 4, from a population in a nonimmunized area of New Brunswick). The remaining 3 patients with unknown vaccine status would have been eligible for publicly funded vaccine because of their ages and locations of residence.

All of the B:ET15 isolates were serotype 2a. Seven were subtype P1.2,5 and 2 were nonsubtypeable (P1.±).

In 1993, 1994, and 1995 there were, respectively, 393, 361, and 305 cases of invasive meningococcal disease in Canada, corresponding to yearly incidences of 1.4, 1.3, and 1.0/100,000, respectively. Of all laboratory-confirmed cases during this period, 39% were caused by group B strains, of which 36.4% were nonserotypeable, 28.7% serotype 4, 16.8% serotype 15, 8.3% serotype 14, 3.4% serotype 2a, and 2.1% serotype 2b. Serogroup C strains accounted for 48.6% of all laboratory-confirmed cases during the same time period. Among these, 87.9% were serotype 2a, 8.9% were nonserotypeable, and 3.2% were other serotypes, including 14, 15, 4, and 2b. Between 1993 and 1995, ET15 accounted for 93% of all serogroup C isolates in Canada.

Discussion

This report describes the appearance, in Canada, of a variant form of a prevalent invasive serogroup C meningococcal genotype (C:2a:P1.2,5:ET15), that instead expresses serogroup B capsular polysaccharide.

Meningococcal strains that are genetically similar to each other but express serogroup B or C capsular polysaccharide antigens have also been described in countries other than Canada [1, 11, 12]. The repeated observation of such variants at different times and places and in different chromosomal backgrounds suggests that they arise frequently in the bacterial population, as meningococcal strains carrying genes specifying different capsular polysaccharides co-colonize the human nasopharynx. The rate at which this occurs has yet to be estimated. In the US Pacific Northwest, capsular switching from serogroup B to C was described among a group of ET5-complex isolates during a 1994–1995 outbreak. Molecular genetic analysis of these isolates suggested that the capsule-switching phenomenon resulted from genetic transformation and recombination at a locus within the capsule-biosynthetic (\textit{syn}) operon [12]. The C-to-B capsular switch reported here is the reverse of this B-to-C switch, but analogous genetic events may have preceded the appearance of B:ET15 isolates in Canada.

Two general types of explanation could be advanced for the recent appearance of these B:ET15 strains. The first invokes a fitness advantage for B-encapsulated variants of a prevalent C-encapsulated genotype. This could occur if the B-encapsulated variants were subject to less intense selection pressure from the immune systems of the population of human hosts, because they carry a polysaccharide antigen that is both different from that of the more prevalent group C strains (to which many potential hosts would already possess antibodies) and less immunogenic [13]. The resulting fitness advantage of the B variants may be modest. However, because the background chromosomal genotypes (here represented by the 2a:P1.2,5:ET15 array of markers) of the two capsular variants would be extremely similar, one might expect the B and C forms to occupy very similar ecologic niches. Thus, one might reasonably expect that whatever factors have led to the recent rapid increase in prevalence of C:ET15 in Canada [3] might also apply to B:ET15, and perhaps that intergenotypic competition between B:ET15 and its C:ET15 counterpart contributed to the appearance of the former.

We also raise the question of whether the recent appearance of invasive disease due to serogroup B:ET15 strains in Canada may be related partly to the widespread use of meningococcal vaccine in response to epidemics of C:ET15 (figure 1). Elementary population genetics theory indicates that the probability of escape from random loss of a new, selectively advantageous
cases of IMD to occur and to be noticed. We plan to test this alternative one effect of mass vaccination might have been to intensify a moderately high rate of transformation and recombination at increase if it does escape such random loss, is directly propor- have caused their recent rise in frequency among cases of IMD, identi®ed after mass immunization in Que Âbec and Saskatche- pothesis, using a molecular population-genetic approach [12].

canadian health units and communities, while Que Âbec and Saskatchewan health of®cials should consider when planning targeted or mass C:ET15. Though circumstances differed in each province prior possible relationship with mass immunization. The potential provinces of British Columbia or Ontario, which used smaller- ued surveillance will be necessary to document further this.

Figure 1. Percentage of all cases of laboratory con®rmed meningo-
coccal disease attributable to serogroup C, electrophoretic type 15 (ET15) in Canada between 1987 and 1996 is shown (solid line) in relation to appearance of cases of serogroup B:ET15 isolates (open bars). Period during which mass immunization campaigns occurred in Canada is bracketed by arrows.

generic variant in a population, as well as its rate of frequency increase if it does escape such random loss, is directly propor- to the magnitude of its selective advantage [14]. Thus, one effect of mass vaccination might have been to intensify selection pressure past a critical threshold, such that the frequency of B:ET15 variants in the nasopharyngeal meningococcal population rose to a point where they began to appear in cases of IMD.

It is suggestive that 7 of 9 invasive B:ET15 isolates were identified after mass immunization in Québec and Saskatchewan, provinces that had carried out the largest campaigns. Québec immunized all persons 6 months to 19 years old (~1,613,000 people) between December 1991 and May 1993, and Saskatchewan immunized all persons 2–19 years old (~258,000 people) in November and December 1993. By providing a large segment of the population with antibody against serogroup C capsular polysaccharide, such immunization could have been another factor influencing the relative fitnesses of closely related strains of N. meningitidis carrying different capsular antigens. It is also noteworthy in this context that invasive B:ET15 strains have not yet been identi®ed in the populous provinces of British Columbia or Ontario, which used small-scale mass immunization campaigns to control epidemic C:ET15. Though circumstances differed in each province prior to immunization, the latter two provinces targeted speci®c health units and communities, while Québec and Saskatchewan provided vaccine to large segments of entire provincial populations.

Another form of selection-based explanation is that vaccination might have in®luenced the representation of B:ET15 strains among those causing IMD, not because of direct competition between meningococcal genotypes in the nasopharyngeal environment but because of natural or vaccine-induced protective immunity to serogroup C invasive disease. It is important to point out, first, that at least 2 and likely 4 of the 9 patients in reported cases of B:ET15 disease did not receive vaccine (see Results). This would mean that vaccine-induced immunity of these individuals per se cannot be a direct contributing factor in their having contracted IMD due to B:ET15 meningococci. Second, although such protective immunity may indeed have contributed to the recent decline in the overall incidence of group C disease in Canada (®gure 1), it is not clear how this factor alone could explain the recent appearance of the B:ET15 variants. If the latter had been present at signi®cant frequencies in the meningococcal population before vaccination, one would expect them to have been observed at that time. However, it should also be clear that it is not possible at present to distinguish conclusively between explanations based on selection acting in the nasopharynx and those based on protective immunity to invasive disease.

Finally, despite the apparent plausibility of a selection-and/or competition-based explanation for the appearance of B:ET15 in Canada, alternative scenarios should also be consid- ered. Foremost among these is the possibility that insuf®cient selective advantage of any kind accrues to B:ET15 variants to have caused their recent rise in frequency among cases of IMD, and that the latter e®ect is simply what one would expect, given a moderately high rate of transformation and recombination at the capsular-polysaccharide locus and high prevalence of a well-studied and closely observed genotype (C:ET15) on which selectively neutral B variants would be most likely eventually to occur and to be noticed. We plan to test this alternative explanation against the above-mentioned selection-based hypo-thesis, using a molecular population-genetic approach [12].

A switch from serogroup C:ET15 to serogroup B:ET15 may be especially signi®cant from a public-health perspective, given the epidemic behavior of C:ET15 and because no vaccine exists against the B capsular polysaccharide. None of the B:ET15 isolates have occurred as part of an epidemic or cluster, and there have been no new cases of invasive B:ET15 infection since September 1995. This suggests that B:ET15 does not have the epidemic potential of C:ET15, at least in Canadian host populations at this time. However, comparisons of the epidemiologic behavior of serogroup B:ET15 and serogroup C:ET15 are limited by the small number of isolates, and continued surveillance will be necessary to document further this behavior in Canada and elsewhere, as well as to study the possible relationship with mass immunization. The potential for capsular switching may be one of the many factors public- health officials should consider when planning targeted or mass immunization for epidemic or endemic meningococcal disease.

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References