Potential capsule switching from serogroup Y to B: The characterization of three such *Neisseria meningitidis* isolates causing invasive meningococcal disease in Canada

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Three group B *Neisseria meningitidis* isolates, recovered from meningococcal disease cases in Canada and typed as B:2c:P1.5, were characterized. Multilocus sequence typing showed that all three isolates were related because of an identical sequence type (ST) 573. Isolates typed as 2c:P1.5 are common in serogroup Y meningococci but rare in isolates from serogroups B or C. Although no serogroup Y isolates have been typed as ST-573, eight isolates showed five to six housekeeping gene alleles that were identical to that of ST-573. This suggested that the B:2c:P1.5 isolates may have originated from serogroup Y organisms, possibly by capsule switching.

Key Words: *Capsule switching;* Neisseria meningitidis; *Serogroup Y*

Commutation potentielle de la capsule du sérogroupe Y à B : Caractérisation de trois isolats de *Neisseria meningiditis* ayant causé une maladie méningococcique invasive au Canada

Trois isolats de *Neisseria meningiditis* du groupe B typés B:2c:P1.5, prélevés chez des sujets atteints de maladie méningococcique au Canada ont été caractérisés. Le typage des séquences multilocus a montré que les trois isolats étaient apparentés en raison d'un type de séquence identique (TS) 573. Les isolats typés 2c:P1.5 sont communs dans les méningocoques du sérogroupe Y, mais rares dans ceux des sérogroupes B ou C. Bien qu'aucun isolat du sérogroupe Y n'ait été typé comme TS-573, huit isolats ont montré de cinq à six allèles de gène domestique identiques à ceux du TS-573. Cela donne à penser que les isolats B:2c:P1.5 peuvent avoir trouvé leur origine dans des organismes du sérogroupe Y possiblement par commutation des capsules.

Neisseria meningitidis is a significant pathogen that causes invasive meningococcal disease (IMD). The average case fatality rate of 9% to 12% remains high despite the availability of effective antibiotics and vaccines (1). Laboratory study and surveillance of *N meningitidis* involves the characterization of a number of surface markers of the bacterium, including its capsule and outer membrane proteins (OMPs). Most epidemiological studies of meningococcal disease rely on differentiating meningococcal isolates based on their serogroup, serotype and serosubtype. Serogrouping is determined by the demonstration of serologically distinct epitopes present on chemically and structurally different capsules. Serotyping and serosubtyping rely on the detection of distinct epitopes present on three of five different classes of OMPs of *N meningitidis*. Serotyping epitopes are found on the class 2 or class 3 OMP (also called PorB) of *N meningitidis*; these OMPs are expressed in a mutually exclusively manner (ie, a strain will only express either a class 2 or class 3 OMP but not both). Serosubtyping epitopes are present on the class 1 OMP (also called PorA). Based on this nomenclature scheme, a strain can therefore be characterized by its antigenic formula; for example, B:15:P1.7,16 refers to serogroup B, serotype 15 and serosubtype P1.7,16.

One of the most important virulence factors of meningococci is the capsular polysaccharide antigen, which is also the basis for serogrouping and is the target antigen for the currently licensed vaccines against A, C, Y and W135 organisms. Of the 13 known serogroups, five (serogroups A, B, C, Y and W135) are responsible for most of the meningococcal disease worldwide (2). In North America, most endemic and epidemic strains belong to serogroups B, C, Y and W135 (3,4). Capsules of serogroups B, C, Y and W135 meningococci contain sialic acid, either as a homopolymer of sialic acids assembled by alpha-2,8 linkages (serogroup B) or alpha-2,9 linkages (serogroup C), or as a heteropolymer of sialic acids with glucose (serogroup Y) or galactose (serogroup W135). Besides demonstrating structural similarities, these four serogroups of meningococci also have very similar capsule polysaccharide synthesis (*cps*) gene loci (5). Because of this similarity, capsule switching has been demonstrated in vivo and in vitro by specific gene replacement within the *cps* loci between different

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serogroups. To date, a number of IMD cases have been described in the literature to be caused by organisms in which capsule switching between serogroup B and C meningococci occurred (6-8).

In the present paper, the authors describe three unusual serogroup B meningococci isolated from separate IMD cases in Nanaimo, British Columbia, that presented with the OMP antigens 2c:P1.5, characteristic of serogroup Y strains found in Canada (4). This antigenic profile prompted the authors to examine the relationship of these three serogroup B strains with antigenically similar serogroup Y organisms isolated in Canada. The authors describe the characterization of these antigenically similar isolates and postulate that the B:2c:P1.5 isolates arose by capsule switching from serogroup Y organisms.

MATERIALS AND METHODS

Brief description of three unusual serogroup B isolates and serogroup Y meningococci

Two meningococcal isolates (Nanaimo 1 and Nanaimo 2) were recovered from related IMD cases (15- and 49-year-old males from the same household) in Nanaimo, a community of 80,000 located on Vancouver Island, British Columbia, in January 2001. The third isolate (Nanaimo 3) was recovered from an unrelated case from the same city that involved a 16-year-old boy who presented to the Nanaimo Regional General Hospital (Nanaimo, British Columbia) in January 2002. Two of the isolates (Nanaimo 2 and Nanaimo 3) were recovered from blood cultures, and the third isolate (Nanaimo 1) was cultured from a cerebrospinal fluid specimen. An outbreak of meningococcal disease in Nanaimo has never been documented, and the vaccination practice in this area does not differ from other areas in the province.

Serogroup Y meningococci were selected from the culture collection of IMD isolates from Health Canada's National Microbiology Laboratory (Winnipeg, Manitoba). These isolates were submitted from provincial and territorial public health laboratories in Canada between 1999 and 2003.

Identification, serogrouping, serotyping and serosubtyping of meningococci

N meningitidis isolates were identified using standard biochemical tests. The serogroup antigens were determined by bacterial agglutination using rabbit serogrouping antisera at the British Columbia Centre for Disease Control (Vancouver, British Columbia) and were confirmed by monoclonal antibody at the National Microbiology Laboratory. Serotyping and serosubtyping were performed by indirect whole-cell ELISA using monoclonal antibodies.

Sequencing of *porB* and *porA* genes to determine the PorB and PorA variable region types

Partial sequences of *porB* and *porA* genes were determined by polymerase chain reaction amplification and sequencing of the variable regions (VR) according to methods described by Sacchi et al (9,10).

Multilocus sequence typing

Multilocus sequence typing (MLST) was performed according to methods described in the *Neisseria* MLST Web site (11).

Pulsed-field gel electrophoresis

DNA fingerprinting by pulsed-field gel electrophoresis (PFGE) of genomic DNA after digestion with the restriction enzyme *NheI* and data analysis were performed as essentially described by Tyler and Tsang (12) with the following minor modifications: 20 h run time; initial switch time of 5.3 s; and final switch time of 34.9 s.

RESULTS

Three *N meningitidis* isolates (Nanaimo 1, Nanaimo 2 and Nanaimo 3) from IMD cases in Nanaimo were typed by both polyclonal and monoclonal antibodies to be serogroup B, with the serotype antigen 2c and the serosubtype antigen P1.5. DNA sequencing of their *porB* genes confirmed that they were serotype 2c because their PorB VR types were identified as C, 2c, 2bb and Db for their VR1 to VR4 regions, respectively (9), showing 100% identity with that of the serotype 2c prototype strain 2396, B:2c:P1.5,2 (GenBank accession number U92911). The PorB VR amino acid sequences of these three B:2c:P1.5 isolates were identical to the sequence of the variable loop V.5 described in the PorB typing database for *N meningitidis* (13). DNA sequencing of the *porA* genes showed that these three strains were identical in terms of their VR1 and VR2 regions. Their PorA VR1 types were 5a (the deduced amino acid sequence was 100% identical to that of strain 2996, B:2b:P1.5,2 [GenBank accession number X57180]) and their PorA VR2 types were 10d (the deduced amino acid sequence was 100% identical to that of strain 2413 as described by Sacchi et al [10]). The PorA VR1-5a and VR2-10d were given the designations of 5-1 and 10-4, respectively, according to the nomenclature for *N meningitidis* PorA variable region database (13).

MLST

The three B:2c:P1.5 strains (Nanaimo 1, Nanaimo 2 and Nanaimo 3) gave an identical MLST profile – sequence type (ST) 573 – which further showed that they were related and probably belonged to the same clone. For comparison, the authors also performed MLST on a sample of serogroup Y isolates that had the serotype and serosubtype antigens of 2c:P1.5 (Table 1). Although none of the Y:2c:P1.5 isolates were typed as ST-573, all had MLST allelic profiles very similar to those exhibited by the B:2c:P1.5 isolates (Nanaimo 1, Nanaimo 2 and Nanaimo 3). In one Y:2c:P1.5 isolate (1999-075; ST-1624), six of seven housekeeping genes examined were identical to those found in the three B:2c:P1.5 isolates (ST-573). The MLST profiles from the remaining eight Y:2c:P1.5 isolates (ST-3923, ST-572 and ST-3980) showed differences of two enzyme alleles from the MLST profiles of the B:2c:P1.5 isolates.

PFGE

Serogroup Y meningococci have DNA fingerprints that are distinct from those of serogroup B and C strains (unpublished data). However, when the B:2c:P1.5 isolates were compared with the Y:2c:P1.5 isolates, they showed striking similarity in their PFGE profiles (Figure 1).

DISCUSSION

The process of capsule switching may arise spontaneously (14) or as a result of some selective advantage conferred on the organisms that have their capsules replaced (6-8). With increasing widespread use of both the tetravalent A, C, Y, W135 polysaccharide and the conjugate meningococcal C vaccines in many countries, one of the ongoing epidemiological concerns is the effect of vaccination on serogroup replacement in

TABLE 1
Comparison of multilocus sequence typing (MLST) profiles of B:2c:P1.5 and Y:2c:P1.5 isolates recovered from invasive meningococcal disease cases in Canada between 1999 and 2004

NML number	Source of specimens	Serogroup: serotype:serosubtype	MLST housekeeping gene alleles							Sequence type (ST)
			abcZ	*adk*	*aroE*	*fumC*	*gdh*	*pdhC*	*pgm*	
2002-214	CSF	Y:2c:P1.5	2	7	6	8	9	7	8	ST-3980
1999-121	Blood	Y:2c:P1.5	2	7	6	17	9	6	98	ST-572
2002-151	CSF	Y:2c:P1.5	2	7	6	17	9	7	8	ST-3923
2002-171	Blood	Y:2c:P1.5	2	7	6	17	9	7	8	ST-3923
2002-183	Blood	Y:2c:P1.5	2	7	6	17	9	7	8	ST-3923
2002-277	Blood	Y:2c:P1.5	2	7	6	17	9	7	8	ST-3923
2003-005	Blood/CSF	Y:2c:P1.5	2	7	6	17	9	7	8	ST-3923
1999-075	Blood	Y:2c:P1.5	2	7	6	17	9	6	8	ST-1624
2001-038	CSF	B:2c:P1.5	2	7	6	40	9	6	8	ST-573
2001-041	Blood	B:2c:P1.5	2	7	6	40	9	6	8	ST-573
2002-037	Blood	B:2c:P1.5	2	7	6	40	9	6	8	ST-573

CSF Cerebrospinal fluid; NML National Microbiology Laboratory (Winnipeg, Manitoba)

N meningitidis. Although the exact mechanism of capsule switching is not yet known, it probably involves some recombination event(s) within the meningococcal *cps* locus (12). The new progeny organisms always bear some resemblance to their parent strains (eg, the same serotype and/or serosubtype antigens; identical or similar ST type and PFGE profile [6-8]).

Our surveillance data indicated that serotype 2c meningococci are rarely found in serogroup B and C organisms. For example, none of the 362 serogroup B meningococci collected by the National Microbiology Laboratory (Winnipeg, Manitoba) between 1977 and 1986 was found to be serotype 2c (15). Similarly, when we examined serogroup B isolates obtained from IMD cases in Canada in 2001 (86 isolates) and 2002 (76 isolates), no serotype 2c isolates were detected other than those described in the present study (unpublished data). In Canada, most of the serogroup C isolates from the past decade were serotype 2a, and the few non-2a isolates were unrelated to serotype 2c (16). In contrast, 33 of 61 (54%) serogroup Y isolates collected from IMD cases in Canada from January 1999 to June 2001 were found to be serotype 2c (4), and one-half of those were found to have the serosubtype antigen P1.5. Indeed, one of the two most common serotype and serosubtype antigen combinations among serogroup Y meningococci is 2c:P1.5, with 2c:P1.5,2 being the other most common combination. A similar observation was also found among the collection of serogroup Y and serotype 2c isolates at the Walter Reed Army Institute of Research (Washington, District of Columbia, USA). Close to one-half of approximately 190 serogroup Y isolates belonged to serotype 2c; among the 103 isolates of serotype 2c, 93 were found to be serogroup Y (WD Zollinger, personal communication). These data suggest that the capsule switching that occurred was from Y to B rather than from B to Y.

One potential factor that may have contributed to the emergence of these B:2c:P1.5 isolates is the overall rising incidence of serogroup Y IMD in both Canada (4) and the United States (3). Therefore, finding three B:2c:P1.5 isolates from the same medium-sized community suggested the possibility of a clonal grouping that might be related to serogroup Y organisms. MLST confirmed that the three B:2c:P1.5 isolates were related and belonged to one unique clone, ST-573. A search of the *N meningitidis* MLST Web site (11) did not find any

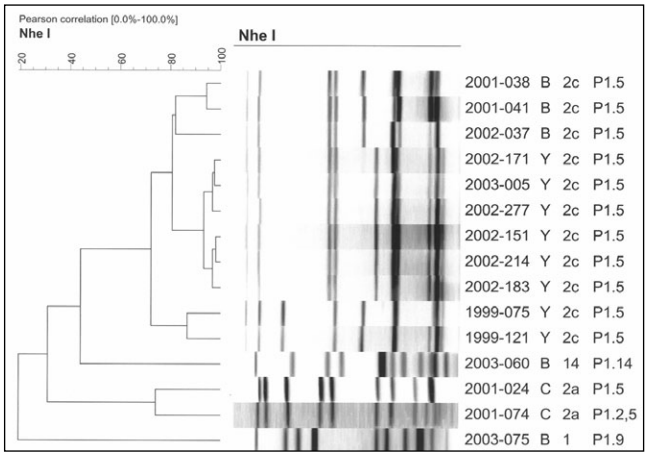

Figure 1) *Pulsed-field gel electrophoresis analysis for comparison of B:2c:P1.5 and Y:2c:P1.5 isolates. The DNA fingerprints were based on* NheI *restriction enzyme digestion of genomic DNA. Samples were identified by their laboratory numbers and their antigenic formula (serogroup:serotype:serosubtype). The dendogram indicates relative similarity of the isolates. Sporadic isolates of serogroup B and serogroup C strains were included as controls*

serogroup B or serogroup C meningococci with either an identical ST or housekeeping gene alleles showing extensive similarity to our B:2c:P1.5 isolates.

Ongoing strain characterization of invasive meningococci collected from IMD cases in Canada has found that most of the serogroup Y organisms can be classified into two clonal groups by MLST (unpublished data). One of the two common clonal groups (ST-3923 and its related STs) is characterized by housekeeping gene alleles showing striking similarity with ST-573, with as many as five to six out of seven of the MLST loci showing a complete match (Table 1). The PFGE data also showed the similarity of the DNA fingerprints of Y:2c:P1.5 and the B:2c:P1.5 isolates. Based on these observations, we postulate that the B:2c:P1.5 isolates probably arose from the Y:2c:P1.5 organisms by a genetic recombination mechanism that involved their *siaD* genes, which encode for the sialyltransferase enzymes that determine the linkages of the sialic acids in serogroup B and Y meningococci (5).

To assess if such isolates had existed before 2001, a search of our records revealed three other serogroup B isolates with the antigenic formula B:2c:P1.5 and belonging to ST 573. These three isolates were from IMD cases from 1999, with two of the isolates recovered from cerebrospinal fluid specimens and the third from blood culture. The three cases, involving a one-year-old boy, a four-year-old boy and a six-month-old girl, occurred between June 1999 and December 1999. All three cases were from British Columbia: one case occurred in Nanaimo and two cases occurred in Prince George, which is located 800 km by road from Nanaimo in central British Columbia. Besides these cases, no similar isolates have since been identified elsewhere in Canada. Whether mass vaccination with meningococcal vaccines against A, C, Y and W135 meningococci will hasten or facilitate the emergence of such B:2c:P1.5 isolates should be carefully monitored. However, our experience with the B:2a:P1.5,2 meningococci belonging to the ET-15 clonal group, which most likely emerged by capsule switching from C:2a:P1.2,5 ET-15 organisms (12), does not suggest rapid or extensive spread of such recombinant strains. Nevertheless, the propensity of meningococci to acquire foreign genetic materials that may favour their spread or transmissibility is of concern. Hence, continued surveillance for the spread of hypervirulent meningococcal clones remains an important public health priority.

Nucleotide sequence accession numbers

The partial sequences of *porB* and *porA* genes of the three B:2c:P1.5 strains have been deposited in GenBank (National Center for Biotechnology Information, USA) with the assigned accession numbers AY660740 to AY660745.

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REFERENCES

1. Rosenstein NE, Perkins BA, Stephens DS, Popovic T, Hughes JM. Meningococcal disease. N Engl J Med 2001;344:1378-88.
2. Peltola H. Meningococcal disease: Still with us. Rev Infect Dis 1983;5:71-91.
3. Rosenstein NE, Perkins BA, Stephens DS, et al. The changing epidemiology of meningococcal disease in the United States, 1992-1996. J Infect Dis 1999;180:1894-901.
4. Tsang RSW, Squires SG, Zollinger WD, Ashton FE. Distribution of serogroups of *Neisseria meningitidis* and antigenic characterization of serogroup Y meningococci in Canada, January 1, 1999 to June 30, 2001. Can J Infect Dis 2002;13:391-6.
5. Claus H, Vogel U, Muhlenhoff M, Gerardy-Schahn R, Frosch M. Molecular divergence of the *sia* locus in different serogroups of *Neisseria meningitidis* expressing polysialic acid capsules. Mol Gen Genet 1997;257:28-34.
6. Alcala B, Arreaza L, Salcedo C, Uria MJ, De La Fuente L, Vazquez JA. Capsule switching among C:2b:P1.2,5 meningococcal epidemic strains after mass immunization campaign, Spain. Emerg Infect Dis 2002;8:1512-4.
7. Perez-Trallero E, Vicente D, Montes M, Cisterna R. Positive effect of meningococcal C vaccination on serogroup replacement in *Neisseria meningitidis*. Lancet 2002;360:953.
8. Stefanelli P, Fazio C, Neri A, Sofia T, Mastrantonio P. First report of capsule replacement among electrophoretic type 37 *Neisseria meningitidis* strains in Italy. J Clin Microbiol 2003;41:5783-6.
9. Sacchi CT, Lemos AP, Whitney AM, et al. Correlation between serological and sequencing analyses of the PorB outer membrane protein in the *Neisseria meningitidis* serotyping system. Clin Diagn Lab Immunol 1998;5:348-54.
10. Sacchi CT, Lemos AP, Brandt ME, et al. Proposed standardization of *Neisseria meningitidis* PorA variable-region typing nomenclature. Clin Diagn Lab Immunol 1998;5:845-55.
11. *Neisseria* MLST. <http://pubmlst.org/neisseria> (Version current at March 31, 2005).
12. Tyler S, Tsang R. Genetic analysis of Canadian isolates of C:2a:P1.2,5 and B:2a:P1.2,5 *Neisseria meningitidis* strains belonging to the hypervirulent clone of ET-15. Can J Microbiol 2004;50:433-43.
13. An electronic resource for the *Neisseria* research community. <http://Neisseria.org> (Version current at March 31, 2005).
14. Frosch M, Meyer TF. Transformation-mediated exchange of virulence determinants by co-cultivation of pathogenic *Neisseriae*. FEMS Microbiol Lett 1992;79:345-9.
15. Ashton FE, Mancino L, Ryan AJ, Poolman JT, Abdillahi H, Zollinger WD. Serotypes and subtypes of *Neisseria meningitidis* serogroup B strains associated with meningococcal disease in Canada, 1977-1989. Can J Microbiol 1991;37:613-7.
16. Law DK, Henderson AM, Tsang RS. DNA sequence analysis of the PorB protein of nonserotypeable serogroup C ET-15 meningococci suggests a potential mutational hot spot on their serotype antigens. J Clin Microbiol 2004;42:2718-23.