Emergence of New Virulent *Neisseria meningitidis* Serogroup C Sequence Type 11 Isolates in France

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In France, there have been variations in the incidence of invasive meningococcal infection due to serogroup C isolates. Infection peaks were observed in 1992 and 2003 that involved isolates of phenotypes C:2a:P1.5,2 and/or C:2a:P1.5, which belong to the sequence type 11 (ST-11) clonal complex. We report an emergence of isolates belonging to the ST-11 clonal complex since 2003. These isolates displayed a new phenotype, C:2a:P1.7,1, caused infections that occurred as clusters, and were associated with increased infection severity and high virulence in mice. These isolates may be responsible for a peak in the incidence of serogroup C meningococcal infection in France, for which there is no routine vaccination to date.

Invasive meningococcal infections are a major public health concern because of the threat of outbreaks and epidemics. Conjugate vaccines against *Neisseria meningitidis* serogroup C have been introduced in routine vaccination procedures in several countries [1]. By contrast, in France, recommendations are for targeted vaccinations alone; they are given to patients who have come into contact with infected individuals and for the control of serogroup C outbreaks [2]. The latter strategy is based on epidemiological and bacteriological surveillance data to detect outbreaks and to tailor vaccination procedures for the exposed population. Indeed, horizontal DNA exchange among meningococcal isolates is frequent. The appearance of variants with enhanced virulence and/or transmissibility may lead to outbreaks among susceptible individuals.

We investigated epidemiological and bacteriological surveillance data combined with pathophysiological analysis with respect to meningococcal infection incidence from 1999 through 2008. We identified a new serogroup C clone with enhanced virulence that may lead to an increase in serogroup C incidence in France and may prompt a change in current vaccination strategies.

Methods. The epidemiological follow-up of patients with invasive meningococcal infection in France entails the mandatory notification of cases to the French institute for public health surveillance (Institut de Veille Sanitaire) and the characterization of invasive strains at the National Reference Center for Meningococci. The case definition includes 1 of the following 4 criteria: (1) a sample collected from a sterile site or from necrotizing skin lesions that tests positive for *N. meningitidis* by means of culture or polymerase chain reaction; (2) detection of gram-negative diplococci in cerebrospinal fluid; (3) purpura fulminans; or (4) a cerebrospinal fluid sample that reveals purulent meningitis associated with a petechial rash and/or positive antigen detection in cerebrospinal fluid, blood, or urine.

All invasive isolates were phenotyped by determining the combination of serogroup, serotype, and serosubtype and genotyped by means of multilocus sequence typing (MLST) and *porA* gene sequencing, as described elsewhere [3, 4]. Alleles, sequence types, and clonal complexes were assigned using the *Neisseria* species MLST database (http://pubmlst.org/neisseria). Clusters were based on at least 2 cases of invasive meningococcal infection occurring within a 6-month interval and the presence of an epidemiological link.

The virulence of each isolate was tested in a transgenic mouse model that expressed human transferrin after intraperitoneal challenge with 5×10^6 colony-forming units (CFUs)/mL, as described elsewhere [5]. Blood samples were collected 2, 6, and 24 h after infection and were plated to determine the number of CFUs per milliliter. We used enzyme-linked immunosorbent assays (Quantikine; R&D Systems Europe) to determine levels of tumor necrosis factor α (TNF-α) and keratinocyto-derived cytokine (KC; the functional murine homologue of human interleukin 8). All animal experimental protocols were approved by the Institut Pasteur Review Board. The apoptosis of Hec-1-B human epithelial cells was assayed as described elsewhere [6]. The differences in the mean of the cytokine levels

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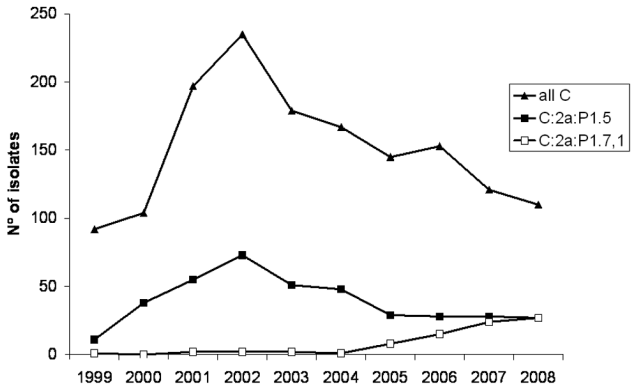

Figure 1. *Neisseria meningitidis* serogroup C isolates recovered from patients in France with culture-confirmed invasive meningococcal infection. The numbers of isolates of serogroup C (all C) and the numbers of isolates showing the phenotypes C:2a:P1.7,1 or C:2a:P1.5 are shown for the period from 1999 through 2008.

and the proportion of apoptosis between groups were tested for significance by use of the paired and 2-tailed Student *t* test and χ^2 test. Results for which $P < .05$ were considered to be statistically significant.

Results. From 1 January 1999 through 31 December 2008, a total of 6528 cases of invasive meningococcal infection were reported to the Institut de Veille Sanitaire. The incidence rate (corrected for underreporting) was 0.98 cases per 100,000 inhabitants in 1999; it increased to 1.43 cases per 100,000 inhabitants in 2002 and 1.61 cases per 100,000 inhabitants in 2003 and then decreased to 1.2 cases per 100,000 inhabitants in 2008. Over the period from 1999 through 2008, the distribution of cases according to serogroup showed that of the 5763 cases of infection with a known serogroup, 3564 (62%) were with serogroup B, 1677 (29%) were with serogroup C, 274 (5%) were with serogroup W-135, 170 (3%) were with serogroup Y, and 71 (1%) were with other serogroups.

The incidence of meningococcal disease attributed to serogroup C showed a statistically significant increase until 2002 (with an incidence rate of 0.5 cases per 100,000 inhabitants and a proportion of serogroup C isolates of 41%) and a subsequent decrease thereafter. Over the same period, the National Reference Center for Meningococci received a total of 1503 serogroup C isolates. The peak observed in 2002 was mainly due to isolates of phenotype C:2a:P1.5 of the sequence type 11 (ST-11) clonal complex; these isolates frequently contained variable regions in the *porA* gene encoding the major outer membrane protein (variable region 1 [VR1], 5-1; variable region 2 [VR2], 10-8). Despite a decrease in the number of invasive serogroup C isolates since 2003, we observed the simultaneous appearance of a new phenotype, C:2a:P1.7,1 (Figure 1). These isolates belong to the ST-11 clonal complex and contain variable regions in the *porA* gene (VR1, 7-1; VR2, 1). Further *fumC* gene sequencing at position 640 showed that the isolates displayed the lineage of ET-15, which is a member of the ST-11 clonal complex [7]. Isolates with C:2a:P1.5 and C:2a:P1.7,1 phenotypes had identical *porB* gene sequences, which confirms that both types of isolates were of the same serotype. A total of 81 C:2a:P1.7,1 isolates have been cultured since 1999, of which 73 (90%) have been isolated since 2005. Indeed, these isolates represented 6.6% of the total number of serogroup C invasive isolates in 2005, 10.3% 2006, 21.9%, in 2007, and 24.5% in 2008.

We used a transgenic mouse model that expressed human transferrin to study the virulence of the new isolates. The new isolates provoked levels of bacteremia that were similar to those observed for C:2a:P1.5/ST-11 isolates 2, 6, and 24 h after intraperitoneal challenge with bacterial suspensions of 5×10^6 CFUs/mL of each tested strain. However, the chemokine KC levels in mice infected with strain C:2a:P1.7,1/ST-11 were statistically significantly higher ($P < .05$) than those in mice in-

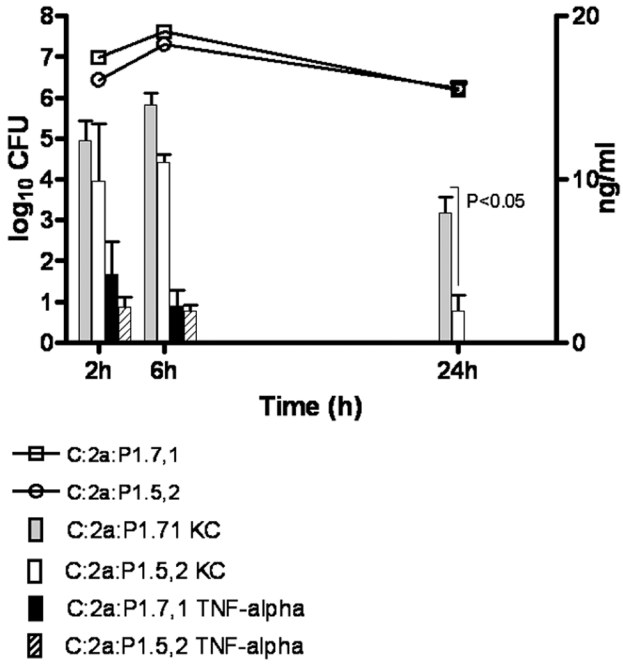

Figure 2. Comparative virulence in transgenic mice that expressed human transferrin of serogroup C strains of *Neisseria meningitidis*, LNP24198 (phenotype C:2a:P1.7,1 of sequence type 11 [ST-11]) and LNP19008 (phenotype C:2a:P1.5,2 of ST-11). After intraperitoneal challenge, colony-forming units (CFUs) were counted by plating blood samples that underwent serial dilution 2, 6, and 24 h after infection. *Points*, Geometric means of the CFU counts in blood from 5 mice per time point per experiment (*left axis*). *Bars*, Levels of tumor necrosis factor α (TNF-α) and keratinocyto-derived cytokine (KC) in blood from transgenic mice infected with strains LNP24198 (C:2a:P1.7,1/ST-11) and LNP19008 (C:2a:P1.5,2/ST-11) (*right axis*). Results are shown as geometric means ± standard errors of the mean of cytokine titers in blood from 5 mice per time point per experiment.

fected with C:2a:P1.5,2 isolates 24 h after infection (Figure 2). Levels of TNF-α (an early inflammatory cytokine) were also slightly higher 2 h after infection. Moreover, levels of apoptosis induced in the Hec-1-B human epithelial cell by C:2a:P1.7,1/ST-11 isolates were higher than those induced by C:2a:P1.5/ST-11 isolates. However, these differences in TNF-α and apoptosis levels between the isolates were not statistically significant (Figure 2 and data not shown).

Matched mandatory notification and microbiological data were available for serogroup C isolates that were obtained from patients with invasive meningococcal infection from 2003 through 2008, which allowed the description of 71 isolates obtained from patients infected with strain C:2a:P1.7,1/ST-11 and comparison with 646 isolates obtained from patients infected with other serogroup C strains. Infection with the C:2a:P1.7,1 strain occurred in all age groups. The mean and median ages of patients infected with strain C:2a:P1.7,1 were 29 and 17 years, respectively (range, 0–94 years) and were similar to mean and median ages of patients infected with other serogroup C strains (26 and 17 years, respectively). The ratio of male patients to female patients was 0.8. Thirty (42%) of the 71 patients infected with C:2a:P1.7,1 isolates had extensive hemorrhagic rash; this proportion was statistically significantly higher than that for patients infected with other isolates (26%; $P = .004$).

Death occurred in 15 of 67 cases of infection with strain C:2a:P1.7,1 with known outcome, corresponding to a case fatality rate of 22%, compared with a case fatality rate of 15% (92 of 626 cases) for cases of infection with other serogroup C isolates with known outcome ($P = .10$). The relative risk of death, adjusted for the presence of extensive hemorrhagic rash, was 1.27 (95% CI, 0.70–2.28).

The emergence of C:2a:P1.7,1/ST-11 isolates occurred simultaneously with the detection of 5 clusters linked to these isolates during the period from 2007 through 2008 among a total of 9 serogroup C invasive meningococcal infection clusters. Two clusters occurred within the same geographical area (western central France). One cluster was reported in April 2007 and involved 3 cases that occurred within an interval of 6 weeks; 1 patient died (age, 11 months). The second cluster was reported in May 2007 and involved 2 cases within an interval of 7 weeks; both patients died (ages, 17 and 20 years). The third and fourth clusters occurred in school settings, and both involved 2 patients who attended the same educational institution but were in different classrooms. For each of these clusters, the cases occurred within 3 weeks of each other, and the health authorities recommended 2 rapid vaccination campaigns against *N. meningitidis* serogroup C that targeted the students and the staff of the 2 schools involved. The fifth cluster corresponded to 2 cases that occurred within 1 week. Both patients (ages, 4 and 3 years) presented with septic shock, and 1 patient died. The patients were living in the same small town (population, 1000 inhabitants). A vaccination campaign was conducted among the 900 inhabitants of this town that were between the ages of 2 months and 19 years.

Discussion. Recent epidemiological data show an overall decrease in the incidence of invasive meningococcal infection due to serogroup C isolates, but our bacteriological findings indicate an emergence of C:2a:P1.7,1 isolates (VR1, 7-1; VR2, 1; ST-11 clonal complex) that are associated with severe cases of invasive meningococcal infection since 2005, as indicated by the high proportion of patients with extensive hemorrhagic rash (Figure 1). Several previous studies have shown that the severity of meningococcal disease is linked to particular phenotypes and genotypes [6, 8]. We showed that serogroup C isolates of phenotypes C:2a:P1.5,2 (VR1, 5; VR2, 2; ST-11 clonal complex) and C:2a:P1.5 (VR1, 5-1; VR2, 10-8; ST-11 clonal complex) were responsible for the increase in incidence of invasive meningococcal infection in France during the period from 2000 through 2005. They were also responsible for several outbreaks that led to several targeted vaccination campaigns [2, 9, 10].

A similar situation was previously observed in Quebec, Canada, in 2001, in which a sudden increase in cases of invasive meningococcal infection due to a unique variant C:2a:P1.7,1/ST-11 strain was observed [11]. The C:2a:P1.7,1 variant has most likely emerged by recombination at the *porA* gene, which encodes the variable region that is targeted by serosubtyping. This type of recombination may have occurred in C:2a:P1.5/ST-11 or C:2a:P1.5,2/ST-11 isolates. This explanation is also favored with respect to the emergence of this phenotype in Canada [11]. Another example of a PorA switching event was reported for a novel ST-11 variant of serogroup C of *N. meningitidis* in Victoria, Australia (VR1, 7-2; VR2, 4) [12]. PorA switching may represent a general mechanism to escape host immune responses [13]. Importantly, the C:2a:P1.7,1 isolates conserved high pathogenesis, as estimated from the case fatality ratios, the proapoptotic effects on epithelial cells, and the high virulence observed in the mouse model. In conjunction with a general mechanism, statistically significantly higher levels of the chemokine KC may be responsible for stronger inflammatory responses that may manifest as an intense influx of polymorphonuclear leukocytes [14].

Our findings have contributed toward the reassessment of meningococcal C vaccination in the immunization schedule and the introduction of conjugate meningococcal C vaccination by the French Health Authorities.

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