Experimental Meningococcal Infection in Mice: A Model for Mucosal Invasion

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A more complete understanding of meningococcal disease has been hampered by the lack of an appropriate animal model. Previous models have utilized injections of meningococci, which precludes the study of nasopharyngeal colonization and invasion. We have developed a model for meningococcal disease in which litters of 5-day-old mice are challenged intranasally with \(10^7\) viable meningococci. Bacteremia is monitored by jugular venous blood cultures, and cerebrospinal fluid is sampled by cisternal punctures. Human disease-associated and carrier strains were compared; nasopharyngeal colonization was similar for these bacteria, but the case-associated strains were much more frequently invasive and caused bacteremia. Twenty-one percent of bacteremic animals had meningitis. There was an age-related susceptibility to infection which correlated inversely with the levels of serum complement. Preinjection of iron dextran increased the number of animals which were bacteremic, the concentration of bacteria in blood, and nasopharyngeal colonization. Noncapsular variants of virulent meningococcal did not colonize nasopharyngeal tissue in vivo, and they were not invasive. This neonatal mouse model mimics meningococcal disease as seen in humans and may be useful in studying the initial events in the pathogenesis of meningococcal disease.

**Neisseria meningitidis**, the meningococcus, remains a major cause of endemic and epidemic bacterial meningitis despite the availability of effective polysaccharide vaccines (13). To study the pathogenesis of meningococcal disease and to test the effectiveness of vaccines, numerous animal models have been developed over the years; these have included experimental meningococcal infection in monkeys (9), mice (16, 18), rabbits (2), chicken embryos (3), and guinea pigs (1, 11). All of these models have required the injection of bacteria either directly into the cerebrospinal fluid, into the bloodstream, or into subcutaneous chambers. It is suspected that some of the illness in the experimental models has been due to the direct injection of toxic bacterial products. Mouse models have become very popular for the study of meningococcal infection; Miller (18) first described the intraperitoneal injection of meningococci in hog gastric mucin, which enhances the virulence of the bacteria. Iron was later found to be an acceptable substitute for mucin (5). These models have been used to test the ability of potential vaccines to prevent either murine death or bacteremia after intraperitoneal injection of meningococci (21). None of these animal models, however, adequately mimics the pathogenesis of meningococcal disease in humans, especially since they bypass the mucosal colonization and invasion steps. In addition to protecting against clinical illness, appropriate vaccines should also have the ability to prevent meningococcal carriage in the nasopharynx and hence eliminate the major reservoir of infection. We have previously studied experimental meningococcal infection in rats, guinea pigs, and mice (25, 26). The most appropriate experimental model involved using neonatal mice which developed nasopharyngeal carriage followed by bacteremia, meningitis, and death.

We describe below meningococcal virulence factors and murine host factors which are important in this model.

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* Corresponding author.
TABLE 1. Comparative colonization and bacteremia after intranasal inoculation of case- and carrier-associated meningococci

<table>
<thead>
<tr>
<th>Source of strain</th>
<th>Iron dextran</th>
<th>Bacteremia</th>
<th>Colonization</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>+</td>
<td>118/354 (52)</td>
<td>144/191 (75)</td>
<td>59/354 (17)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>110/397 (28)</td>
<td>86/270 (32)</td>
<td>3/397 (0.7)</td>
</tr>
<tr>
<td>Carriers</td>
<td>+</td>
<td>11/94 (12)</td>
<td>31/82 (38)</td>
<td>0/94 (0)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>6/83 (7)</td>
<td>42/93 (45)</td>
<td>2/83 (2)</td>
</tr>
</tbody>
</table>

Data are given as the number of animals with bacteremia (or colonization or mortality)/total, with the percentage in parentheses. The statistical significance values of the comparisons were as follows. (i) For iron-pretreated animals (case strains versus controls not pretreated), bacteremia, colonization, and mortality, P < 0.001. (ii) For iron-pretreated animals (carriers versus case strains), bacteremia and colonization, P < 0.001, and mortality, P < 0.01. (iii) For iron-pretreated animals (carrier strains versus controls not pretreated), bacteremia, colonization, and mortality, not significant. (iv) For animals with no iron pretreatment (carrier versus case strains), bacteremia, P < 0.05, and colonization and mortality, not significant.

Nine strains: serogroup B type 2 (five strains), serogroup C type 2 (three strains), and nongroupable (one strain).

Seven strains: serogroup B (five strains), serogroup X (one strain), serogroup 29e.Z (1 strain). None was serotype 2.

Laboratories, Inc., Wilmington, Mass., and were atraumatically inoculated intranasally with 10^7 meningococci as previously described (26). Briefly, bacterial suspensions containing 10^7 CFU/ml were aspirated into 0.5-ml glass syringes to which were attached 5-mm-long, blunt, 30-gauge needles. Without any anesthesia, 0.01 ml was atraumatically inoculated into one nostril. Blood cultures were taken at 3, 24, and 48 h by puncturing the internal jugular vein with a 25-gauge needle. Samples (0.01 ml) of blood were withdrawn and placed on agar plates. Nasal cultures were obtained by washing the nasal passages with 0.05 ml of brain heart infusion broth, which was then immediately placed on agar plates.

At 3 to 6 h before intranasal inoculation, some animals also received intraperitoneal injections of iron dextran (Imferon, Pírons Corp. Ltd., Scarborough, Ontario) at a dosage of 250 mg of iron per kg. Control animals received saline.

Cerebrospinal fluid was sampled by inserting a 25-gauge needle into the nape of the neck and then into the cisterna magna (19). This procedure could be done repeatedly on an animal without mortality.

After inoculations all animals were observed and weighed daily. Deaths were tabulated, and selected animals underwent pathological examination.

**Complement studies.** Cobra venom factor from *Naja naja*, obtained from Cordis Laboratories, Miami, Fla., was reconstituted in phosphate-buffered saline, and portions were frozen at -80°C. At 24 h before intranasal instillation of meningococci, mice received 200 U of the venom factor per kg. At 24 h after injection there was no C3 present, as measured by a sensitive alternate complement pathway assay employing ^51^Cr-labeled erythrocytes (17). In addition, A/J mice which were deficient in the fifth component of complement (Jackson Laboratories, Bar Harbor, Maine) were challenged with meningococci as described above.

**Statistical methods.** Fourfold tables were constructed, and the chi-square values (χ²) were determined by using Yates’ correction for continuity.

**RESULTS**

Comparative colonization and bacteremia. Five-day-old litters of mice were inoculated intranasally with strains derived from human patients with meningitis or carriers (Table 1). In the animals which had not been pretreated with iron dextran, the rates of bacteremia were significantly higher in pups in which the disease-associated isolates were instilled intranasally. Despite the higher rates of bacteremia, nasopharyngeal colonization rates and mortality were not significantly different in the groups which were challenged with either carrier- or case-derived isolates. Intraperitoneal injection of iron dextran significantly enhanced rates of bacteremia, colonization, and mortality in those mice which were challenged with the disease-associated isolates. Iron dextran did not alter these parameters in the mice challenged with carrier strains. Of the mice which were bacteremic from case-derived strains, 21% (4 of 19) had infected cerebrospinal fluid as determined by cisternal puncture (26).

To ensure that bacteria were not being directly injected into mucosal tissue or even into the bloodstream, an alternate form of instillation was used. Using a Pasteur pipette, 100 μl of meningococci was dropped onto the external nares. The mice quickly aspirated the fluid into the internal nares. Animals which were challenged in this manner had identical results to those which had intranasal instillation with a blunt needle (data not shown).

We then determined the rates of bacteremia in relationship to the numbers of bacteria instilled intranasally. In the absence of iron, an inoculum of at least 10^3 disease-associated bacteria was required to produce any detectable bacteremia (Fig. 1). This increased to 20% when 7 × 10^8 bacteria were used. Injections of iron dextran significantly enhanced rates of bacteremia and reduced the minimum infective dose so that even after challenge with 700 bacteria, 10% of animals were bacteremic.

Quantitative blood cultures were also done to determine the level of bacteremia under different circumstances. With the virulent strain B16B6, almost 90% of the animals which were bacteremic had less than 10^3 organisms per ml of blood (Fig. 2). Injection of iron dextran not only increased rates of bacteremia (Table 1) but also increased the concentration of bacteria in those with positive blood cultures (Fig. 2). The mortality was highest in those animals with more than 10^4 bacteria per ml of blood.

![FIG. 1. Comparison of rates of bacteremia in neonatal mice 24 h after intranasal instillation of N. meningitidis B16B6 (○). Iron dextran was given intraperitoneally to another group of animals at the time of intranasal challenge (●). Each point represents the mean and standard deviation of the results from a minimum of four litters (at least 45 pups).](http://iai.asm.org/Downloaded/from.http://iai.asm.org/)
The duration of bacteremia and colonization was assessed by doing repeated blood and nasal cultures at 3 h and then daily for 4 days. No further colonization or bacteremia was detectable by day 4 (age 9 days) (Table 2).

**Role of the capsule.** Encapsulated and noncapsular variants of strains M986 and 3006 were tested for their ability to colonize the nasopharynx and invade into the bloodstream (Table 3). Rates of bacteremia were markedly reduced in the noncapsular variants compared with the parent strains. A small number of mice became bacteremic after receiving the unencapsulated variant of strain 3006; however, approximately 5% of colonies reverted to the parent encapsulated form as detected on antiserum agar plates. No group B polysaccharide was detectable on strain M986. The noncapsular variants of both strains also colonized the nasopharynx to a significantly lesser extent than the parental strains.

**Role of serum complement.** We previously found that, in this model, there was an inverse relationship between the age of the mice and susceptibility to meningococcal bacteremia (26). Since humans may be susceptible to meningococcal disease because of complement deficiency (22), we wanted to determine whether the increased susceptibility of mice was also due to low complement levels. Serum complementary activity was measured at different ages and was found to be undetectable during the first week of life, rising to one-fifth of the adult levels by the second week of life. With equivalent numbers of male and female mice, the 50% hemolytic complement levels were 150, 30, and <5 U/ml at 6 weeks, 10 days, and <10 days of age, respectively. Rates of bacteremia are known to diminish in this model by 10 days, and bacteremia does not occur at 6 weeks of age.

To confirm the absence of serum complement at 5 days of age, cobra venom factor was injected intraperitoneally, and mice were challenged intranasally with the virulent strain B16B6 (Table 4). Cobra venom factor eliminated total hemolytic complement from adult animals by 24 h. After preinjection of neonatal mice with cobra venom factor, there was no enhancement of rates of bacteremia, levels of bacteremia, nasal colonization, or mortality. Similarly, there was no significant change in mortality when strain B16B6 was instilled into mice which have a congenital deficiency of C5. However, there was an increase in rates of bacteremia (25% versus 69%) as well as colonization (35% versus 100%).

**DISCUSSION**

There have been many descriptions of animal models for meningococcal disease ever since *N. meningitidis* was clearly associated with epidemic cerebrospinal meningitis (28). The most popular model has been the mouse bacteremia model, which requires intraperitoneal injections of meningococci alone (16), in hog gastric mucin (18), or in iron dextran (5).

None of the previously described experimental models for meningococcal disease fully satisfies the criteria of an ideal model. Some suggested criteria for an experimental animal model (14) are as follows: (i) the portal of entry and route of dissemination must be similar to those in human disease, (ii) bacteria which are virulent for humans should also be virulent for the experimental animals, (iii) the course of disease should be predictable, (iv) it should be reproducible, (v) experimental lesions should be similar to those in human

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**TABLE 2. Duration of bacteremia and colonization in neonatal mice**

<table>
<thead>
<tr>
<th>Time after i.n. instillation (h)</th>
<th>Bacteremia</th>
<th>Colonization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No iron</td>
<td>With iron</td>
</tr>
<tr>
<td>3</td>
<td>6/40 (15)</td>
<td>ND</td>
</tr>
<tr>
<td>24</td>
<td>16/100 (16)</td>
<td>104/174 (60)</td>
</tr>
<tr>
<td>48</td>
<td>21/103 (20)</td>
<td>61/120 (51)</td>
</tr>
<tr>
<td>72</td>
<td>2/78 (3)</td>
<td>2/32 (6)</td>
</tr>
<tr>
<td>96</td>
<td>0/78 (0)</td>
<td>0/40 (0)</td>
</tr>
</tbody>
</table>

* Data are given as the number of animals with bacteremia (or colonization) per total, with the percentage in parentheses. *N. meningitidis* B16B6 was used.

* i.n., Intranasal.

* ND, Not done.

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**TABLE 3. Comparison of colonization and invasiveness of encapsulated and noncapsular meningococcal variants**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Description</th>
<th>Bacteremia</th>
<th>Nasal colonization</th>
</tr>
</thead>
<tbody>
<tr>
<td>M986 (B/2a,7)*</td>
<td>Encapsulated</td>
<td>14/40 (35)*</td>
<td>18/36 (50)*</td>
</tr>
<tr>
<td></td>
<td>Noncapsular variant</td>
<td>0/54 (0)*</td>
<td>3/54 (6)*</td>
</tr>
<tr>
<td>3006 (B/2b)*</td>
<td>Encapsulated</td>
<td>17/37 (46)</td>
<td>37/37 (100)</td>
</tr>
<tr>
<td></td>
<td>Noncapsular variant</td>
<td>7/76 (9)</td>
<td>17/76 (22)</td>
</tr>
</tbody>
</table>

* Data are given as the number of animals with bacteremia (or colonization) per total, with the percentage in parentheses.

* (Serogroup/serotype).

* P < 0.001, compared with noncapsular variant.

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**TABLE 4. Effects of cobra venom factor on neonatal mice**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bacteremia</th>
<th>Nasal colonization</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>24/99 (24)</td>
<td>1.4 ± 2.6</td>
<td>29/97 (30)</td>
</tr>
<tr>
<td>Cobra venom factor</td>
<td>55/223 (25)</td>
<td>1.8 ± 2.5</td>
<td>19/68 (28)</td>
</tr>
</tbody>
</table>

* Strain B16B6 was injected intranasally. Except for bacterial concentration, data are given as the number of animals with bacteremia (or colonization or mortality) per total, with the percentage in parentheses.

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**FIG. 2.** Intranasal inoculation of 5-day-old pups with *N. meningitidis* B16B6. Mice which were not bacteremic were eliminated from this analysis. Quantitation of the degree of bacteremia was done on animals which had received meningococci alone or together with iron dextran. There was a 24% (24 of 99) rate of bacteremia without iron dextran and a 73% (81 of 111) rate of bacteremia in pups treated with iron.
disease, (vi) the techniques should be relatively simple, and (vii) the pathophysiologic events must be similar to those occurring in humans (8). It is doubtful that any one animal model for meningococcal disease can meet all of these requirements.

Unfortunately none of the previously described models includes a mucosal colonization phase followed by bacterial invasion; this has precluded a study of the initial events in meningococcal disease. Similar problems had existed with models for Haemophilus influenzae until the development of the neonatal rat model, which utilizes intranasal instillation of bacteria (19).

We have described a neonatal mouse model for meningococcal disease in which the intranasal inoculation of bacteria is followed by a phase of nasopharyngeal colonization (25, 26). Virulent and avirulent strains attach to and colonize the murine nasopharynx to the same degree, but it is mainly those strains which are virulent for humans which invade through the nasal mucosa and cause bacteremia. About 20% of bacteremic animals do develop culture-positive purulent meningitis. Histopathologic studies indicate that smaller numbers of animals develop leptomenigitis and ventriculitis (26). There is an age-related susceptibility to meningococcal disease in this model, with resistance increasing after the first week of life; this resistance was correlated with increasing levels of serum complement. Protection against meningococcal disease in humans is also correlated with the presence of serum bactericidal activity. Complement-deficient humans are at increased risk for systemic meningococcal disease (22); similarly, we found that congenital C5-deficient mice had enhanced rates of bacteremia.

Injection of iron dextran increased rates of bacteremia, level of bacteremia, and nasopharyngeal colonization, but only for those strains which are intrinsically virulent. It is well known that iron enhances the virulence of many bacteria (4). Host iron is not readily available to bacteria because it is strongly bound to iron-binding proteins, and there is only an extremely small amount of free iron available in serum unless extrinsic iron is provided. Pathogenic neisseriae such as the meningococcus can utilize iron which is bound to transferrin; however, infection is associated with hypoferremia, which reduces the amount of iron available in the transferrin iron pool. Virulence is enhanced when iron saturation of transferrin is maintained at high levels by exogenous iron (15).

In this neonatal mouse model, the most virulent strains were encapsulated and had the serotype 2 antigen. Carrier-associated strains, however, did not invade despite the fact that all were encapsulated. None had the serotype 2 antigen. In humans, the serotype 2 antigen is also a major virulence determinant in invasive strains; carrier strains may be encapsulated or unencapsulated but rarely have the serotype 2 antigen (10). Factors responsible for meningococcal adherence are poorly defined, although pili appear to mediate adherence to human pharyngeal cells in vitro (24) and the A or C polysaccharide inhibits attachment to and agglutination of human erythrocytes in vitro (23). In the present study, organisms with the group B polysaccharide on their surface were more efficient in colonizing the nasopharynx in vivo than the noncapsular variants. Studies in humans with the A and C polysaccharide vaccines do suggest that anti-polysaccharide antibodies can similarly reduce pharyngeal colonization (12, 20). This implies that the group B polysaccharide may mediate meningococcal attachment in vivo. Other explanations are possible. (i) An important adhesin might be lost during the preparation of the noncapsular variant. (ii) Invasion may allow enhanced nasopharyngeal colonization by a "reverse invasion" procedure whereby bacteria from the bloodstream recolonize the nasopharynx; this is unlikely since the colonization rates are similar for the invasive and noninvasive strains. (iii) "Serum factors" may kill some meningococci in the nasopharynx. The in vivo colonization is enhanced by iron dextran. The mechanism is uncertain, but may involve some of the above factors, an interaction with the adhesin, increased growth rate, or a change in bacterial production of an adhesive molecule.

It is now possible to study in more detail the early phase of meningococcal disease including nasopharyngeal colonization, the poorly understood invasion through the mucosa, and ultimately bacteremia and meningitis. In addition, one can also study the ability of vaccines and passively transfused antibodies to inhibit mucosal colonization.

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LITERATURE CITED