Gonorrhea affects 150 million people worldwide annually. One of the most serious clinical forms of gonococcal infections is disseminated gonococcal infection (DGI) (36). DGI affects 1 to 3% of patients with gonorrhea (2, 7, 12, 18, 25, 31). Further, women infected with human immunodeficiency virus (HIV) are at increased risk of contracting DGI (3, 5, 17, 23, 32). Gonorrhea during pregnancy increases the risk of DGI (9, 33). Gestational DGI increases the risk of perinatal infant morbidity and mortality (6, 24, 27). Infected mothers may transmit Neisseria gonorrhoeae to their newborns during pregnancy or delivery or in the postpartum period (1, 11, 13, 27). The prevalence of prenatal gonococcal infection has been estimated at about 7.5% among high-risk populations in the United States (1). Newborns exposed to gonorrhea may develop systemic illnesses, including septicemia and arthritis. Neonatal sepsis is a life-threatening disease (1). The factors involved in the transmission of N. gonorrhoeae from the mother to the fetus during pregnancy are not characterized. The absence of an adequate experimental model hampers understanding of the mechanisms of transmission of N. gonorrhoeae to the fetus during pregnancy. Identification of the host and bacterial factors that increase risk for neonatal gonococcal sepsis would be important to understand the mechanism of gestational infection and to develop novel preventive approaches.

A nonprimate animal model of gonococcal bacteremia in rat pups was recently developed (28). In this model, intraperitoneal (i.p.) inoculation of serum-resistant (serR) N. gonorrhoeae JC1, preincubated with human complement C1q, resulted in bacteremia that lasted 6 to 7 days (28). Preincubation of strain JC1 with C1q, instead of the initiation of killing via activation of the classical complement pathway, protected N. gonorrhoeae from the bactericidal effect of rat pup serum both in vitro and in vivo (28).

The purpose of this investigation was to evaluate (i) whether serR strains of N. gonorrhoeae that express C1q-mediated virulence may be transmitted from a pregnant mother to the fetus and (ii) whether human complement C1q may enhance transmission.

In the first set of experiments, we investigated whether pregnant rats are susceptible to N. gonorrhoeae infection. Three strains of N. gonorrhoeae, JC1 from DGI, 2005 from pelvic inflammatory disease (PID), and 1658 from local infection (LI), were used to infect pregnant rats. Selection of these three strains was based on significantly different clinical virulence to humans, which has been shown to correlate with C1q-mediated peritonitis to rat pups (Table 1). Eighty-six Sprague-Dawley rats were infected on day 20 of their pregnancy by i.p. inoculation with three different N. gonorrhoeae strains originating from patients with PID, DGI, and LI. Each group was divided into two subgroups consisting of those inoculated with 5 \times 10^6 CFU of N. gonorrhoeae preincubated for 15 min at 37°C with 80 μg of C1q/ml or bovine serum albumin (BSA) (control). Human C1q isolated from human serum was shown to be pure by sodium dodecyl sulfate–5.6% polyacrylamide gel electrophoresis and by double immunodiffusion against a goat anti-C1q monospecific antiserum (Cytotect, San Diego, Calif.) (20). The C1q was shown to be active by standard hemolytic assay. BSA (catalog no. A3156) and nonimmune goat serum (catalog no. S2007) were purchased from Sigma (St. Louis, Mo.).

Bacterial suspension in buffered saline was prepared from piliated (P+) and opaque (OP+) colonies (75% P+, OP+). Twenty-four hours postinoculation, blood samples were collected from pregnant rats and cultured. Pregnant rats were sacrificed 24 h postinoculation, the uterus was opened immediately, and the number of live and/or infected fetuses was evaluated. Ten-microliter blood samples were collected from fetuses by heart puncture without anticoagulant, and dilutions were made immediately and subcultured on modified Thayer-Martin agar plates in triplicate and incubated for 48 h in 5% CO₂. Bacterial infection was evaluated by counting CFU per milliliter of blood. Five pregnant control rats received 200 μl of buffer with C1q (80 μg/ml) i.p. to determine whether C1q alone influenced pregnancy outcome. Results showed that none of the three N. gonorrhoeae strains JC1, 2005, or 1658, pretreated with BSA, were recovered from the blood of the pregnant rats. Pretreatment of tested strains of N. gonorrhoeae with C1q did not result in infection of the pregnant rats themselves (Table 2); however, the fetuses of these pregnant rats were infected. It is not clear why adult rats are resistant to infection, but one possible explanation is that they have bactericidal antibody to N. gonorrhoeae, while pups do not.

Table 2 shows that 100% of live fetuses of pregnant rats...
inoculated with *N. gonorrhoeae* JC1 and 2005 pretreated with C1q had positive blood cultures, while none inoculated with strain 1658 pretreated with C1q had positive blood cultures. Blood cultures of dead fetuses were negative. Furthermore, the fetuses of pregnant rats inoculated with BSA-pretreated strain JC1, 2005, or 1658 showed negative blood cultures.

Figure 1 shows quantitative blood cultures of fetuses obtained from one litter. The mean values, expressed as CFU/ml, of fetal blood for strains JC1 (DGI), 2005 (PID), and 1658 (LI) were 10⁵, 10⁴, and 0, respectively.

These results demonstrate that *N. gonorrhoeae* strains associated with DGI or PID were able to spread from the pregnant rats to the fetuses. These two strains possess a 344-bp DNA fragment of sac-4, conferring resistance to *N. gonorrhoeae* to human and rat pup sera and virulence to rat pups in the presence of C1q (29). The fetuses were not infected in utero when the pregnant rat was challenged with ser⁺ LI strain missing the 344-bp DNA fragment of sac-4 or with BSA-pretreated *N. gonorrhoeae* (DGI, PID, and LI).

The next set of experiments was designed to investigate the effects of C1q concentration on bacteremia and fetal death. There was an 18-fold increase in CFU/ml in the blood of fetuses infected with JC1 and a 7.6-fold increase in the blood of fetuses infected with strain 2005 treated with a higher concentration of C1q (120 μg/ml) and reached statistical significance for both strains. For statistical evaluation of the associations between two factors, the Fisher exact test was utilized to determine the significance level. *P* values less than 0.05 implied the existence of a statistically significant association between the tested factors. When pregnant rats were inoculated with C1q (80 μg/ml)-pretreated DGI and PID strains, 38.4 and 33.3% of the fetuses, respectively, died (*P* > 0.75). With an increase in the C1q concentration (120 μg/ml), the death rate among the fetuses increased to 45.3% (DGI) and 33.7% (PID), respectively, but did not reach statistical significance (*P* > 0.75). Incidentally, human gonococcal infection during pregnancy is also associated with 35% infant mortality (27). It is important that blood cultures from the dead fetuses were negative. Although the reason for this result is not clear at this time, it is possible that *N. gonorrhoeae* cannot survive in dead animals. Alternatively, fetal death may have been caused by complement and/or proinflammatory cytokines that could be induced by lipopoligosaccharide released from killed *N. gonorrhoeae* in the maternal bloodstream. This hypothesis is supported by previous findings that systemic administration of bacterial lipopolysaccharide to pregnant rats induced fetal resorption or fetal death (4, 10, 37).

The rate of fetal bacteremia correlated with C1q concentration. The results suggest that C1q not only supported the spread of *N. gonorrhoeae* strains carrying the 344-bp DNA fragment of the peritoneal cavity of the pregnant rat to the fetus but also increased morbidity and mortality.

**Human C1q is known to activate rat complement (28) and also protected ser⁺ *N. gonorrhoeae* from the killing effect of rat pup complement (28). It is, therefore, conceivable that ser' strains inoculated with human C1q activate complement on the surface of bacteria in vivo without bacterial death. Further activation of complement in infected fetuses (15) may contribute to the proinflammatory responses via cytokines, activation of neutrophils, and the enhancement of vasopermeability and, therefore, increase virulence to the fetus (9, 12, 22).

Although the precise role of the complement in the pathogenesis of gonococcal sepsis is not clear (8, 21, 27, 34, 35, 40), in human bacteremia, complement played an essential role in septic shock (14, 16, 20, 26, 30, 34, 38, 39). It is also known that

**TABLE 1. Relevant phenotype and genotype of strains used for experimental infection**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Clinical diagnosis</th>
<th>Serum sensitivity</th>
<th>sac-4&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Serotype</th>
<th>Auxotype</th>
<th>Source</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>JC1</td>
<td>DGI</td>
<td>R</td>
<td>+</td>
<td>1A</td>
<td>Arg</td>
<td>Houston, Tex.</td>
<td>22</td>
</tr>
<tr>
<td>2005</td>
<td>PID</td>
<td>R</td>
<td>−</td>
<td>1B3</td>
<td>Pro</td>
<td>Atlanta, Ga.</td>
<td>23</td>
</tr>
<tr>
<td>1658</td>
<td>LI</td>
<td>S</td>
<td>−</td>
<td>1B4</td>
<td>Proto</td>
<td>Atlanta, Ga.</td>
<td>23</td>
</tr>
</tbody>
</table>

<sup>a</sup> R, serum resistant; S, serum sensitive.

<sup>b</sup> Detection of 344-bp DNA fragment of sac-4 region by PCR; −, negative by PCR.

**TABLE 2. Transmission of *N. gonorrhoeae* from pregnant rats to fetuses<sup>a</sup>**

<table>
<thead>
<tr>
<th>Strain</th>
<th>C1q&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Total no. of fetuses&lt;sup&gt;c&lt;/sup&gt;</th>
<th>% Dead fetuses&lt;sup&gt;d&lt;/sup&gt;</th>
<th>% Rat fetuses with positive blood culture&lt;sup&gt;e&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>JC1</td>
<td>−</td>
<td>117</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>114</td>
<td>38.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>100&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>2005</td>
<td>−</td>
<td>112</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>115</td>
<td>33.3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>100&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>1658</td>
<td>−</td>
<td>110</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>113</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Live fetuses.

<sup>b</sup> These data represent nine litters from three independent experiments.

<sup>c</sup> N. gonorrhoeae pretreated with C1q (80 μg/ml), −, N. gonorrhoeae pretreated with BSA (80 μg/ml).

<sup>d</sup> Blood culture negative.

<sup>e</sup> Three litters of rats, including three pregnant rats and 34 ± 5 (mean ± standard deviation) number of fetuses in each group, were sacrificed and bled 24 h postinoculation. The percentage of pregnant rats with positive blood cultures was 0.

**FIG. 1. Recovery of *N. gonorrhoeae* from fetuses' blood 24 h after i.p. inoculation of rats on day 20 of pregnancy with the following three types of *N. gonorrhoeae* strains: DGI (JC1), PID (2005), and LI (1658).**
inhibition of the biologic effects of complement in baboons with sepsis attenuates the lethal complications (38). It remains to be investigated whether inhibition of the biologic effects of complement C1q in pregnant rats may successfully prevent neonatal sepsis.

Overall, we propose that the model showing C1q-dependent transmission of ser" N. gonorrhoeae from mother to fetus may be relevant for the evaluation of pathogenesis of gestational gonococcal infection and fetal morbidity. Future studies are planned to evaluate the molecular mechanisms responsible for the C1q-dependent and mother-fetus transmission of N. gonorrhoeae and the inhibition of this process before the onset of neonatal sepsis and stillbirth.

REFERENCES


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