Maternal Protection against *Escherichia coli* K1 Infection in a Neonatal Rat Model

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Abstract. Maternal protection against *Escherichia coli* K1 meningitis in a neonatal rat model was studied. Mothers immunized with formalin-killed and live *E. coli* K1 had detectable levels of antibody in both serum and colostrum. After orogastric challenge with *E. coli* K1, 34% of pups born to and nursed by immunized mothers were found to be bacteremic compared to 60% of those born to and nursed by nonimmunized mothers. Thus, mothers immunized with *E. coli* K1 organisms prior to delivery can provide protection against subsequent development of K1 meningitis in the neonate.

*Escherichia coli* species that contain K1 capsular polysaccharide antigen have been shown to be an important cause of neonatal meningitis [9, 10]. *Escherichia coli* K1 meningitis in the neonate continues to have a high morbidity and mortality even with appropriate antibiotic therapy [8]. For this reason, research in this disease must focus on the virulence-enhancing mechanism of the K1 polysaccharide [5, 11-13], and the potential for immunologic prophylaxis of the neonate against *E. coli* K1 meningitis. Passive immunization is known to present some particular difficulties since the K1 polysaccharide is weakly immunogenic in humans [2].

In the present study we use a model of *E. coli* K1 meningitis in neonatal rats to test the hypothesis that mothers immunized with *E. coli* K1 organisms prior to delivery can provide protection against subsequent development of K1 meningitis in the neonate.

Materials and Methods

ICR strain female white rats were used. Rats were bled by tail vein and serum tested for antibody to K1 polysaccharide. A direct hemagglutination technique was used in which serum is serially diluted and added to sheep red blood cells coated with purified K1 polysaccharide. (K1 polysaccharide was prepared by Cetavlon extraction by the method of Gotschlich et al. [4].) No antibody to K1 polysaccharide was detected in any of three female rats. The rats then received a regimen of active immunization as follows: day 0— injection by tail vein of 0.3 ml 10$^8$ formalin-killed *E. coli* 018 K1; day 5—0.5 ml of 10$^6$ 018 K1, formalin-killed; days 8, 12, 16—0.2 ml of overnight broth culture.

After challenge, antibody titers were assayed by hemagglutination technique in the three rats. These rats were bred with ICR strain white male rats. All three mothers delivered without difficulty.

Five-day-old infant pups were challenged with 07 K1 *E. coli* as follows: 0.1 ml (10$^7$-10$^8$) *E. coli* K1 on day 5 and 0.05 ml (10$^7$-10$^8$) on day 6. Pups were challenged by force-feeding through an orogastric tube (Popper and Sons, New Hyde Park, NY). Infant pups from six control mothers were also challenged. On the eighth day after birth (two days after challenge of *E. coli* K1), blood samples were taken from the lateral tail vein (0.1 ml) and plated on blood and MacConkey media for determination of bacteremia.

In two mother rats (A and B) and one control mother, antibody to K1 polysaccharide was determined in milk. The rats were sacrificed after protection studies were completed. Mammary glands were dissected and diced. A 1:2 solution of colostrum and sterile saline was made. Solution was centrifuged at 6000 rpm for 30 min. Lipid and cells were separated from milk. Hemagglutination was determined using 0.1 ml of serially diluted "milk," and 0.1 ml K1 coated sheep red blood cells.

Results

Mothers immunized with formalin-killed and live *E. coli* K1 had detectable levels of antibody in both serum and colostrum as shown in Table 1. Control mothers did not have anti-K1 antibody detectable. After orogastric challenge, the number of pups born to immunized mothers who were found to be bacte-
Table 1. Antibody response of mother rats to Escherichia coli K1 challenge

<table>
<thead>
<tr>
<th>Antibody titer to K1 polysaccharide in serum (log 2)</th>
<th>Antibody titer to K1 polysaccharide in milk (log 2)</th>
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</thead>
<tbody>
<tr>
<td>Mother A</td>
<td>4</td>
</tr>
<tr>
<td>Mother B</td>
<td>2</td>
</tr>
<tr>
<td>Mother C</td>
<td>5</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
</tr>
</tbody>
</table>

remic with *E. coli* K1 was 11/32 or 34% (litter 1–4/10 = 40%, litter 2–3/10 = 30%, litter 3–4/12 = 33%). Under the same conditions, 35 of 58 pups (60%) born to nonimmunized mothers developed bacteremia (*P* < 0.05 chi square) (Table 2).

**Discussion**

This study shows that mothers who have developed antibody titers to K1 polysaccharide by immunization with whole organisms can protect neonates against *E. coli* K1 infection. Neonates of mothers with prior antibody production to K1 were less likely to develop bacteremia after orogastric challenge of *E. coli* K1 than neonates of control mothers. Previous studies have shown that non-K1 *E. coli* given by orogastric challenge to neonatal rats will not produce bacteremia [1, 3]. The mechanism of protection may be transplacental transmission of IgG antibody or passive transfer of IgA antibody from breast milk to neonate. Wolberg and Dewitt [14] and Kaijser and Olling [6] have shown that anti-K1 antibodies produced by whole bacteria challenge protected against experimental infection of peritonitis in mice and pyelonephritis in the rabbit. Cross et al. have recently demonstrated a murine monoclonal antibody to K1 polysaccharide that has a killing effect on all *E. coli* K1 strains tested [2].

Vaccination with *E. coli* K1 may be a potential approach to preventing neonatal meningitis. However, vaccination of humans with Group-B meningococcal polysaccharide (cross reactive with K1 polysaccharide) alone has failed to produce bactericidal levels of antibody [15]. Human beings with *E. coli* K1 bacteremia have not developed increased levels of antibody to K1 [2]. A vaccine component of mannose-sensitive pili has recently been reported to protect neonatal rats from *E. coli* K1 meningitis after being given to mothers of the challenged neonates [7].

Further research with this animal model may be useful in developing a vaccine given to mothers to protect against neonatal *E. coli* K1 meningitis.

Table 2. Incidence of bacteremia in neonatal rats challenged with *Escherichia coli* K1

<table>
<thead>
<tr>
<th>Group</th>
<th>Neonates bacteremic/number tested</th>
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<tbody>
<tr>
<td>Born to and nursed by immunized mothers</td>
<td>11/32 (34%)</td>
</tr>
<tr>
<td>Born to and nursed by nonimmunized mothers</td>
<td>35/58 (60%)*</td>
</tr>
</tbody>
</table>

*P* < 0.05 chi square.

**Literature Cited**


