Additive Protective Effects of Colostral Antipili Antibodies in Calves Experimentally Infected with Enterotoxigenic Escherichia coli

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With oral infection of calves by an enterotoxigenic Escherichia coli strain carrying K99, F41, and FY adhesins, colostrums from cows vaccinated against either K99+F41 or FY did not provide protection, but a mixture of the two colostrums did. The association of antibodies directed against the different adhesins is more effective than antibodies directed against one adhesin alone for colostral protection against enterotoxigenic E. coli carrying several adhesins.

The vaccination of pregnant cows with K99 (or F5; see reference 20 for a discussion of antigen nomenclature) Escherichia coli provides good colostral protection of calves against K99 enterotoxigenic E. coli (ETEC) (2, 5, 19, 24). Other pericellular structures of ETEC are also important in the colonization of the small intestine. We previously described a pilus provisionally designated FY which could be associated with K99 and F41 (6, 9). This FY pilus was later described with the designation Att25 by Pohl et al. (21, 22). E. coli carrying both K99 and FY pili represent nearly 10% of bovine ETEC isolated in France (8) and 30% of that isolated in Belgium (21). To study the respective roles of K99+F41 and FY pili, calves were infected with ETEC carrying K99, F41, and FY, and the protective role of anti-K99,F41 and anti-FY colostral antibodies was studied both monospecifically and in association.

The vaccination of two groups of four Friesian cows was done subcutaneously at month 7 of gestation (1.8 × 1010 E. coli killed by formaldehyde in a 5-ml suspension solution containing 21 mg of NaCl, 0.7 mg of aluminium hydroxide, and 0.3 mg of saponin). Nonvaccinated cows supplied a control colostrum (C); cows vaccinated with E. coli O9:K30:H-,K99,F41 supplied anti-K99,F41 colostrum (A), and cows vaccinated with E. coli O2:K7:H42,FY supplied anti-FY colostrum (B). Colostrum A+B was obtained by mixing equal amounts of colostrums A and B. After mixing, the colostrums were divided into 2-liter flasks. The colostrums were then frozen (−20°C) pending further use. Calves receiving colostrums C, A, B, or A+B were challenged with E. coli O101:K32:H9,K99,F41,FY.

The experimental infection was done with Friesian calves in a sterile isolator. Before they were 5 h old, the calves consumed 2 liters of colostrum, and then they consumed the bacterial inoculum (5 × 1010 to 1011 E. coli). They were later fed sterilized (ultra high temperature) whole milk from cows. The presence of the infectious strains (adonitol−) in the feces was checked for daily by the method of Contrepois and Gouet (7).

Agglutinins were assayed on the serocolostrums obtained after reenet coagulation by tube seroagglutination of bacteria (2 × 109/ml) grown at 37°C in a glucose Minca medium (10). E. coli B41 (O101:K99,F41) and the E. coli O101:K32:H+,FY were used to titrate, respectively, the anti-K99,F41 and anti-FY agglutinins.

The two control calves died of dehydration after severe diarrhea (Table 1). The same occurred with the three calves that received colostrum B. Two of the three calves that received colostrum A died from dehydration. The third survived after severe diarrhea lasting 2 days and clinical signs of dehydration. The four calves that received the colostrum A+B mixture were protected. Two did not become ill, and the other two had passing bouts of diarrhea without any clinical sign of dehydration.

According to H. W. Moon (14), immunological protection is assured by the antibodies that act against the pili and, to a lesser extent, against capsular K antigens. Those that act against O antigens and flagella seem to have a minor role. Accordingly, the H9 antigen of the infecting strain was not taken into consideration. Assay of the agglutinins that act against the O101 and K32 antigens showed that colostrums C, A, and B were comparable (data not shown).

Anti-K99,F41 vaccination increased eightfold the amount of agglutinins directed against these antigens. However, all the cows, including those from groups C and B, produced anti-K99,F41 agglutinins. This can be explained by the anti-K99 vaccination given 2 years earlier to all the cows in the herd, information we obtained after the experiment. Under natural conditions, only a low proportion of cows produce the anti-K99 antibodies (1, 4). The anti-FY vaccination increased 4- and 16-fold the anti-FY agglutinin level in group B cows compared with group A and C cows. However, all the cows had a relatively high titer of anti-FY agglutinins (log2 = 10). This was not entirely unexpected, as the K99+F41 strains are not rare (8, 21), and FY is a potent immunogen (6). Anti-FY agglutinin titer was high in the batch of colostrum A (log2 = 12) because, unfortunately, two of the four cows had high anti-FY agglutinin levels in their colostrum.

Under similar experimental conditions, the colostrums enriched in anti-K99 antibodies protected the calves from E. coli K99 infection, but E. coli B41 (5, 18) or B44 (4, 24) were most frequently involved. As regards our infecting strain, protection was practically null. This indirectly suggests that FY has a specific role in colonizing the small intestine of the calf. However, FY is still not the major adhesin, because colostrum B did not protect the calves. Good protection was obtained only when the antibodies directed against K99+F41 and FY of colostrums A and B were associated.

The differences between the colostral agglutinin titers which were protective and those which were not were only

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TABLE 1. Clinical evolution of calves infected with *E. coli* O101:K32:H9,K99,F41,FY

<table>
<thead>
<tr>
<th>Calf no.</th>
<th>Colostrum type and ant (liters)</th>
<th>Agglutinin titers (log2)</th>
<th><em>E. coli</em> infectious dose (×10⁹)</th>
<th>Calf age at inoculation (h)</th>
<th>Diarrhea after oral infection</th>
<th>Time of death after inoculation (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C (2)</td>
<td>7</td>
<td>10</td>
<td>4</td>
<td>12</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>C (2)</td>
<td>7</td>
<td>10</td>
<td>4</td>
<td>12</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>A (2)</td>
<td>10</td>
<td>12</td>
<td>8</td>
<td>16</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>A (2)</td>
<td>10</td>
<td>12</td>
<td>8</td>
<td>12</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>A (2)</td>
<td>10</td>
<td>12</td>
<td>8</td>
<td>15</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>B (2)</td>
<td>7</td>
<td>14</td>
<td>8</td>
<td>12</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>B (2)</td>
<td>7</td>
<td>14</td>
<td>8</td>
<td>16</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>B (2)</td>
<td>7</td>
<td>14</td>
<td>8</td>
<td>15</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>A + B (2)</td>
<td>10</td>
<td>14</td>
<td>8</td>
<td>18</td>
<td>−</td>
</tr>
<tr>
<td>10</td>
<td>A + B (2)</td>
<td>10</td>
<td>14</td>
<td>8</td>
<td>30</td>
<td>−</td>
</tr>
<tr>
<td>11</td>
<td>A + B (2)</td>
<td>10</td>
<td>14</td>
<td>10</td>
<td>18</td>
<td>−</td>
</tr>
<tr>
<td>12</td>
<td>A + B (2)</td>
<td>10</td>
<td>14</td>
<td>8</td>
<td>2.5</td>
<td>18</td>
</tr>
</tbody>
</table>

four- or eightfold. Except for Altmann and Makkur (3) and Nagy (19) who measured, respectively, 100- and 20-fold augmentation of colostral agglutinins after vaccination, other researchers (2, 24, 25) have used an enzyme-linked immunosorbent assay or passive hemagglutination or its inhibition, and so comparison is not possible. However, by using the colostrum of vaccinated cows with anti-K99,F41 titers similar to these and by challenging calves with homologous (B41) or heterologous (B44) ETEC, we previously obtained, respectively, complete (5) or 50% (4) protection. Another point that should be made here is that agglutinins are only a part of the colostral antibodies. With the Coombs anti-globulin test (5) or enzyme-linked immunosorbent assay (4), these anti-K99 titers are not always correlated with those of agglutinins. This is not surprising if these antibodies belong to the bovine immunoglobulin G1 class, which has poor agglutinating properties (13). Therefore, agglutinin titers are indicative only of antibody response and are not necessarily strictly correlated with protection. However, we think that using animals with lower initial agglutinin levels would probably provide a clearer demonstration of the additive protection.

It is known that K99 is not the only surface antigen of the ETEC involved in colonizing the small intestine of the calf. For example, capsular antigens have been shown to be more important than K99 pilus (11, 23) for some strains. F41 (15) seems to be an important factor in colonization of the small intestine, as demonstrated previously in the piglet (16), the lamb (17), and the calf (26). As regards type 1 pilus (F1), which is carried in association with other adhesive pili by some ETEC, conflicting results have been obtained concerning in vivo biosynthesis and the protective effects of antibodies (12, 27). The results obtained here indirectly suggest that F1 can be an in vivo colonization factor of this ETEC. In vitro, the ETEC carrying both the K99 and F1 pili adhere to the enterocytes more strongly than do the ETEC with only K99 (6, 9) pili.

Colostral antibodies did not eliminate the infecting strain from the intestine, for the latter was present in the dominant colibacillary flora for more than 8 days in the calves that survived. Those animals that are protected by colostral antibodies therefore remain an important source of contamination.

As regards vaccination policy, it does not appear desirable to give preference to monovalent K99 formulas. Valences corresponding to capsular antigens and to the pilus most frequently encountered in bovine ETEC should be associated.

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LITERATURE CITED


