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Abstract

Titers of antibody against Escherichia coli in human milk and in the sera of 11 breast-fed infants, 6 bottle-fed infants and 9 infants in the post-weaning period were measured by the passive hemagglutination method. High antibody titers were observed in human milk in the first 4 days after parturition, but the titer decreased rapidly thereafter. None of the healthy, breast-fed infants had detectable serum antibodies, while a breast-fed infant with a perianal E. coli abscess had antibodies. On the other hand, 4 of the 6 bottle-fed infants and all of the 9 infants in the post-weaning period had antibodies. The significance of these results was discussed.

KEYWORDS: breast-fed infant, bottle-fed infant, E. coli antibody

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Serum Antibodies to *Escherichia coli* in Breast-fed and Bottle-fed Infants

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Titers of antibody against *Escherichia coli* in human milk and in the sera of 11 breast-fed infants, 6 bottle-fed infants and 9 infants in the post-weaning period were measured by the passive hemagglutination method. High antibody titers were observed in human milk in the first 4 days after parturition, but the titer decreased rapidly thereafter. None of the healthy, breast-fed infants had detectable serum antibodies, while a breast-fed infant with a perianal *E. coli* abscess had antibodies. On the other hand, 4 of the 6 bottle-fed infants and all of the 9 infants in the post-weaning period had antibodies. The significance of these results was discussed.

Key words : breast-fed infant, bottle-fed infant, *E. coli* antibody

It has been accepted for many years that the morbidity and mortality are lower in breast-fed infants than in those fed by other means (1-4). Tassovatz and Kotsitch reported that serial outbreaks of enteropathogenic *E. coli* infections in neonatal nurseries ceased after changing from bottle feeding to breast feeding (5). Several investigators have indicated differences in intestinal bacterial colonization, especially that of *E. coli*, between breast-fed and bottle-fed infants (6-8). However, the difference in the host response to *E. coli* between these two groups of infants is yet to be elucidated.

The present communication reports the titers of antibody to *E. coli* in the colostrum of mothers and in the serum of breast-fed and bottle-fed infants.

Materials and Methods

Colostrum, milk and serum. Colostrum and milk were collected from 15 mothers over a period of 11 days after parturition. The samples were removed of fats by centrifugation at 3000 rpm for 10 min at 4°C. Sera were obtained from 25 healthy infants, an infant with a perianal abscess, 8 healthy children and 5 healthy adults. The collected milk and sera were stored at −20°C. Prior to use, they were heated at 56°C for 30 min to inactivate the proteolytic enzymes.

Sheep red blood cells and lipopolysaccharide of E. coli. Sheep red blood cells (SRBC) were purchased from Tokyo-Kessei Corp., Tokyo, Japan. The lipopolysaccharide of *E. coli*, serotype 026 : B6, which is known to cause enterocolitis in infants and children, was obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A.

Hemagglutinating antibodies to E. coli. The passive hemmagglutination test was done according to the method described by Neter and Kenny (9-10). The microtitration technique employed is briefly described as follows : SRBC stored at 4°C in Alsever's solution were washed 3 times with phosphate buffered saline (PBS) and coated with *E. coli* lipopolysaccharide at 37°C for 1 h. Then, a 2.5% suspension of antigen-coated SRBC was added to serial twofold dilutions of milk and sera, starting from a dilution of 1 : 2 or 1 : 10. The reaction was carried out at room temperature for 2 h and at 4°C overnight. The highest dilution of

milk or sera in which agglutination was just perceptible was taken as the antibody titer. The control titration was done with uncoated SRBC, but nonspecific agglutination of SRBC was not observed with any milk or serum sample.

Results

Fig. 1 shows the changes in titers of antibody against *E. coli* in human postpartum milk. The breast milk obtained during the early postpartum period (2-5 days) showed high antibody titers, which subsequently decreased rapidly.

Fig. 2 shows the titers of antibody to *E. coli* in the serum of breast-fed infants, bottle-fed infants, children and adults. Significantly high antibody titers were noted in all of the adults and children 2 years of age or older. Infants who were 7 months or older and in the post-weaning period had antibodies. The difference between the samples from breast-fed infants and those from bottle-fed infants was striking. No antibodies were detected in any of the 10 healthy, breast-fed infants younger than 7 months of age. A breast-fed infant with a perianal *E. coli* abscess had an antibody titer of 1 : 4. On the contrary, positive antibody titers were detected in 4 of 6 bottle-fed or mixed-fed infants of similar ages. Two of these positive cases had titers of 1 : 20, which was the same as that observed in adults.

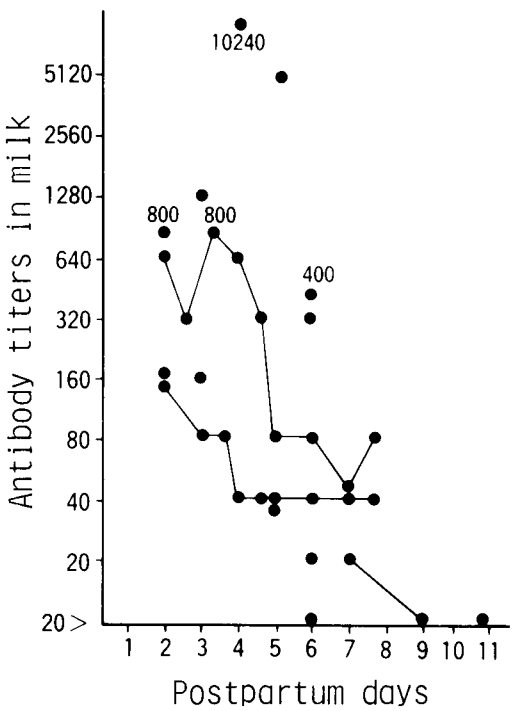

Fig. 1 Titers of antibody to *E. coli*, 026 : B6, in human milk. Circles connected by a solid line represent milk antibody titers from the same subject.

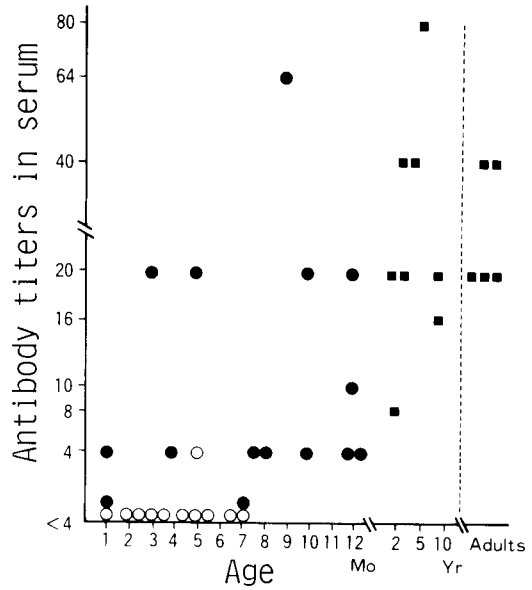

Fig. 2 Serum titers of antibody to *E. coli*, 026 : B6, in breast-fed infants (○), bottle-fed, mixed-fed and post-weaning infants (●), and children and adults (■).

Discussion

A correlation between the levels of antibodies against *E. coli* in human colostrum and the number of coliform bacteria in the stools of breast-fed newborns has been demonstrated by Michael *et al.* (7). They suggested that the antibody to *E. coli* in the colostrum might lead to a prevalence of lactobacilli over *E. coli* in the feces of breast-fed

infants during the first four days of life. The result of the present studies with the breast milk obtained during the postpartum period was essentially the same as theirs, confirming their inference. However, there still remains the question whether the antibodies against *E. coli* in the breast-milk have any beneficial effects more than four days postpartum. In the present studies, none of the breast-fed infants except one infant with a perianal *E. coli* abscess, had detectable antibodies against *E. coli* within 7 months of birth, whereas 67% of the bottle- or mix-fed infants developed detectable serum antibodies against *E. coli* within this period. These results suggest that during the first 7 months after birth, breast-fed infants are not exposed to *E. coli,* whereas almost all of the bottle-fed infants are exposed to *E. coli* in the early period of infancy.

Recent *in vitro* studies have shown that human milk inhibits the adherence of *E. coli* to epithelial cells. This effect appears to be due to secretory IgA antibodies to *E. coli* in human milk (11). However, it is still not known why infection with *E. coli* is almost completely avoided in breast-fed infants for as long as 7 months after birth, when secretory IgA or other anti-infective factors in human milk are thought to be markedly diminished. In relation to this question, Ogra and Ogra have suggested that the prevention of *E. coli* infections mainly depends on the total amount of IgA antibodies taken by infants rather than their concentration in the milk (12). It is also considered currently that, in a rather early stage of infancy, the anti-infective properties of human milk are replaced by the secretory IgA system in the intestine of infants themselves (13).

It thus appears conceivable that the succession from milk IgA to locally synthesized IgA in the intestine protects the breast-fed infants from infections of *E. coli* for a period as long as 7 months.

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