Short Communication

Effects of Orally Administered Bovine Lactoferrin on the Faecal Enterobacteriaceae of SPF Mice Fed Milk

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The number of faecal Enterobacteriaceae of mice fed only bovine milk increased greatly. In addition, when mice were fed milk supplemented with bovine lactoferrin (bLF), the number of faecal Enterobacteriaceae decreased to the level in faeces of mice fed ordinary solid diets. This result is considered to be caused by the bacteriostatic effect of bovine lactoferrin in vivo.

Lactoferrin, an iron-binding protein prominently contained in animal colostrum and mature milk, has been found to have bacteriostatic activity against bacteria which require iron for growth. It has frequently been suggested that lactoferrin plays a part in the defense of breast-fed infants against bacterial infections. Though many studies have been reported concerning the bacteriostatic effects of lactoferrin in vitro, there have been no reports confirming the in vivo effects of lactoferrin. In order to examine the bacteriostatic effects of bLF in vivo, the number of faecal Enterobacteriaceae of mice fed bLF-containing milk was examined.

At first, the influence of milk feeding on faecal Enterobacteriaceae was examined, and then the effects of bLF added in milk were examined. bLF (iron saturation 14.5%) was prepared from fresh skim milk (Morinaga Milk Industry Co., Zama City, Japan) by the method of Law and Reiter. After 7 days on an ordinary diet, SPF mice (Balb/c, female, 4 weeks old, 10 mice/group) were fed commercial milk (pasturized at 130°C, 2 s) ad libitum using a feeding pack for the next 7 days. The milk diet was then replaced with milk containing 5% bLF for the next 7 days. Fresh faeces were collected separately from each mouse and diluted with ice-cold saline. Diluted samples were cultured on plates of DHL agar (deoxycholate–hydrogen sulfide–lactose agar, Eiken Chemical Co., Tokyo, Japan) and Enterobacteriaceae were enumerated after 24 h incubation at 37°C. After two days of milk feeding, the number of faecal Enterobacteriaceae increased 100 times more than that of control mice fed the ordinary diet continuously (Fig. 1). Though the tendency for faecal bacteria including Enterobacteriaceae in mice and rats to be increased by milk feeding has been reported previously, the cause of the increase was not known. The addition of 5% bLF to the milk decreased the number of faecal Enterobacteriaceae to a level close to that of the control mice. The removal of bLF caused a rapid increase of bacterial numbers. During this experiment, the intake of milk or milk containing bLF

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**Fig. 1.** Effects of Administered Bovine Lactoferrin on the Number of Enterobacteriaceae in Feces of SPF Mice.

The first group of mice (Balb/c, female, 4 weeks old, 10 mice/group) were fed an ordinary diet (commercial pellets) and water during the experiment as control (○). The second group were fed an ordinary diet and 5% bLF in water solution during days 8 to 21 but an ordinary diet and water for the other days (●). The third group were fed milk during the experiment (□) and the fourth group were fed milk containing 5% bLF during days 8 to 21 but milk for the other days (▲). Milk, or milk containing 5% bLF, or 5% bLF water solution, was fed using a sterile bag and a sterile nozzle. From fresh faeces diluted with ice-cold saline bacteria were counted using DHL agar medium after 24 h of incubation at 37°C. The number of Enterobacteriaceae is expressed as the logarithm of colony forming units (CFU) per gram of faeces.

**Fig. 2.** Effects of Administered Bovine Lactoferrins with Different Iron Saturation on the Initial Proliferation of Enterobacteriaceae in Feces of SPF Mice.

Each group of mice was fed an ordinary diet and water (○), milk (●), milk containing 5% native bLF (□), milk containing 5% apo-bLF (■), or milk containing 5% holo-bLF (▲).

*Abbreviations: bLF, bovine lactoferrin; SPF, specific pathogen free.*
was about 15 g per mouse (each weighing 18 to 20 g) per day. There was no significant differences between the body weights of each group.

The effects of concentration and iron-saturation of bLF on the number of faecal Enterobacteriaceae were examined (Figs. 2 and 3). Apo-bLF (iron saturation 2.3%) and holo-bLF (iron saturation 97.6%) were prepared by dialysis of native bLF against citric acid and iron citrate solutions, respectively, followed by dialysis against distilled water. After a week of milk feeding (at day 7), each group of mice was separately fed milk contained native bLF at concentrations ranging from 0.5% to 5.0%, or apo- or holo-bLF at concentrations of 5%. The other conditions were as described above. The change in the number of faecal Enterobacteriaceae was examined during the first two days after administration of milk containing bLF (Fig. 2). At day 8, the faecal Enterobacteriaceae of the milk-fed groups increased to a level 10 times higher than that of the control group fed an ordinary diet. At day 9, the bacterial number in the feces of groups administered bLF (including apo- and holo-) remained at the same levels at day 8, while that of the milk-fed group increased to a level 100 times higher than that of the control group. There was no significant difference in the faecal bacterial numbers between the groups fed milks containing native, apo-, or holo-bLF. This suppressive effect of bLF against intestinal Enterobacteriaceae appeared to be independent of the degree of iron saturation of bLF. Presumably the antimicrobial mechanism of bLF obtained herein may involve the bactericidal domain of bLF which has been recently reported and named lactoferrin.7 The dose response of the number of faecal Enterobacteriaceae to bLF is shown in Fig. 3. The number of faecal Enterobacteriaceae decreased with bLF concentration from 0.5% to 2%, and remained constant for the mice fed milk with containing more than 2% bLF. Compared with the faecal bacterial numbers at day 11, there was a tendency for the bacterial numbers to decrease in these groups fed bLF-containing milk at day 18. Especially, the faecal bacterial numbers of the group fed milk containing 0.5% bLF decreased greatly and, compared to the group fed milk, the difference in bacterial numbers was significant. Therefore, it was demonstrated that feeding milk containing 0.5% bLF could induce a gradual decrease of intestinal Enterobacteriaceae. In the case of mice fed commercial pellets, the addition of bLF into drinking water could not exert this effect, even at a high concentration of 5% bLF (Fig. 1). Commercial diets contain a variety of dietary factors as dietary fibers and minerals which may act to mask the effect of bLF. Feeding mice only cow’s milk that lacks some of these factors caused a steep rise in the number of Enterobacteriaceae. If the change of intestinal flora caused by the change from a solid diet to a milk diet is common in animals, it is expected that the addition of bLF to milk might decrease Enterobacteriaceae in the gut of human infants fed infant formula. The intestinal flora of human infants is formed rapidly during the first week after delivery. Escherichia coli is the most common bacterium in the phase of primary infection before the establishment of the intestinal flora.8 Infants ingest a considerable amount of lactoferrin from colostrum during this term. Lactoferrin may have the potential to suppress the extraordinary increase of pathogenic bacteria in new-born infants which have immature intestines.

The effects of bLF on the intestinal flora of mice fed milk is being investigated further.

References