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Depression of Activity of Intestinal Mucosal Alkaline Phosphatase with Gastrointestinal Microorganisms

YASUO KAWAI,* TAKAHIKO ISHII, MASAMI MOROTOMI, AND MASAHIKO MUTAI

Department of Intestinal Microbiology, Yakult Institute for Microbiological Research, 1796 Yaho, Kunitachi, Tokyo, Japan

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Depression of intestinal alkaline phosphatase activity can be caused by the formation of the microbial ecosystem with several kinds of viable microorganisms in rats.

We have been studying the relationships between gastrointestinal microflora and host rats (2, 3). In a previous paper (1), we reported that the influence of intestinal microflora on the activity levels of some enzymes was observed in the gastrointestinal tracts of various kinds of gnotobiotic rats associated with bacteria indigenous to conventional rats; in particular, the activity levels of some mucosal enzymes in the upper small intestine were depressed by gastrointestinal bacteria. The alkaline phosphatase activity level of the upper small intestine in various gnotobiotic rats was intermediate between the levels in germfree and conventional rats (1). Thus, it was found that intestinal microflora was important in the regulation of the enzyme activity level in the digestive tract of the host. The following experiments were designed to determine what kinds of indigenous bacteria were responsible for such depression of the mucosal enzyme activity as seen in the upper small intestines of gnotobiotic rats. Groups of male germfree rats (five rats per group), Fischer strain 344, 6 weeks old, were monoassociated with one of the following six kinds of indigenous intestinal microorganisms isolated from conventional rats (2): *Escherichia coli* N-1, *Staphylococcus epidermidis* N-1, *Streptococcus faecalis* subsp. *liquefaciens* Y-5, *Bacteroides vulgatus* GAM-8, gram-positive fusiform bacterium EG-1, and *Lactobacillus fermentum*. These rats were designated GB1. A similar group of five rats, designated GB6, was inoculated with a mixture of 18 strains of lactobacilli (*L. acidophilus, L. fermentum,* and *L. murini*) and the other five strains cited above. Rats were kept for 2 weeks in vinyl isolators sterilized with peracetic acid (2).

The activity level of upper small intestinal alkaline phosphatase in GB6 rats was significantly lower than that in germfree rats and higher than that in conventional rats (Table 1, experiment 1). Thus, the association with the six kinds of indigenous intestinal bacteria (GB6) depressed the activity level of alkaline phosphatase in the upper small intestine of rats. This result confirmed our previously reported data (1). However, no influence of bacteria on the enzyme activity level was observed in any of the monoassociated GB1 rats. Thus, association with a mixture of bacteria of indigenous gastrointestinal microorganisms, but not monoassociation with any of the individual strains tested, depressed the activity level of alkaline phosphatase in the upper small intestine of rats.

In gnotobiotic and conventional rats, gastrointestinal bacteria form specific microbial ecosystems (2) in which the localization of microorganisms was seen. To examine whether the viable bacteria are needed to depress the activity of mucosal alkaline phosphatase in the upper small intestine, we administered heat-killed cells of the six kinds of bacteria to germfree rats. Bacteria were administered, via stomach tubes, each day for the first 3 days and thereafter every

TABLE 1. *Specific activities of mucosal alkaline phosphatase in the upper small intestine in germfree, conventional, and gnotobiotic rats*

Expt	Rats	Sp act (μmol of *p*-nitrophenol/15 min per mg of protein)[a]
1	Germfree	30.56 ± 1.10
	Conventional	15.63 ± 0.65
	GB6	20.68 ± 1.98
	GB1, *E. coli*	29.29 ± 1.89
	GB1, *Lactobacillus*	34.17 ± 1.23
	GB1, *Staphylococcus*	31.25 ± 3.14
	GB1, *Bacteroides*	31.46 ± 2.19
	GB1, *Streptococcus*	34.89 ± 1.02
	GB1, fusiform bacteria	28.72 ± 0.71
2	Germfree rats administered dead cells of six kinds of bacteria[b]	28.99 ± 2.21

[a] Mean ± standard error.
[b] These bacteria are the same kinds as in GB6 rats.

other day for the next 2 weeks. In this case, the activity was not depressed significantly, as seen in GB6 rats (Table 1, experiment 2). This result indicates that viable bacteria are necessary for depression of mucosal alkaline phosphatase activity in the upper small intestine of rats.

The population levels of various bacteria in the contents of the upper small intestine were 10^7 to 10^8 in the conventional and various gnotobiotic rats, except in GB1 rats with fusiform bacteria (Table 2). This result indicates that the depression of alkaline phosphatase activity in the upper small intestine was not influenced directly by the presence of bacteria in that region. Although the population level of lactobacilli was the highest among the indigenous bacteria in the contents of the upper small intestine in conventional, GB6, and GB1 rats with lactobacilli, the activity of alkaline phosphatase in these rats was at different levels (Table 1). Therefore, the depression of alkaline phosphatase activity was not due to the dominant bacteria in the region. Yolton and Savage (4) reported that activity levels of duodenal alkaline phosphatase in specific-pathogen-free mice was strikingly lower than the level in germfree mice, and that monoassociation with indigenous *Bacteroides* sp. reduced the activity of alkaline phosphatase to a level of intermediate between that of germfree and specific-pathogen-free mice. In our experiments, the alkaline phosphatase activity was depressed by association with a mixture of the six kinds of indigenous bacteria (GB6) but was not depressed by monoassociation with any of these microorganisms (GB1). Furthermore, our results showed that alkaline phosphatase activity was not significantly reduced by administration of heat-killed cells of the six kinds of indigenous bacteria (Table 1, experiment 2).

These results strongly indicate that the formation of ecosystems with several kinds of the viable microorganisms is important for the depression of mucosal alkaline phosphatase activ-

TABLE 2. *Distribution of indigenous bacteria in the contents of the digestive tract in conventional and gnotobiotic rats mixed, or monoassociated, with six kinds of indigenous intestinal microorganisms isolated from conventional rats*

Rats	Digestive tract contents in:	log/g (wet wt) of contents						
		E. coli	*Lactobacillus*	*Staphylococcus*	*Bacteroides*	*Streptococcus*	Fusiform bacteria	Total anaerobic bacteria
Conventional	Stomach	4.5 ± 0.4	8.4 ± 0.3	0.6–3.6	1.3–3.7	5.0 ± 1.0		ND[a]
	Upper SI[b]	1.1–3.3	6.6 ± 0.7	ND	ND	0.7–3.4		ND
	Lower SI	6.6 ± 0.8	8.1 ± 0.4	1.4–3.8	0.7–3.7	6.4 ± 0.7		8.2 ± 0.4
	Cecum	6.4 ± 1.5	8.6 ± 0.4	1.3–3.7	8.1 ± 0.4	6.3 ± 0.8		10.3 ± 0.2
CB6	Stomach	5.1 ± 0.6	9.0 ± 0.2	4.1–4.8	1.2–3.8	5.9 ± 0.7	ND	
	Upper SI	3.3–3.8	7.5 ± 0.5	2.9–3.6	2.3–3.7	3.6–4.2	ND	
	Lower SI	7.7 ± 0.8	9.4 ± 0.3	5.6 ± 0.9	4.2–5.0	7.3 ± 0.6	ND	
	Cecum	8.7 ± 0.1	9.3 ± 0.2	6.2 ± 1.0	9.8 ± 0.2	8.8 ± 0.2	9.2 ± 0.2	
GB1, *E. coli*	Stomach	6.9 ± 0.7						
	Upper SI	8.1 ± 0.3						
	Lower SI	8.5 ± 0.5						
	Cecum	9.1 ± 0.3						
GB1, *Lactobacillus*	Stomach		7.6 ± 0.3					
	Upper SI		7.2 ± 0.2					
	Lower SI		8.0 ± 0.5					
	Cecum		8.0 ± 0.3					
GB1, *Staphylococcus*	Stomach			7.3 ± 0.7				
	Upper SI			7.3 ± 1.0				
	Lower SI			7.6 ± 0.2				
	Cecum			7.9 ± 0.4				
CB1, *Bacteroides*	Stomach	-			6.4 ± 1.5			
	Upper SI				7.4 ± 0.8			
	Lower SI				7.0 ± 1.0			
	Cecum				9.6 ± 0.3			
GB1, *Streptococcus*	Stomach					8.0 ± 1.0		
	Upper SI					8.1 ± 0.6		
	Lower SI					9.2 ± 0.3		
	Cecum					9.2 ± 0.3		
GB1, fusiform bacteria	Stomach						ND	
	Upper SI						ND	
	Lower SI						ND	
	Cecum						7.9 ± 0.2	

[a] ND, Not detected ($<10^3$).
[b] SI, Small intestine.

ity in the upper small intestine of rats.

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