Fecal Immunoglobulin A (IgA) Antibodies to Yersinia enterocolitica in Mice Orally Vaccinated and Infected

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ABSTRACT. Fecal immunoglobulin-A (IgA) antibodies to Yersinia enterocolitica serovar O3 strain were detected in the mice orally immunized with formalin-killed organisms. Y. enterocolitica O3 organisms were inhibited to colonize in the intestines of the mice producing fecal IgA. The fecal IgA antibodies were detected in the mice orally infected with the bacteria. When IgA was produced in the mice infected, they ceased shedding the organisms in their feces.—Key words: immunoglobulin A (IgA), oral vaccination, Y. enterocolitica.


There are some reports on detection of human serum IgA-antibodies to Yersinia enterocolitica [1, 6]. Very little is known, however, about IgA-class Yersinia antibodies in the intestines of man and animals. Fecal IgA-class Yersinia antibodies have not been reported and this is the first report on the detection of such antibodies.

It is known that fecal excretion of pathogenic Y. enterocolitica is associated with Ca²⁺ dependency coded by a virulent plasmid and that nonpathogenic Y. enterocolitica is not excreted in the murine feces [5]. It is not known, however, whether or not the fecal excretion of the bacteria varies depending on the age and the strain of the mouse. Fecal excretion of Y. enterocolitica is suppressed in the mice vaccinated orally with viable organisms [7] or orally with killed organisms [4]. The role of IgA played in these phenomena, however, is still unknown. This paper deals with the production of specific fecal IgA in the mice orally vaccinated with killed Y. enterocolitica and in those infected with the viable organisms.

MATERIALS AND METHODS

Bacterial strain: The bacterial strain used was Ca²⁺ dependent Y. enterocolitica serovar O3, biovar 3 [3] isolated from a brown rat. Its biochemical characteristics have been reported [3]. It was demonstrated by the method of Kado and Liu [2] that this strain harbored a 42 megadalton virulence-associated plasmid. In addition, Escherichia coli JC-2, Salmonella serovar typhimurium ATCC 13311 and Staphylococcus aureus 209P were used for ELISA antigens. These bacteria were cultured on Trypticase soy agar (BBL) for 48 hr at 25°C and stored at −80°C in a mixture of equal volumes of calf serum and a 10% lactose solution.

Animals, challenge and quantitative direct culture: Fourteen female 4-week-old SPF ICR mice (Shizuoka Agric. Coop. Assoc. Lab., Hamamatsu, Japan) were used. The mice were shown not to harbor Yersinia species by culturing their feces before the experiment. A frozen stock of the bacteria was thawed and mixed with physiological saline to a concentration of 10⁷ viable organisms/0.1 ml. The mice were intragas-
trically challenged with 0.1 ml of the mixture through a gastric feeding tube. Quantitative direct culture of feces was made as before [4]. Salmonella-Shigella agar (Eiken, Tokyo) was used as a selective medium for *Y. enterocolitica* as before [4], since MacConkey agar did not inhibit swarming colonies.

**Vaccination:** Vaccination was carried out by slight modification of the methods of Yanagisawa *et al.* [8]. The organisms grown on Trypticase soy agar (BBL) for 48 hr at 25°C were suspended in physiological saline. A final concentration of 1% (v/v) formaldehyde was added to the suspension which was maintained at room temperature for 24 hr. To remove formaldehyde, the formalin-killed cells were centrifuged and resuspended in sterile physiological saline to a final concentration of 500 mg (wet weight)/ml. This suspension was used as the vaccine. Four doses of 100 mg of the killed organisms were given orally to mice through a gastric feeding tube at 4-day intervals.

**Extract of feces:** Feces were collected everyday from each mouse caged individually. The fecal pool was weighed and suspended in 4 volumes of PBS consisting of 0.01 M Na₂HPO₄-NaH₂PO₄ and 0.15 M NaCl, pH 7.2, ground with a glass rod, and centrifuged at 720×g for 5 min to obtain the supernatant for ELISA.

**Procedures for ELISA:** The formalin-killed bacteria were suspended at concentrations of 12.5, 25, 50, 100, and 200 μg/ml in carbonate-bicarbonate (pH 9.6) containing 1.59 g of Na₂CO₃, 2.93 g of NaHCO₃, and 0.2 g of NaN₃ per liter. The wells of microtiter plates (Dynatech Lab., Alexandria, VA, U.S.A.) were each coated with 0.05 ml of the antigen suspension, incubated for 1 hr at 25°C and coated with 0.3 ml of 1% (w/v) bovine serum albumin for 15 min at 25°C. Each well was added with 0.05 ml of fecal extract, incubated for 1 hr at 25°C, washed once with 0.02% Tween 20 in 0.02 M imidazole buffered saline, and then incubated with 0.05 ml of peroxidase-labelled goat anti-mouse IgA (α) at a concentration of 0.5 μg/ml for 1 hr at 25°C. After additional five washes with PBS-Tween, peroxidase was assayed by the addition of 0.05 ml of an ABTS (2,2’-azino-di-[3-ethylbenzthiazolin sulfonate (6)]) substrate solution (KPL). The absorbance was read with a microplate photometer MTP12 (Corona Electric Co., Tokyo) with a 405 nm filter.

**RESULTS**

The optimal concentration of the antigen for adsorption was 25 μg/ml (wet weight) as shown in Fig. 1. The values are means of duplicate experiments. Higher concentrations of the antigen did not increase the sensitivity of the assay. Lower concentrations resulted in decreased sensitivity. The antigen concentration of 25 μg/ml was,
therefore, used in the following experiments.

The time course of the IgA response in feces of the mice immunized with killed organisms is shown in Fig. 2. The data are the means of determinations in five mice with standard deviations. The data with each mouse were the means of duplicate ELISA experiments. The IgA level began to increase in the vaccinated mice in 21 days after the first vaccination, maintained the high level for 10 days and then decreased thereafter (Fig. 2). On the other hand, the IgA levels against E. coli, Salmonella serovar typhimurium or S. aureus did not rise during the experimental period. From feces of all the mice orally challenged with viable Y. enterocolitica O3 organisms, no bacteria were recovered on the 7th day after the challenge. During the experimental period unimmunized mice did not produce IgA antibodies to Y. enterocolitica.

Fig. 3 shows the excretion of IgA in the feces of unvaccinated mice after challenge with $10^7$ viable organisms of Y. enterocolitica O3 strain. In all the four mice challenged, IgA began to increase about 20 days after the challenge, when the bacteria in feces began to decrease in number. In three mice, IgA decreased immediately after the first rise and increased again as the bacteria disappeared from their feces.

**DISCUSSION**

In the present study, IgA was detected in the feces of the mice immunized with killed Y. enterocolitica O3 organisms. The fecal specimens containing IgA against O3 organisms did not react to unrelated bacteria, but reacted to only the O3 organisms. The IgA excreted in the feces of mice, therefore, was considered to be specific against Y. enterocolitica.

It is known that the protection against intestinal colonization of challenged Y. enterocolitica is attained by oral vaccination with killed organisms [4]. In the present study, IgA production was demonstrated in the mice orally vaccinated with killed organisms. Furthermore, the mice producing IgA were protected against intestinal colonization of Y. enterocolitica. Therefore, IgA should be involved in the protective mechanisms against intestinal colonization of Y. enterocolitica induced by oral vaccination with the homologous killed organisms.

IgA against Y. enterocolitica was detected in the mice orally infected with the viable organisms. IgA titers did not rise in any of the four mice infected with Y. enterocolitica, while the bacteria colonized in the intestines and were excreted in the feces. When the IgA titers rose, the bacteria disappeared.
from the feces of all mice. These facts suggest that IgA is involved in the mechanisms of termination of the intestinal colonization of *Y. enterocolitica*.

Although the IgA titer decreased immediately after the rise of IgA titer in two of the four mice infected with viable organisms, such decrease was not clearly observed in the other two mice. It is not known whether different mechanisms of IgA production are involved between the mice orally inoculated with viable and those with killed organisms.

Since oral vaccination with killed organisms of O3 strain induced IgA production in the murine intestines, further studies are

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Fig. 3. Detection of fecal IgA in the mice orally challenged with $10^7$ viable organisms of *Y. enterocolitica*. ●, ELISA absorbance value; ○, the number of the bacteria per gram of feces.
warranted to find whether oral vaccination with other serovars induce IgA production as does O3 strain.

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REFERENCES


要約

Yersinia enterocolitica を経口投与されたマウスにおける糞便中 IgA の検出：下野和之・金子賢一・松谷秀樹・小川益男（東京農工大学農学部家畜衛生学教室）——Yersinia enterocolitica O3菌のホルマリン死菌を経口投与したマウスにおいて、同菌に対する特異的 IgA が糞便中に検出された。IgA 産生のマウスに Y. enterocolitica O3生菌攻撃を行うと腸管への定着が阻止された。同様に生菌を経口投与したマウスについても IgA が糞便中に検出された。これらのマウスでは IgA の産生が見られると腸管への定着は直ちに終了した。