Location of *Edwardsiella tarda* Antigens in Tissues of Eel (*Anguilla japonica*) after Intramuscular Injection

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The fate of *Edwardsiella tarda* antigens, crude lipopolysaccharide (LPS) and formalin-killed cells (FKC) which was injected intramuscularly in eel, *Anguilla japonica*, was investigated. Location of the antigens was observed using fluorescent antibody technique. After injection, crude LPS was detected in the liver, kidney and spleen at 30 min and 1 h, in the heart and liver at 3 and 6 h, but not detected in any tissues at 12 h. FKC were detected in the gill, liver and kidney at 30 min, in the gill, stomach, intestine, liver and kidney at 1 h, in the intestine, heart, liver, kidney and spleen at 3 h, in the liver, kidney and spleen at 6 h, and in the kidney at 12 h. FKC disappeared from all tissues except the kidney until 12 h. Intracellular specific fluorescence was observed in the cells of the heart and liver of both fish groups injected with crude LPS or FKC. These findings suggest that after injection, the antigens are rapidly accumulated mainly in the liver and kidney, then decomposed or excluded.

To develop an effective vaccine against edwardsiellosis for cultured Japanese eel, *Anguilla japonica*, and European eel, *A. anguilla*, immune response of eel has been studied employing a culture filtrate (SALATI *et al.*, 1983), heat-killed or formalin-killed cells (FKC) (SONG and KOU, 1977; SONG and KOU, 1981; SALATI *et al.*, 1983), lipopolysaccharide (LPS) and polysaccharide (SALATI *et al.*, 1984; SALATI and KUSUDA, 1985) and lipid (SALATI and KUSUDA, 1986) of *Edwardsiella tarda*.

SALATI *et al.* (1983 and 1984) found that *E. tarda* crude LPS and crude polysaccharide possessed higher protective immunogenicity than whole cell preparation against experimental challenge of eel. SALATI and KUSUDA (1986) and SALATI *et al.* (1987) further showed that *E. tarda* crude LPS enhanced the phagocytic activity of eel leukocytes.

To examine the difference of eel's immune response due to different antigens, this paper describes location and fate of *E. tarda* antigens, crude LPS and FKC, in tissues of eel after intramuscular injection.

Materials and Methods

Experimental Fish

Japanese eel, *Anguilla japonica*, weighing about 100 g were maintained at 25°C in 60 l tanks with recirculating system and aeration. The fish were not fed during the experiments.

Bacterium

Edwardsiella tarda strain EF-1 was originally isolated in June 1977 from the kidney of a diseased Japanese eel from eel ponds in Fukuroi, Shizuoka Prefecture, Japan.

Antigen Preparation

Lyophilized EF-1 was precultured in Brain Heart Infusion (Difco) at 37°C for 18 h, then inoculated in the same medium and incubated at 37°C for 48 h with shaking. After harvest by centrifugation, the bacterial cells were washed with distilled water. From a portion of the crude LPS was extracted by Westphal's phenol-water method as previously described (SALATI and KUSUDA, 1985) and adjusted to the concentration

at 10 mg/ml in physiological saline.

Another portion of the cells was resuspended in physiological saline at a concentration of 100 mg/ml and formalin was added at a concentration of 0.5%. After a 24 h incubation at 4°C, FKC suspension was obtained.

Antigen Injection and Fish Sampling

One hundred microliter of the crude LPS solution or FKC suspension were injected to the fish intramuscularly. Three fish from both groups were collected 30 min, 1, 3, 6 and 12 h after injection.

Fluorescent Antibody Preparation and Staining

Rabbit anti-*E. tarda* immunoglobulin G was purified using DEAE Affi-Gel Blue chromatography (Bio-Rad) and labeled with fluorescein isothiocyanate as previously described (KAWAHARA *et al.*, 1986; KAWAHARA and KUSUDA, 1987). Fixation of organs, section and fluorescent antibody staining were performed by the method of KAWAHARA and KUSUDA (1988).

Results

The changes in the location of crude LPS in eel organs are summarized in Table 1. After injection, crude LPS was found in the liver, kidney and spleen at 30 min and 1 h, in the heart

Table 1. Location of *Edwardsiella tarda* crude lipopolysaccharide (LPS) in eel organs at selected time intervals after intramuscular injection

Organ	Hours after injection				
	0.5	1	3	6	12
Gill	—	—	—	—	—
Stomach	—	—	—	—	—
Intestine	—	—	—	—	—
Heart	—	—	+	⊕	—
Liver	⊕⊕	⊕⊕	⊕	⊕	—
Kidney	+	+	—	—	—
Spleen	+	+	—	—	—
Gall bladder	—	—	—	—	—
Musculature	—	—	—	—	—

+: presence of crude LPS, —: absence of crude LPS, ⊕: fluorescence in the cells of eel tissues. Concentration of crude LPS: ++ > +.

Fig. 1. *Edwardsiella tarda* crude lipopolysaccharide accumulated in the cells of liver 30 min after intramuscular injection. Arrows show fluorescent cells. Bar = 80 μm.

Table 2. Location of *Edwardsiella tarda* formalin-killed cells (FKC) in eel organs at selected time intervals after intramuscular injection

Organ	Hours after injection				
	0.5	1	3	6	12
Gill	+	+	—	—	—
Stomach	—	+	—	—	—
Intestine	—	+	+	+	—
Heart	—	—	⊕	—	—
Liver	⊕+	⊕+	⊕+	⊕	—
Kidney	+++	++	+	+	+
Spleen	—	—	+	+	—
Gall bladder	—	—	—	—	—
Musculature	—	—	—	—	—

+: presence of FKC, —: absence of FKC, ⊕: fluorescence in the cells of eel tissues. Concentration of FKC: +++ > ++ > +.

and liver at 3 and 6 h, and not found in any organs at 12 h. Crude LPS was not detected in the gill, stomach, intestine, gall bladder and musculature throughout the periods. Intracellular fluorescence was observed in the heart at 6 h, and in the liver from 30 min (Fig. 1) to 6 h.

The changes in the location of FKC in eel organs are summarized in Table 2. After injection, FKC was found in the gill, mostly in the

Fig. 2. *Edwardsiella tarda* formalin-killed cells stained with fluorescent antibody in the kidney 30 min after intramuscular injection. Arrows show *E. tarda* cellular antigen. Bar = 40 μ m.

chloride cells, liver and kidney (Fig. 2) at 30 min, in the gill, liver, kidney, lamina propria of the stomach and intestine at 1 h, in the intestine, heart, liver, kidney and spleen at 3 h, in the intestine, liver, kidney and spleen at 6 h, and only in the kidney at 12 h. FKC were not observed in the gall bladder and musculature throughout the period. Intracellular specific fluorescence was found in the heart at 3 h, and in the liver from 30 min to 6 h.

Discussion

After injection, both antigens, LPS and FKC located commonly in the liver, kidney and spleen. Accumulation of both antigens was higher in the liver than in the other organs, but the antigens disappeared from the liver before 12 h as well as most of the other organs. Digestive activity of the liver may be great.

Comparing the fate of antigens in the spleen, crude LPS appeared faster than FKC. The time lag before the appearance of FKC may be caused by the lipid of *E. tarda*, which acts as an immunosuppressant in the eel (SALATI and KUSUDA, 1986). The number of fluorescent cells in the liver of eel inoculated with crude LPS was

greater than that of eel inoculated with FKC. These results indicate that crude LPS stimulates directly the leukocytes, and is more liable to be phagocytosed than FKC. Therefore, crude LPS is more effective than FKC as an immunogen against edwardsiellosis for the eel.

Crude LPS and FKC antigens disappeared from the tissues before 12 h after injection which is faster than the case of *Pasteurella piscicida* (KAWAHARA and KUSUDA, 1988). They reported that FKC, sonicated cells and crude LPS of *P. piscicida* remained in several tissues for 3 days after immersion of yellowtail, *Seriola quinqueradiata*, with those antigens. The difference in the remaining time between *E. tarda* antigens in eel and *P. piscicida* antigens in yellowtail may be due to antigen characters and host's immune response.

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