— Note —

***Corynebacterium kutscheri* Invasiveness of the Gastrointestinal Tract in Young Mice**

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Corynebacterium kutscheri invasiveness of the tissues of the gastrointestinal tract in mice was investigated by the fluorescent antibody technique. The invasion was clearly recognized in 3-week-old mice infected intragastrically along with gastric mucin and a subcutaneous dose of cortisone; 10^7 organisms caused a notable invasion of the gastric and duodenal tissues. Furthermore, marked bacterial invasion of the large intestine appeared in mice challenged perorally with 7×10^7 organisms.

In mice and rats infected naturally with *Corynebacterium kutscheri* (CK), development of visceral abscesses, swelling of the large intestine, and hemorrhage or ulceration in the colon have been observed [1,4-6]. However, the bacterial invading behavior in the tissues of the gastrointestinal (G. I.) tract of infected animals has not been investigated [4-7].

The purpose of the present investigation was to examine bacterial invasiveness of G. I. tissues using weanling mice and with an immunofluorescence procedure.

Strains: Two CK strains, CK-1 and R-11 derived from a mouse and a rat, respectively, and strains of various other species, i. e., 1 strain each of *A. aerogenes*, *B. subtilis*, *Bacteroides* sp., *E. coli*, *Lactobacillus* sp., *P. aeruginosa*, *P. vulgaris*, and *S. aureus*, were applied for oral infection and/or to confirm the specificity of the fluorescein-labeled antibody.

Fluorescein isothiocyanate-labeled antibody: Immune serum having an agglutination titer of 1:16,000 against strain CK-1 was obtained from a rabbit subcutaneously inoculated twice with 5ml of formalin-

killed CK suspension (McFarland Scale No. 3) with aluminum hydroxide gel. The crude gammaglobulin was labeled with fluorescein isothiocyanate according to the method of Kawamura [2]. The conjugates were adsorbed twice with a mouse liver powder, and their specificity was confirmed using visceral organs of mice free from CK and smears of the forenamed various test organisms.

Specimens and fluorescent staining: Procedures of immunohistologic examinations were described in a previous paper [3].

Oral challenge experiment: Three- or four-week (WK)-old SPF mice (ICR, male) were used and kept in isolation with sterilized food. Oral exposure was conducted with a single 0.2ml dose of a 3-day culture in trypticase soy broth of strain CK-1 ($1-7 \times 10^7$ organisms) suspended in an equal volume of a 5% neutral hog gastric mucin suspension; the dose was instilled into the stomach through a catheter, followed by a 2.5 mg subcutaneous injection of cortisone acetate. Immunohistologic examinations were done at intervals of several days, and recovery of the bacterium

was effected on trypticase soy agar plates for confirming the infection of the lung, liver, spleen, and kidney. The serum agglutination titer was calculated using a microtiter technique.

The results were as follows :

1. Disparity in the bacterial invasiveness between 3- and 4-WK-old mice. Both groups of mice were orally challenged with the bacterial suspension (4×10^7 organisms).

The results are shown in Table 1. In the 3-WK-old mice, the invasion into the G. I. tissues was rare on the whole although a few CK appeared later in the mucous membranes of the stomach and caecum in occasional mice. In 3-WK-old mice that received cortisone, however, three of six parts of the G. I. tract that were examined, the stomach, duodenum and caecum, showed evidence of bacterial invasion. There was

Table 1. Disparity in the effect of cortisone acetate on gastrointestinal infection of *C. kutscheri* between 3- and 4-week(wk)-old mice orally infected with 4×10^7 organisms.

Tissue	3-wk-old		3-wk-old*	4-wk-old*	
	PED 2 to 6	PED 9 to 19	PED 2 to 6	PED 2 to 6	PED 10 to 15
Stomach	0 ^a (0) /4 ^b	3 (0) /8	4 (1) /5	0 (2) /7	0 (0) /5
Duodenum	1 (1) /4	2 (1) /8	3 (4) /5	1 (3) /7	0 (2) /5
Jejunum	0 (0) /4	1 (0) /8	1 (2) /5	0 (2) /7	0 (0) /5
Ileum	0 (0) /4	1 (0) /8	0 (1) /5	1 (3) /7	0 (0) /5
Caecum	0 (0) /4	3 (0) /8	3 (0) /5	2 (4) /7	0 (2) /5
Colon	0 (2) /4	1 (0) /8	0 (5) /5	0 (5) /7	0 (2) /5

* : Mice subcutaneously administered with a dose (2.5mg) of cortisones acetate.

a : No. of mice with *C. kutscheri* in gastrointestinal tissues.

b : No. of mice examined.

() : No. of mice with *C. kutscheri* in contents.

PED : Postexposure day.

Table 2. Local infection of the gastrointestinal tract in 3-week-old mice orally infected with 10^7 *C. kutscheri* organisms under the influence of cortisone.

Tissue	Postexposure day					
	1	3	5	7	10, 20	Total
Stomach	2*(2) 0** /6	3 (0) 1 /6	5 (0) 1 /5	4 (2) 2 /5	2 (0) 0 /8	16 (4) 4 /30
Duodenum	2 (1) 0 /6	3 (2) 0 /6	3 (3) 0 /5	4 (1) 0 /5	2 (0) 0 /8	14 (7) 0 /30
Jejunum	0 (1) 1 /6	0 (0) 1 /6	1 (2) 0 /5	1 (0) 0 /5	0 (0) 0 /8	2 (2) 2 /30
Ileum	0 (1) 0 /6	1 (0) 0 /6	1 (0) 0 /5	0 (0) 0 /5	1 (1) 0 /8	3 (2) 0 /30
Caecum	1 (1) 0 /6	3 (2) 1 /6	2 (0) 1 /5	3 (0) 0 /5	3 (1) 1 /8	12 (4) 3 /30
Colon	0 (0) 0 /6	1 (1) 0 /6	1 (0) 0 /5	2 (0) 1 /5	1 (2) 0 /8	5 (3) 1 /30

* : No. of mice with *C. kutscheri* in the epithelium or lamina propria.

** : No. of mice with *C. kutscheri* in the muscularis.

() : No. of mice with *C. kutscheri* in contents.

Denominator : No. of mice examined.

an abundant accumulation of CK in the colonic contents of every mouse. Moreover, the submucosa in the caecum of a mouse was invaded remarkably at 6 days postinfection (Fig. 5). The CK was recovered from almost all viscera, and a rapid abscess formation was recognized in several organs.

On the other hand, the invasion of the G. I. tissues in 4-WK-old mice was negligible in spite of the use of the immunosuppressant. The 2 cortisone treated mouse groups developed a tendency to local aggregation of CK in the contents of both the caecum (Fig. 4) and colon (Fig. 8).

2. Invasion-site in the alimentary tract of 3-WK-old mice. Two challenge trials in weanling mice were repeated in order to examine the pathology in more detail. (A) Thirty 3-WK-old mice were given a dose of 10^7 organisms, and their G. I. tracts were examined at 6 periods in 20 days. The results are shown in Table 2. The bacterial invasion proceeded notably in the stomach and duodenal tissues at 3 to 7 days of infection; many bacilli proliferated in the mucous membrane of the fundic glands of the stomach (Fig. 1, 2). Several nodular clumps of specific fluorescent (SF)-positive bacilli emerged at times in the muscularis

mucosae and glandular epithelium (Fig. 3). In the duodenum, the mucosal epithelium and lamina propria frequently harbored bacterial cells. Above all, in the tissues of the latter, many clumps of SF-positive bacilli appeared occasionally, forming an orbicular shape of various sizes. Subsequently, the invasion of these tissues decreased remarkably. Otherwise, the sporadic infection was often seen in the caecum, particularly in the muscularis; the invasion of the tissues was visible in some mice even at days 13, 14 and 20 postinfection (Fig. 6). Abscesses or bacterial clumps were often formed in several organs (liver, heart, lung or kidney), and recovery of CK was obtained by cultivation from them, in 5 to 11 days postinfection. Subsequently, there was neither macroscopic changes in the viscera nor recovery of the CK from the viscera by cultivation, and a serum agglutination titer (1:8) developed at the final examination. (B) Further observation of the bacterial invasiveness of the large intestinal tissues was effected with a large dose of CK; an oral challenge with 7×10^7 organisms was performed by the same method. The observation period was 20 days. The results are shown in Table 3.

Table 3. Infection of the large intestine of 3-week-old mice orally administered with a large dose (7×10^7 organisms) of *C. kutscheri* under the influence of cortisone.

		Postexposure day				
		Large intestine		5	10	20
Caecum	Contents	3/10		3/4		1/3
	Crypt	1/10		3/4		1/3
	Epithelium or lamina propria	8/10	16*/40** (40)***	2/4	9/16 (56)	4/12 (33)
	Muscle layers	4/10		1/4		0/3
Colon	Contents	5/10		4/4		3/3
	Crypt	0/10		3/4		1/3
	Epithelium or lamina propria	5/10	12/40 (30)	3/4	14/16 (88)	7/12 (58)
	Muscle layers	2/10		4/4		1/3

* : Totally positive sites of intestines.

** : Total No. of mice examined.

*** : Percent.

Many more SF-positive bacteria were found in the colon than in the caecum, and there was bacterial invasion of both intestinal tissues (Fig. 7, 9, 10). However, the colon tissues appeared to show delayed bacterial penetration compared with the caecum. Low agglutination titers (1:8-1:16) were produced in the mice at the final examination.

In these trials, the effect of cortisone at the early age of 3 weeks progressed remarkably. This might have been brought about by a strong suppression in the production of copr-antibody. In addition, the mucin-coated organisms are effective in eliminating the bactericidal action of the gastric secretions. The localization of CK in the large intestine was reported previously [8]. The present results clearly demonstrated the invasiveness of this pathogen in the lower parts of the G. I.

tract in young mice.

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Corynebacterium kutscheri の幼若マウス消化管に対する侵襲態度

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Corynebacterium kutscheri のマウス消化管に対する侵襲態度を免疫蛍光法でしらべた。コーチゾンとムチンを併用した3週齢時の経口感染により、消化管組織へ

の侵襲が明瞭に顕われた。 10^7 個の菌の経口感染で胃底部、十二指腸への侵入増殖を見、また 7×10^7 個の菌の投与によって盲、結腸への侵襲が顕著となった。

Explanation of Figures

- Fig. 1 Invasion by *C. kutscheri* (CK) of the epithelium tunica mucosa in pars glandularis of the stomach.
- Fig. 2 Progressive invasion by CK of the glandular epithelium and muscular layer about the gastric glands.
- Fig. 3 Formation of nodular clumps of CK in the fundic glandular area of the stomach.
- Fig. 4 Great accumulation of CK in the caecal canal.
(oral infection in a 4-week-old mouse)
- Fig. 5 Invasion of CK in the submucosa of the caecum.
- Fig. 6 CK (arrow) invade the muscular layer of the caecum.
- Fig. 7 Clumps of CK developing in the lamina propria in the colon.
- Fig. 8 Great numbers of CK in the colon canal.
(oral infection in a 3-week-old mouse)
- Fig. 9 Pervasively CK-invaded site in the tunica mucosa of the colon.
- Fig. 10 Local infection of CK in the tunica mucosa of the colon.
(All figures : 400 magnifications)