Salmonella typhimurium線毛,非線毛株のマウスに対する感染性の再検討

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BRIEF NOTE

Further Studies on the Infectivity to Mice of *Salmonella typhimurium* Strains with or without Fimbriae

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The previous study on the infectivity of freshly isolated *S. typhimurium* to mice [3] demonstrated that fimbriate descendants of the organism had higher infectivity than nonfimbriate descendants upon oral administration, and that the proportion of fimbriate cells in the fimbriate descendants was correlated with the infectivity to mice, while the two descendants showed almost the same infectivities upon intraperitoneal inoculation.

The present report deals with comparison of the infectivity to mice between fimbriate and nonfimbriate *S. typhimurium* organisms both being preserved strains different from those used in the previous study [3] performed to reconfirm the previous findings.

Five-week-old, SPF female mice of the NIH (gpc) strain raised in Funabashi Farm were used in this experiment. For each inoculum, mice were divided into 5 to 7 groups each consisting of 5 animals. Care and management of the inoculated mice were performed in essentially the same manner as reported previously [3]. The animals were observed for a period of 14 days after oral administration and 8 days after intraperitoneal injection. A total of 13 *S. typhimurium* strains isolated from feces of carrier dogs or chickens, that had been subcultured for 5 to 12 years, were used as inocula. Of six isolates from dog feces, four were fimbriate and the other two nonfimbriate. Of seven isolates from chickens, five were fimbriate and the other two nonfimbriate. The fimbriate and nonfimbriate strains were confirmed by the hemagglutination test with horse or guinea pig erythrocytes and by electron microscopy as reported previously [3]. To count the fimbriate cells of a fimbriate strains, 100 to 300 cells were examined by electron microscopy. All the fimbriate and nonfimbriate strains were cultured in Duguid-Gillies's broth [2] at 37°C for 48 hr. Ten-fold serial dilutions were made of each culture with phosphate-buffered saline (pH 7.0). A one-milliliter portion of an appropriate dilution containing 10^2–10^6 cells was given orally to each mouse of a group through a stomach catheter. A 0.5-m*l* portion of certain dilutions (10^0–10^6 cells/0.5 m*l*) was inoculated intraperitoneally into each of another group of mice. After oral or intraperitoneal inoculation, bacteriological, pathological and serological examinations for O, H and fimbrial agglutinins were made in the same way as described previously [3] on mice died

Table 1. Infectivity of the fimbriate and nonfimbriate strains of *S. typhimurium* to mice upon oral and intraperitoneal inoculation

Source	Strain	No.	% of fimbriate cells	Oral inoculation					Intraperitoneal inoculation
				Died of sepsis	Surviving infection	not infected	ID_{50} (Log_{10})	95 % confidence intervals in ID_{50}	LD_{50} (Log_{10})
Carrier-dog feces	F	1	75	3	10	7	3.9	3.5—4.5	2.3
		13	68	3	7	10	4.5	3.8—5.2	2.6
		14	73	2	11	7	4.3	3.7—4.8	2.3
		46	77	2	5	8	4.1	3.6—4.6	2.5
	NF	12	0	0	1	19	≧6.3	5.8—6.7	3.0
		58	0	0	4	16	≧5.9	5.3—6.5	2.7
Chickens	F	149	63	4	9	12	3.9	3.5—4.5	2.7
		180	57	0	5	20	≧5.5	4.7—6.3	5.1
		181	60	0	3	22	≧5.9	5.3—6.5	4.5
		442	75	9	9	7	3.3	2.7—3.9	2.7
		448	72	6	9	10	3.5	2.8—4.1	2.5
	NF	92	0	1	4	20	≧5.7	5.0—6.4	3.4
		443	0	2	6	17	≧5.3	4.5—5.9	3.1

F : Fimbriate strain, NF : Nonfimbriate strain.

during the observation period or sacrificed on the 14th day. After oral administration, such mice were regarded as being infected that died of sepsis with *S. typhimurium* or showed the specific antibody with or without recovery of the organism and gross lesions at the time of necropsy. As reported in the previous paper [3], 50% infective dose (ID_{50}) after oral administration and 50% lethal dose (LD_{50}) after intraperitoneal inoculation were calculated. For each strain, adherence of the fimbriate and nonfimbriate organisms to the isolated ileal mucosa of mice was examined by scanning electron microscopy [5].

Upon oral administration of four fimbriate strains from dog feces, no significant difference in the ID_{50} value at the 5% level was seen among strains (Table 1). In mice given orally nonfimbriate strains, the ID_{50} value was large compared with that of the fimbriate strains, and the difference in the value was significant between the two. On the contrary, when mice were inoculated intraperitoneally, fimbriate and nonfimbriate strains gave nearly the same LD_{50} values. Upon oral administration of five fimbriate strains from chickens, two strains (Nos. 180 and 181) showed larger ID_{50} values than those o fthe remaining three strains. The ID_{50} value of these two strains differed significantly from not only the other three fimbriate strains but also from nonfimbriate strains, even though the strains contained 57 to 60% of fimbriate cells. Upon intraperitoneal inoculation, three fimbriate and two nonfimbriate strains gave nearly the same LD_{50} values, but two fimbriate strains with low infectivity to mice upon oral administration gave large values. The LD_{50} values of the aged strains were generally larger than those of the fresh isolates reported previously [3]. In all the fimbriate strains except two from chickens, there was no relationship between the oral infectivity to mice and the proportion of fimbriate cells.

The surviving mice that proved infection with fimbriate strains from the dog feces or chickens had the fimbrial, O and H anti-

bodies. The fimbrial agglutinin titer ranged from 1:10 to 1:40, and O and H titers from 1:10 to 1:80. The dead mice had such gross lesion as enlargement and small, white, irregular foci in the liver; 70% of them showed also lesions in the spleen and mesenteric lymph node. *S. typhimurium* organisms administered were recovered from all the sites examined (the heart blood, liver, lung, kidney, mesenteric lymph node and intestinal contents). Of 68 survivals with infection, 56 were positive in both formation of gross lesions and the recovery of the organism, and eight only in the recovery of the organism. None of the mice given orally the nonfimbriate strains from dog feces died, but three of 10 mice given 10^6 cells of such strains from chickens died of sepsis. All 15 surviving mice with infection harbored the organism in the mesenteric lymph node, intestinal contents or liver, and showed O and H agglutinins (1:10 to 1:40) but none of them had fimbrial antibody. Another experiment in mice [4] demonstrated that the infectivity of *S. typhimurium* organisms having lost the fimbriae by fimbrial phase variation [1] became significantly lower than that of the same strain with fimbriae when administered orally. On the contrary, upon intraperitoneal inoculation, the fimbriate and nonfimbriate organisms showed nearly the same infectivities.

By scanning electron microscopy, a number of *S. typhimurium* organisms adhering to the surface of villi of the isolated ileum were seen with all the fimbriate strains examined (Fig. 1), and the fimbriae extended from the bacterial surface being in contact with microvilli (Fig. 2), while no organisms of the nonfimbriate strains were found on the epithelium of the ileum.

These results suggest that the adhesive property of the fimbriae to the mucosal surface participating in the initial stage of intestinal infection differentiates the infectivity of fresh isolates and preserved strains as well. There were two exceptional fimbriate strains from chickens that showed low infectivity to mice in both oral and intraperitoneal inoculation comparing with that of the nonfimbriate strains. In the exceptional strains, therefore, it seems likely that the fimbria is not an important determinant of infectivity to mice unlike O or lipopolysaccharide (LPS) somatic antigen.

References

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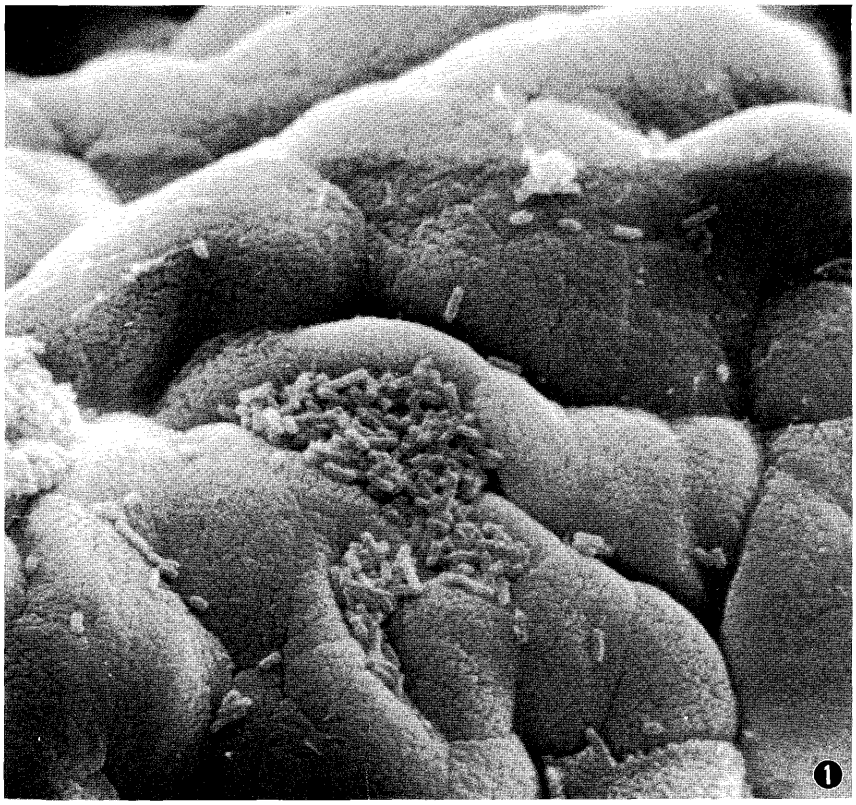
要　約

Salmonella typhimurium 線毛，非線毛株のマウスに対する感染性の再検討（短報）：田中　饒（国立予防衛生研究所獣疫部）——犬由来（保存株）の線毛4株の経口投与による ID_{50} 値は，同由来非線毛2株のそれより小さく，両者の間に有意差を生じた．一方腹腔内接種による LD_{50} 値は両者共にほぼ同様であった．鶏肉由来（保存株）の経口投与実験では線毛5株のうち，2株の LD_{50} 値および ID_{50} 値は残りの線毛3株および同由来非線毛2株より大きく，有意差がみられた．線毛3株と非線毛2株における ID_{50} 値および LD_{50} 値の間には犬由来株でみられたと同様の有意差があった．In vitro の実験で，走査電顕の観察から線毛による回腸粘膜の microvilli との接触による菌体の付着像がみられた．以上から，前報において新鮮分離株でみられたように由来の異なる保存株においても線毛の有無による感染性に差異を生じ，この原因は線毛の腸管粘膜付着能によるものと推測された．

Explanation of Figures

Fig. 1. Numerous fimbriate *S. typhimurium* organisms having adhered to villus of the mouse ileum (Scanning electron micrograph. ×4,300).

Fig. 2. Bacterial adherence of fimbriate *S. typhimurium* to villus of the mouse ileum. Fimbriae extending from the organisms are in contact with microvilli (Long, thick and curved filaments are flagella) (Scanning electron micrograph. ×25,000).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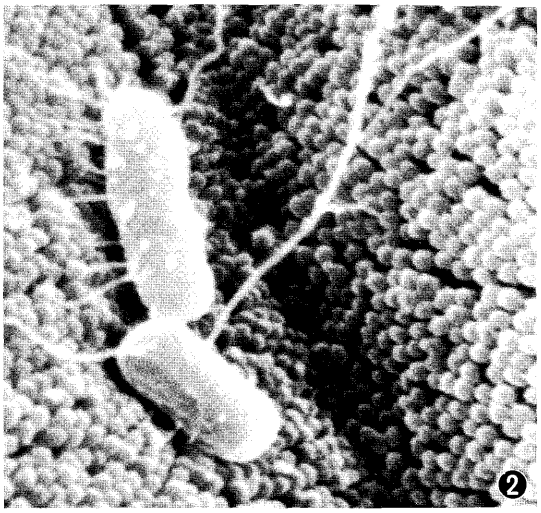
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