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Intact Motility as a *Salmonella typhi* Invasion-Related Factor

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Invasiveness of *Salmonella typhi* was investigated. At first, we introduced Tn*5* into the chromosome of a wild-type *S. typhi* strain, GIFU 10007, and screened the independent Tn*5* insertion mutants for noninvasive (Inv$^-$) strains. During the first half of this work, we obtained 4 Inv$^-$ strains from 1,338 independent Tn*5* mutants. The four were either nonflagellate (Fla$^-$), nonmotile (Mot$^-$), or nonchemotactic (Che$^-$). We then isolated more Fla$^-$, Mot$^-$, or Che$^-$ mutants and examined the invasiveness of these mutants. Sixty-three spontaneous or Tn*5* insertion motility mutants, i.e., Fla$^-$, Mot$^-$, or Che$^-$, were independently isolated from the wild-type strain GIFU 10007; all of them were noninvasive. Motile revertants isolated from some of these mutants showed the same invasiveness as the parent strain. P22-mediated transductional crosses were carried out between some of the motility mutants (as the recipients) and the Fla$^-$ reference strains of *S. typhimurium* with known deletion sites on the genome (as the donors). The mutational sites of the *S. typhi* mutants were assigned almost evenly to the three flagellar gene regions (regions I, II, and III) of *S. typhimurium*. The invasiveness of the motile recombinants obtained from the transduction assays was examined. The restoration of intact motility resulted in the restoration of invasiveness. Thus, we conclude that intact motility is an invasion-related factor of *S. typhi*. The relationship of Vi antigen to the invasiveness of *S. typhi* was also studied. Vi-negative mutants with intact motility remained invasive, whereas all 63 Inv$^-$ spontaneous or Tn*5* mutants were Vi positive. Therefore, Vi antigen was not related to the invasiveness of *S. typhi*.

One crucial step in the pathogenesis of enteroinvasive bacteria is the ability of the bacteria to penetrate the epithelial cell border of the intestinal mucosa and multiply within the epithelial cells. Extensive studies have been made employing cell culture techniques to approach the invasiveness of enteroinvasive bacteria such as *Shigella* spp. (10, 14, 15, 19, 27, 29, 30), *Salmonella* spp. (11–13, 18, 21), invasive *Escherichia coli* (2, 4, 24, 28), and *Yersinia enterocolitica* (34); a strong correlation between in vivo virulence and invasiveness in HeLa or HEp-2 cell cultures has been revealed. The invasiveness of most of the bacteria studied to date is suggested to be associated with plasmids.

The invasive mechanism of bacteria without plasmids, such as *Salmonella typhi*, on the other hand, has been poorly documented. *S. typhi* is so peculiar that among the ca. 2,000 genetically closely related serovars in the genus *Salmonella* (26), only *S. typhi* and a few others (e.g., *S. paratyphi* A, etc.) have the ability to elicit typhoid fever in human beings by invading the epithelial cells of the intestine. Vi antigen has been thought to be an important factor in the pathogenicity of *S. typhi*. In our previous (35) and present studies, however, this factor was shown to have nothing to do with the invasiveness of *S. typhi*. To identify the invasive factor(s) of *S. typhi*, we introduced Tn*5* into the chromosome of *S. typhi* and screened independent Tn*5* insertion mutants for noninvasive (Inv$^-$) strains. The first half of this work told us that invasiveness was somehow related to intact motility of the bacteria. So we isolated more motility mutants, i.e., nonflagellate (Fla$^-$), nonmotile (Mot$^-$), or nonchemotactic (Che$^-$) mutants, and investigated the relationship between the invasiveness and the intact motility of these mutants. The results presented in this report demonstrate that intact motility is an invasion-related factor of *S. typhi*. The mutational sites of some of the Inv$^-$ mutants were mapped; they were almost evenly distributed over all of the three regions containing the flagellar genes of *Salmonella typhimurium*.

MATERIALS AND METHODS

Bacterial and bacteriophage strains. The clinical isolates and NCTC strains and their Vi-antigen negative (Vi$^-$) derivatives of *S. typhi* used in this study and the deletion strains of *S. typhimurium* used for gene mapping are listed in Table 1. SJW 7371 and SJW1518 have deletions which together cover region I of *S. typhimurium* flagellar genes; deletions of SJW 1399 and SJW 1368 and deletions of SJW 1411, SJW 1572, and SJW 1556 cover regions II and III, respectively. The flagellotropic bacteriophage χ (25) was used to select Fla$^-$ or Mot$^-$ strains. Bacteriophage P22 was used in transduction experiments.

Media. Bacteria were grown in heart infusion (HI) broth (Difco Laboratories, Detroit, Mich.), in L broth (5 g of NaCl, 10 g of Bacto-Peptone [Difco], 5 g of yeast extract, and 1 g of glucose for 1 liter), on HI agar (Difco), or on MacConkey agar (Eiken Chemical Co., Tokyo). When required, media were supplemented with kanamycin, (50 μg/ml), nalidixic acid (50 μg/ml), or ampicillin (100 μg/ml). For the isolation and identification of Fla$^-$, Mot$^-$, and Che$^-$ mutants, plates of Gard semisolid agar (9) or soft nutrient gelatin agar (NGA) (36) were employed.

Cell lines. HeLa S3 (ATCC certified cell line 2.2) or Intestine 407 (ATCC certified cell line 6) cells were obtained from Dainippon Seiyaku Co., Osaka, and cultured in Dulbecco modified Eagle medium (DMEM; Nissui Seiyaku Co., Tokyo) supplemented with 10% fetal calf serum (Hyclone Laboratories, Logan, Utah) and 2 mM L-glutamine. Dulbecco phosphate-buffered saline without calcium chloride and magnesium chloride (Nissui Seiyaku Co., Tokyo) was used to wash the cells.

Tranpositional mutagenesis. GIFU 10793, a Nal^r derivative of GIFU 10007 containing the plasmid pCHR81*rep*(Ts)::Tn*5* (31) (Table 1), was streaked onto a MacConkey agar plate containing kanamycin (KM-MacConkey) and incubated at

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TABLE 1. Bacterial strains

Strain	Description
S. typhi	
GIFU 10007	Isolated from a typhoid fever patient (35)
GIFU 10007-1	Nal^r derivative of GIFU 10007
GIFU 10793	GIFU 10007-1 harbouring plasmid pCHR81*rep*(Ts)::Tn*5* (31)
GIFU 10387	Vi$^-$ mutant of GIFU 10007
NCTC 8385	Ty2
GIFU 11725	Vi$^-$ mutant of NCTC 8385
NCTC 8395	Derivative of NCTC 8385; rough, Fla$^-$
GIFU 11727	Vi$^-$ mutant of NCTC 8395
GIFU 9931	Isolated from a typhoid fever patient
GIFU 11726	Vi$^-$ mutant of GIFU 9931
S. typhimurium	
SJW 1103	Phase 1 stable derivative of TM2 (37)
SJW 1518[a]	Derivative of SJW 1103; Fla$^-$
SJW 7371[a]	Derivative of SJW 1103; Fla$^-$
SJW 1368[a]	Derivative of SJW 1103; Fla$^-$
SJW 1399[a]	Derivative of SJW 1103; Fla$^-$
SJW 1411[a]	Derivative of SJW 1103; Fla$^-$
SJW 1556[a]	Derivative of SJW 1103; Fla$^-$
SJW 1572[a]	Derivative of SJW 1103; Fla$^-$

[a] These were used as indicator strains for the determination of *fla* gene location. See Fig. 3 and references 36 and 37 for detailed regions of their deleted genes.

30°C overnight. Several colonies were picked up and each was streaked on two KM-MacConkey plates with one plate incubated at 30°C and the other incubated at 42°C for 36 to 48 h. One colony that grew at 30°C but not at 42°C was transferred into 1-ml samples of HI broth and incubated at 37°C for 12 h. Each culture was diluted to 10^6-fold in saline, and 0.1 ml was spread on a KM-MacConkey plate. Plates were incubated at 42°C until colonies appeared. Since plasmid pCHR81*rep*(Ts)::Tn*5* cannot replicate at 42°C, growth of the strains on a kanamycin plate at 42°C means the transfer of Tn*5* from the plasmid to the chromosome of the bacteria. This was confirmed by the absence of the plasmid from the strains which had become kanamycin resistant. Colonies from different plates grown at 42°C were regarded as independent. One colony was picked up from each plate, numbered with the prefix IV, and subcultured for an invasion assay.

Isolation and characterization of Tn*5* insertion Fla$^-$, Mot$^-$, and Che$^-$ mutants. During the first half of this work, we obtained 4 Inv$^-$ strains from 1,338 independent Tn*5* insertion mutants. The four were either Fla$^-$ (IV340, IV1104), Mot$^-$ (IV867), or Che$^-$ (IV985) (see Results). Then we isolated more motility mutants. One colony of GIFU 10793, which could grow at 30°C but not at 42°C, was cultured in HI broth at 30°C overnight. A 10-μl sample of this culture was inoculated on the top of semisolid agar contained in a 5- by 50-mm tube (1 ml per tube). The samples were closed in a moist box and incubated at 42°C for 96 h. At this time, the bacteria with normal motility would go deep to the bottom of the tube, and the top was expected to be rich in the bacteria without motility or chemotaxis. Bacteria on the top were streaked onto an NGA plate so that some of the colonies would be separated from each other by a distance of at least 1 cm. The plates were incubated at 37°C for 1 or 2 days until different colonies could be distinguished by their detailed appearance under a stereoscopic microscope (Fig. 1). For each potential Fla$^-$, Mot$^-$, or Che$^-$ strain, we examined the motility or swimming behaviors microscopically as described by Macnab and Ornston (23) by using the exponential-phase cultures. Sometimes we checked the motility or the chemotaxis of the strains on a Gard plate, on which motile strains (even Che$^-$ mutants) spread better (Fig. 1). Confirmation of the distinction between Fla$^-$ and Mot$^-$ mutants was obtained by the visualization of the flagella in the light microscope by the staining technique of Leifson (20).

Isolation and characterization of spontaneous Fla$^-$ and Mot$^-$ mutants. GIFU 10007 was inoculated into 0.5-ml amounts of L broth, and a drop of phage χ suspension (ca. 10^{10} PFU ml^{-1}) was added to each culture. After overnight incubation at 37°C, each culture was spread on an NGA plate. Inspection of the colonies was as described above. Only one colony was picked up from each plate. With this method, Che$^-$ mutants were also isolated. Independent mutants isolated by this method were numbered with the prefix PK.

Isolation of motile revertants from Fla$^-$ or Mot$^-$ mutants. A 10-μl sample of exponential-phase culture of an Fla$^-$ or Mot$^-$ strain was spotted on the center of a well of a 24-well tissue culture plate containing Gard semisolid agar and incubated at 37°C. When swarms appeared, bacteria were picked from the edges of the swarms and purified on an HI or MacConkey plate.

Immobilization of the motile, invasive strains by agglutination with antiflagellar sera. As an alternative way of demonstrating the relationship between the motility and invasiveness of *S. typhi*, we immobilized the parent strains GIFU 10007 and NCTC 8385 by agglutination with antiflagellar sera. The antisera were added into the 8-h cultures at dilutions of 10-, 20-, 40-, or 80-fold. The cultures were incubated for another 10 min and prepared for the invasion assay as stated above with the same 8-h cultures without antiserum as controls.

Complementation tests. Complementation tests were performed by P22-mediated transduction as described by Yamaguchi et al. (37) with the deletion strains of *S. typhimurium* listed in Table 1 as the donors and the *S. typhi* mutants as the recipients.

Invasion assay. HeLa cell culture and invasion assays were performed as described previously (35). Intestine cells were treated in the same way as HeLa cells. Briefly, the cells were maintained in DMEM no. 1 (50 μg of kanamycin per ml) at 37°C in a 5% CO_2 humidified atmosphere. Cells were trypsinized and seeded into the chambers of a tissue culture chamber slide with eight chambers (Miles Scientific, Div. Miles Laboratories, Inc., Naperville, Ill.) at 4×10^4 cells per chamber in 0.4 ml of DMEM no. 1. At such a concentration, a nearly confluent cell monolayer could be obtained in 48 h.

Tn*5* insertion or spontaneous mutants obtained as described above were incubated in 1 ml of HI broth at 37°C for 12 h, diluted 10-fold in DMEM no. 3 (antibiotic free), and incubated for 0.5 h. Medium was removed from the cell monolayers, and 0.4 ml of the bacterial suspension was added. After incubation, bacteria were removed by aspiration, and the cell monolayers were washed three times with Dulbecco phosphate-buffered saline without calcium chloride and magnesium cloride. The walls of the chambers were taken off, and slides were fixed in methyl alcohol, stained with Giemsa solution, and observed microscopically.

Centrifugation of the bacteria onto HeLa or intestine cell monolayers. The supernatant of the cell monolayers was replaced by the bacterial suspension described above, and the caps of the chambers were fixed tightly with a tape onto the chamber slides. Then the chamber slides were centri-

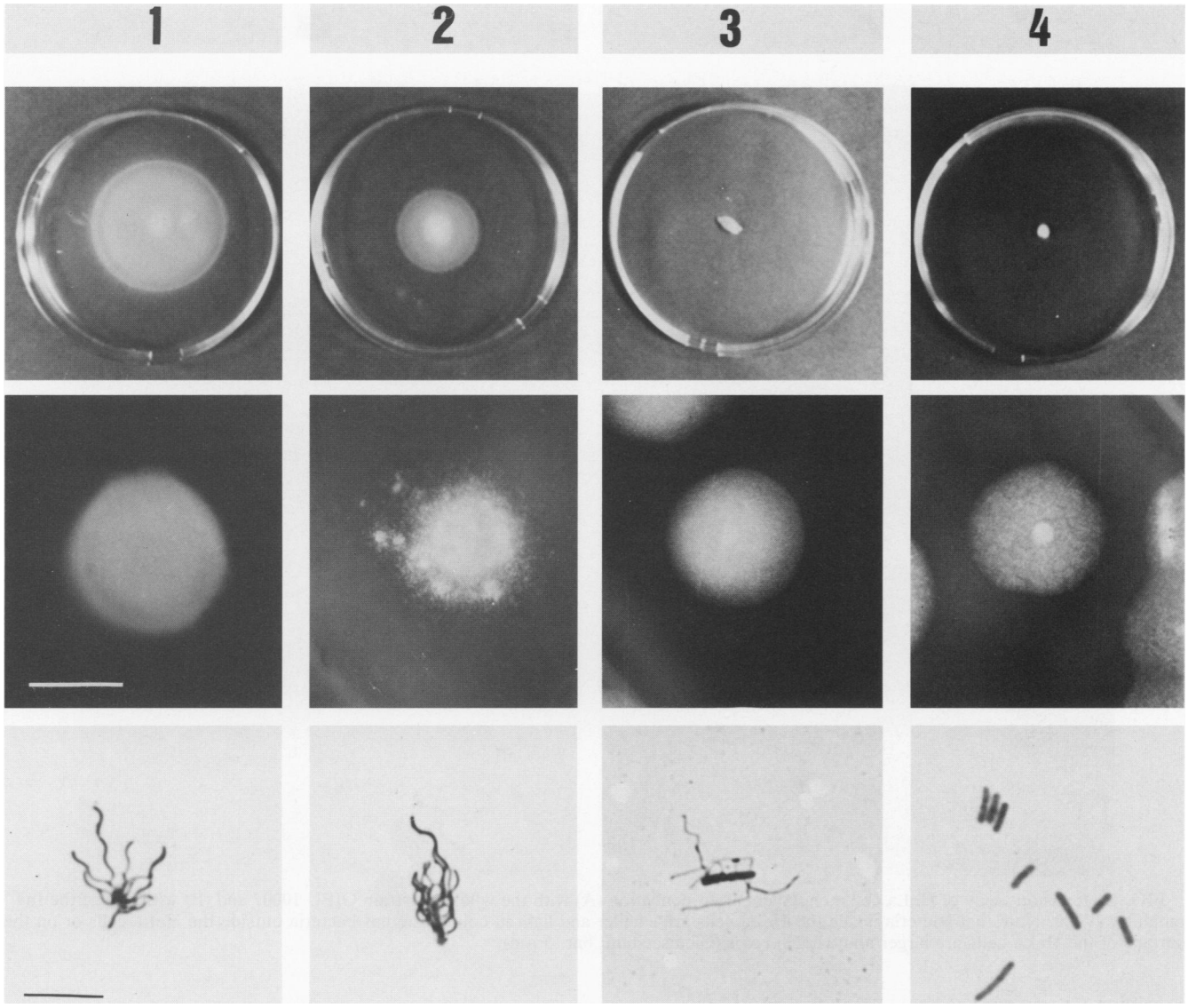

FIG. 1. Phenotypic differentiation between the wild-type strain and the motility mutants. Top row, Motility observed on a Gard plate; middle row, colony morphology on an NGA plate (bar, 1 mm); bottom row, flagellation visualized by Leifson staining (bar, 5 μm). Strains: 1, wild-type strain GIFU 10007; 2, Che$^-$ mutant exemplified by IV985; 3, Mot$^-$ mutant exemplified by IV867; 4, Fla$^-$ mutant exemplified by IV340.

fuged at 160 × *g* or 20 × *g* at 37°C for 10 min. After centrifugation, the cultures were incubated at 37°C in a 5% CO_2 humidified atmosphere for 1 h. Washing, fixing, and staining were as described above.

RESULTS

Identification of the Fla$^-$, Mot$^-$, and Che$^-$ mutants. On an NGA plate, mutants without motility (Fla$^-$ and Mot$^-$) formed compact colonies with sharp edges and no swarms or migration regions around the colonies could be seen. The difference between Fla$^-$ and Mot$^-$ was a dense center, which was present in the colonies of Fla$^-$ but not Mot$^-$ mutants. We confirmed the NGA plate results by directly observing the motility under a microscope and visualizing the flagellation of these mutants (Fig. 1; see below). Colonies of Che$^-$ mutants, on the other hand, showed different morphologies. They could spread on a Gard or NGA plate but to a much limited extent compared with that of the wild-type strains. In some Che$^-$ strains, many small colonies or migration clusters could be seen radiating from the center of the spreads (Fig. 1). Under a microscope, many of the Che$^-$ mutants showed motility as active as that of the wild-type strains. However, the motility was aberrant, with a high tumbling frequency. When tethered to slides (23), cells of the Che$^-$ mutants mainly rotated in one direction: clockwise or counterclockwise.

Invasiveness of the strains. With a multiplicity of infection of ca. 500:1, invasion by the wild-type strain GIFU 10007 could be observed as early as 10 min in the case of HeLa cells and 1 h in the case of intestine cells after bacterial inoculation (Fig. 2A). In this study, the invasive strains invaded both HeLa and intestine cells and the Inv$^-$ strains invaded neither HeLa nor intestine cells.

All of the motile Vi$^-$ mutants and their wild-type parents were invasive. NCTC 8395 and its Vi$^-$ derivative GIFU

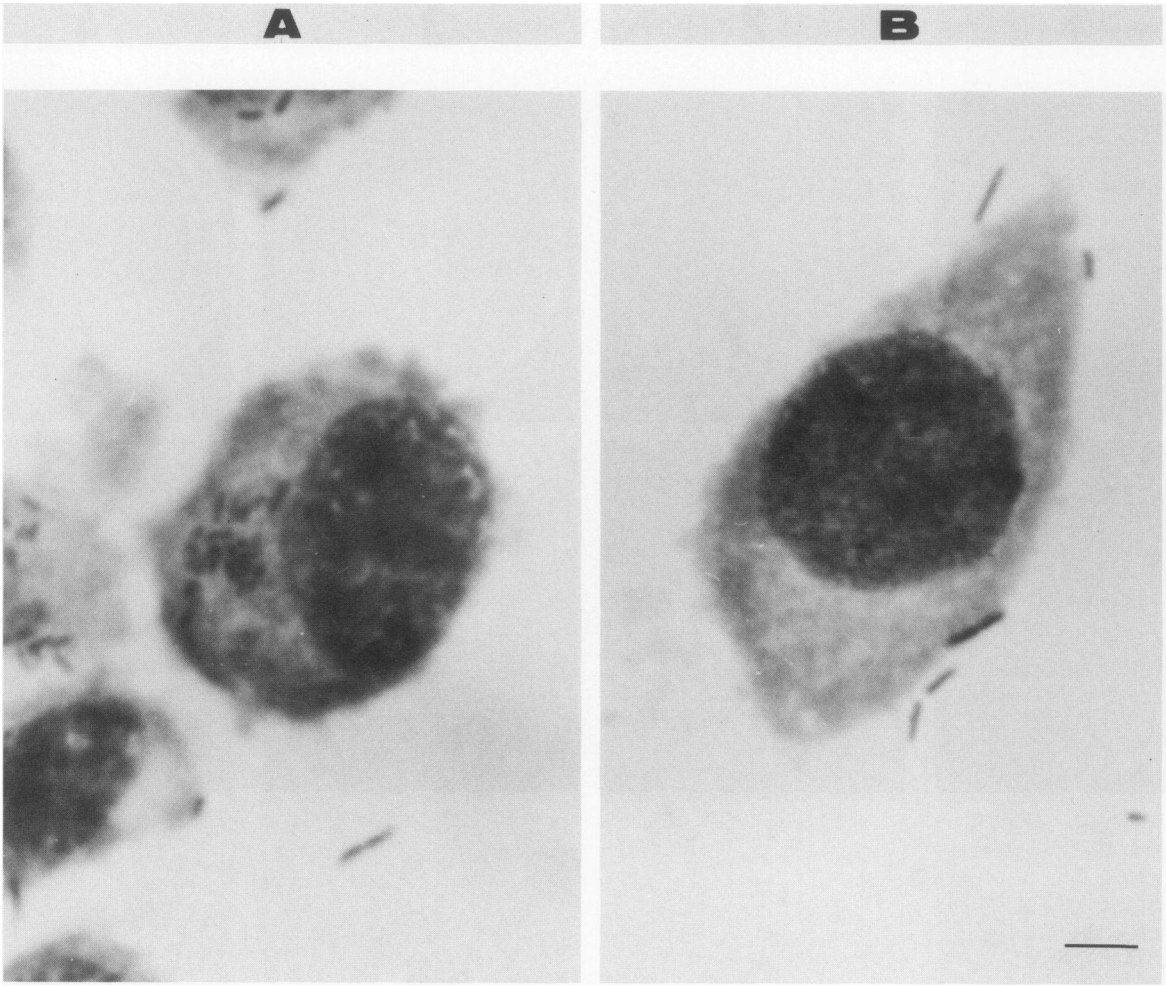

FIG. 2. Invasion assay of HeLa cell monolayers after incubation (A) with the wild-type strain GIFU 10007 and (B) with one of the Inv$^-$ mutants, IV340. Note that bacteria inside the HeLa cells are smaller and light in color, whereas bacteria outside the HeLa cells or on the surface of the HeLa cells are larger and dark in color (Giemsa stain; bar, 5 μm).

11727, both being Fla$^-$, were noninvasive (Table 2). Besides the first four Tn*5* mutants, i.e., IV340, IV867, IV985, and IV1104, we isolated one more Fla$^-$, one more Mot$^-$, and seven more Che$^-$ Tn*5* insertion mutants. All of these were noninvasive (Fig. 2B). Among the spontaneous mutants isolated, there were 29 Fla$^-$, 13 Mot$^-$, and 8 Che$^-$ strains, all of which were noninvasive (Table 3).

To confirm the inability of these mutants to invade HeLa or intestine cells, we prolonged the incubation time from 1 h

TABLE 2. Invasiveness of Vi$^-$ *S. typhi* mutants and their Vi$^+$ parents

Strain	Vi	Invasiveness[a]
GIFU 10007	+	+
GIFU 10387	−	+
NCTC 8385	+	+
GIFU 11725	−	+
NCTC 8395	+	−
GIFU 11727	−	−
GIFU 9931	+	+
GIFU 11726	−	+

[a] Both to HeLa and intestine cells.

TABLE 3. Noninvasive spontaneous or Tn*5* insertion mutants[a] of GIFU 10007

Phenotype	Spontaneous mutants	Tn*5* insertion mutants
Fla$^-$	PK1, PK3, PK4, PK7,[b] PK10,[b] PK11, PK12, PK18, PK 25,[b] PK28, PK30,[b] PK31, PK33,[b] PK35,[b] PK37,[b] PK38, PK46, PK48, PK49, PK51,[b] PK53, PK55, PK57, PK108,[b] PK109,[b] PK114,[b] PK116,[b] PK147,[b] PK154	IV340, IV1104, IV1416
Mot$^-$	PK2,[b] PK5,[b] PK6, PK13, PK23, PK36, PK39, PK45, PK215,[b] PK232,[b] PK234,[b] PK249, PK252[b]	IV867, IV1377
Che$^-$	PK308, PK309, PK314, PK315, PK316, PK332, PK334, PK347	IV985, IV1417, IV1419, IV1421, IV1422, IV1423, IV1429, IV1430

[a] All of the mutants are Vi$^+$.
[b] From these strains, motile revertants were isolated; all revertants were invasive.

to 3 or 24 h. No invasion, however, was seen in either of the conditions. We also tried centrifuge-assisted attachment by impacting the bacteria on HeLa or intestine cell monolayers through centrifugation. Nevertheless, invasion was not seen even after centrifugation.

GIFU 10007 and NCTC 8385 became noninvasive when immobilized by agglutination with the antiflagellar sera.

In any case, adhesion of the noninvasive strains, mutated or immobilized, flagellated or nonflagellated, seemed to be conserved, for we noted that a HeLa cell was often surrounded by dozens of bacterial cells of any kind of the Inv$^-$ strains.

Visualization of the flagella. The invasive strains were normally flagellated. Although no flagella could be seen in most of the Fla$^-$ mutants, a small percentage of the cells of some leaky Fla$^-$ strains showed a couple of flagella, somewhat short, with or without curves. Mot$^-$ mutants usually had flagella very hard to distinguish from those of the wild-type strains, although some strains indeed showed paralyzed flagella, that is, flagella without curves (Fig. 1). Curly flagella were a feature of some Che$^-$ mutants (23), but most of the flagella were similar to those of the wild strains, i.e., with seemingly normal waveform, when visualized by Leifson staining.

Mapping of the flagellar genes by P22-mediated transduction and the invasiveness of the recombinants. With some of the Inv$^-$ mutants, we conducted complementation tests to map the mutational sites, employing the deletion mutants of *S. typhimurium* listed in Table 1. The mutational sites of the Inv$^-$ mutants tested were distributed almost evenly over all three regions of the flagellar genes of *S. typhimurium* (Fig. 3). All of the motile transductional recombinants became invasive.

Invasiveness of the motile revertants. Most of the motile revertants were isolated with frequencies of 1×10^{-7} to 5×10^{-9} per generation. These included 15 of the 29 spontaneous Fla$^-$ mutants and 5 of the 13 spontaneous Mot$^-$ mutants. From one Mot$^-$ mutant, PK2, motile revertants were isolated with a frequency of 10^{-10} per generation. We failed to obtain motile revertants from the other motility mutants even after an incubation period of up to 5 days. All of the motile revertants showed the phenotype of the parent strain and were invasive (Table 3).

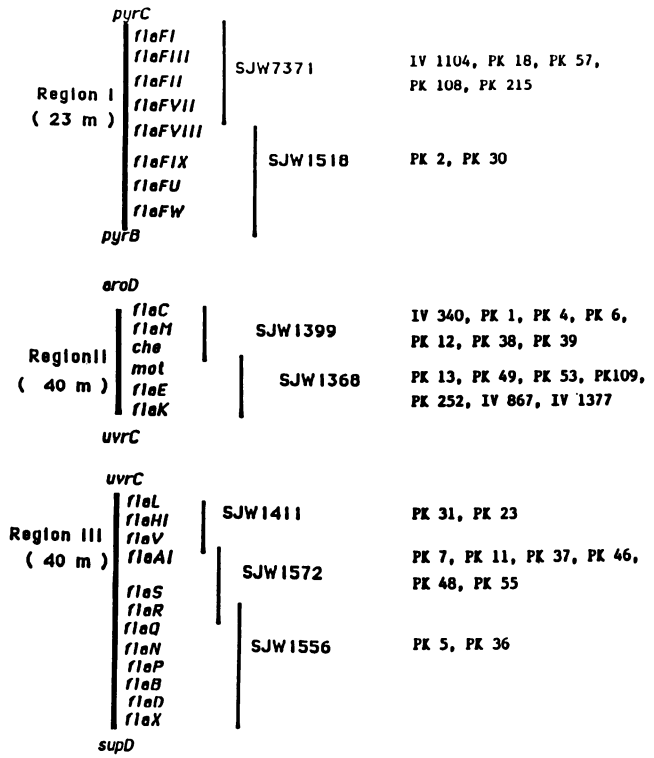

FIG. 3. Assignment of the mutational flagellar gene sites of the Inv$^-$ mutants. The flagellar gene regions of *S. typhimurium* are shown on the left; the *S. typhi* Inv$^-$ mutants whose mutational sites were mapped to the respective regions of the *S. typhimurium* linkage map are listed on the right.

DISCUSSION

It is more than one century since *S. typhi* was isolated and recognized as the causative agent of human typhoid fever (5, 8). However, the pathogenic mechanisms of the virulence factors of *S. typhi* are still not well understood.

The Vi antigen of *S. typhi*, on the other hand, has been the subject of extensive investigations since the 1930s as a factor of outstanding importance in the pathogenesis of and immunity to typhoid fever. Isolates of *S. typhi* from the blood of patients with typhoid fever invariably possess the vi antigen (6). Felix and Pitt (6) showed that the most virulent strains of *S. typhi* are those that develop both the Vi and the O antigens in maximum quantities; anti-Vi antibody alone, without accompanying anti-O antibody, is sufficient to protect mice effectively against infection with strains of the highly virulent O$^+$ Vi$^+$ type. The Vi antigen seems to serve not as an invasive factor prerequisite to the internalization of enteroinvasive bacteria by the epithelial cells but as a protective factor for the O antigen against the action of the natural or immune O antibody or complement, thereby protecting the bacterial cell against phagocytosis and the bactericidal action of serum. We already reported that, of six Vi$^+$ strains from clinical isolates, only three were highly invasive to HeLa cells (35). In the present study, all 13 Inv$^-$ Tn*5* mutants and 50 Inv$^-$ spontaneous mutants were Vi$^+$; meanwhile, all of the Vi$^-$ mutants, except the nonflagellate GIFU 11727, remained invasive (Table 2). Thus, it seemed that Vi antigen had nothing to do with the invasiveness of *S. typhi*.

In the present work, on the other hand, invasiveness seemed to be associated with intact motility. None of the Inv$^-$ Tn*5* or spontaneous mutants had normal motility or chemotaxis. Although we do not know whether the motile revertants isolated were by back-mutation or by suppression, maybe some by one and some by the other, the restoration of the wild-type motility did result in the restoration of invasiveness. All of our Inv$^-$ mutants were thought to have only single mutations, as evidenced by the reversion as well as the complementation tests. In addition, we performed Southern hybridization tests with the Tn*5* mutants to check the numbers of the Tn*5* insertions, with the chromosome DNAs cut by *Eco*RI and the Tn*5* probe labeled with biotin. Each of the Tn*5* mutants showed only one band hybridized by the probe; however, unfortunately, we have not got the contrast between the bands and the background sharp enough to take a photo. In recent years, motility has been recognized as a virulence factor for some of the flagellated bacteria such as *Campylobacter jejuni*, *Vibrio cholerae*, and *Pseudomonas aeruginosa* (3, 16, 32, 38). In view of the fact that the mutational sites of the flagellar genes of the Inv$^-$ *S. typhi* mutants in this study were distributed almost evenly over all three regions of the flagellar genes of

S. typhimurium, we tend to conclude that intact motility is an invasion-related factor of *S. typhi*.

Jones et al. (17) made an extensive study of the invasion of HeLa cells by *S. typhimurium*. According to them, the internalization of *S. typhimurium* by HeLa cells consists of a sequence of three phases: reversible attachment, irreversible attachment, and internalization. Reversible attachment is facilitated by the motility of the bacteria, without which the contact of the bacteria with the HeLa cells would be very poor, and thus the attachment and the internalization would be impossible. The nonmotile mutants of *S. typhimurium* generated by UV mutagenesis or other methods, therefore, cannot be internalized by HeLa cells. To help the bacteria come into contact with the HeLa cells, Jones et al. centrifuged the bacteria with the HeLa cells. The nonmotile mutants were thus internalized by the HeLa cells as effectively as the wild-type strains.

With our Inv$^-$ mutants, however, internalization of the bacteria could not be obtained either by centrifuge-assisted attachment or by prolongation of incubation time; this suggested that, unlike *S. typhimurium*, *S. typhi* requires intrinsic, intact motility for invading the epithelial cells, and thus motility could not be substituted for or mimicked by the mechanical force.

Chemotaxis, for which motility is necessary, is interesting more and more workers because of its role in the interaction between some enteric pathogens and the mucosa. Allweiss et al. (1) noted that chemotaxis is important for the bacteria to attach to the mucosa. Uhlman and Jones (33) emphasized chemotaxis as a factor in interactions between HeLa cells and *S. typhimurium*, because a diffusible attractant released by HeLa cells greatly increased the collision frequency and hence the attachment of chemotactic *S. typhimurium*. Although it seems hard to conceive that collision frequency increased by chemotaxis is greater than that increased by centrifugation, we want to approach this question in the future.

Another factor that is generally assumed to be a prerequisite for invasion is adherence (7). Lindquist et al. (22) demonstrated that mannose-sensitive fimbriae promote the binding of *S. typhimurium* to rat enterocytes. By using mannose-sensitive fimbriae, *S. typhimurium* attaches to enterocytes and perturbs the microvilli, causing their degeneration and possibly the release of chemoattractants. Bacterial cells respond to the chemoattractants, swarm to the target cell, associate with it, and cause its eventual destruction. We have not ruled out the possibility that adherence by using, say, the fimbriae would be a factor for invasiveness. During the screening of the *S. typhi* mutants, we noted that a HeLa cell was often surrounded by dozens of bacterial cells of an Inv$^-$ strain, indicating that the failure of invasiveness might be in some of the steps after adherence. We wonder whether a strain that is defective in adherence by something like the fimbriae but intact in motility could invade the HeLa or intestine cells, or, if not, whether invasion of the cells by such a strain could be obtained by centrifuge-assisted attachment.

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