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The Attenuated Phenotype of a *Salmonella typhimurium flgM* Mutant Is Related to Expression of FliC Flagellin

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The *flgM* gene of *Salmonella typhimurium* encodes a negative regulator of flagellin synthesis that acts by inhibiting the flagellum-specific sigma factor FliA (σ^{28}), but only when a mutation in a flagellar basal body, hook, or switch gene is present. We previously showed that FlgM is also necessary for the virulence of *S. typhimurium* in the mouse model of typhoid fever and proposed that FlgM is required to modulate the activity of the FliA sigma factor, which, in turn, regulates a gene involved in virulence. In this investigation, we observed that (i) the in vitro generation times of *flgM* mutant and wild-type strains of *S. typhimurium* were indistinguishable, as were the amounts of flagellin produced by the strains; (ii) the 50% lethal doses of *fliA* mutant and wild-type strains of *S. typhimurium* were similar in orally infected mice; and (iii) inactivation of the FliA-regulated flagellin gene *fliC* in an *flgM S. typhimurium* mutant resulted in a virulent phenotype. Therefore, we now conclude that expression of the FliC flagellin subunit in an *flgM* strain is responsible for the attenuated phenotype of an *flgM* mutant and that FliA does not appear to positively regulate virulence genes in *S. typhimurium*. Our results suggest that the normal regulation of flagellum synthesis appears to be necessary for virulence and that there may be an advantage conferred in vivo by expression of a particular flagellar phenotype of *S. typhimurium*.

The synthesis of flagella in *Salmonella typhimurium* is regulated in a cascade manner by several classes of genes (17). Thus, the expression of one class of genes is required for the transcription of the subsequent class(es) of genes (Fig. 1). Moreover, *S. typhimurium* is capable of phase variation, whereby the organism switches between antigenically distinct flagellar types H1 (phase 1 [FliC]) and H2 (phase 2 [FljB]) (24). Both of the flagellin genes are late class genes. The *flgM* gene of *S. typhimurium*, a middle class gene, encodes a negative regulator of flagellin synthesis (10) that inhibits the flagellum-specific sigma factor FliA, σ^{28} (20). This inhibition is phenotypically apparent only in the presence of a flagellar basal body, hook, or switch gene mutation (10). We recently showed that FlgM is also necessary for the virulence of *S. typhimurium* in the mouse model of typhoid fever (23), even though flagella per se are not a prerequisite for *S. typhimurium* pathogenicity in this model (4, 18). Although the precise role of FlgM in virulence is not known, FlgM is probably required to modulate the activity of the FliA sigma factor, since mutation of *fliA* in an *flgM* background renders the strain virulent (23).

Additional lines of evidence that support a link between the flagella regulatory system and bacterial virulence expression are as follows. First, Akerley and Miller reported that the *flaA* gene of *Bordetella bronchiseptica* is regulated by the BvgAS virulence control system (1). This finding led these investigators to propose a relationship between virulence and motility in *B. bronchiseptica*. Akerley et al. also found that inappropriate expression of flagella interferes with virulence of *B. bronchiseptica* (1). Second, Gardel and Mekelanos found that *Vibrio cholerae motB* mutants, which are flagellate but nonmotile, produce increased levels of cholera toxin and toxin-coregulated pilus (25). Third, a σ^{28}-like promoter precedes virulence-associated genes in different genera of bacteria. For example, the *lcrD* gene of *Yersinia pestis* and the *Shigella flexneri mxiA* gene, an *lcrD* homolog, contain σ^{28}-like promoters (2). However, the importance of the *lcrD* and *mxiA* genes in virulence remains to be proven.

On the basis of our earlier results, we previously proposed four models to address the relationship between the FlgM-FliA regulatory system in *S. typhimurium* and virulence; i.e., FliA could (i) positively regulate a virulence factor, provided that the virulence factor was also regulated by σ^{70}; (ii) positively regulate a factor that is inhibitory to virulence; (iii) positively regulate a repressor of a virulence factor; or (iv) act as both an activator of flagellar genes and a repressor of a gene involved in virulence. The role of FlgM in all of these proposed models would be to modulate the activity of the FliA sigma factor. The following experiments were designed to test some of these possibilities.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used in this study are shown in Table 1.

Media and enzymes. Strains were grown in L broth (per liter: 10 g of tryptone, 5 g of yeast extract, 5 g of NaCl). Chloramphenicol, kanamycin, and tetracycline (Sigma Chemical Co., St. Louis, Mo.) were added to the medium at final concentrations of 15, 25, and 10 μg/ml, respectively, as needed. Motility agar contained the following (per liter): 10 g of tryptone, 5 g of NaCl, and 0.35% Bacto-Agar.

Analysis of flagellins. Surface flagellin was prepared from cultures either by the acid-base denaturing method of Ibrahim et al. (15) or mechanical shearing (18) with an Omni homogenizer. The cells were then sonically disrupted to release intracellular flagellin. The total protein concentration of the bacterial extracts was determined with a bicinchoninic acid assay kit (Pierce Chemical Co., Rockford, Ill.), and the samples were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Western blotting (immunoblotting) of the gels was done as described previously (8), with monoclonal antibody 15D8 as the probe (IGEN, Rockville, Md.). This antibody recognizes most flagella of members of the family *Enterobacteriaceae*. Immunoblots were developed with enhanced chemiluminescence reagents (Amersham). Strains that phenotypically expressed one predominant flagellar type were isolated from motility

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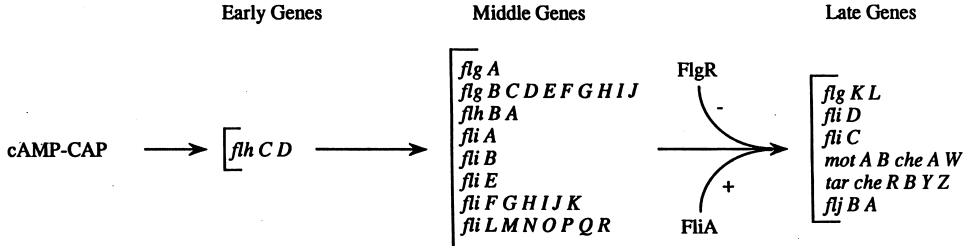

FIG. 1. Synthesis of flagella in *S. typhimurium*, as well as in *Escherichia coli*, is regulated in a classwise fashion (17). The *flgM* (FlgR in figure) and *fliA* gene products regulate the expression of several late class flagellar genes. The late class encodes products involved in motility and chemotaxis. Two of these late flagellar genes, *fliC* (phase 1 [H1]) and *fljB* (phase 2 [H2]), encode the alternate subunits of the flagellum and are normally expressed at very high levels. cAMP, cyclic AMP; CAP, catabolite gene activator protein. Figure reprinted from reference 10 with permission of K. Hughes.

agar that contained the heterologous antiserum (1:500 dilution of salmonella H antiserum i or antiserum single factor 2; Difco, Detroit, Mich.).

Transductions. Bacteriophage P22HT *int* was used as described previously (6) to transduce the *fljB5001*::MudJ, *fliC*::Tn*10*, *fliC5050*::MudJ, and *fliA5059*::Tn*10*dTc mutations into strain SL3201 or strain SL7395.

Assay for mouse virulence. Animal experiments were carried out according to the principles outlined in the *Guide for the Care and Use of Laboratory Animals* (19). The assay for mouse virulence has been described in detail (23). Briefly, C57BL/6J mice (6- to 8-week-old females; Jackson Laboratories, Bar Harbor, Maine) were injected intraperitoneally (i.p.) with approximately 700 CFU of the test strain and observed for 3 weeks. This dose was chosen to distinguish between virulent and attenuated strains, since 700 CFU is above the i.p. 50% lethal dose (LD_{50}) of the virulent strains used in this study (i.p. LD_{50}, ~20 CFU) and below the LD_{50} of attenuated strains (i.p. LD_{50}, ~10^5 CFU).

Determination of LD_{50}s. The LD_{50}s were determined as follows. Groups of five mice were infected orally or i.p. with graded doses of bacteria ranging over 5 logs. The exception to the use of five animals per dose were the LD_{50} studies done with the virulent strains SL3201 and SL7395 *fliC*::Tn*10*dTc. For these strains, the i.p. LD_{50}s were determined with three groups of mice and three doses of bacteria (i.p. LD_{50}s, <20 CFU). The challenge inocula were enumerated by viable counts. Orally infected mice were observed for 30 days, and i.p.-infected mice were observed for 21 days. The method of Reed and Muench was used to estimate the LD_{50} (22).

RESULTS

Growth rates. Kutsukake and Iino observed that a frameshift mutation in *flgM* in *S. typhimurium* KK1004 (strain KK1004 is LT2 [Δ*H2* region] and strain KK1301 is LT2 [ΔH2 region] *flgM101*) resulted in a much reduced growth rate (16). Therefore, we determined the growth rates of SL3201 and SL7395 by optical density and CFU counts to assess whether slow growth was the cause of the attenuation of our *flgM* mutant. The wild-type strain, SL3201, and its *flgM* mutant, SL7395, had nearly identical in vitro growth curves and had doubling times of approximately 30 min (Fig. 2). SL3201 and SL7395 also had similar growth rates in minimal medium (data not shown).

Motility of the *flgM* mutant. Wild-type strain SL3201 and *flgM* mutant SL7395 appeared equally motile in bacterial wet mounts. However, they behaved differently when stabbed into motility agar. SL7395 was less motile (i.e., formed a much smaller swarm) than SL3201. Kutsukake and Iino did not comment on the motility characteristics of their *flgM* mutant strain, but they did observe an increase in flagellation of the *flgM* mutant in their background *S. typhimurium* strain (16).

Oral LD_{50} of *fliA* mutants. We previously reported that mutation of the flagellin-specific sigma factor gene, *fliA*, could overcome the virulence defect of an *flgM* mutant (23). To determine whether FliA is directly involved in virulence, the oral LD_{50}s of two different *S. typhimurium* strains with mutations in *fliA* were compared with those of *fliA*$^+$ parent strains. Mutation of *fliA* did not significantly alter the oral LD_{50} of either strain (Table 2).

Virulence of flagellin mutants. One model to explain the relationship between the *flgM-fliA* regulatory system and virulence involves the FliA-regulated expression of a factor that attenuates the strain. Because the increased activity of the FliA sigma factor in an *flgM* mutant results in elevated expression of the late class of flagellar genes (11, 14, 16), we asked whether mutations in one or both of the flagellin subunit genes *fljB* (phase 1 [H1]) and *fliC* (phase 2 [H2]) could overcome the attenuated phenotype of an *flgM* mutant. Strains were constructed that contained mutations in one or both of the flagellin genes in the presence or absence of an *flgM* mutation. These strains were then tested for virulence in mice. Mutations in one or both flagellin genes had no effect on the virulence of the *flgM*$^+$ parent (Table 3). However, mutation of *fliC*, alone or in

TABLE 1. Bacterial strains used in this study

Salmonella strain	Relevant characteristic(s)	Source or reference
SL3201	Mouse virulent; Fim$^-$	B. A. D. Stocker (13)
ST39	SL3201*flg25zcd-907*::Tn*10*, attenuated, Mot$^-$	5
SL7395	SL3201 *flgM5096*::Tn*10*dCm; attenuated, Mot$^+$	23
TML	Mouse virulent; Fim$^+$	9
TH714	*fljB5001*::MudJ	K. Hughes (10)
TH1467	*fliC*::Tn*10*	K. Hughes
TH1077	*fliC5050*::MudJ	K. Hughes (10)
TH1479	*fliA5059*::Tn*10*dTc	K. Hughes

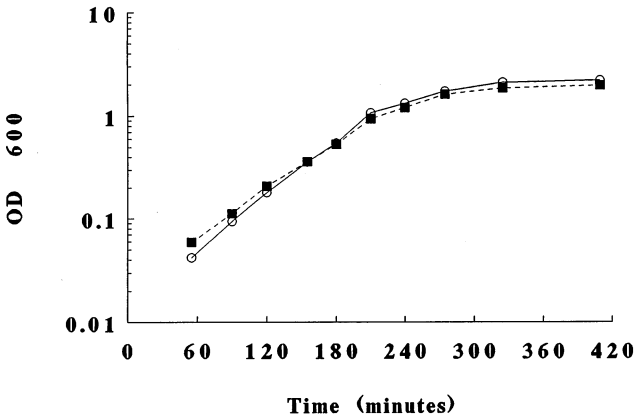

FIG. 2. Growth rates of strain SL3201 and its *flgM* derivative SL7395 in L broth. ○, SL3201; ■, SL7395. The corresponding numbers of CFU per milliliter for SL3201 and SL7395, respectively, were as follows: 90 min, 2.18×10^7 and 1.94×10^7; 155 min, 1.19×10^8 and 9.4×10^7; 210 min, 2.5×10^8 and 4.82×10^8; and 275 min, 9.62×10^8 and 8.1×10^8. OD 600, optical density at 600 nm.

TABLE 2. LD_{50}s of *fliA* mutants administered orally

Strain	Motility	Oral LD_{50} (CFU)
SL3201	Fla^+	2×10^5
SL3201*fliA*	Fla^-	6×10^4
TML	Fla^+	1×10^5
TML*fliA*	Fla^-	1×10^5

combination with mutation of *fljB*, suppressed the attenuated phenotype of the *flgM* mutant, i.e., the *flgM fliC* and *flgM fliC fljB* mutants were virulent. The growth rates of the strains were also measured, and no differences between the mutants and the parent strain were found (data not shown). The motility phenotypes of the $flgM^+$ and *flgM* strains were not altered by the introduction of a single mutation in *fljB* or *fliC* (data not shown).

Analysis of flagellins produced in the presence and absence of FlgM. The amounts of flagellin produced by the $flgM^+$ and *flgM* strains intracellularly and on the surface of the organisms were compared to determine whether FliC accumulated in the *flgM* mutant. This analysis was performed because we speculated that accumulation of flagellin might be detrimental to the bacterium and thus might explain the attenuated phenotype of an *flgM* mutant. The intracellular flagellin concentration was assessed by Western analysis (Fig. 3A). No accumulation of flagellin was seen in the *flgM* mutant background compared with that in the wild type. Surface flagellin was also analyzed. Since the majority of the protein isolated by homogenization of the bacteria was flagellin (Fig. 3B and 4), the amount of surface protein visualized by PAGE was normalized to CFU. The strains tested included derivatives of SL3201 and SL7395 that expressed one predominant flagellin. The strains were subcultured after growth through either motility agar (for single-flagellin-gene mutants) or motility agar that contained antiserum specific for each flagellin. Similar amounts of flagellin were isolated from all of the strains tested, with the exception of SL7395 *fliC*::Tn*10* ($FljB^+$), which produced slightly less flagellin than the other strains (Fig. 3B). Therefore, attenuation of SL7395 was not due to intracellular accumulation or increased surface expression of FliC or FljB flagellins.

Virulence of strains that express FliC or FljB. Because *S. typhimurium* is able to switch between production of two different flagellar types, we did not know which, if any, flagellin was expressed by some of the strains tested in the experiments presented in Table 3. Therefore, to determine whether there was an advantage conferred in vivo by expression of a particular flagellar phenotype of *S. typhimurium*, we repeated virulence assays with the flagellin mutants and control strains after determining which flagellar type was predominant at the time of inoculation. Surface flagella were isolated from a fraction of the inoculating bacterial culture and visualized by SDS-PAGE to determine the major flagellar type (example shown in Fig. 4). Since a low-dose inoculum was used to infect the mice (~700 CFU i.p.), the major flagellin seen on the gels should correspond to the type expressed by the majority of the infecting bacteria. In the absence of a flagellin gene mutation, the *flgM* mutant was attenuated (Table 4). However, in the presence of an *fliC* mutation, the *flgM* mutant which produced FljB flagellin was virulent. This finding was consistent with results of our previous experiments presented in Table 3. Mutation of both flagellin genes, which resulted in a lack of flagella, also rendered the *flgM* mutant virulent.

Derivatives of the *flgM* mutant SL7395 that expressed predominantly FljB or FliC were isolated and tested for virulence in mice. Motility agar that contained serotype-specific antisera was used to immobilize bacteria that expressed FljB or FliC; bacteria that could swim through the agar were confirmed to express predominantly the heterologous flagellin. The *flgM* strains were both attenuated irrespective of the flagellar serotype initially expressed (Table 4).

LD_{50} of flagellin mutants. The LD_{50}s of *flgM*-flagellin mu-

TABLE 3. Virulence of *flgM* mutant in the presence and absence of mutations in flagellin gene(s) after i.p. inoculation

Strain and relevant genotype	No. of dead mice/ no. infected[a]	Mean (± SD) no. of days to death
SL3201	13/15	8 ± 2
SL3201 *fljB*::MudJ	14/15	8 ± 2
SL3201 *fliC*::Tn*10*	14/15	11 ± 5
SL3201 *fljB*::MudJ *fliC*::Tn*10*	14/15	6 ± 1
SL7395 *flgM*	0/15	NA[b]
SL7395 *flgM fljB*::MudJ	0/15	NA
SL7395 *flgM fliC*::Tn*10*	15/15	9 ± 1
SL7395 *flgM fljB*::MudJ *fliC*::Tn*10*	14/15	7 ± 1

[a] Number of dead mice/number of mice infected with approximately 700 CFU. These data were compiled from three experiments.
[b] NA, not applicable.

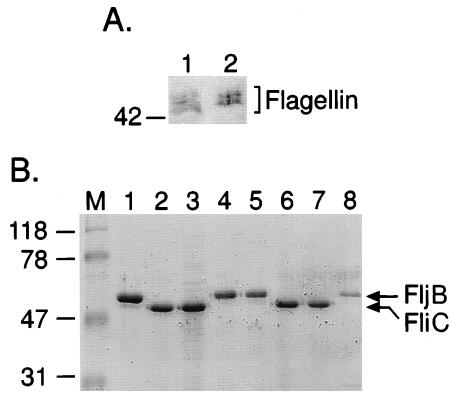

FIG. 3. (A) Western blot of flagellin with a monoclonal antibody that reacts with both FljB and FliC. Intracellular flagellin was isolated after removal of surface flagella by an acid-base treatment. Lane 1, SL3201 ($flgM^+$); lane 2, SL7395 (*flgM* mutant); marker, 42 kDa. The autoradiograph was computer scanned and labeled with Aldus Freehand on a Macintosh computer. (B) Coomassie-stained SDS-PAGE (10% polyacrylamide) of surface flagellin from 10^8 CFU of the following strains. Lanes: 1, SL3201 ($FljB^+$); 2, SL3201 ($FliC^+$); 3, SL3201*fljB*::MudJ ($FliC^+$); 4, SL3201*fliC*::Tn*10* ($FljB^+$); 5, SL7395 ($FljB^+$); 6, SL7395 ($FliC^+$); 7, SL7395*fljB*::MudJ ($FliC^+$); 8, SL7395*fliC*::Tn*10* ($FljB^+$); M, molecular mass markers (kilodaltons). The Coomassie-stained gel was computer scanned and labeled with Adobe Photoshop and Aldus Freehand on a Macintosh computer.

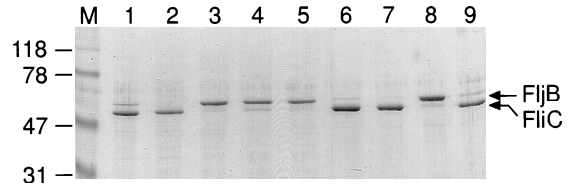

FIG. 4. Coomassie-stained SDS-PAGE (10% polyacrylamide) of surface flagellin. Lanes: 1 and 6, SL3201; 2 and 7, SL3201*fljB*::MudJ; 3 and 8, SL3201*fliC*::Tn*10*; 4 and 9, SL7395; 5, SL7395*fliC*::Tn*10*; M, molecular mass markers (kilodaltons). The Coomassie-stained gel was computer scanned and labeled with Adobe Photoshop and Aldus Freehand on a Macintosh computer.

TABLE 4. Virulence of *flgM* mutant that predominantly expresses one flagellar serotype after i.p. inoculation

Strain and relevant genotype	Input phenotype[a]	No. of dead mice/ no. infected[b]	Mean (± SD) no. of days to death[c]
SL3201	FliC$^+$≫FljB$^+$	5/5	6.6 ± 2.6
SL3201 *fliC*::Mud	FljB$^+$	4/5	10 ± 5
SL7395 *flgM*	FljB$^+$>FliC$^+$	0/14	NA[c]
SL7395 *flgM fljB*::Mud	FliC$^+$	0/10	NA
SL7395 *flgM fliC*::Tn*10*	FljB$^+$	15/15	8.2 ± 2.2
SL7395 *flgM fliC*::Mud	FljB$^+$	4/5	11 ± 2
SL7395 *flgM fliC*::Tn*10 fljB*::Mud	Fla$^-$	5/5	7.8 ± 5.2
SL7395 *flgM*[d]	FliC$^+$	0/5	NA
SL7395 *flgM*[d]	FljB$^+$	0/5	NA

[a] Input phenotype determined by PAGE visualization of flagellin isolated from the inoculum.
[b] Number of dead mice/number of mice infected with approximately 700 CFU.
[c] NA, not applicable.
[d] Strains passaged through antiserum-agar to isolate bacteria that predominantly expressed FljB or FliC flagellin.

tants administered i.p. or orally were determined. These studies were designed to examine the influence of the route of inoculation on the virulence of each flagellin mutant in a wild-type background and to more quantitatively assess the influence of flagellin type on the virulence of the *flgM* strain after i.p. inoculation. Previous studies revealed that the attenuating effect of what we now know is an *flgM* mutation is evident after oral as well as i.p. inoculation (5).

Mutation of either flagellin gene in the *flgM*$^+$ parent strain had little or no effect on the LD_{50}s as determined after oral or i.p. inoculation (Table 5). The LD_{50}s of the *flgM* mutant and the *flgM fljB* strain administered i.p. were similar at about 10^5 CFU. In contrast, mutation of the *fliC* gene in the *flgM* background resulted in a dramatic decrease in the LD_{50} to a value comparable to that of the wild type (<20 CFU).

Growth rates of strains that express FliC or FljB. Neither mutation of a flagellin gene nor expression of a particular flagellin affected the in vitro growth rates of the strains (data not shown).

DISCUSSION

To begin to interpret any effects that mutations in the *flgM*, *fliA*, *fljB*, or *fliC* genes might have on *S. typhimurium* virulence in mice, we first compared individual strains with single mutations in each of those genes with the wild-type parent strain SL3201. We found no differences in the in vitro generation times when we compared the parent strain with *flgM*, *fliA*, and flagellin mutant strains. Therefore, the in vivo attenuation of the *flgM* strain is not simply due to an alteration of the in vitro growth rate. These results are in contrast to those of Kutsukake and Iino, whose *flgM* mutant grew slowly in vitro (16). The growth defect of their *flgM* mutant was relieved by mutation of *flhD*, *fliA*, or *fliC* (16). The discrepancy in phenotype of our *flgM* mutant and that of Kutsukake and Iino may reflect the fact that our mutant contains an insertion in *flgM*, while Kutsukake and Iino studied an *flgM* frameshift mutant. The strain used by Kutsukake and Iino also contains a deletion of the *fljB fljA* genes. It is possible that deletion of *fljA*, which encodes the repressor of *fliC*, is deleterious to an *flgM* mutant.

TABLE 5. LD_{50}s of *flgM* and flagellin mutants

Strain and relevant genotype	Input phenotype[a]	LD_{50} (CFU)	
		i.p.	Oral
SL3201	FliC$^+$≫FljB$^+$	<20	1×10^5
SL3201 *fljB*::Mud	FliC$^+$	<20	5×10^5
SL3201 *fliC*::Tn*10*	FljB$^+$	<20	1×10^6
SL7395 *flgM*	FljB$^+$=FliC$^+$	2×10^5	$>10^7$
SL7395 *flgM fljB*::Mud	FliC$^+$	1×10^5	$>10^7$
SL7395 *flgM fliC*::Tn*10*	FljB$^+$	<20	2×10^4

[a] Input phenotype determined by PAGE visualization of flagellin isolated from the inoculum.

We also measured the intracellular pools of flagellin and the amounts of surface flagellin produced by the wild-type *S. typhimurium* strain and the *flgM* mutant. The *flgM* mutant produced the same amount of flagellin or less relative to the wild-type strain and did not accumulate flagellin intracellularly. Thus, the attenuation of the *flgM* mutant strain SL7395 cannot be explained by overproduction of flagellin.

We previously proposed a model in which the *fliA* component of the flagellar regulatory pathway positively regulates the expression of a factor that is inhibitory to *S. typhimurium* virulence. We now conclude that FliA is not directly required for pathogenicity of *S. typhimurium* on the basis of our finding that mutation of *fliA* did not affect the oral LD_{50}s of two unrelated *S. typhimurium* strains. However, we did show that the FlgM-FliA regulatory pathway is involved in virulence. Indeed, we found that mutation of the *fliC* flagellin gene, but not the *fljB* flagellin gene, could overcome the attenuated phenotype of the *flgM* strain. Thus, the abnormally regulated production of FliC flagellin in an *flgM* mutant appears to interfere with virulence for reasons that are as yet unexplained. We believe that by studying an *flgM* mutant, we have unmasked a process whose regulation by *flgM* is critical for the virulence of a wild-type strain. Other examples of one phenotype that conceals the presence of another phenotype are the discovery of the zonula occludens toxin in a cholera toxin mutant of *Vibrio cholerae* (7) and the recent report by Parsot et al. of the secretion and formation of filaments by *Shigella flexneri ipaB* or *ipaD* mutants (21).

We determined whether the virulent phenotype of the *flgM fliC* mutant could be altered by introduction of a multicopy plasmid that contains a flagellin gene. Plasmids that encoded *fliC* or *fljB* had the general effect of attenuating normally virulent strains, regardless of the *flgM* allele, without affecting the in vitro growth rates of the transformants (data not shown). These results are not surprising in light of the complex regulation of the flagellar regulon.

The capability of *S. typhimurium* to undergo phase variation and thus switch between the production of two different flagellin types must be considered when evaluating the differences in virulence that we found between *flgM fliC* and *flgM fljB* strains.

Gillen and Hughes showed that the in vitro switching rates between flagellar phases range from 1/240 to 1/5,900 per cell per generation (10). These rates of switching, taken with the small numbers of CFU used (<700 CFU) in our experiments and the visualization of the flagellar type produced, suggest that very few if any of the infecting organisms that were mutated or selected to express a single flagellar serotype expressed the heterologous flagellin. Furthermore, the bias in the direction of switching that favors the *fliC*(ON) orientation (4- to 20-fold) (10) should not affect our initial inoculum because we enriched for and screened for the expression of a particular flagellin type.

One explanation for the differences in virulence between the various *flgM* and/or flagellin gene mutants that we studied is the possibility that the in vivo switching rates are very different from those measured in vitro. The results of virulence experiments in which the *S. typhimurium* inoculum was selected by passage through antiserum-agar are consistent with this possibility. Such a divergence in the in vitro and in vivo switching rates would be intriguing and would suggest a selection in vivo for or against particular flagellins. We are currently designing experiments and reagents with which to address these issues. One study that supports a dominant role for a specific flagellar serotype in *Salmonella* virulence was described by Grossman et al. (12). In that report, an analysis of the two *S. typhi* strains endemic to Indonesia suggested a link between flagellar serotype and severity of illness (12).

Previous studies showed that mutations in *fliD*, which encodes the flagellar cap protein, result in increased expression of the late flagellar operons (16, 17). A recent report from Yokoseki et al. elucidates the mechanism behind the increased expression of these operons; mutations in *fliD* result in enhanced export of FlgM and, consequently, a lower intracellular concentration of FlgM (26). This absence of FlgM leads to an increase in expression of the late flagellar genes. Moreover, Bäumler et al. found that an *fliD* mutant of *S. typhimurium* was attenuated in the mouse model of typhoid fever (3). The findings of Yokoseki et al. and Bäumler and colleagues, taken together with our results, suggest that *fliD* mutants are attenuated because the *fliD* mutant is phenotypically $FlgM^-$.

Although we and others have shown that flagella are not required by *S. typhimurium* for virulence in the mouse model of typhoid fever (4, 18), flagella may be required for the development of gastroenteritis in humans. Indeed, *Salmonella* strains associated with human disease typically are flagellate. Flagella may also have a role in the environment or in another niche that *S. typhimurium* encounters in vivo. Our results suggest that expression of different flagellin types might influence the outcome of *S. typhimurium* infection.

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