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Synergistic Effect of Mutations in *invA* and *lpfC* on the Ability of *Salmonella typhimurium* To Cause Murine Typhoid

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Penetration of the intestinal mucosa at areas of Peyer's patches is an important first step for *Salmonella typhimurium* to produce lethal systemic disease in mice. However, mutations in genes that are important for intestinal invasion result in only moderately decreased virulence of *S. typhimurium* for mice. Here we report that combining mutations in *invA* and *lpfC*, two genes necessary for entry into Peyer's patches, results in a much stronger attenuation of *S. typhimurium* than inactivation of either of these genes alone. An *S. typhimurium invA lpfC* mutant was 150-fold attenuated by the oral route of infection but was fully virulent when the intestine was bypassed by intraperitoneal challenge of mice. During mixed-infection experiments, the *S. typhimurium invA lpfC* mutant showed a strong defect in colonizing Peyer's patches and mesenteric lymph nodes. These data suggest that mutations in *invA* and *lpfC* deactivate distinct pathways for intestinal penetration and colonization of Peyer's patches. While the *inv*-mediated pathway is widely distributed, the *lpf* operon is absent from many phylogenetic groups within the genus *Salmonella*. To investigate how acquisition of the *lpf*-mediated pathway for mucosal penetration contributed to evolution of virulence, we studied the relationship between the presence of the *lpf* operon and the pathogenicity for mice of 18 isolates representing 14 *Salmonella* serotypes. Only strains possessing the *lpf* operon were able to cause lethal infection in mice. These data show that both the *invA*- and *lpfC*-mediated pathways of intestinal perforation are conserved in mouse virulent *Salmonella* serotypes.

Salmonella typhimurium causes a systemic infection in mice known as murine typhoid. After oral inoculation, the bacteria must first penetrate the intestinal wall in order to reach systemic sites of infection and cause disease. The primary sites of *S. typhimurium* penetration after oral challenge are the Peyer's patches of the distal ileum (7, 17). The major pathway by which *S. typhimurium* is able to enter these organs is by invasion of epithelial cells (19, 36).

Several genes necessary for invasion have been identified by studying bacterial entry into cultured epithelial cell lines (5, 9, 14, 18, 20, 22, 35). Most of these genes are located at centisome 63 on the *S. typhimurium* physical map in a 40-kb DNA region, designated *Salmonella* pathogenicity island 1 (SPI 1) (26). SPI 1 is present in all phylogenetic lineages of the genus *Salmonella* but is absent from close relatives such as *Escherichia coli* (23, 26). The genes located on SPI 1 encode a contact-dependent secretion system that induces changes in epithelial cell signaling, ultimately resulting in induction of macropinocytosis and bacterial entry (8, 10, 11, 13, 21, 27, 30, 36). *S. typhimurium* strains that carry mutations in the *inv*, *spa*, *org*, or *prg* gene, all of which are located on SPI 1, are unable to enter cultured epithelial cells (5, 9, 14, 18). An *S. typhimurium invA* mutant has a reduced ability to colonize Peyer's patches after oral challenge of mice (9). In studies using ligated ileal loops, it has been shown that *S. typhimurium* strains carrying mutations in *orgA* or *invA* are unable to selectively invade and destroy M cells in the follicle-associated epithelium of murine Peyer's patches (19). Furthermore, mutations in *invA* and *orgA* result in an increased 50% lethal dose (LD_{50}) of *S. typhimurium* for mice only after oral infection, not if the intestine is bypassed by the intraperitoneal route of infection (9, 18). These data clearly demonstrate that SPI 1 plays an important role during invasion of the intestinal mucosa. However, *S. typhimurium* strains carrying mutations in SPI 1 are still able to cause lethal infection in mice when administered orally at higher doses, indicating that alternate pathways to penetrate the intestinal epithelium must still be operational (9, 18). Does *S. typhimurium* possess virulence genes that mediate SPI 1-independent penetration of the mucosa, or is the residual amount of translocation across the intestinal epithelium observed for *invA* mutants the result of passive transport, e.g., by M cells?

To address this question, we investigated the synergistic action of SPI 1 and a second virulence gene cluster, the *lpf* operon, during colonization and penetration of the intestine. The *lpf* operon is located at centisome 80 on the *S. typhimurium* physical map and encodes long polar fimbriae (3). An *S. typhimurium lpfC* mutant has a reduced ability to adhere to and colonize murine Peyer's patches (4). Like mutations in SPI 1, a mutation in *lpfC* causes an attenuation if mice are infected orally but not if they are challenged by the intraperitoneal route of infection. These data indicate that mutations in *invA* and *lpfC* both impede similar steps during infection, namely, penetration of the intestinal epithelium and colonization of Peyer's patches. Here we investigate the contribution of SPI 1 and the *lpf* operon to mucosal invasion and characterize an *S. typhimurium invA lpfC* double mutant.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Natural isolates of different *Salmonella* serotypes were obtained from the *Salmonella* reference collection B (SARB) and have been reported recently (6). *S. typhimurium* mutants and parental strains used in this study are listed in Table 1. *E. coli* TA One Shot and

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TABLE 1. *S. typhimurium* strains used in this study

Strain	Genotype	Reference
IR715	ATCC 14028 Nal^r	34
AJB75	IR715 *invA*::TnPhoA	This study
AJB83	IR715 *invA*::TnPhoA *lpfC*::Cm^r	This study
AJB93	IR715 *lpfC*::Cm^r	This study
χ3642	SR-11 *invA*::TnPhoA	9

TABLE 2. Virulence of *S. typhimurium* mutants for mice

Strain	Relevant genotype	LD_{50} (CFU) after intragastric infection
IR715	Wild type	6.0×10^5
AJB75	*invA*	9.6×10^6
AJB83	*lpfC invA*	9.3×10^7
AJB93	*lpfC*	3.5×10^6

[a] For all strains, the LD_{50} after intraperitoneal infection was <10 CFU/mg.

DH5α were purchased from Invitrogen and Gibco BRL, respectively. All bacteria were cultured in Luria-Bertani (LB) broth or on plates (32). If appropriate, antibiotics were included at the following concentrations: nalidixic acid, 50 μg/ml; chloramphenicol, 30 μg/ml; kanamycin, 100 μg/ml; and carbenicillin, 100 μg/ml. Analytical-grade chemicals and enzymes were purchased from Sigma and Boehringer Mannheim, respectively.

Construction of mutants. AJB75 was constructed by transducing *invA*::TnPhoA from *S. typhimurium* χ3642 (9) into strain IR715 (34), using bacteriophage KB1*int*. A chloramphenicol resistance cassette was constructed by digestion of plasmid pBC SK (Stratagene) with *Bst*YI, and a 1.2-kb fragment containing the chloramphenicol acetyltransferase (Cm^r) gene was cloned into *Xho*I-restricted pUC4K (KIXX; Pharmacia) after filling in of the *Xho*I ends with Klenow polymerase. The resulting plasmid, pCMXX, was digested with *Eco*RI to excise the Cm^r gene. The Cm^r gene was cloned into the *Eco*RI restriction site located within the *lpfC* open reading frame in plasmid pMS1054 (3). The resulting *lpfC* allele (which is disrupted by insertion of the Cm^r gene) was cloned into the *Sma*I site of suicide vector pGP705 (37), using *Pst*I followed by Klenow polymerase treatment. The resulting plasmid, pJV100, was introduced into *E. coli* S17-1 λ*pir* (33) and then conjugated into *S. typhimurium* AJB75 and IR715. Exconjugants were selected on LB-nalidixic acid-chloramphenicol plates. One exconjugant sensitive to tetracycline (vector) and one resistant to chloramphenicol were chosen from each conjugation and designated AJB83 and AJB93, respectively. Insertional inactivation of *invA* in strains AJB75 and AJB83 was confirmed by Southern hybridization with an *invA*-specific DNA probe. Chromosomal DNA of AJB83 and AJB93 was analyzed by Southern hybridization with the *lpfC*-specific DNA probe to confirm insertional inactivation.

Isolation of chromosomal DNA and Southern hybridization. The primers and conditions for PCR amplification of internal parts of the *invA* and *spvR* open reading frames have been described previously (24, 28). The *invA* and *spvR* PCR products were cloned into the vector pCRII and transformed into *E. coli* TA One Shot (TA-cloning kit; Invitrogen). Plasmids were isolated by using ion-exchange columns from Qiagen. To generate nucleotide probes, DNA fragments were labeled by random-primed incorporation of digoxigenin-labeled dUTP, using a DNA labeling and detection kit (nonradioactive) from Boehringer Mannheim. An *lpfC*-specific DNA probe has been described previously (4). Isolation of chromosomal DNA was performed as recently described (1). Chromosomal DNA of *Salmonella* serotypes from the SARB collection was restricted with *Eco*RI, and the fragments were separated on a 0.5% agarose gel. Southern transfer of DNA onto a nylon membrane was performed as previously described (1). Hybridization was performed at 65°C in solutions without formamide. A 15-min wash was performed at room temperature in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate. Subsequently membranes were washed for 15 min at 65°C in 0.2× SSC–0.1% sodium dodecyl sulfate. Hybrids were detected by using a labeling and detection kit (nonradioactive) from Boehringer Mannheim.

Animal experiments. Prior to infection of mice, all bacteria were cultured as static overnight cultures in LB broth, harvested by centrifugation, and resuspended in sterile saline. Six- to eight-week-old female BALB/c mice were used throughout this study.

Virulence of *S. typhimurium* mutants was tested by infecting groups of four mice intragastrically or intraperitoneally with serial 10-fold dilutions of bacterial cultures in a 0.2-ml volume. Mortality was recorded at 28 days postinfection, and the LD_{50} was calculated by the method of Reed and Muench (29). Levels of mouse virulence of different *Salmonella* serotypes that are part of the SARB collection were compared by infecting groups of two mice intragastrically with bacterial cultures that had been adjusted to about the same optical density at 576 nm (OD_{576}). In all experiments, the bacterial titer of the inoculum was determined by spreading serial 10-fold dilutions on agar plates and determining CFU.

Numbers of bacteria in feces and colonization of organs were investigated by mixed infection of a group of 10 mice intragastrically with 1.6×10^7 bacteria/mouse in a 0.2-ml volume. The mixture contained approximately equal numbers of IR715 (3.4×10^6), AJB75 (4.8×10^6), and AJB83 (3.6×10^6), and AJB93 (4.4×10^6). Prior to infection, fecal pellets from all 10 mice were suspended in phosphate-buffered saline (PBS) and plated on LB-nalidixic acid plates to ensure that the indigenous microflora is sensitive to nalidixic acid. At days 1, 3, and 5 postinfection, two fecal pellets were collected from each mouse for enumeration of bacterial counts. Fecal pellets were weighed, homogenized in 1 ml of PBS, and plated on appropriate antibiotic plates to determine CFU/milligram of feces for each strain. At 5 days postinfection, mice were sacrificed, and internal organs (the three Peyer's patches proximal to the cecum, mesenteric lymph node, and spleen) were collected and homogenized in 5 ml of PBS, using a Stomacher (Tekmar, Cincinnati, Ohio). Dilutions were plated on LB plates containing the appropriate antibiotics, and significance of differences observed was determined by using a paired-difference test.

RESULTS

Virulence of an *S. typhimurium invA lpfC* mutant for mice. Strain IR715 is a nalidixic acid-resistant (Nal^r) derivative of the *S. typhimurium* wild-type strain ATCC 14028 which is fully virulent for mice (34). IR715 was used to construct isogenic mutants in which *invA* (AJB75), *lpfC* (AJB93), or both genes (AJB83) were inactivated. For construction of strains that carry mutations in *invA* by KB1*int* transduction, we used the *invA*::TnPhoA allele of *S. typhimurium* χ3642 that has been characterized previously (9). The *lpfC* gene was inactivated by insertion of a Cm^r gene, using marker exchange with an *lpfC*::Cm^r allele located on suicide plasmid pJV100. Mouse virulence of the *invA* (AJB75), *lpfC* (AJB93), and *invA lpfC* (AJB83) mutants was compared with that of the wild type (IR715) by the intragastric route of infection. The *S. typhimurium lpfC* mutant (AJB93) had a sixfold-increased LD_{50} compared to its parent (IR715) (Table 2). These data therefore confirm previous studies using a *S. typhimurium* mutant strain which carried a kanamycin resistance (Km^r) gene inserted in *lpfC* (4). A mutation in *invA* (AJB75) resulted in a 16-fold increased LD_{50} compared to IR715. Galán and coworkers reported a slightly stronger attenuation for an *invA* mutant (about 50-fold) (9). This difference may result from the use of different *S. typhimurium* wild-type strains (ATCC 14028 versus SR-11) or the use of different BALB/c mouse lineages. However, consistent with previous findings, the *S. typhimurium invA* mutant (AJB75) exhibited a stronger degree of attenuation than the *lpfC* mutant (AJB93). Most importantly, the introduction of an *lpfC* mutation into the *S. typhimurium invA* mutant resulted in a dramatic reduction of virulence. Compared with the isogenic wild-type (IR715), strain AJB83 (*invA lpfC*) possessed an about 150-fold-increased LD_{50}. These data clearly demonstrate that the degree of attenuation observed in *invA* (AJB75) and *lpfC* mutants (AJB93) can be potentiated by combining both mutations in one strain.

In a second experiment, levels of virulence of IR715, AJB75, AJB83 and AJB93 were compared by challenging mice intraperitoneally (Table 2). This route of infection bypasses the intestine, where the initial phase of infection takes place. Consistent with previous reports, mutations in *invA* and *lpfC* did not attenuate *S. typhimurium* when mice were challenged intraperitoneally, demonstrating that these genes are not required to cause systemic disease by this route of infection (4, 9). Similarly, the *S. typhimurium invA lpfC* mutant (AJB83) was not attenuated by the intraperitoneal route of infection. These data suggest that AJB83 is impaired at a step during infection not encountered by *S. typhimurium* when administered intra-

peritoneally, such as the intestine, Peyer's patches, or mesenteric lymph nodes.

Mixed-infection studies. We and other investigators have found that bacterial numbers recovered from individual mice infected with the same dose of *S. typhimurium* may vary over a wide range, thereby complicating analysis of the experimental results. This problem can be avoided by performing mixed infections. Mixed-infection experiments allow for a direct comparison between mutant and wild type because both are recovered from the same animal. This type of analysis has been successfully used in the past to determine that mutations in *invA* and *lpfC* both affect colonization of murine Peyer's patches (4, 9). We therefore performed a mixed-infection experiment to pinpoint the step during infection at which an *S. typhimurium invA lpfC* mutant (AJB83) is deficient. A group of 10 mice was infected intragastrically with approximately equal numbers of IR715 (wild type, Nal^r), AJB75 (*invA*, Nal^r Km^r), AJB83 (*invA lpfC*, Nal^r Km^r Cm^r), and AJB93 (*lpfC*, Nal^r Cm^r). The four strains are distinguishable by their different antibiotic resistances. Fecal pellets were collected at 1, 3, and 5 days postinfection, and each dilution was spread on LB-nalidixic acid, LB-nalidixic acid-kanamycin, LB-nalidixic acid-chloramphenicol, and LB-nalidixic acid-chloramphenicol-kanamycin plates. Bacterial counts from these plates were used to calculate CFU/milligram of feces for IR715, AJB75, AJB83, and AJB93. The limit of detection was about 0.1 CFU/mg of feces. Strains carrying a mutation in *invA* (AJB75 and AJB83) were absent from feces more frequently than the wild type. However, for each strain, the bacterial numbers recovered from feces varied over a wide range (from 10^{-1} to 10^5 CFU/mg of feces) between different mice (Fig. 1). There was no significant difference in numbers between the wild type (IR715) and the different mutants ($P > 0.1$). However, since CFU recovered from feces were highly variable, interpretation of these results is difficult and the possibility that mutations in *invA lpfC* have an effect on the ability of *S. typhimurium* to colonize fecal contents cannot be excluded.

At day 5 postinfection, organs (Peyer's patches, mesenteric lymph nodes, and spleen) from mice were collected, and dilutions of organ homogenates were spread onto LB-nalidixic acid, LB-nalidixic acid-kanamycin, LB-nalidixic acid-chloramphenicol, and LB-nalidixic acid-chloramphenicol-kanamycin plates. Bacterial numbers recovered from organs of different mice showed less variation than the fecal counts. The limit of detection was 5 CFU/organ. Strain AJB93 (*lpfC*) was recovered in significantly lower numbers from Peyer's patches than its parent (IR715) ($P < 0.05$) (Fig. 2). The *S. typhimurium invA* mutant (AJB75) was recovered in significantly lower numbers than IR715 from Peyer's patches ($P < 0.025$), mesenteric lymph nodes ($P < 0.05$), and spleen ($P < 0.1$). These results confirm previous studies which show that mutations in *invA* and *lpfC* cause a defect early during infection (4, 9). However, a mutation in *invA* (AJB75) had a more pronounced effect on colonization of Peyer's patches than a mutation in *lpfC* (AJB93), suggesting that the major pathway of intestinal penetration is encoded by SPI 1. Strain AJB83 (*invA lpfC*) was found in significantly lower numbers than IR715 in Peyer's patches ($P < 0.025$), mesenteric lymph nodes ($P < 0.05$), and spleen ($P < 0.1$). Furthermore, the numbers recovered from Peyer's patches and mesenteric lymph nodes were lower for the *S. typhimurium invA lpfC* mutant (AJB83) than for the isogenic *invA* mutant (AJB75). These data show that an *S. typhimurium invA lpfC* mutant is impaired in performing a step early during infection, namely, the colonization of Peyer's patches and mesenteric lymph nodes. An *S. typhimurium invE* mutant will invade epithelial cell lines in vitro if added along with wild-type bacteria, suggesting that during mixed-infection experiments, cross-complementation between bacterial strains could occur (12). However, the fact that AJB93 (*lpfC*), AJB75 (*invA*), and AJB83 (*invA lpfC*) were recovered in lower numbers from Peyer's patches than IR715 (wild type) suggests that cross-complementation did not rescue mutant bacteria in this experimental setup.

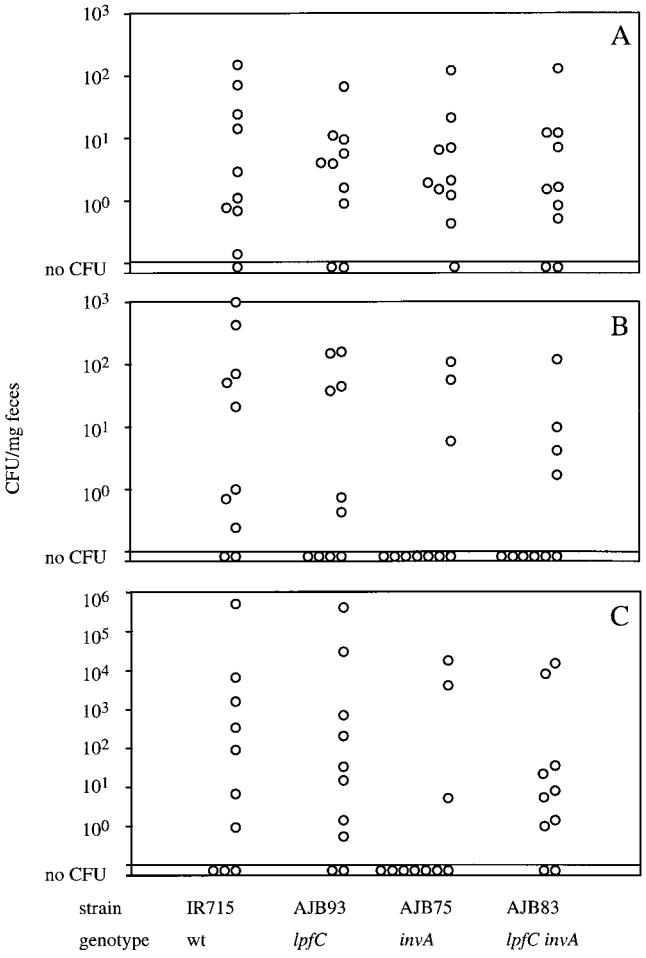

FIG. 1. Numbers of bacteria recovered from feces after mixed infection of mice with approximately equal numbers of IR715 (wild type [wt]), AJB93, AJB75, and AJB83. Fecal pellets were collected, and bacterial counts were determined at 1 (A), 3 (B), and 5 (C) days postinfection. Circles represent data obtained from individual mice. The limit of detection was about 0.1 CFU/mg of feces.

Correlation between mouse virulence of *Salmonella* serotypes and the presence of virulence genes. While SPI 1 is well conserved among *Salmonella* serotypes (23, 28), the *lpf* operon is absent from several phylogenetic lineages within the genus *Salmonella* (2). If both the SPI 1- and *lpf*-mediated pathways for mucosal penetration are important adaptations for infection of mice, then these gene clusters should both be present in all *Salmonella* serotypes that can cause lethal murine infections. We therefore investigated whether the *lpf* operon is present in serotypes other than *S. typhimurium* that are virulent for mice. To this end, mice were infected with 18 natural *Salmonella* isolates representing 14 different serotypes, and virulence was recorded 28 days postinfection (Fig. 3). Six

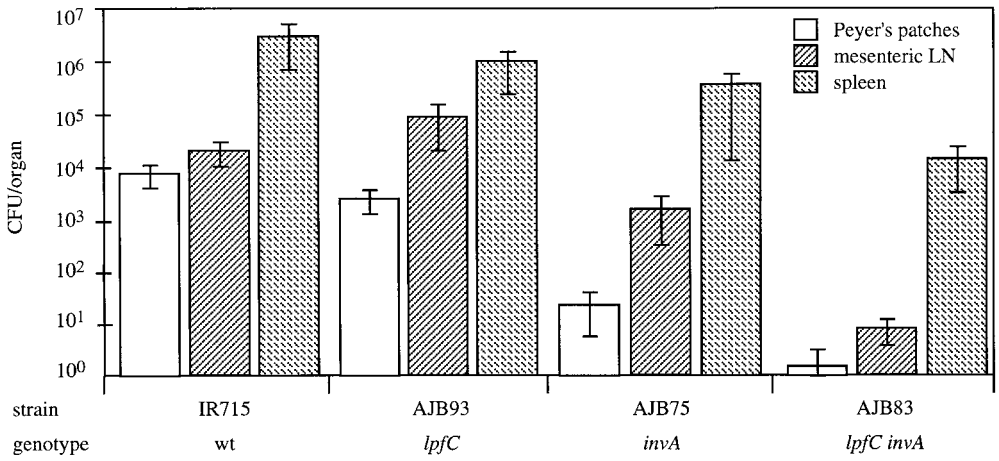

FIG. 2. Numbers of bacteria recovered from Peyer's patches, mesenteric lymph nodes (LN), and spleen of mice 5 days postinfection. Mice were infected with a mixture consisting of approximately equal numbers of IR715, AJB93, AJB75, and AJB83. Bars represent the means ± standard errors.

strains, including *S. typhimurium* Tm1, *S. pullorum* Pu3, *S. dublin* Du1, *S. enteritidis* En1, *S. choleraesuis* Cs1, and *S. paratyphi C* Pc2, were able to cause fatal infection in mice.

The presence of the *lpf* operon and the *Salmonella* pathogenicity island 2 (SPI 2) among the strains shown in Fig. 3 has been reported previously (2, 16). To determine the distribution of two other virulence gene clusters, SPI 1 and the *spv* operon, chromosomal DNA of these strains was hybridized with *invA*- and *spvR*-specific DNA probes, respectively (Fig. 3). Comparative analysis of these data revealed that all strains capable of producing lethal infection in mice possess SPI 1, SPI 2, the *spv* operon, and the *lpf* operon. Of the 12 avirulent strains, 11 did not contain *spvR* and 4 did not contain the *lpf* operon. These data show that the *lpf*- and SPI 1-mediated pathways for penetration of the mucosa are well conserved among mouse-virulent *Salmonella* serotypes, suggesting that synergistic action of the encoded virulence factors is important for mouse virulence of natural isolates.

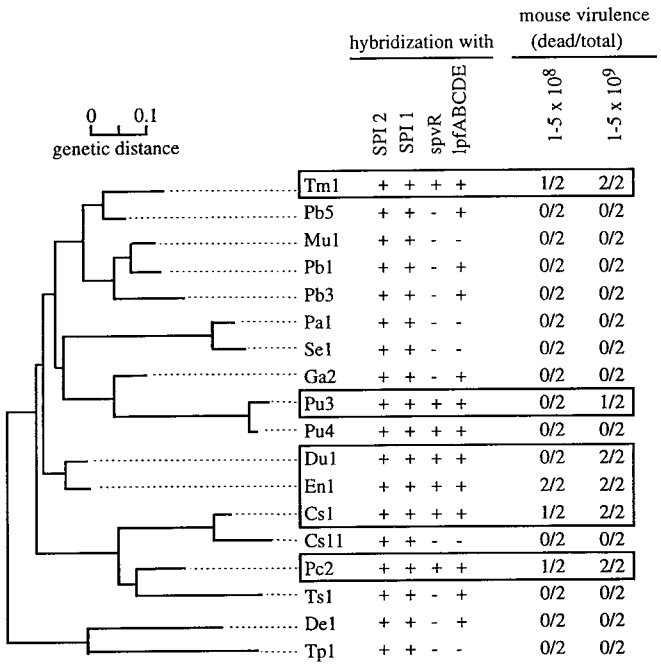

FIG. 3. Phylogenetic distribution of virulence genes and virulence of different *Salmonella* serotypes for mice. The bacterial isolates used in this experiment have been described previously (6). The left side shows a dendrogram reflecting the phylogenetic relatedness of these strains as reported by Boyd et al. (6). The distribution of SPI 2 and *lpfABCDE* among these isolates has been reported previously (2, 16). The distribution of *invA* and *spvR* among these strains was determined in this study and is shown in the center. +, hybridization signal; −, no hybridization signal. The results of animal experiments are shown on the right. For each strain, two groups of two mice were infected with the indicated dose, and virulence was recorded 28 days postinfection. Strains that were able to produce lethal infection are boxed. Cs, *S. choleraesuis*; De, *S. derby*; Du, *S. dublin*; En, *S. enteritidis*; Ga, *S. gallinarum*; Pa, *S. paratyphi A*; Pb, *S. paratyphi B*; Pc, *S. paratyphi C*; Pu, *S. pullorum*; Se, *S. sendai*; Tm, *S. typhimurium*; Tp, *S. typhi*; Ts, *S. typhisuis.*

DISCUSSION

Penetration of the intestinal mucosa appears to be essential for *S. typhimurium* to cause lethal infection in mice. For instance, secretion of monoclonal anti-*Salmonella* immunoglobulin A (IgA) into the intestinal tract provides protection from oral challenge with *S. typhimurium*. Production of IgA does not protect against intraperitoneal challenge, as mice remain fully susceptible to *S. typhimurium* administered by this route of infection. Rather, the IgA-mediated protection is due to immune exclusion at the mucosal surface, which prevents entry of *S. typhimurium* into Peyer's patches (25). These data imply that blockage of mucosal penetration results in avirulence of *S. typhimurium* for mice by the oral route of infection. However, none of the mutations that affect *S. typhimurium* invasion and colonization of Peyer's patches completely abolishes its ability to cause lethal infection in mice. In fact, *S. typhimurium* strains that carry mutations in *invA* or *lpfC*, two genes involved in mucosal penetration and colonization of Peyer's patches, retain considerable mouse virulence (Table 2; Fig. 2). The modest degree of attenuation of *S. typhimurium invA* or *lpfC* mutants implies that alternate routes of mucosal penetration are still operational in these strains (4, 9). Here we report that combining mutations in *lpfC* and *invA* has a synergistic effect on intestinal penetration and on mouse virulence. Mixed-infection studies showed that combining mutations in *invA* and *lpfC* further reduced the ability of *S. typhimurium* to colonize murine Peyer's patches and mesenteric lymph nodes (Fig. 2). Consistent with a defect in the intestinal phase of infection, the *S. typhimurium invA lpfC* mutant was strongly attenuated (150-

fold) by the oral route of infection but was fully virulent when administered intraperitoneally (Table 2). These data are evidence for the redundancy or synergistic action of virulence factors that function in penetration of the intestinal barrier by *S. typhimurium*. The fact that the *invA lpfC* mutant was still able to cause lethal infection in mice when administered orally at high doses indicates that *S. typhimurium* is able to cross the intestinal mucosa by yet another route, possibly via M-cell-mediated sampling of the intestinal content.

The increased intragastric LD_{50} of the *invA lpfC* mutant compared to *S. typhimurium* strains that carry mutations in only *invA* or *lpfC* suggests that SPI 1 and the *lpf* operon determine alternate routes for entering murine Peyer's patches. Of these two pathways, the one mediated by SPI 1 appears to be more important, as mutations in *invA* attenuate to a greater degree than those in *lpfC*. Consistent with its important role in intestinal penetration, SPI 1 has been shown to be present in all phylogenetic lineages of the genus *Salmonella* (23). In contrast, in a study on the distribution of the *lpf* genes among *Salmonella* serotypes, we found that this operon was present in only about half (44 of 90) of the strains tested (2). The restricted distribution of the *lpf* operon among *Salmonella* serotypes may be indicative of its role during infection of only a limited number of host species. The results presented here suggest that synergistic action of SPI 1 and *lpf* in penetration of the intestinal wall confers an advantage during infection of mice.

The development of host adaptations of *Salmonella* serotypes was accompanied by acquisition of host range factors by horizontal gene transfer (2). The *spv* operon is the only gene cluster identified to date that encodes a host range factor of *Salmonella* serotypes for infection of rodents (31). The *spv* genes were acquired by horizontal transfer, in fact in a plasmid-mediated event, as this operon is located on a large plasmid (15). All *Salmonella* serotypes that cause murine typhoid carry the *spv* genes on a plasmid (31). Similar to *spv*, the *lpf* operon was acquired in the genus *Salmonella* by way of horizontal gene transfer (2). Furthermore, we show here that all *Salmonella* isolates that were able to cause lethal infection in mice possessed the *lpf* operon. However, among 11 strains that were unable to cause lethal infection in mice, 7 also carried the *lpf* operon. These data show that like SPI 1, the *lpf* operon is present in but is not limited to mouse-virulent *Salmonella* serotypes.

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