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The *Caenorhabditis elegans* ABL-1 Tyrosine Kinase Is Required for *Shigella flexneri* Pathogenesis

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Shigellosis is a diarrheal disease caused by the gram-negative bacterium *Shigella flexneri*. Following ingestion of the bacterium, *S. flexneri* interferes with innate immunity, establishes an infection within the human colon, and initiates an inflammatory response that results in destruction of the tissue lining the gut. Examination of host cell factors required for *S. flexneri* pathogenesis in vivo has proven difficult due to limited host susceptibility. Here we report the development of a pathogenesis system that involves the use of *Caenorhabditis elegans* as a model organism to study *S. flexneri* virulence determinants and host molecules required for pathogenesis. We show that *S. flexneri*-mediated killing of *C. elegans* correlates with bacterial accumulation in the intestinal tract of the animal. The *S. flexneri* virulence plasmid, which encodes a type III secretory system as well as various virulence determinants crucial for pathogenesis in mammalian systems, was found to be required for maximal *C. elegans* killing. Additionally, we demonstrate that ABL-1, the *C. elegans* homolog of the mammalian c-Abl nonreceptor tyrosine kinase ABL1, is required for *S. flexneri* pathogenesis in nematodes. These data demonstrate the feasibility of using *C. elegans* to study *S. flexneri* pathogenesis in vivo and provide insight into host factors that contribute to *S. flexneri* pathogenesis.

The gram-negative bacterium *Shigella flexneri* is the causative agent of shigellosis, a diarrheal disease which affects up to 150 million people annually (22). The study of shigellosis in vivo has been hampered by the lack of a suitable model system. Unlike humans, mice do not develop intestinal disease upon ingestion of *S. flexneri*. While recent developments of mouse models using newborn mice (17) and infection paired with intraluminal injection of interleukin-8 (34) are promising, their utility in the study of host cell factors involved in shigellosis remains to be determined.

Although *Shigella* is believed to specifically infect primates, several reports indicate that invertebrates, such as flies and nematodes, may serve as vectors of the bacterium (5, 12, 16, 25, 28, 41, 42). These results indicate that the host range of the pathogen may be broader than is suspected and open the possibility of using invertebrates to study conserved host-pathogen interactions that can be translated to mammalian systems. *Caenorhabditis elegans* has become a well-established model invertebrate for the study of bacterial pathogenesis and innate immunity. As in mammals, peristalsis, low pH, lytic enzymes, and antimicrobial substances prevent microbial colonization of the *C. elegans* intestine. However, pathogenic bacteria are capable of proliferating and killing *C. elegans* using different mechanisms. The lists of bacterial pathogens that induce nematode killing include both gram-negative pathogens such as *Salmonella enterica*, *Pseudomonas aeruginosa*, and *Serratia marcescens* and gram-positive bacteria such as *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Enterococcus faecalis* (reviewed in references 1, 4, and 33). The relatively simple innate immune system of *C. elegans* and the number of traits that facilitate genetic and genomic analysis using this organism, including a hermaphroditic lifestyle and short 2- to 3-week life span, have nurtured rapid advances into the understanding of *C. elegans* innate immunity during the last few years (26, 30, 31). In addition, the nematode has also been used to study the mechanisms by which specific virulence factors interact with host molecules to promote bacterial pathogenesis (14, 39).

Here we demonstrate that *S. flexneri* kills *C. elegans* by an infection-like process that requires live bacteria, correlates with the bacterial accumulation in the intestine of the animals, and requires the *S. flexneri* virulence plasmid which is crucial for pathogenesis in mammalian systems. In addition, we have shown that *C. elegans* ABL-1 is required for *S. flexneri*-induced killing, as loss of ABL-1 through mutation, RNA interference (RNAi) ablation of expression, or pharmacological inhibition results in extension of nematode life span on *S. flexneri*. These observations suggest that *C. elegans* can be used to study host-pathogen interactions between *S. flexneri* and invertebrates, some of which may also take place in mammals.

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MATERIALS AND METHODS

Bacterial strains and reagents. The wild-type strain *S. flexneri* 2457T was a generous gift from Marcia Goldberg (Harvard University). The virulence plasmid-cured *S. flexneri* Δinv strain has been described previously (10). *S. flexneri* was grown on tryptic soy broth (Difco) agar plates containing 0.5% Congo Red. *Escherichia coli* OP50 (7) and *Salmonella. enterica* serovar Typhimurium strains SL1344 and SL1344-GFP (3) have been described. All bacterial strains were

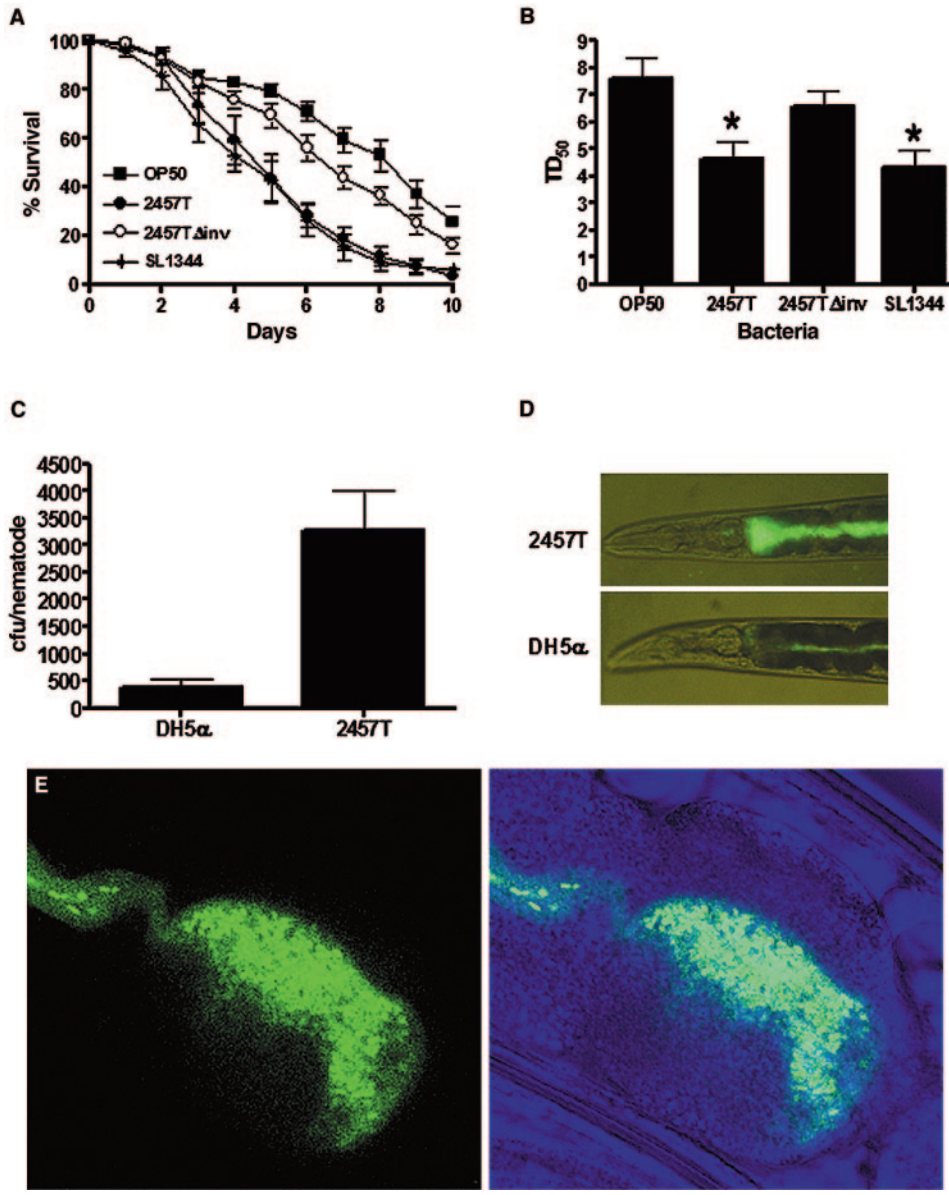

FIG. 1. *S. flexneri* kills *C. elegans* and accumulates in the nematode intestine. (A) Young adult hermaphrodite N2 nematodes were fed on lawns of either *E. coli* OP50, *S. flexneri* 2457T, *S. flexneri* 2457TΔinv, or *S. enterica* serovar Typhimurium SL1344 at 25°C, and nematodes were scored daily for survival. The survival curve represents data from 5 independent experiments, each using 20 nematodes. (B) The time required for nematodes to die (TD_{50}) was calculated using the survival data in panel A, representing data from five independent experiments. Asterisks indicate significant differences compared to data for OP50 ($P < 0.05$). (C) Young adult nematodes were fed on lawns of either *E. coli* DH5α or *S. flexneri* 2457T expressing GFP for 48 h and mechanically disrupted. Diluted lysates were plated on LB-ampicillin plates, and colonies were quantified in order to calculate *S. flexneri* cells associated with individual nematodes. These data represent 3 independent experiments, each using 20 nematodes. (D) Nematodes fed either *E. coli* DH5α or *S. flexneri* 2457T expressing GFP for 48 h were analyzed using a fluorescence stereomicroscope. (E) Confocal images show the anterior intestine of an animal fed *S. flexneri* 2457T expressing GFP for 48 h.

grown overnight at 37°C in Luria broth (LB). STI571, a generous gift of Brian Druker, was added to the modified nematode growth (NG) agar media, at noted concentrations. Bacterial lawns grown on plates containing STI571 were not visually different from lawns grown on control plates, and growth curves of bacteria grown on LB or LB containing STI571 were not different, indicating that the drug does not have any effect on bacterial growth at the concentrations used in this study.

Nematode strains and maintenance of nematodes. *C. elegans* strain Bristol N2 was maintained as hermaphrodites at 20°C, grown on modified NG agar plates (0.35% instead of 0.25% peptone), and fed with *E. coli* strain OP50 as described previously (36). The *abl-1*(*ok171*) mutant strain XR1 was obtained from the *Caenorhabditis* Genetics Center (University of Minnesota). Worms were observed under a Leica MZ7.5 dissecting microscope. RNAi *abl-1* nematodes were prepared by feeding, as described previously (23).

***C. elegans* killing assays.** Bacterial lawns used for killing assays were prepared by plating 10 μl of the overnight culture on modified NG agar medium in 35-mm dishes. Plates were grown overnight at 37°C and cooled to room temperature before use. Young adult hermaphrodite nematodes were transferred to bacterial lawns and incubated at 25°C. The worms were transferred to fresh lawns each day for 3 days to separate adults from progeny. Worm mortality was scored over time, and a worm was considered dead when it no longer responded to touch. Worms that died as a result of getting stuck to the wall of the plate were excluded

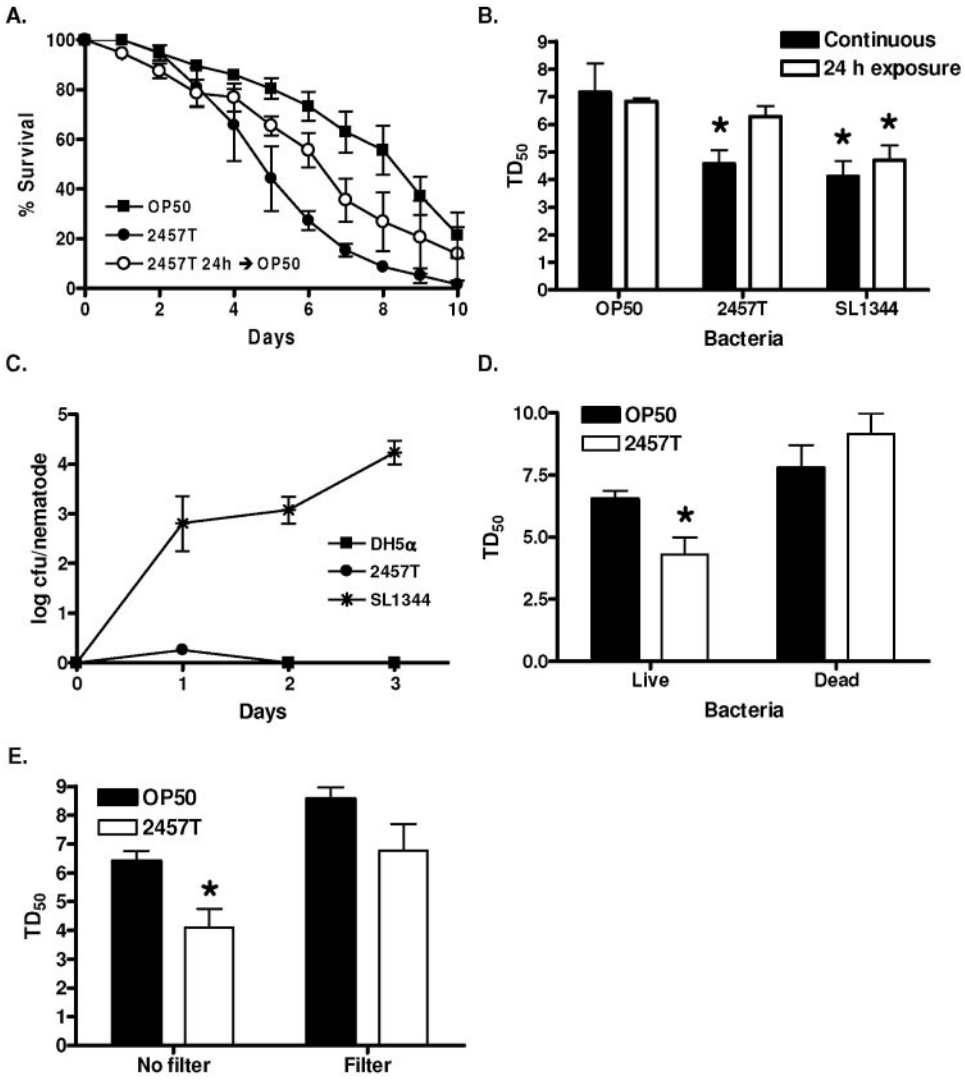

FIG. 2. The mechanism of *S. flexneri*-mediated *C. elegans* killing is distinct from that of *S. enterica* and *P. aeruginosa*. (A) Young adult hermaphrodite nematodes were fed on lawns of either *E. coli* OP50 (squares) or *S. flexneri* 2457T (closed circles) continuously or fed on *S. flexneri* 2457T for 24 h and then transferred to OP50 for the duration of the assay (open circles). Nematodes were scored daily for survival. The survival curve represents data from three independent experiments, each using 20 nematodes. (B) The time required for nematodes to die (TD_{50}) was calculated from the survival data in panel A, representing data from three independent experiments. Asterisks indicate significant differences, compared to results for OP50 ($P < 0.05$). (C) Young adult hermaphrodite nematodes were fed on *E. coli* DH5α-GFP (squares), *S. flexneri* 2457T-GFP (circles), or *S. enterica* serovar Typhimurium SL1344-GFP (asterisks) for 24 h and then transferred to lawns of *E. coli* OP50. Nematodes were removed every 24 h and mechanically disrupted to release internalized bacteria. Diluted lysates were plated on LB-ampicillin plates, and colonies were scored in order to quantify *S. flexneri* cells associated with individual nematodes. (D) Young adult hermaphrodites were fed on either live or UV-killed *E. coli* OP50 or *S. flexneri* 2457T and scored daily for survival. The time for nematodes to die (TD_{50}) was calculated from the survival data. Asterisks indicate significant differences, compared to results for OP50 ($P < 0.05$). (E) Young adult nematodes were fed on either *E. coli* OP50 or *S. flexneri* 2457T or on *E. coli* OP50 plated on agar plates incubated with *E. coli* OP50 or *S. flexneri* 2457T separated by a 0.45-μm filter. The nematodes were scored daily for survival, and the time required for nematodes to die (TD_{50}) was calculated from the survival data. Asterisks indicate significant differences, compared to results for OP50 ($P < 0.05$).

from the analysis. The time required for 50% of the nematodes to die (TD_{50}) was calculated using GraphPad Prism software (version 4.01) using the following equation: $Y = \text{Bottom} + [\text{Top} - \text{Bottom}]/[1 + 10^{(\log EC_{50} - X)}(\text{Hill slope})]$, where X is the logarithm of days, Y is the average of dead worms (3), and EC_{50} is the midpoint of the curve. The TD_{50} data were analyzed using the paired t test, with a P value of <0.05 denoting a significant result. The relative mortality was calculated using the following equation: (TD_{50} wild-type nematode on pathogen/TD_{50} test nematode on pathogen)/(TD_{50} wild-type nematode on *E. coli*/TD_{50} test nematode on *E. coli*) (2).

Bacterial accumulation assay. To examine bacterial accumulation, young adult hermaphrodites were transferred to *E. coli* DH5α-GFP or *S. flexneri* 2457T-GFP bacterial lawns prepared on modified NG agar plates containing ampicillin (50 μg/ml) and were incubated at 25°C for 48 h. The ampicillin resistance of both *E. coli* DH5α-GFP or *S. flexneri* 2457T-GFP is given by the pSMC21 plasmid (6) they carry. Nematodes were then serially transferred to plates containing *E. coli* OP50, in order to remove any external green fluorescent protein (GFP)-expressing bacteria. The nematodes were washed, lysed in phosphate-buffered saline (PBS) containing 0.1% Triton X-100, and mechanically disrupted using a pestle. The worm lysates were diluted and plated on LB plates containing ampicillin (50 μg/ml). The plates were incubated overnight at 37°C. Colonies were quantified and used to calculate the number of bacteria per nematode. Alternatively, nematodes were anesthetized with sodium azide

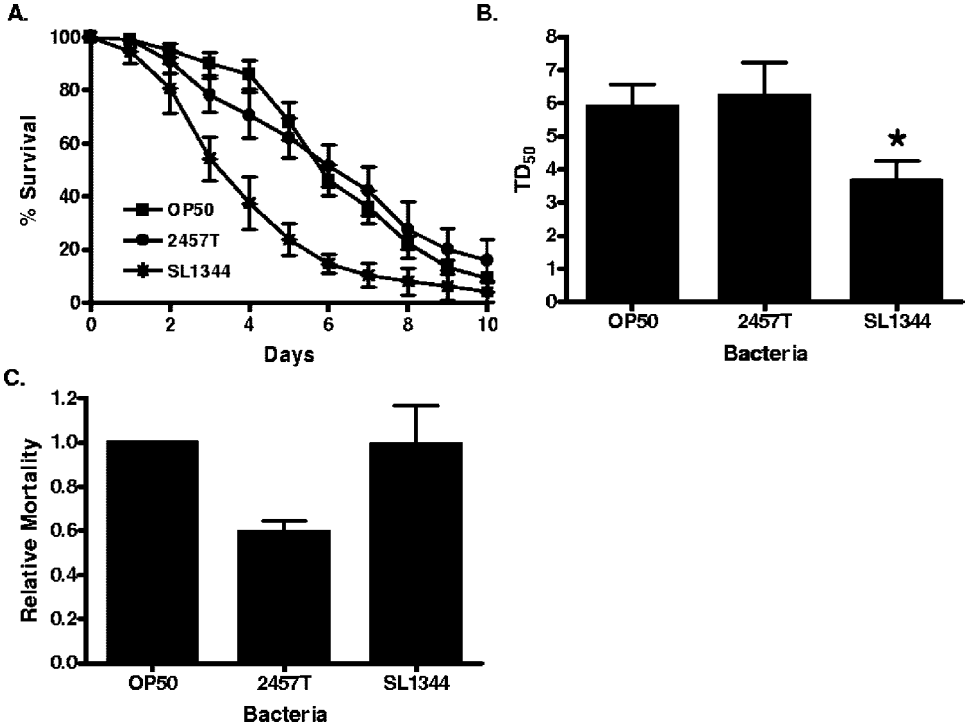

FIG. 3. ABL-1 mutant nematodes are resistant to *S. flexneri*-mediated *C. elegans* killing. (A) Young adult hermaphrodite *abl-1(ok171)* nematodes were fed on lawns of either *E. coli* OP50, *S. flexneri* 2457T, or *S. enterica* serovar Typhimurium SL1344 at 25°C, and nematodes were scored daily for survival. The survival curve represents data from 5 independent experiments, each using 20 nematodes. These experiments also included analysis of N2 nematodes, shown in Fig. 1. (B) The time required for nematodes to die (TD_{50}) was calculated from the survival data in panel A, representing data from three independent experiments. The asterisk indicates significant difference compared to results for OP50 ($P < 0.05$). (C) The relative mortality was calculated using the TD_{50} values for N2 and *abl-1(ok171)* nematodes in order to normalize the survival rates of the nematodes grown on pathogens compared to that of nematodes fed on *E. coli* OP50 (2).

and analyzed by confocal microscopy, as described previously (3). To examine persistent colonization, young adult hermaphrodites were transferred to *E. coli* DH5α-GFP, *S. enterica* 1344-GFP, or *S. flexneri* 2457T-GFP bacterial lawns prepared on modified NG agar plates containing ampicillin (50 μg/ml) and were incubated at 25°C for 24 h. Nematodes were transferred to lawns of *E. coli* OP50 for 1 h and then transferred to new plates containing OP50. The worms were transferred to new plates every 24 h and lysed at various time points. The lysates were diluted, plated on LB plates containing ampicillin (50 μg/ml), and examined as described above.

UV treatment of bacteria. Bacteria on lawns were killed by irradiation using the UV Stratalinker autosetting (1,200 μJ × 100) for 1 min. As a control, a sample of killed bacteria was transferred to LB and grown overnight at 37°C to check for viable bacteria. Experiments were discarded if growth was detected.

RESULTS

***S. flexneri* killing of *C. elegans* correlates with bacterial accumulation in the intestine and requires the virulence plasmid.** To study *S. flexneri* interactions with invertebrates, we examined whether *S. flexneri* was capable of killing *C. elegans*. As shown in Fig. 1A, wild-type N2 nematodes died more quickly when fed on the *S. flexneri* strain 2457T than when fed on *E. coli* OP50, the usual food source for propagating *C. elegans* in the laboratory. Nematodes grown on *S. flexneri* die with kinetics similar to those observed for animals infected with *S. enterica*, which has been previously shown to be pathogenic to *C. elegans* (3, 27). The TD_{50} when feeding on *S. flexneri* lawns ($TD_{50} = 4.64 \pm 0.62$) was calculated in five independent experiments and determined to be significantly shorter than that for nematodes feeding on *E. coli* ($TD_{50} = 7.61 \pm 0.76$) (Fig. 1B).

Most of the *S. flexneri* virulence determinants are located in a large 220-kb virulence plasmid (8, 40) required for full pathogenicity in mammals (32). The *S. flexneri* virulence plasmid encodes a type III secretory system (TTSS) critical for the translocation of a variety of virulence factors that facilitate bacterial pathogenesis by modulating actin cytoskeleton dynamics and innate immunity. To examine whether the virulence plasmid was required for *C. elegans* killing, we examined the life span of nematodes fed plasmid-cured *S. flexneri* (10). Indeed, the nematodes fed the plasmid-cured 2457TΔinv strain exhibited a longer life span ($TD_{50} = 6.56 \pm 0.59$) than nematodes infected with wild-type *S. flexneri*, suggesting that the virulence plasmid of *S. flexneri* is required for full pathogenicity in *C. elegans*. These observations provide evidence that *S. flexneri* kills *C. elegans* using virulence mechanisms important for pathogenesis in mammalians systems and that nematodes may be used to analyze host factors involved in early stages of the pathogenic process.

C. elegans is susceptible to a number of bacterial pathogens, which kill the nematodes using a variety of mechanisms. Under high-osmolarity conditions, *Pseudomonas aeruginosa* PA14 produces phenazines, which are toxic to the nematode (29). Other bacteria, like *S. enterica*, establish a persistent infection within the gut of the nematode (3, 27). First we examined

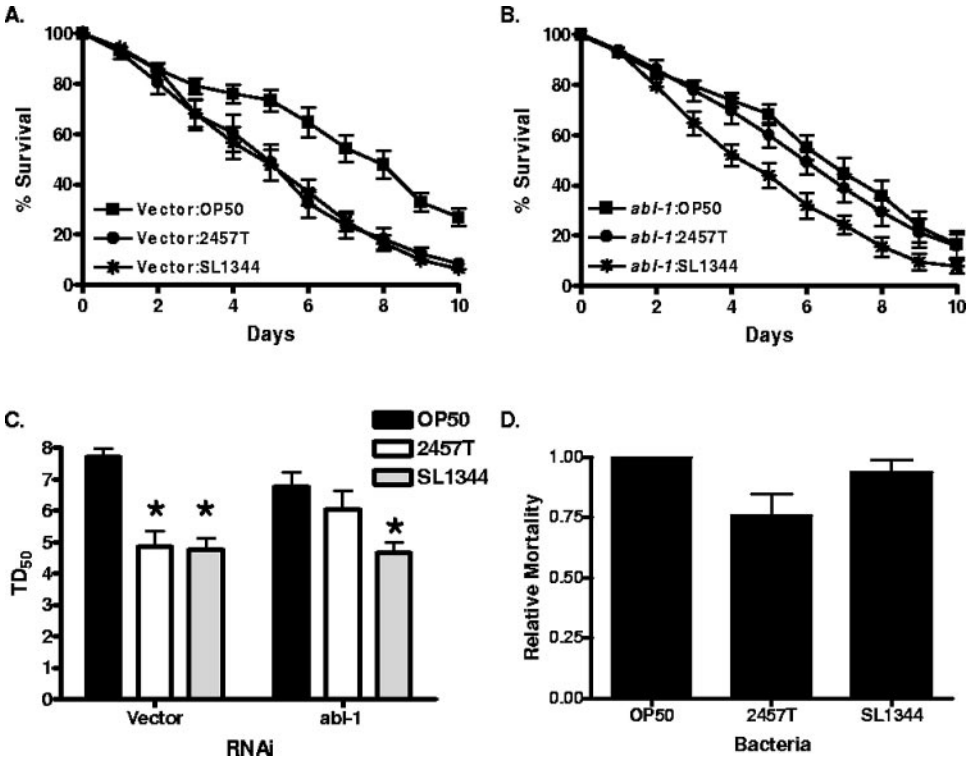

FIG. 4. ABL-1 knockdown nematodes are resistant to *S. flexneri*-mediated *C. elegans* killing. (A and B) Vector (panel A) or *abl-1* RNAi (panel B) young adult hermaphrodite nematodes were fed on lawns of either *E. coli* OP50, *S. flexneri* 2457T, or *S. enterica* serovar Typhimurium SL1344 at 25°C, and nematodes were scored daily for survival. The survival curve represents data from 9 independent experiments, each using 20 nematodes. (C) The time for nematodes to die (TD_{50}) was calculated from the survival data in panels A and B. Asterisks indicate significant differences, compared to results for OP50 ($P < 0.05$), representing data from nine independent experiments. (D) The relative mortality was calculated using the TD_{50} values for the vector and *abl-1* RNAi nematodes in order to normalize the survival rates of the nematodes grown on pathogens compared to that of nematodes fed on *E. coli* OP50 (2).

whether *S. flexneri* killing of *C. elegans* correlates with bacteria accumulation in the intestine. The profile of bacterial accumulation in the gut was examined by scoring the number of live bacteria in the gut and following the accumulation of bacteria expressing GFP in the gut of the animals by direct observation under the fluorescence microscope (3). As shown in Fig. 1C, *S. flexneri* 2457T accumulated in the nematodes to a much greater extent than *E. coli* DH5α. Consistent with these results, Fig. 1D shows that after 48 h of continuous feeding on *S. flexneri*, the intestinal lumen of the nematodes is distended and full of intact bacteria (upper panel). In contrast, reduced numbers of intact bacteria were observed when the worms were fed *E. coli* DH5α-GFP for 48 h, and the lumen was not as highly distended (Fig. 1D, lower panel). Figure 1E shows a confocal image that indicates that *S. flexneri* does not invade the intestinal cells of the nematode and remains in the intestinal lumen, which is filled with GFP from disrupted bacteria.

Characterization of the *S. flexneri*-mediated killing of *C. elegans*. Since we observed N2 nematodes to have a similarly shortened life span when fed *S. flexneri* or *S. enterica*, we examined whether *S. flexneri*-mediated killing resembled the *C. elegans* killing induced by *S. enterica*. Short exposures to *S. enterica*, as little as 5 h, result in a persistent and lethal infection of *C. elegans* that correlates with bacterial replication in the intestinal lumen (3). To determine whether *S. flexneri* was capable of persistently colonizing the *C. elegans* intestine, we exposed *C. elegans* to *S. flexneri* for 24 h and then transferred the nematodes back to *E. coli* OP50 and compared their survival rate to that of nematodes continuously exposed to either *E. coli* OP50 or *S. flexneri* 2457T. Nematodes exposed to *S. flexneri* for 24 h exhibited an extended life span compared to those fed *S. flexneri* for the duration of the assay (Fig. 2A and B), suggesting that, in contrast to the case with *S. enterica*, long-term exposure to *S. flexneri* is required for maximal killing. We also exposed nematodes to either *E. coli* DH5α, *S. flexneri* 2457T, or *S. enterica* SL1344 for 24 h and then transferred back to *E. coli* OP50. Nematodes were removed every 24 h and mechanically disrupted to release internalized bacteria, which were quantified in a plating assay. While the numbers of internalized *S. enterica* increased over time, we did not observe a similar increase in either DH5α or *S. flexneri* (Fig. 2C). Similarly, *S. flexneri* expressing GFP was not observed in the intestine when nematodes were analyzed by fluorescence microscopy (data not shown), indicating that *S. flexneri* cannot persistently colonize the intestine of the nematode.

We next examined whether *S. flexneri* might kill *C. elegans* using a mechanism that involves diffusible toxins. Under conditions of high osmolarity, *P. aeruginosa* PA14 produces diffusible toxins and remains pathogenic to the nematodes following either antibiotic or heat-mediated killing of the bacteria

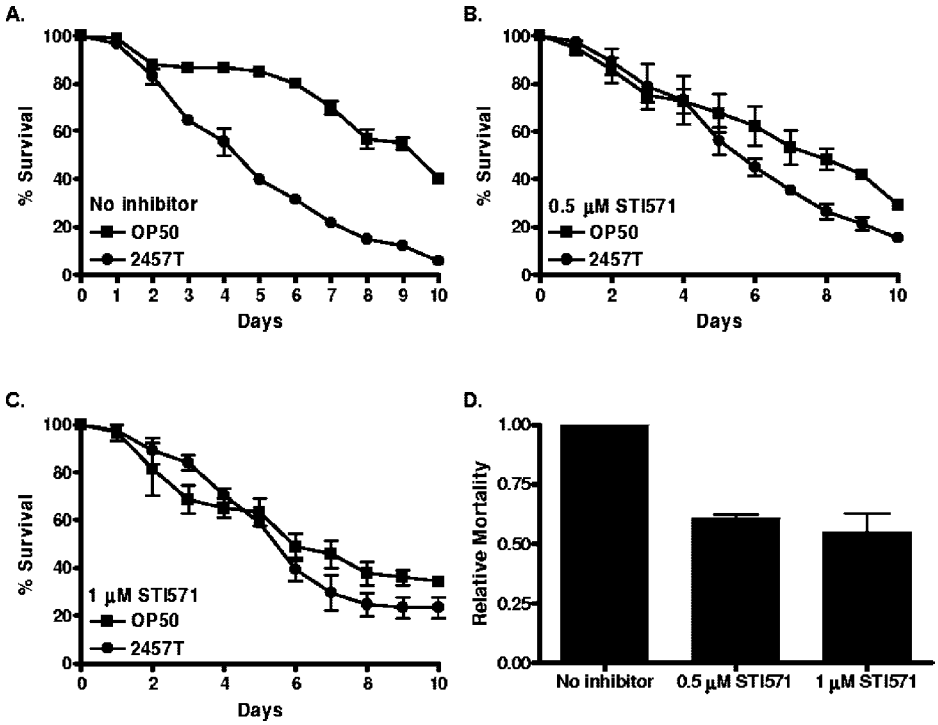

FIG. 5. Nematodes treated with STI571 are resistant to *S. flexneri*-mediated *C. elegans* killing. (A to C) Young adult hermaphrodite nematodes were fed on lawns of either *E. coli* OP50 or *S. flexneri* 2457T at 25°C in the presence of PBS (A) or 0.5 μM (B) or 1 μM (C) STI571, and nematodes were scored daily for survival. The survival curve represents data from 3 independent experiments, each using 20 nematodes. (D) The relative mortality was calculated using the TD_{50} values (representing data from three independent experiments) for the PBS and STI571-treated nematodes, in order to normalize the survival rates of the nematodes grown on *S. flexneri* 2457T compared to that of nematodes fed on *E. coli* OP50 (2).

(29, 38). First we examined whether live *S. flexneri* was required for *C. elegans* killing by feeding nematodes on lawns of either live or UV-killed *S. flexneri*. While nematodes were susceptible to live *S. flexneri*, there was no corresponding decrease in life span induced by the UV-killed *S. flexneri* (Fig. 2D). Next we analyzed whether *S. flexneri* might produce a lethal diffusible toxin. Lawns of *E. coli* OP50 and *S. flexneri* 2457T were plated on 0.45-μm filters atop modified NG agar plates, so that any potential toxins produced by the bacteria will diffuse through the filters into the agar. The filters were removed, and *E. coli* OP50 was added to the plates to prevent starvation. Young adult nematodes were added to the plates and analyzed for shortened life span. As shown in Fig. 2E, we did not observe a decreased life span of the nematodes fed on the *S. flexneri* 2457T filter plates, suggesting that *S. flexneri* does not kill *C. elegans* by producing a potent, diffusible toxin.

Use of the *S. flexneri-C. elegans* pathogenesis system to study the role of Abl kinases in defense response against *S. flexneri* infection. Using a cell culture system, it has been previously demonstrated that loss of Abl kinase activity affects the virulence phenotype of *S. flexneri* (9, 10). Here we use *C. elegans* as a whole-animal model host to determine whether the Abl kinases are required for *S. flexneri* infection in vivo. While there are two mammalian Abl tyrosine kinases, *C. elegans* has only one homologue, ABL-1 (20). The full-length ABL-1 protein exhibits 32% sequence identity and 62% sequence similarity to human c-Abl. The catalytic domain retains the highest degree of homology, at 67% identity, and retains sensitivity to pharmacological inhibitors, such as STI571. The ABL-1 protein retains the SH2 and SH3 domains to a high degree of similarity but is less conserved at the C terminus (15).

To test the hypothesis that loss of ABL-1 would reduce nematode susceptibility to *S. flexneri*, we first compared *S. flexneri* killing of wild-type N2 nematodes to worms carrying the *abl-1(ok171)* deletion allele. This mutant strain was created by UV irradiation and trimethylpsoralen treatment, resulting in a deletion of exons 8 to 12 and loss of expression of full-length ABL-1 (15). In contrast to N2 nematodes examined within the same experiments (data shown in Fig. 1A and B), the *abl-1(ok171)* nematodes exhibited no significant difference in life span when fed *S. flexneri* (TD_{50} = 6.23 ± 1.00), compared to results with *E. coli* (TD_{50} = 5.90 ± 0.68) (Fig. 3A and B). Indeed, the relative mortality of *abl-1(ok171)* nematodes infected with *S. flexneri* is 41% lower than the relative mortality of N2 nematodes (Fig. 3C). The relative mortality has the advantage of normalizing any observed change in longevity of the *abl-1(ok171)* animals feeding on *S. flexneri* to any change in longevity when feeding on *E. coli* and, therefore, takes into account changes in life span due to a general modification in fitness rather than a specific defect in innate immunity against *S. flexneri*. The enhanced resistance to *S. flexneri* exhibited by *abl-1(ok171)* animals is specific, since the nematodes remained susceptible to *S. enterica* (TD_{50} = 3.67 ± 0.60), and the relative mortality of *abl-1(ok171)* animals is comparable to that of wild-type animals when infected with *S. enterica* (Fig. 3A).

We also examined nematodes in which ABL-1 levels were

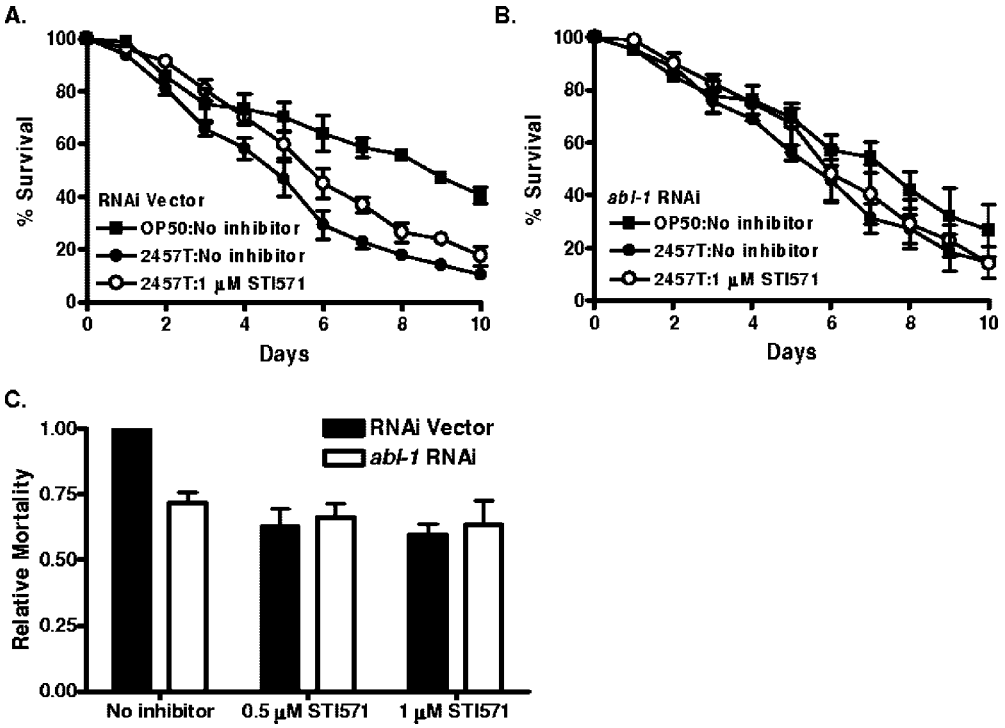

FIG. 6. STI571 is targeting ABL-1 during *S. flexneri* infection. (A and B) Vector (A) or *abl-1* RNAi (B) young adult hermaphrodite nematodes were fed on lawns of either *E. coli* OP50 or *S. flexneri* 2457T at 25°C in the presence of PBS (filled symbols) or 1 μM STI571 (open symbols), and nematodes were scored daily for survival. The survival curve represents data from 4 independent experiments, each using 20 nematodes. (C) The relative mortality was calculated using the TD_{50} values (representing data from four independent experiments) for the PBS and STI571-treated nematodes, in order to normalize the survival rates of the nematodes grown on *S. flexneri* 2457T compared to that of nematodes fed on *E. coli* OP50 (2).

diminished by RNAi (23) for their susceptibility to *S. flexneri*. Nematodes fed RNAi vector exhibited a significantly decreased life span when fed *S. flexneri* (TD_{50} = 4.84 ± 0.2) than when fed *E. coli* (TD_{50} = 7.82 ± 0.15) (Fig. 4A and C). However, nematodes with ablated ABL-1 levels did not show a significant decrease in life span on *S. flexneri* (TD_{50} = 5.97 ± 0.7) compared to results on *E. coli* (TD_{50} = 6.8 ± 0.2) (Fig. 4B and C). The ABL-1 RNAi nematodes exhibited a 33% decrease in relative mortality on *S. flexneri*, while there was no significant decrease when fed *S. enterica* (Fig. 4D), indicating that ABL-1 is specifically required for *S. flexneri* pathogenesis in *C. elegans*.

ABL-1 kinase activity is required for *S. flexneri*-mediated killing of *C. elegans*. To examine whether ABL-1 kinase activity might be required for *S. flexneri*-induced killing of *C. elegans*, we used STI571, a pharmacological inhibitor of the mammalian Abl kinases. STI571 has been demonstrated to inhibit *C. elegans* ABL-1 (15). STI571 was incorporated into the bacterial media upon which lawns of *E. coli* and *S. flexneri* were grown. When N2 nematodes were exposed to *S. flexneri*, there was an increase in life span of those grown in the presence (TD_{50} = 5.62 ± 0.23) of 1 μM STI571 compared to results for those grown in the absence of STI571 (TD_{50} = 4.37 ± 0.14) (Fig. 5A to C). Indeed, *S. flexneri* feeding in the presence of STI571 reduced the relative mortality of the nematodes by 39% and 45% at the 0.5 μM and 1 μM concentrations (Fig. 5D). To verify that ABL-1 was being specifically targeted by STI571, we analyzed the effect of the inhibitor on nematodes in which *abl-1* expression had been inhibited by RNAi. Nematodes fed the RNAi vector had a similar response to untreated N2 nematodes, in that STI571 treatment reduced *S. flexneri*-induced killing (Fig. 6A and C). However, treatment of *abl-1* knockdown nematodes did not result in a significant enhancement of resistance to *S. flexneri* above that already observed following RNAi ablation of ABL-1 expression (Fig. 6B and C). This observation demonstrates that STI571 prevents *S. flexneri*-induced killing of the nematodes through the specific inhibition of ABL-1 activity, rather than through a nonspecific mechanism.

DISCUSSION

Our results indicate that the invertebrate *C. elegans* can be efficiently killed by *S. flexneri* with kinetics comparable to that of other human pathogens such as *S. enterica*. Both *S. enterica* and *S. flexneri* accumulate within the gut of the nematode; however, *S. flexneri* does not establish a persistent infection, suggesting that *S. enterica* and *S. flexneri* exhibit distinct mechanisms of nematode killing. We have also demonstrated that the *S. flexneri* virulence plasmid, which encodes a type III secretory system as well as various virulence determinants crucial for pathogenesis in mammalian systems, is required for maximal *C. elegans* killing (Fig. 1). Since *S. flexneri* TTSS-related genes are expressed at 37°C but not at 25°C, which is the temperature used to infect *C. elegans*, it is likely that other *S. flexneri* virulence-related genes play a role in *C. elegans*

killing. However, since the nematodes are transferred every day to fresh *S. flexneri* lawns grown at 37°C until day 4 of the assay, we cannot completely rule out the possibility that TTSS-related genes play a role in *C. elegans* killing.

Moreover, we have demonstrated that the tyrosine kinase ABL-1 is required for maximal *S. flexneri* pathogenesis in the nematode, as disruption of ABL-1 by mutagenesis, RNAi, or pharmacological inhibition reduces *S. flexneri*-mediated killing of *C. elegans* (Fig. 3 to 6). The kinase may act as an inhibitor of innate immune responses against *S. flexneri*. Although we have shown that germ line apoptosis protects *C. elegans* from *S. enterica* infection (2) and *abl-1(ok171)* animals exhibit higher levels of germ line apoptosis (15), it is unlikely that this is the reason for the increased resistance to *S. flexneri* of ABL-1-deficient animals. *S. flexneri* does not induce germ line apoptosis to the extent to which *S. enterica* does (data not shown), and higher levels of germ line apoptosis do not appear to confer resistance to bacteria (2). In addition, ABL-1-deficient animals are not more susceptible to *S. enterica*-mediated killing. Another possibility is that ABL-1 is a component of the host machinery required for the activation of *S. flexneri* virulence factors that enter the *C. elegans* cells. It has been proposed that IpaC interacts with and activates Src, suggesting that IpaC might be a target of host cell tyrosine kinases (13), and that the TTSS protein Tir from enteropathogenic *E. coli* is phosphorylated by host cell tyrosine kinases (37). At this point in our investigation, we cannot distinguish between the possibilities that ABL-1 negatively regulates an *S. flexneri*-specific defense response or that it is required to activate *S. flexneri* virulence factors.

In addition to the differences in growth temperature between nematodes and mammals, a second limitation of the system to model *S. flexneri* pathogenesis is the lack of bacterial internalization in the intestinal cells of *C. elegans*. Despite this limitation, our results indicate that *C. elegans* can be used to study some aspects of host-pathogen interactions that can be translated to mammalian systems, in addition to interactions that may occur in nature between enteric pathogens and invertebrates. Human enteric pathogens have been found to be associated with free-living nematodes (41, 42), and *C. elegans* has been suggested to transport *Salmonella* to fruits and vegetables (11, 24). It is known that *Shigella* gets in contact with invertebrates such as flies (5, 16, 25, 28) and that it is ingested and defecated by free-living saprozoic nematodes (12). Thus, although the interaction between *C. elegans* and *S. flexneri* in the petri dish is artificially caused, the interaction between the two organisms may take place in nature.

In conclusion, we have demonstrated that *Shigella* kills *C. elegans* by an active process that requires the virulence plasmid and correlates with the bacterial accumulation in the intestine. Based on the results reported here and various studies that indicate that some of the key features underlying host-pathogen interactions have ancient origins (1, 18, 19, 21, 35), we believe that the *C. elegans*-*S. flexneri* pathogenesis system may be useful for studying putative natural interactions between *Shigella* and invertebrates. The extent to which those interactions can be translated to mammalian systems remains to be determined.

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