HOST TOLERANCE

A secreted bacterial peptidoglycan hydrolase enhances tolerance to enteric pathogens

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The intestinal microbiome modulates host susceptibility to enteric pathogens, but the specific protective factors and mechanisms of individual bacterial species are not fully characterized. We show that secreted antigen A (SagA) from *Enterococcus faecium* is sufficient to protect *Caenorhabditis elegans* against *Salmonella* pathogenesis by promoting pathogen tolerance. The NlpC/p60 peptidoglycan hydrolase activity of SagA is required and generates muramyl-peptide fragments that are sufficient to protect *C. elegans* against *Salmonella* pathogenesis in a *tol-1*–dependent manner. SagA can also be heterologously expressed and secreted to improve the protective activity of probiotics against *Salmonella* pathogenesis in *C. elegans* and mice. Our study highlights how protective intestinal bacteria can modify microbial-associated molecular patterns to enhance pathogen tolerance.

Dysbiosis of the gut microbiota is associated with metabolic disorders, inflammatory bowel disease, and increased pathogen susceptibility (*1*). Nonetheless, individual bacterial species and factors involved in host protection have been difficult to characterize (*2*). *Enterococci* are lactic acid bacteria associated with the intestinal microbiome of diverse species ranging from humans to flies and can attenuate host susceptibility to enteric pathogens, including *Salmonella* (*3*, *4*). Nonpathogenic strains of *Enterococcus faecium* have been used as probiotics, but their protection mechanisms are unclear (*5*). Because *E. faecium* can colonize the *Caenorhabditis elegans* intestine without causing apparent disease (*6*), we used *C. elegans* as a model organism (*7*) to elucidate the protective mechanism (or mechanisms) underlying *E. faecium* probiotic activity. To investigate whether *E. faecium* can attenuate enteric bacterial pathogenesis in *C. elegans*, we developed a treatment-infection assay with *Salmonella enterica* serovar Typhimurium (fig. S1A), which causes persistent intestinal infection and death in *C. elegans* (*8*–*10*). In our assay, *E. faecium*–treated animals appeared less fragile and more motile than did control *Escherichia coli* OP50–treated animals after *S.* Typhimurium infection (fig. S1B). *C. elegans* survival was increased in animals fed *E. faecium* before infection as compared with animals fed *E. coli* OP50 or *Bacillus subtilis* 168 (Fig. 1A and fig. S1C). Multiple strains of *E. faecium*, including a pathogenic strain, were able to inhibit *S.* Typhimurium pathogenesis (fig. S1, D and E). *E. faecium*–treated animals were also more resistant to the intrinsic pathogenesis of *E. coli* OP50 (fig. S1F) as well as pathogenesis caused by *Enterococcus faecalis* V583 (fig. S1G) (*6*). These results suggest that the mechanism of protection is conserved among *E. faecium* strains and is active against diverse enteric pathogens.

We next analyzed the effect of *E. faecium* on *S.* Typhimurium colonization and persistence. Fluorescence imaging of mCherry–*S.* Typhimurium 3 days after infection showed comparable *S.* Typhimurium colonization with or without *E. faecium* treatment (Fig. 1B and fig. S1H). Viable *S.* Typhimurium [colony-forming units (CFU)] recovered from lysed worms revealed a ~2 log decrease in *S.* Typhimurium colonization 1 day after infection in *E. faecium*–treated *S.* Typhimurium–infected animals (Fig. 1C). However, by 3 days after infection, *S.* Typhimurium titer was similar in OP50- and *E. faecium*–treated *S.* Typhimurium–infected animals (Fig. 1C). To determine whether this transient decrease in *S.* Typhimurium colonization represented niche competition early in our assay, we monitored *E. faecium* CFU throughout the infection assay (fig. S1I). Whereas *E. faecium* initially colonized worms to ~10^5 CFU/worm, *E. faecium* numbers decreased to ~10 to 10^2 CFU/worm 1 day after infection, demonstrating that the transient decrease in *S.* Typhimurium colonization was not concomitant with an increase in *E. faecium* load. Electron microscopy of worm transverse sections 4 days after infection revealed substantial degradation of the intestinal microvilli in OP50-treated *S.* Typhimurium–infected animals as compared with uninfected or *E. faecium*–treated animals (Fig. 1D). In OP50-treated *S.* Typhimurium–infected animals, bacteria had escaped the intestinal lumen and caused extensive tissue damage (Fig. 1D, middle). In contrast, *E. faecium*–treated *S.* Typhimurium–infected animals contained a similar bacterial load to the intestinal lumen and showed no apparent tissue damage (Fig. 1D, right), suggesting improved epithelial barrier integrity. These results demonstrate that *E. faecium* does not prevent *S.* Typhimurium colonization or replication but may enhance host tolerance to pathogens.

We next explored whether specific factors produced by *E. faecium* were sufficient for protection against *S.* Typhimurium pathogenesis. *E. faecium* culture supernatant was as effective as live bacterial cultures in inhibiting *S.* Typhimurium pathogenesis (Fig. 2A). Activity of the supernatant was sensitive to proteinase-K treatment, trichloroacetic acid precipitation, and 10-kDa size exclusion (fig. S2, A to C), leading us to analyze the protein composition of *E. faecium* culture supernatant by means of mass spectrometry (fig. S2, D and E, and table S1). This revealed a number of secreted proteins and an enrichment of peptidoglycan remodeling factors (Fig. 2B). We focused on secreted antigen A (SagA), the most abundant protein identified in the supernatant (Fig. 2B), which encodes a putative secreted NlpC/p60 peptidoglycan hydrolase that is essential for *E. faecium* viability (*11*). Imaging of animals treated with *E. faecium*–expressing mCherry under the *sagA* promoter (*psagA:mcherry*) showed that *E. faecium* expresses SagA in vivo (Fig. 2C). Treatment of animals with recombinant SagA-His_6 purified from either *E. coli* BL21-RIL(DE3) or *E. faecium* Com15 was sufficient to inhibit *S.* Typhimurium pathogenesis (Fig. 2, D and E; fig. S3; and table S2). All sequenced *E. faecium* strains encode a *sagA* ortholog in their genomes, whereas sequenced *E. faecalis* strains do not. We inserted *sagA-his*$_6$ into the *E. faecalis* OG1RF chromosome to generate *E. faecalis–sagA* (figs. S4 and S5). Treatment of *C. elegans* with *E. faecalis–sagA* attenuated *S.* Typhimurium pathogenesis comparably with *E. faecium*, whereas treatment with wild-type *E. faecalis* was not protective (Fig. 2F and fig. S6A). *S.* Typhimurium load was similar across all infected conditions, demonstrating that *E. faecalis–sagA* does not inhibit *S.* Typhimurium colonization in vivo but rather improves pathogen tolerance (fig. S6B). SagA expression also counteracted the intrinsic pathogenesis of *E. faecalis* OG1RF (fig. S6C) (*6*). These results demonstrate that SagA is sufficient to enhance host tolerance against bacterial pathogens.

The protective activity of *E. faecium* against multiple enteric pathogens suggested that SagA may engage host pathways to limit pathogenesis. A survey of *C. elegans* immunity-associated mutants indicated no major role for the p38 MAPK/Pmk-1 pathway (*12*, *13*), the transforming growth factor–β (TGF-β)–like/Dbl-1 pathway (*14*), the insulin-like receptor/Daf-2 pathway (*15*), or the Npr-1–mediated pathogen avoidance pathway (fig. S7) (*16*, *17*). *C. elegans* encodes one homolog of Toll-like receptor (TLR), *tol-1* (*18*). *C. elegans* lacking the *tol-1* TIR signaling domain [*tol-1(nr2033)*] exhibit defective pathogen avoidance to *S. marsescens* (*19*) and increased susceptibility to *S.* Typhimurium infection (*20*). We assessed SagA-mediated protection in *tol-1(nr2033)* animals and found that neither *E. faecium* nor *E. faecalis–sagA* were protective against *S.* Typhimurium infection in this mutant background, which suggests that SagA

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enhances pathogen tolerance through *tol-1* signaling (Fig. 2G).

To evaluate the mechanism of SagA protection (*21*), we generated an active site mutant as well as a C-terminal domain truncation of the NlpC/p60 hydrolase domain (Fig. 3A and fig. S8A). Neither mutant was able to inhibit *S.* Typhimurium pathogenesis, indicating that the NlpC/p60 hydrolase activity is required for SagA-mediated protection (Fig. 3B). SagA did not affect *S.* Typhimurium colonization of *C. elegans* or directly attenuate *S.* Typhimurium growth or virulence mechanisms

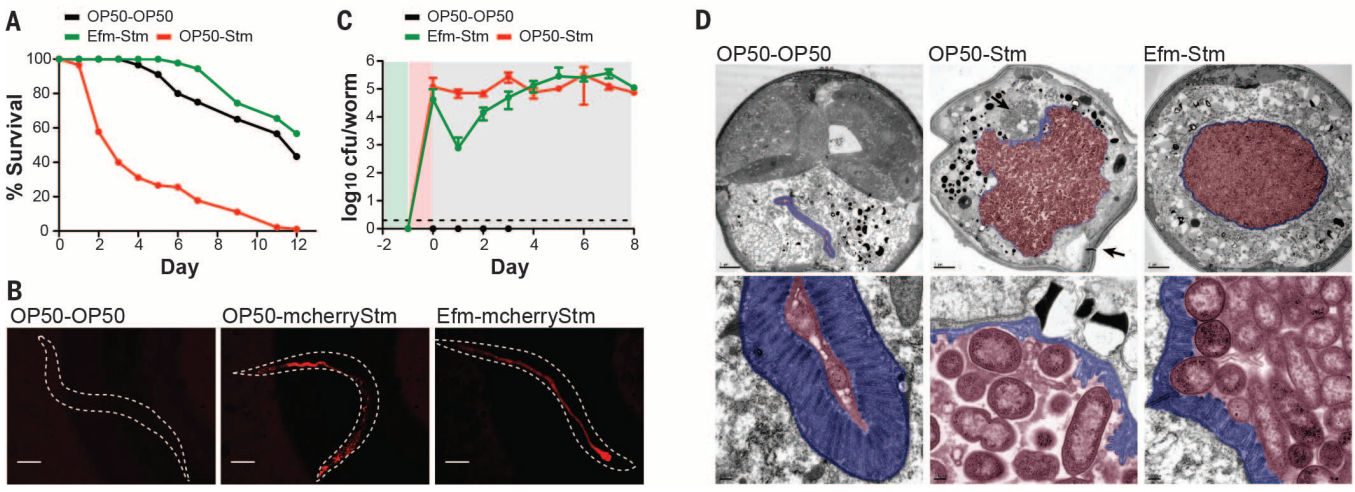

Fig. 1. *E. faecium* induces host tolerance to *S.* Typhimurium. (**A**) Survival curve showing *E. faecium* (Efm, Com15)–mediated inhibition of *S.* Typhimurium (Stm, 14028) pathogenesis ($P < 10^{-10}$). The legend indicates treatment-infection. Control worms were fed *E. coli* OP50 for both the treatment and infection stages of the assay. For *C. elegans* survival curves in all figures, significance was calculated by means of log-rank test with Bonferroni correction for multiple comparisons. Data points represent mean survival from 90 worms from a representative experiment independently replicated at least twice. (**B**) Fluorescence images of *C. elegans* infected with Stm-expressing plasmid-encoded mcherry (mcherryStm) at 3 days after infection. The dashed lines indicate an outline of the worm body. Scale bars, 100 μm. (**C**) Stm CFU measured in *C. elegans* throughout the infection assay. Data points represent average CFU from five worms ± SD of two independent experiments. The dashed line indicates detection limit. The background shading represents stage of the treatment-infection assay. Green indicates treatment, red indicates infection, and gray indicates *E. coli* (OP50) feeding. (**D**) Electron microscopy of transverse sections of *C. elegans* (top) and magnification of intestinal region (bottom) at 4 days after infection. The intestinal microvilli are highlighted blue; the intestinal lumen is highlighted red. (Top middle) The top arrow indicates bacteria that have breached the epithelial barrier, and the bottom arrow indicates loss of overall turgidity. Scale bars, 5 μm (top row) and 200 nm (bottom row).

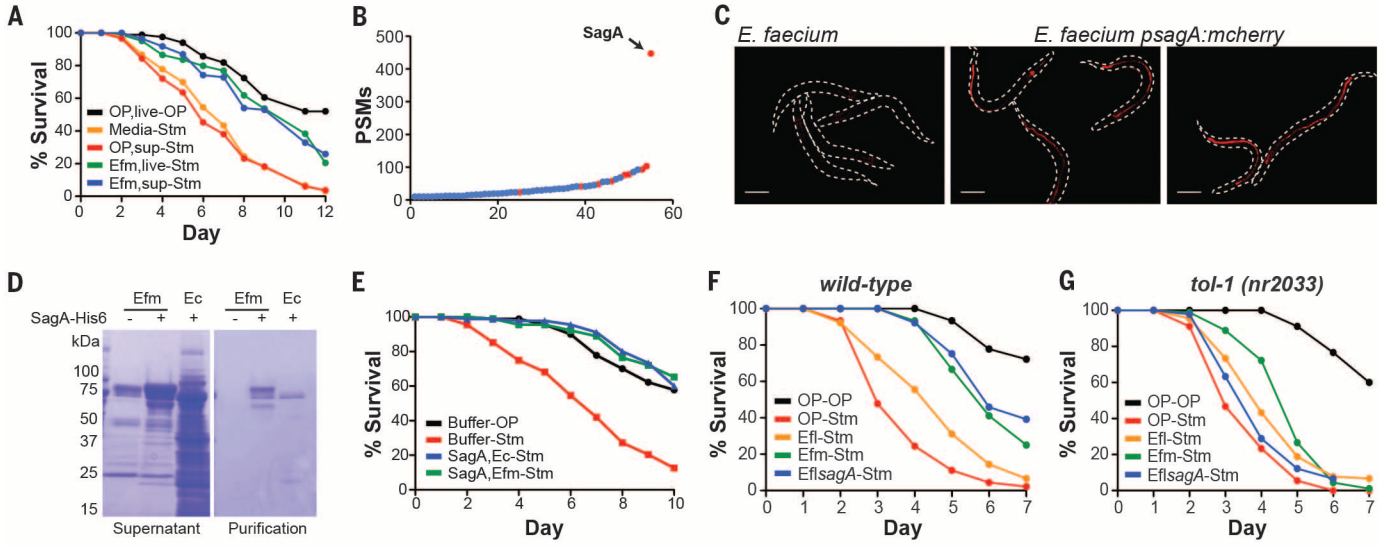

Fig. 2. SagA is sufficient for inducing pathogen tolerance in a *tol-1*–dependent manner. (**A**) Survival curve showing that both *E. faecium* culture supernatant (Efm, sup) ($P < 10^{-6}$) and live *E. faecium* culture (Efm, live) ($P < 10^{-7}$) inhibit *S.* Typhimurium (Stm)–induced death. OP50 culture supernatant (OP, sup) is not protective ($P = 1$). (**B**) Summary of proteins identified in Efm culture supernatant by means of mass spectrometry with at least 10 peptide spectrum matches (PSMs). Proteins involved in peptidoglycan remodeling are in red (table S1). The *x* axis represents arbitrary protein number. (**C**) Fluorescence images of *C. elegans* treated for 1 day with wild-type Efm or Efm-expressing mcherry under the sagA promoter (psagA:mcherry). The dashed lines indicate an outline of the worm body. Scale bars, 200 μm. (**D**) Coomassie stained SDS–polyacrylamide gel electrophoresis of culture supernatants and SagA-His_6 purifications from *E. faecium* Com15 (Efm) and *E. coli* BL21-RIL(DE3) (Ec). (**E**) Survival curve showing that SagA-His_6 purified from either *E. coli* BL21-RIL(DE3) (SagA, Ec) ($P < 10^{-10}$) or *E. faecium* Com15 (SagA, Efm) ($P < 10^{-10}$) inhibits Stm pathogenesis. (**F**) Survival curve from a continuous infection assay (fig. S6A) showing that *E. faecalis* (Efl, OG1RF)–sagA inhibits Stm pathogenesis ($P < 10^{-10}$) similarly to Efm (Com15) ($P = 1$) as compared with *E. faecalis* (Efl, OG1RF) and OP50. (**G**) Survival curve from a continuous infection assay showing that Efl-*sagA* ($P = 0.053$) does not inhibit Stm pathogenesis in *tol-1(nr2033) C. elegans*.

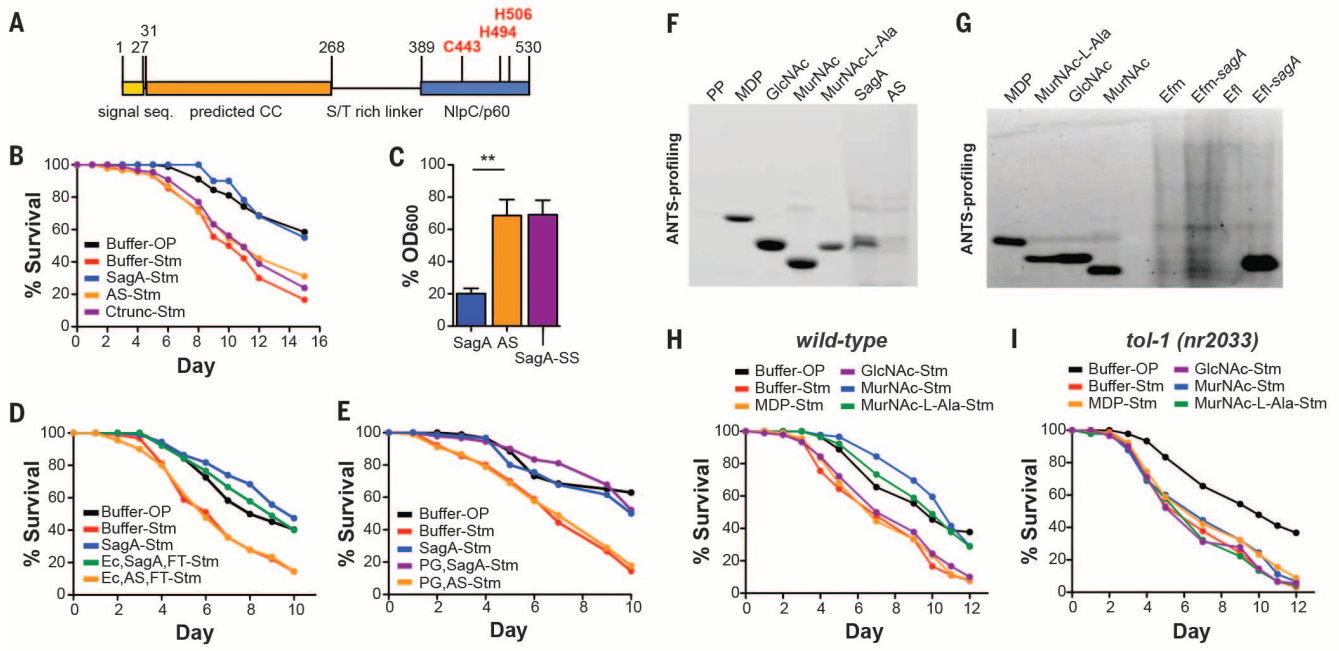

Fig. 3. Enzymatic activity of SagA is required for enhancing pathogen tolerance. (**A**) Schematic of SagA domain organization. The signal sequence is yellow, a predicted coiled-coil (CC) domain is orange, and the NlpC/p60-type hydrolase domain is blue. Active site residues are in red type. (**B**) Survival curve showing that SagA inhibits *S.* Typhimurium (Stm) pathogenesis ($P < 10^{-10}$), whereas an active site mutant (AS) and C-terminal truncation mutant (Ctrunc) do not ($P = 0.42$ and 0.98, respectively). (**C**) OD_{600} of *E. coli* BL21-RIL(DE3) expressing SagA, the active site mutant, or cytoplasmically localized SagA (SagA-SS) 1 hour after induction. Bars represent mean ± SEM from three independent experiments. Significance was calculated by means of unpaired *t* test. $^{**}P < 0.01$. (**D**) Survival curve showing that 5-kDa-MWCO column-filtered *E. coli* culture supernatants expressing SagA-His_6 (Ec, sagA-FT) inhibit Stm pathogenesis ($P < 10^{-4}$), whereas filtered *E. coli* culture supernatants expressing the active site mutant (Ec, AS-FT) do not ($P = 1$). (**E**) Survival curve showing that purified *E. coli* peptidoglycan treated with SagA (PG, SagA) can inhibit Stm pathogenesis ($P < 10^{-10}$), whereas *E. coli* peptidoglycan treated with the active site mutant (PG, AS) cannot ($P = 1$). (**F**) ANTS visualization of *E. coli* culture supernatants expressing SagA-His_6 or the active site mutant. A sugarless pentapeptide (PP) shows ultraviolet signal specificity. (**G**) ANTS visualization of peptidoglycan fragments in Efm, Efm-*sagA*, Efl, and Efl-*sagA* culture supernatants. (**H**) Survival curve showing that treatment with MurNAc ($P < 10^{-5}$) or MurNAc-L-Ala ($P < 10^{-10}$) can inhibit Stm pathogenesis, whereas MDP ($P = 1$) and GlcNAc ($P = 1$) are not protective. (**I**) Survival curve showing that MurNAc ($P = 1$) and MurNAc-L-Ala ($P = 0.61$) do not inhibit pathogenesis in *tol-1(nr2033) C. elegans*.

(fig. S8, B to E). In culture, recombinant SagA had no effect on *E. coli* growth rate (fig. S9A), but induction of SagA expression caused a decrease in culture optical density (OD) (Fig. 3C and fig. S9, B and C), indicating cell lysis. In contrast, expression of the active site mutant or cytoplasmically localized SagA did not induce *E. coli* cell lysis (Fig. 3C and fig. S9, B and C). These data suggest that although exogenous addition of SagA is not bacteriolytic, SagA is a functional hydrolase that can cleave peptidoglycan when targeted to the periplasm.

We hypothesized that SagA generates peptidoglycan fragments responsible for enhancing pathogen tolerance. Consistent with this hypothesis, we found that the flow-through from 5-kDa molecular weight cut-off (MWCO) column-filtered culture supernatants of *E. coli* expressing SagA, but not the active site mutant, protected *C. elegans* from *S.* Typhimurium pathogenesis (Fig. 3D and fig. S10A), which suggests that lower-molecular-weight products of SagA enzymatic activity are sufficient for protection. To test whether SagA-generated *E. coli* peptidoglycan fragments can protect *C. elegans* from *S.* Typhimurium, we digested purified *E. coli* peptidoglycan with lysozyme and either SagA or the active site mutant then filtered the digests to exclude protein. *C. elegans* treated with the SagA peptidoglycan digests survived similarly to SagA-treated animals, whereas active site mutant digests failed to attenuate pathogenesis (Fig. 3E). These results suggest that SagA-generated peptidoglycan fragments, and not SagA itself, are responsible for enhancing pathogen tolerance.

To identify the peptidoglycan fragment (or fragments) generated by SagA, we analyzed filtered bacterial culture supernatants by means of 8-aminonaphthalene-1,3,6-trisulfonic acid (ANTS) labeling and gel-based profiling (*22*, *23*). From *E. coli* expressing SagA, we detected a SagA-specific product that migrated similarly to the synthetic peptidoglycan fragments MurNAc-L-Ala and GlcNAc, but not to MurNAc-L-Ala-D-Glu (MDP) or MurNAc (Fig. 3F). ANTS analysis of *E. faecium*, *E. faecalis*, and *E. faecalis–sagA* peptidoglycan extracts revealed that SagA expression alters the muropeptide profile (fig. S11). From *E. faecalis–sagA* culture supernatant, we detected an ANTS-labeled product that comigrates with MurNAc (Fig. 3G), which suggests that heterologous SagA expression induces muropeptide shedding in both *E. coli* and *E. faecalis*. In contrast, 10-kDa-MWCO–filtered *E. faecium* culture supernatant did not yield detectable levels of MurNAc-L-Ala or MurNAc (Fig. 3G) and was not protective when administered to *C. elegans* (fig. S2C). *E. faecium* that expresses SagA endogenously is likely resistant to SagA-induced peptidoglycan shedding. Because SagA is abundantly secreted by *E. faecium* (fig. S4 and tables S1 and S4) and is protective after purification (Fig. 2, D and E), soluble SagA may hydrolyze extracellular peptidoglycan fragments derived from digested bacteria in vivo. Indeed, incubation of purified *E. coli* peptidoglycan with lysozyme and recombinant SagA, but not the active site mutant, yielded a peptidoglycan cleavage product with similar mobility to that of MurNAc-L-Ala (fig. S10B). These data suggest that heterologous expression of SagA in bacteria can remodel bacterial peptidoglycan (fig. S11), induce shedding of small muropeptide fragments (Fig. 3, F and G), and cleave extracellular peptidoglycan when secreted (fig. S10B). We next assessed the protective activity of SagA-generated peptidoglycan fragments, MurNAc and MurNAc-L-Ala, as well as GlcNAc and MDP. Treatment of *C. elegans* with either MurNAc or MurNAc-L-Ala was sufficient to inhibit *S.* Typhimurium pathogenesis, whereas treatment with MDP or GlcNAc was not (Fig. 3H). MurNAc and MurNAc-L-Ala were not protective in *tol-1(nr2033)* animals (Fig. 3I), which suggests that *tol-1* is required for mediating host protection in response to these peptidoglycan fragments. These data are consistent with the activity of muropeptides in mammals (*24*, *25*) but show that MurNAc-L-Ala and MurNAc are the minimal peptidoglycan components that enhance pathogen tolerance in *C. elegans*.

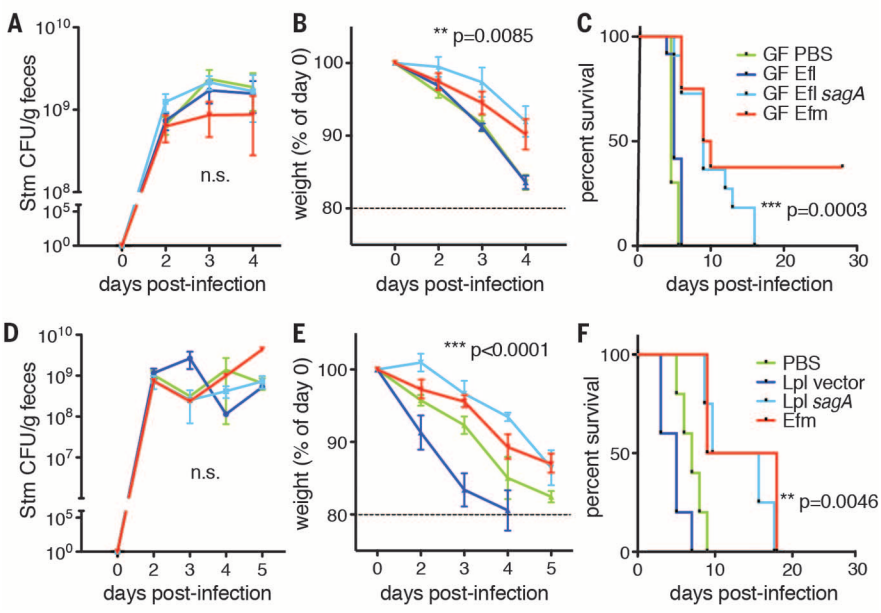

Fig. 4. *E. faecium* and SagA enhance pathogen tolerance in mice. (**A** to **C**) Germ-free (GF) C57BL/6 mice were orally gavaged with 10^8 CFU *E. faecalis* (Efl), Efl-expressing *sagA* (Efl-*sagA*), or *E. faecium* (Efm) 7 days before oral infection with 10^2 CFU *S.* Typhimurium (Stm). (A) Stm CFU in feces, (B) weight loss, and (C) survival are shown. Pooled data are from four independent experiments, $n = 10$ to 14 mice per group. (**D** to **F**) Mice were given an ampicillin, metronidazole, neomycin, and vancomycin (AMNV) antibiotic cocktail for 14 days and colonized with 10^8 CFU *L. plantarum* (Lpl) harboring an empty plasmid vector (Lpl-vector) or a *sagA* plasmid (Lpl-*sagA*) or 10^8 CFU Efm before oral infection with 10^6 CFU Stm. (D) Stm CFU in feces, (E) weight loss, and (F) survival are shown. Pooled data are from two independent experiments, $n = 2$ to 5 mice per group. [(A), (B), (D), and (E)] Mean ± SEM, 2-way analysis of variance, *P* value shown comparing *sagA*-expressing Efl or Lpl to wild type (WT) or vector controls, respectively. n.s., not significant. [(C) and (F)] Log-rank analysis, *P* value shown comparing Efm, *sagA*-expressing Efl, or Lpl to WT or vector controls, respectively. $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ for all analyses. Comparisons with no asterisk had $P > 0.05$ and were not considered significant.

We next evaluated SagA-mediated protection against *Salmonella* pathogenesis in mice. Germ-free mice were monocolonized with *E. faecium*, *E. faecalis*, or *E. faecalis–sagA* 7 days before infection with *S.* Typhimurium. *Enterococcus* and *Salmonella* load were measured in the feces, and mouse survival was tracked. All *Enterococcus* strains were similarly recovered from the feces after gavage, indicating efficient intestinal colonization (fig. S12). Consistent with our results in *C. elegans*, *S.* Typhimurium CFU in the feces were similar across all conditions throughout infection (Fig. 4A), which suggests that *E. faecium* does not inhibit *Salmonella* colonization. Remarkably, mice gavaged with *E. faecium* or *E. faecalis–sagA* before infection exhibited reduced weight loss and prolonged survival, with a median survival of 9 days, as compared with that of *E. faecalis*–treated mice (Fig. 4, B and C). Although *Enterococci* are used as probiotics in livestock, their pathogenic potential makes them problematic for use in humans (*26*). We thus introduced *sagA* into a nonpathogenic probiotic, *Lactobacillus plantarum* (*27*), and confirmed its expression and secretion (fig. S13). *sagA*-expressing *L. plantarum* significantly prevented weight loss and improved survival in an antibiotic-induced *S.* Typhimurium infection model compared with *L. plantarum* (Fig. 4, D to F, and fig. S14). These results indicate that SagA is sufficient to attenuate *Salmonella* pathogenesis in mammals and is protective even when expressed by other probiotic bacteria.

We demonstrate that *C. elegans* is an effective model with which to explore the protective mechanisms of intestinal bacteria and show that SagA from *E. faecium* is sufficient to protect *C. elegans* and mice from enteric pathogens. Our results suggest that the NlpC/p60 hydrolase activity of SagA generates distinct peptidoglycan fragments that may activate host immune pathways to enhance epithelial barrier integrity and confine pathogens to the intestinal lumen, ultimately promoting tolerance to infection (fig. S15). Our analysis of *E. faecium* and engineered SagA-expressing bacterial strains in mice suggests that SagA also improves intestinal epithelial barrier integrity to limit bacterial pathogenesis in mammals (*28*). The protective activity of *E. faecium* and SagA in mice requires the TLR signaling adaptor MyD88, the peptidoglycan pattern recognition receptor NOD2, and the C-type lectin RegIIIγ (*28*). These results together suggest that *E. faecium* and SagA may function through evolutionarily conserved pathways to enhance epithelial barrier integrity and protect animals from enteric pathogens. Last, this study suggests that bacterial NlpC/p60-type peptidoglycan hydrolases (*29*–*32*) can enhance host tolerance to pathogens and that these enzymes could be used to improve the activity of existing probiotics.

REFERENCES AND NOTES

1. K. Honda, D. R. Littman, *Annu. Rev. Immunol.* **30**, 759–795 (2012).
2. C. G. Buffie, E. G. Pamer, *Nat. Rev. Immunol.* **13**, 790–801 (2013).
3. F. Lebreton, R. J. L. Willems, M. S. Gilmore, in *Enterococci: From Commensals to Leading Causes of Drug Resistant Infection*, M. S. Gilmore, D. B. Clewell, Y. Ike, N. Shankar, Eds. (Boston Massachusetts Eye and Ear Infirmary, 2014).
4. C. Staley, G. M. Dunny, M. J. Sadowsky, *Adv. Appl. Microbiol.* **87**, 147–186 (2014).
5. C. M. Franz, M. Huch, H. Abriouel, W. Holzapfel, A. Gálvez, *Int. J. Food Microbiol.* **151**, 125–140 (2011).
6. D. A. Garsin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 10892–10897 (2001).
7. J. E. Irazoqui, J. M. Urbach, F. M. Ausubel, *Nat. Rev. Immunol.* **10**, 47–58 (2010).
8. A. Haraga, M. B. Ohlson, S. I. Miller, *Nat. Rev. Microbiol.* **6**, 53–66 (2008).
9. A. Aballay, P. Yorgey, F. M. Ausubel, *Curr. Biol.* **10**, 1539–1542 (2000).
10. A. Labrousse, S. Chauvet, C. Couillault, C. L. Kurz, J. J. Ewbank, *Curr. Biol.* **10**, 1543–1545 (2000).
11. F. Teng, M. Kawalec, G. M. Weinstock, W. Hryniewicz, B. E. Murray, *Infect. Immun.* **71**, 5033–5041 (2003).
12. D. H. Kim *et al.*, *Science* **297**, 623–626 (2002).
13. R. P. Shivers, T. Kooistra, S. W. Chu, D. J. Pagano, D. H. Kim, *Cell Host Microbe* **6**, 321–330 (2009).
14. G. V. Mallo *et al.*, *Curr. Biol.* **12**, 1209–1214 (2002).
15. D. A. Garsin *et al.*, *Science* **300**, 1921 (2003).
16. E. Z. Macosko *et al.*, *Nature* **458**, 1171–1175 (2009).
17. K. L. Styer *et al.*, *Science* **322**, 460–464 (2008).
18. N. Pujol *et al.*, *Curr. Biol.* **11**, 809–821 (2001).
19. E. Pradel *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 2295–2300 (2007).
20. J. L. Tenor, A. Aballay, *EMBO Rep.* **9**, 103–109 (2008).
21. M. Firczuk, M. Bochtler, *FEMS Microbiol. Rev.* **31**, 676–691 (2007).
22. S. Y. Li, J. V. Höltje, K. D. Young, *Anal. Biochem.* **326**, 1–12 (2004).
23. K. D. Young, *J. Bacteriol.* **178**, 3962–3966 (1996).
24. D. J. Philpott, M. T. Sorbara, S. J. Robertson, K. Croitoru, S. E. Girardin, *Nat. Rev. Immunol.* **14**, 9–23 (2014).
25. J. Royet, R. Dziarski, *Nat. Rev. Microbiol.* **5**, 264–277 (2007).
26. B. Lund, C. Edlund, *Clin. Infect. Dis.* **32**, 1384–1385 (2001).
27. L. M. Dicks, M. Botes, *Benef. Microbes* **1**, 11–29 (2010).
28. V. A. Pedicord *et al.*, *Sci. Immunol.* 10.1126/sciimmunol.aai7732 (2016).
29. W. Vollmer, B. Joris, P. Charlier, S. Foster, *FEMS Microbiol. Rev.* **32**, 259–286 (2008).
30. F. Yan *et al.*, *J. Clin. Invest.* **121**, 2242–2253 (2011).
31. F. Yan *et al.*, *Gastroenterology* **132**, 562–575 (2007).
32. F. Yan *et al.*, *J. Biol. Chem.* **288**, 30742–30751 (2013).

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/353/6306/1434/suppl/DC1
Materials and Methods
Figs. S1 to S15
Tables S1 to S4
References (*33*–*50*)

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