Commensal microbes provide first line defense against *Listeria monocytogenes* infection

Simone Becattini,[1] Eric R. Littmann,[2] Rebecca A. Carter,[1] Sohn G. Kim,[1] Sejal M. Morjaria,[3] Lilan Ling,[2] Yangtsho Gyaltshen,[2] Emily Fontana,[2] Ying Taur,[2,3] Ingrid M. Leiner,[1] and Eric G. Pamer[1,2,3]

[1]Immunology Program, Sloan Kettering Institute, [2]Lucille Castori Center for Microbes Inflammation and Cancer, Molecular Microbiology Core Facility, and [3]Infectious Diseases Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY

Listeria monocytogenes **is a foodborne pathogen that causes septicemia, meningitis and chorioamnionitis and is associated with high mortality. Immunocompetent humans and animals, however, can tolerate high doses of *L. monocytogenes* without developing systemic disease. The intestinal microbiota provides colonization resistance against many orally acquired pathogens, and antibiotic-mediated depletion of the microbiota reduces host resistance to infection. Here we show that a diverse microbiota markedly reduces *Listeria monocytogenes* colonization of the gut lumen and prevents systemic dissemination. Antibiotic administration to mice before low dose oral inoculation increases *L. monocytogenes* growth in the intestine. In immunodeficient or chemotherapy-treated mice, the intestinal microbiota provides nonredundant defense against lethal, disseminated infection. We have assembled a consortium of commensal bacteria belonging to the Clostridiales order, which exerts in vitro antilisterial activity and confers in vivo resistance upon transfer into germ free mice. Thus, we demonstrate a defensive role of the gut microbiota against *Listeria monocytogenes* infection and identify intestinal commensal species that, by enhancing resistance against this pathogen, represent potential probiotics.**

INTRODUCTION

Listeria monocytogenes is a Gram-positive, facultative intracellular bacterium that can contaminate food which, upon ingestion, can result in infection of a wide range of animals, including livestock and humans (Vázquez-Boland et al., 2001). The severity and extent of *L. monocytogenes* infection is determined by the virulence of the bacterial strain and the host's immune status. Ingestion of *L. monocytogenes*–contaminated food by immune-competent individuals is often limited to gastroenteritis that resolves in a few days, with clearance of the pathogen from the intestine (Dalton et al., 1997; Aureli et al., 2000). Pregnant women, infants, older adults, and immunocompromised individuals, particularly cancer patients, are at risk for systemic *L. monocytogenes* infections (Vázquez-Boland et al., 2001; Swaminathan and Gerner-Smidt, 2007). The remarkable capacity of *L. monocytogenes* to infect the placenta (Lecuit, 2005; Gessain et al., 2015) can result in septic abortion and neonatal infection, whereas in immunocompromised adults, bacteremia and meningo-encephalitis are the major syndromes associated with *L. monocytogenes* infection (Goulet et al., 2012). Patients with cancer have some of the highest incidences of systemic *L. monocytogenes* infection (Mook et al., 2011; Goulet et al., 2012), and, whereas cell-mediated immune function may be compromised in these patients (Pamer, 2004), predisposing factors in humans to *L. monocytogenes* infection remain incompletely defined.

L. monocytogenes outbreaks are associated with food contamination (CENTERS FOR DISEASE CONTROL AND PREVENTION (CDC), 2016); however, most listeriosis cases that occur in immunocompromised hosts are sporadic and likely result from ingestion of low numbers of bacteria that are known to contaminate many foods meant to be cooked before ingestion (Pinner et al., 1992; Schuchat et al., 1992). In the general population, exposure to *L. monocytogenes* and asymptomatic clearance are believed to occur several times per year (Grif et al., 2003).

Mice are considered relatively resistant to oral infection with *L. monocytogenes*, but can develop disseminated infection upon oral inoculation with very high doses of *L. monocytogenes* (Bou Ghanem et al., 2012; Hoelzer et al., 2012). Dissemination from the gut requires *L. monocytogenes* to traverse the intestinal epithelium, either by transcytosis of M cells into Peyer's patches (MacDonald and Carter, 1980; Marco et al., 1997; Pron et al., 1998; Corr et al., 2006; Chiba et al., 2011; Bou Ghanem et al., 2012), or by binding of the bacterial invasion protein internalin A (InlA) with its ligand, E-cadherin (E-cad; Lecuit et al., 1999). The relative resistance of mice, compared with humans, to oral infection with *L. monocytogenes* results, in part, from reduced affinity of InlA for murine, as opposed to human E-cad. Indeed,

Correspondence to Simone Becattini: becattis@mskcc.org;Eric G. Pamer: pamere@mskcc.org

Abbreviations used: FMT, fecal microbiota transplantation; MNVC, metronidazole-neomycin-vancomycin-clindamycin; Strepto, Streptomycin.

The Rockefeller University Press $30.00
J. Exp. Med. 2017 Vol. 214 No. 7 1973–1989
https://doi.org/10.1084/jem.20170495

the efficiency of murine *L. monocytogenes* infection can be increased by transgenic expression of human E-cad in mice (Lecuit et al., 2001) or mutagenesis of InlA to increase its affinity for murine E-cad (Wollert et al., 2007). Nevertheless, even with optimized InlA/E-cad affinities, murine infection requires high doses of orally administered *L. monocytogenes*, suggesting that other mechanisms limit in vivo *L. monocytogenes* virulence (Lecuit et al., 2001; Wollert et al., 2007; Bou Ghanem et al., 2012).

The intestinal microbiota provides resistance against orally acquired bacterial pathogens (Buffie and Pamer, 2013). Commensal microbes that constitute the microbiota directly inhibit potential pathogens by producing bacteriocins and by nutrient depletion and also indirectly by induction of host defense pathways, such as expression of defensins and bactericidal C-type lectins. We hypothesized that resistance of mice and humans to *L. monocytogenes* infection might depend on the intestinal microbiota and that antibiotic-mediated damage to the microbiota, in particular in immunocompromised hosts, would increase susceptibility to *L. monocytogenes* infection. Herein, we show that an intact, diverse microbiota inhibits *L. monocytogenes* growth and that antibiotic administration to mice before *L. monocytogenes* inoculation decreases the inoculum required for systemic infection to as few as 100 CFUs. Antibiotic-mediated damage to the microbiota enables *L. monocytogenes* to expand in the host intestine and to traverse the epithelial barrier. This has particularly dramatic consequences for immunocompromised or chemotherapy-treated mice, promoting lethal infection even with small inocula. We have identified a subset of commensal bacteria that confer protection against oral *L. monocytogenes* infection, suggesting that the microbiota of vulnerable hosts can be augmented to enhance resistance against this important pathogen.

RESULTS

Antibiotic treatment results in increased susceptibility to *L. monocytogenes* infection

To determine the extent to which the intestinal microbiota provides resistance against oral infection with *L. monocytogenes*, we treated C57BL/6 mice from The Jackson Laboratory with one dose of clindamycin, followed by oral gavage 24 h later with a sublethal dose of *L. monocytogenes*. Clindamycin treatment markedly increased the duration and magnitude of *L. monocytogenes* carriage in the intestinal lumen and tissue (Fig. 1 A). The density of *L. monocytogenes* CFUs in the intestinal lumen directly correlated with pathogen burden in the intestinal wall (Fig. 1 B). Colonies recovered on selective growth plates were further screened by PCR for the *L. monocytogenes p60* gene (Fig. S1). The kinetics of luminal clearance of *L. monocytogenes* were similar in WT and *Rag1*$^{-/-}$ mice, indicating that B and T lymphocytes of the adaptive immune system do not contribute to pathogen elimination between days 1 and 6 (Fig. 1, C and D). Some *Rag1*$^{-/-}$ mice continued to excrete *L. monocytogenes* in their feces 10 d after inoculation, possibly as a result of defective CD8^{+} T cell-mediated clearance of systemic infection (Andersson et al., 1998; Bregenholt et al., 2001).

A single dose of streptomycin, which markedly enhances murine susceptibility to *Salmonella* infection (Bohnhoff and Miller, 1962), or a cocktail of four antibiotics (metronidazole, neomycin, vancomycin, and clindamycin [MNVC]), also resulted in robust expansion of *L. monocytogenes* (Fig. 1 E) and increased morbidity (Fig. 1 F,G). At day 3 after infection, edema, inflammatory cell infiltration, and epithelial cell shedding were detected in the intestinal tissue of infected antibiotic-treated, but not PBS-treated, mice (Fig. 1 F). Weight loss, the combined pathology score (Abt et al., 2015), and mortality were consistently increased in antibiotic-treated mice (Fig. 1 G). Treatment of mice with streptomycin induced the highest level of susceptibility, potentially because *L. monocytogenes* 10403s, the strain used in these studies, is highly resistant to this antibiotic, whereas residual neomycin, vancomycin, and clindamycin might inhibit *L. monocytogenes* (Fig. S2). We detected high bacterial loads in spleen and liver of antibiotic-treated mice (unpublished data), indicating that antibiotic-mediated compromise of the microbiota predisposes to severe, disseminated *L. monocytogenes* infection by enabling pathogen expansion in the intestinal lumen and increasing penetration into the intestinal tissue and the systemic circulation.

Small inocula or gut-resident *L. monocytogenes* can promote infection upon dysbiosis

Inoculation of mice with as few as 10^2 CFUs ($\sim 10^{-7}$ * LD_{50}) after streptomycin treatment resulted in high-density colonization of the intestinal lumen with weight loss, signs of distress, and diarrhea and fecal shedding persisting for over 10 d (Fig. 2 A,B and not depicted). Despite the low inoculum size, *L. monocytogenes* penetrated the epithelium, infiltrated mesenteric lymph nodes, and spread to spleen and liver in antibiotic-treated, but not PBS-treated, mice (Fig. 2 C). These results indicate that microbiota-mediated colonization resistance against *L. monocytogenes* is a major contributor to the high level of murine resistance to oral infection.

Roughly 0.5–5% of the human population transiently and asymptomatically carries low levels *L. monocytogenes* in the gastrointestinal tract (Bojsen-Moller, 1964; Kampelmacher and van Noorle Jansen, 1969; MacGowan et al., 1994; Cobb et al., 1996; Grif et al., 2003). To determine whether antibiotic-mediated microbiota depletion in mice with low-level *L. monocytogenes* colonization of the gut could lead to blooming of *L. monocytogenes*, we orally infected mice and fecal pellets were cultured daily until *L. monocytogenes* was undetectable (detection limit = 100 CFU/g feces). Once *L. monocytogenes* became undetectable in feces, it could not be cultured from intestinal and abdominal organs, including the gallbladder, which was previously suggested to be a reservoir for *L. monocytogenes* in infected BALB/c mice (Hardy et al., 2004; Bou Ghanem et al., 2012; Fig. 2 D), but appears

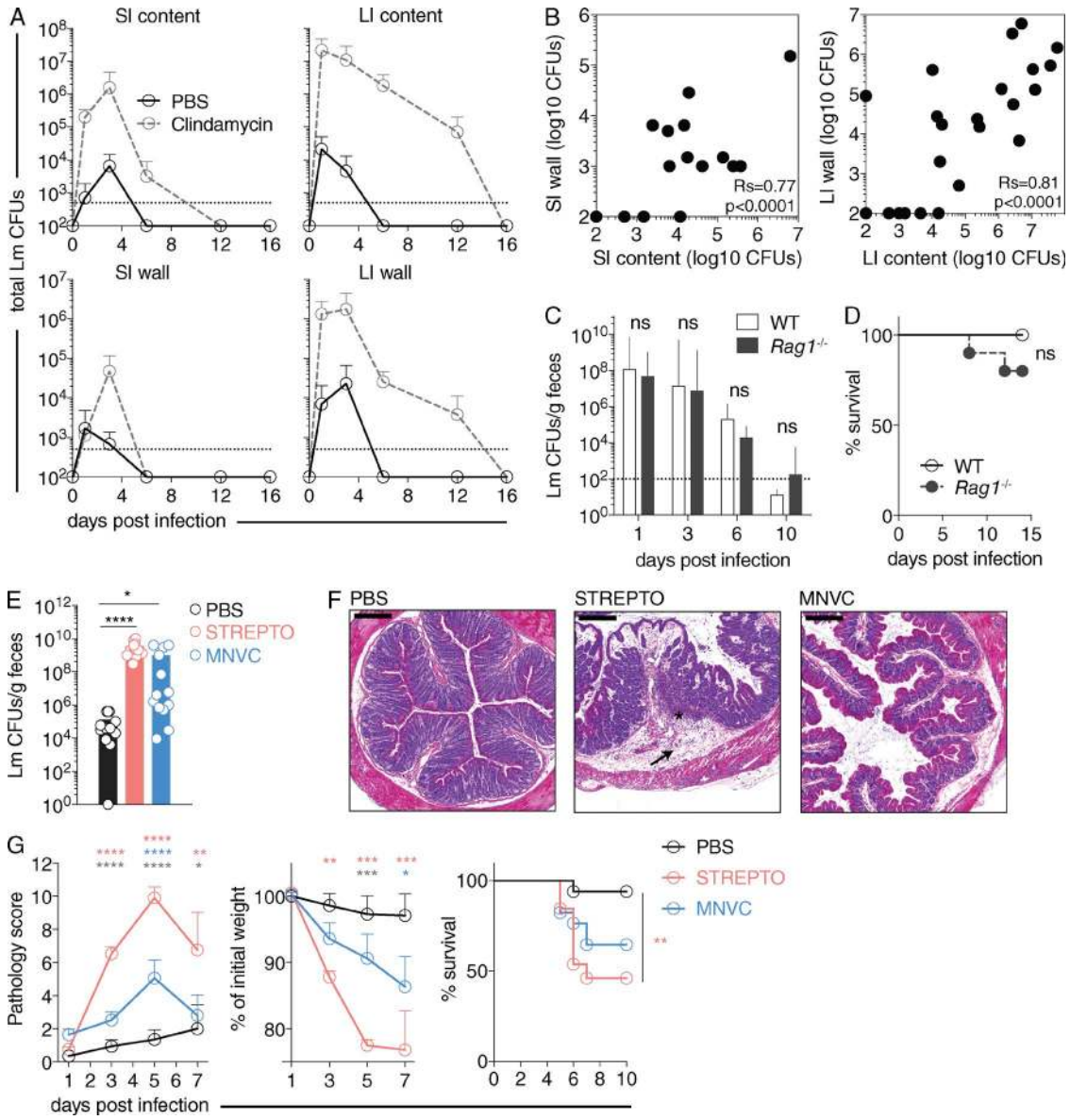

Figure 1. **Antibiotic treatment predisposes to severe *L. monocytogenes* infection.** (A) *L. monocytogenes* (Lm) burden in antibiotic-treated mice. WT mice were treated with a single i.p. injection of clindamycin or PBS and infected orally 24 h later with 10^7 Lm 10403s CFUs. At each time point, animals were euthanized and the total number of Lm CFUs was determined by plating homogenized organs or intestinal content (n = 4 per time point, from two independent experiments). (B) Spearman correlation between Lm CFUs recovered from the intestinal content and wall of mice shown in A, for small and large intestine. (C and D) WT and *Rag1*$^{-/-}$ (Rag) mice were cohoused for 3 wk, then injected i.p. with a single dose of clindamycin and infected 24 h later with 10^7 Lm CFUs. Survival (C) and fecal shedding (D) of Lm were monitored over time (geometric means + geometric SD are shown; n = 10 per group; from two independent experiments). (E) Lm burden in feces 1 d after infection with 10^8 Lm CFUs of mice treated with either streptomycin or a combination of metronidazole, neomycin, vancomycin, and clindamycin (MNVC). Antibiotic treatment was terminated 1 d before infection in both cases (n = 9–13; Kruskal-Wallis test with Dunn's multiple comparison correction). (F) Representative H&E staining of colonic tissue from mice treated as in E, 3 d after infection (arrows indicate edema and stars indicate cellular infiltration; Bar, 200 µm). (G) Weight loss, pathology score (see Materials and methods) and survival of mice treated as in E (n = 13–17 per group; means + SD; Two way ANOVA with Tukey's multiple comparison test and Log-Rank (Mantel-Cox) for survival). *, P < 0.05; **, P < 0.005; ***, P < 0.001; ****, P < 0.0001.

less permissive to the growth of the pathogen in C57BL/6 mice, at least at relatively low doses of infection (Bou Ghanem et al., 2012). Upon virtual clearance, mice were treated with streptomycin, which resulted in a marked bloom of *L. monocytogenes* (Fig. 2 E). Administration of streptomycin at different times after infection demonstrated that a high proportion of mice harbor *L. monocytogenes* 11 d (~80%), 16 d (~40%), and 21 d (~20%) after infection (Fig. 2 F), longer than pre-

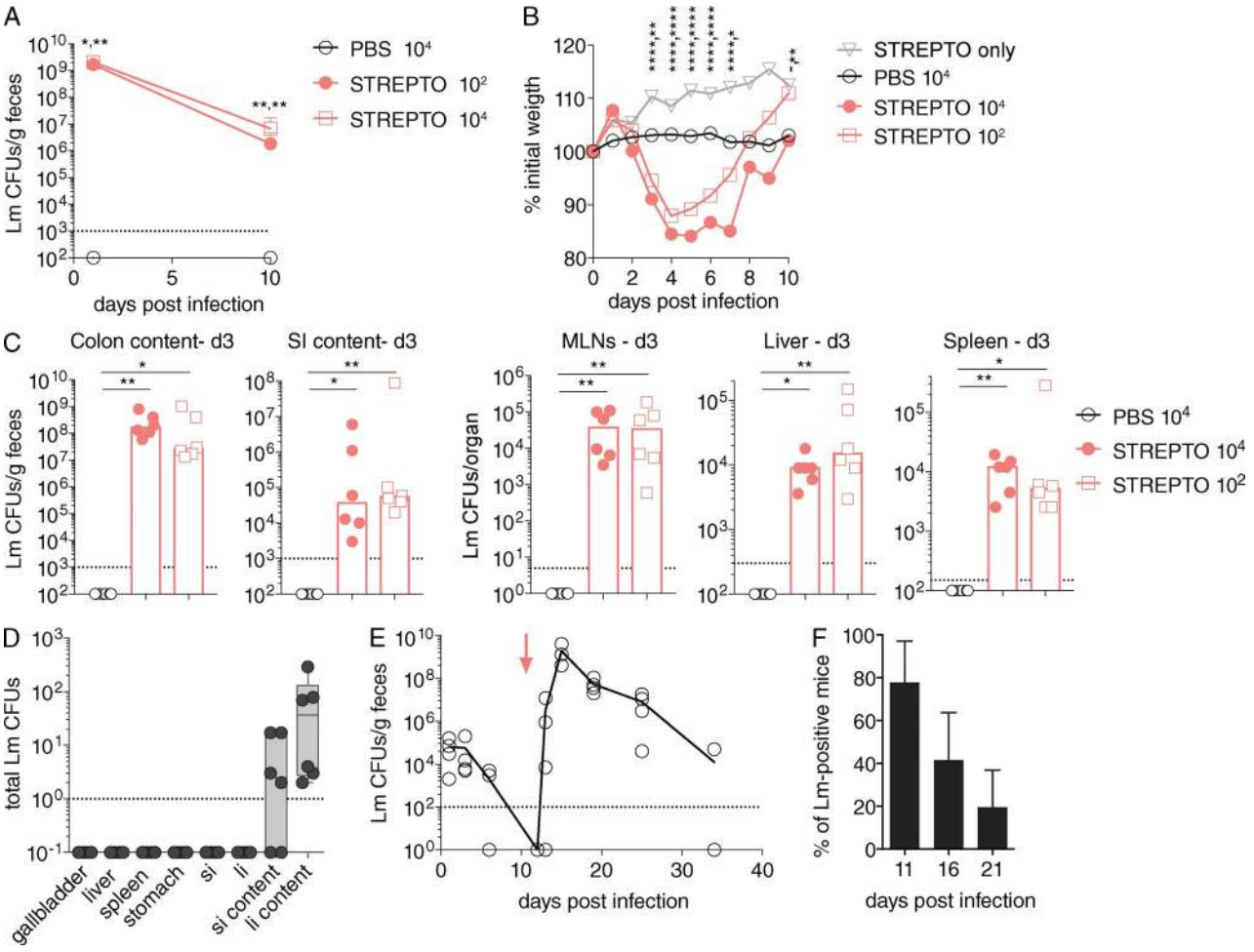

Figure 2. **Small *L. monocytogenes* oral inocula spread systemically in antibiotic-treated mice.** (A) WT mice were treated with one dose of oral streptomycin or PBS and infected 24 h later with 10^4 or 10^2 (only streptomycin group) CFUs of *L. monocytogenes* (Lm). Lm fecal shedding is shown over time (mean + SD; $n = 8$ per group; from two independent experiments). (B) Weight loss for animals shown in A ($n = 7$–8; $n = 3$ for streptomycin-only group; from two independent experiments; two Way ANOVA with Tukey's multiple comparisons; a comma separates significance values for STREPTO × 10^4 vs. PBS × 10^4 groups and STREPTO × 10^2 vs. PBS × 10^4 groups, respectively). (C) Lm burden in the depicted compartments at 3 d after infection, same conditions as in A ($n = 6$; from two independent experiments; Kruskal-Wallis test with Dunn's multiple comparisons). (D) Mice infected with 10^8 Lm particles were euthanized 1 d after negative fecal cultures for Lm, and whole organs/intestinal contents were homogenized and plated for Lm detection ($n = 6$; shown are only mice for which colonies where detected). (E) Kinetics of Lm fecal shedding in mice infected with 10^8 Lm CFUs. Lm presence in the feces was monitored over time, and mice were administered 1 dose of streptomycin (salmon arrow) on the first day after fecal cultures became negative for Lm ($n = 4$; one representative of three experiments shown). (F) Percentages of mice bearing Lm at the depicted time points after infection, as assessed using the approach described in E, except that in this experiment, mice were maintained in wire floor cages to prevent coprophagy ($n = 10$ per group; from three different experiments). *, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.001$; ****, $P < 0.0001$.

viously appreciated (Huleatt et al., 2001). Thus, despite negative fecal cultures, residual *L. monocytogenes* bacteria can undergo expansion when microbiota-mediated colonization resistance is impaired by antibiotic administration.

The gut microbiota provides nonredundant protection against *L. monocytogenes* in immunocompromised hosts

To investigate the contribution of the microbiota to resistance against oral *L. monocytogenes* infection in immunocompromised hosts, we infected *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ (Raggc) mice, which lack T and B cells, as well as NK cells and innate lymphoid cells (ILCs), and are known the be highly susceptible to oral *L. monocytogenes* infection (Bregenholt et al., 2001). We confirmed that Raggc mice orally inoculated with *L. monocytogenes* doses that are sublethal for WT mice rapidly lose weight and succumb to infection (Fig. 2 A and not depicted). Interestingly, although it was originally proposed that lack of T cells accounted for marked susceptibility, we only detected increased susceptibility to oral *L. monocytogenes* infection in *Rag1*$^{-/-}$*Ifng*$^{-/-}$ double KO mice, but not *Rag1*$^{-/-}$ or *Rag1*$^{-/-}$*Il17*$^{-/-}$ mice, suggesting that type 1 ILCs or NK cells, rather than T cells, protect against *L. monocyto-*

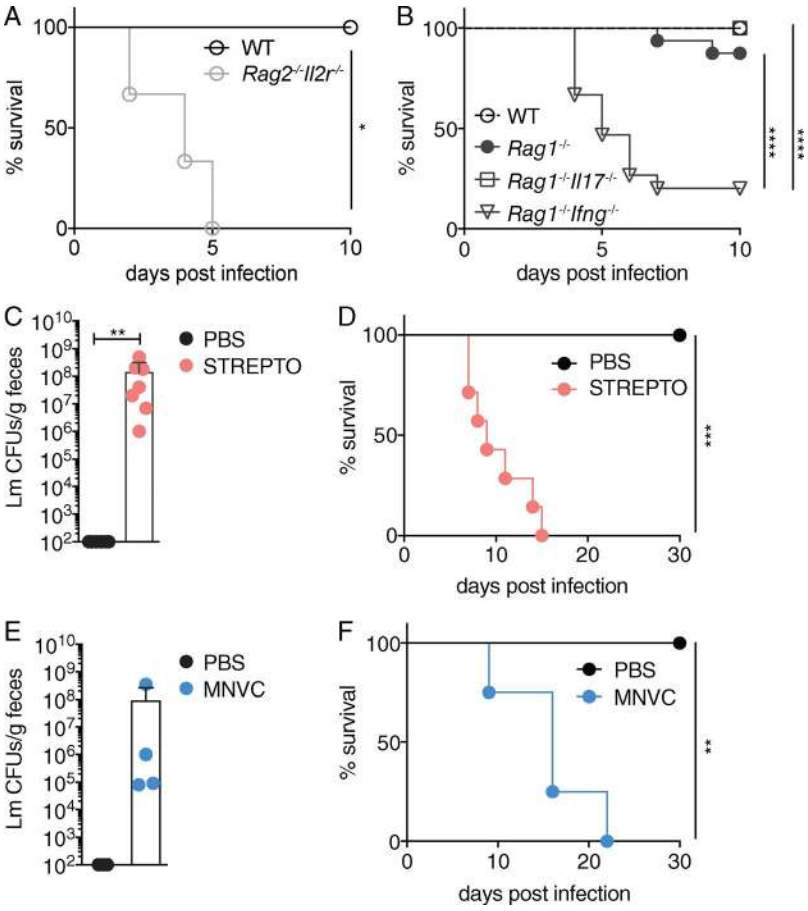

Figure 3. **Antibiotic treatment predisposes hosts with congenic immunodeficiency to lethal listeriosis.** (A) *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ (Raggc) and WT mice were cohoused for 3 wk and infected orally with 10^8 *L. monocytogenes* (Lm) CFUs. Survival was monitored overtime (n = 3; similar results were obtained with a lower infectious dose). (B) Mice of the depicted strain were cohoused for 3 wk, and then challenged orally with 10^8 Lm CFUs. Survival was monitored overtime (n = 9–15; from three independent experiments). (C–F) Raggc mice were administered the depicted antibiotics or PBS and infected 24 h later with 10^4 Lm CFUs. (C and F) Show Lm CFUs in feces 1 d after infection (mean + SD), (D and F) show survival rates (n = 6–7 for [A and B]; n = 4 for [C and D], from three and two independent experiments, respectively; Mann-Withney test for CFU comparison and Log-Rank [Mantel-Cox] test for survival.) *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001.

genes in these settings(Fig. 2 B). This result is consistent with previous demonstrations of the importance of early innate sources of IFN-γ for effective immune responses in systemic *L. monocytogenes* infection (Dunn and North, 1991 Ladel et al., 1996; Thäle and Kiderlen, 2005), although NKs have also been shown to reduce resistance during early phases of *Listeria* infection after intravenous inoculation of WT mice (Teixeira and Kaufmann, 1994; Viegas et al., 2013; Clark et al., 2016). To determine the role of the microbiota in immunocompromised, highly susceptible hosts, we treated Raggc mice with PBS or antibiotics (streptomycin, MNVC) and infected them orally with a sublethal *L. monocytogenes* inoculum (Fig. 3, C–F). Strikingly, all antibiotic-treated Raggc mice succumbed to infection, whereas all PBS-treated Raggc mice survived (Fig. 3, D and F). This indicates that the intestinal microbiota in immunocompromised hosts provides nonredundant, first line defense against *L. monocytogenes* infection.

Anticancer chemotherapy and antibiotics synergize in predisposing the host to listeriosis

Cancer patients have the highest incidence of severe *L. monocytogenes* infection (Gillespie et al., 2009; Mook et al., 2011; Fernández Guerrero et al., 2012; Goulet et al., 2012), with some types of cancer resulting in infection rates increased by 1,000-fold (Goulet et al., 2012). Although the immune-suppressive effects of cancer chemotherapy and radiation therapy might contribute to enhanced susceptibility, experimental evidence for their impact on *L. monocytogenes* infection is lacking. Furthermore, a recent study demonstrated that cancer chemotherapy alters the gut microbiota (Viaud et al., 2013), which may increase susceptibility to infections. Review of patients admitted to MSKCC with a diagnosis of *L. monocytogenes* infection in the past 20 yr (Fig. S3) demonstrated that the majority had been treated with multiple chemotherapeutic agents, corticosteroids, or antibiotics. To determine whether chemotherapy can predispose to *L. monocytogenes* dissemination after oral infection, we treated C57BL/6 mice with doxorubicin and cyclophosphamide, a commonly used chemotherapy combination for a variety of tumors (Alyamkina et al., 2010; Mainetti et al., 2013), and subsequently infected with *L. monocytogenes*. Chemotherapy reduced the total number of circulating cells approximately fourfold, with decreased circulating B cells, $CD8^+$ and $CD8^-$ T cells, ILCs/NKs, and monocytes (Fig. 4, A–C). Severity of *L. monocytogenes* infection was markedly augmented by chemotherapy administration, with increased morbidity over a range of inoculum doses (not depicted), and 100% mortality (Fig. 4 D). Chemotherapy administra-

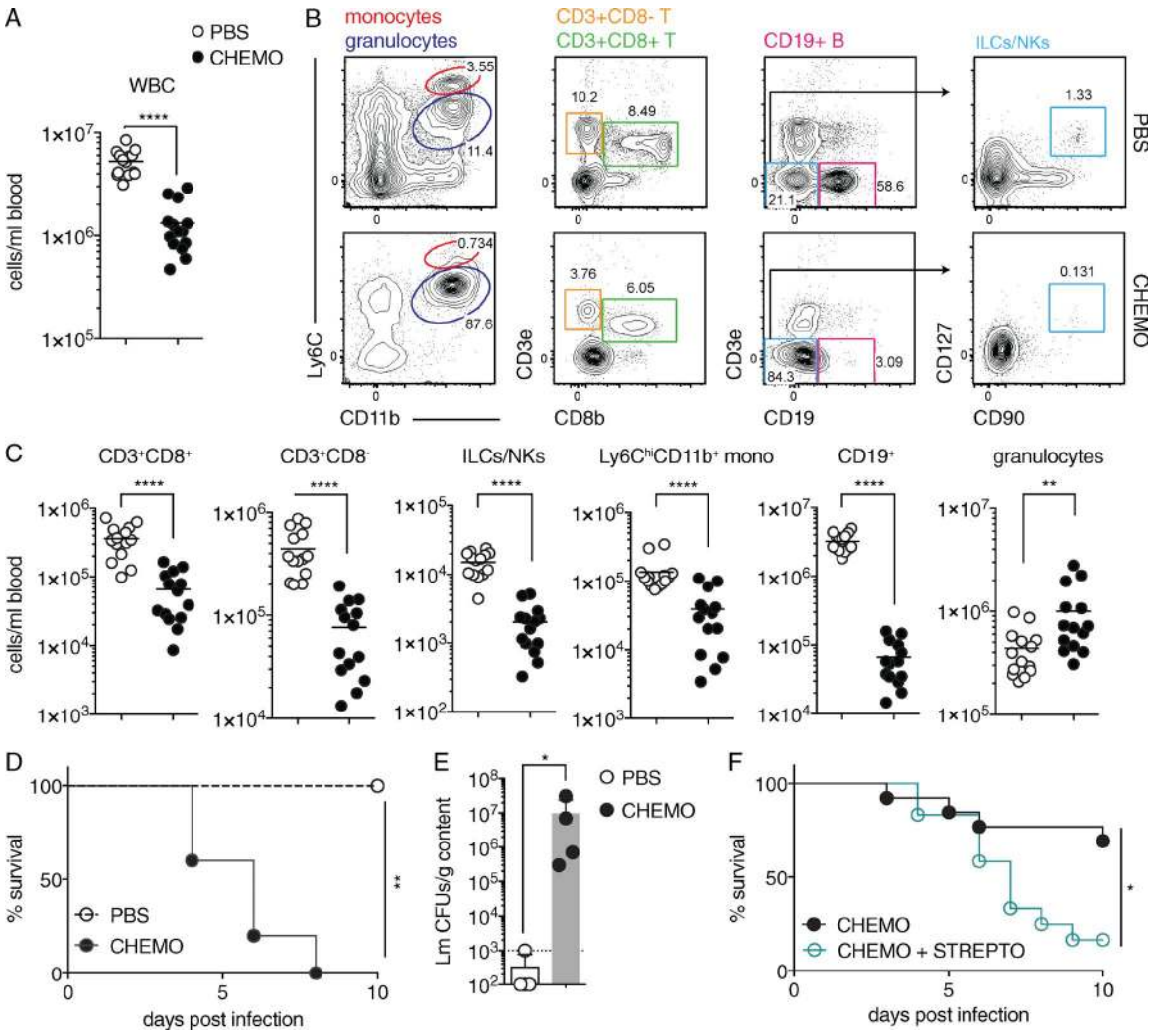

Figure 4. **Anticancer chemotherapy and antibiotics synergistically enhance susceptibility to *L. monocytogenes* infection.** (A) Mice were administered combined chemotherapy (CHEMO) composed of cyclophosphamide and doxorubicin, or PBS, injected twice i.p. (on day 0 and 7). On day 8 mice were bled and the white blood cell (WBC) count was determined ($n = 14$; from three independent experiments). (B) Representative FACS plots of cells obtained as in A and stained for markers of interest. (C) Cell numbers for circulating leukocytes identified as in B from mice shown in A ($n = 14$; lines represent means). (D) Survival of PBS- vs. CHEMO-treated mice infected 1 d after second treatment (d8) with 10^6 *L. monocytogenes* CFUs ($n = 5$). (E) Lm burden in the colonic content of mice treated and infected as in D with 10^8 Lm CFUs, 1 d after infection. Shown is one representative of two experiments ($n = 4$). (F) Mice were treated as in A, administered either PBS or streptomycin on day 7 (concomitant with the second CHEMO administration) and infected 24 h later with 10^4 Lm CFUs ($n = 12–13$; Mann-Whitney test in A, C, and E; Log-Rank test in D and F. *, $P < 0.05$; **, $P < 0.005$; ****, $P < 0.0001$).

tion resulted in higher *L. monocytogenes* CFUs in the large intestine lumen 24 h after infection (Fig. 4 E), suggesting that drug-induced dysbiosis or inflammation facilitates *L. monocytogenes* growth, contributing to greater dissemination. Of note, cyclophosphamide treatment has been shown to reduce the abundance of bacterial strains belonging to the order Clostridiales in a mouse model (Viaud et al., 2013). To determine whether antibiotic-induced microbiota perturbation increases susceptibility of chemotherapy-treated mice to oral *L. monocytogenes* infection, chemotherapy recipients were treated with streptomycin or PBS before infection with a low *L. monocytogenes* inoculum. In concordance with results obtained in Raggc mice, streptomycin treatment accentuated chemotherapy-induced susceptibility and resulted in increased mortality rates (Fig. 4 F).

Commensal microbes efficiently antagonize *L. monocytogenes* ex vivo

Antibiotic-mediated depletion of commensals can reduce mucosal and systemic immune defenses (Caballero and Pamer, 2015; Becattini et al., 2016) and commensal-driven, MyD88-mediated stimuli induce RegIIIγ production by the intestinal epithelium and reduce *L. monocytogenes* growth in the small intestine (Brandl et al., 2007, 2008). Alternatively,

intestinal commensal bacteria can also directly inhibit pathogens by competing for nutrients or producing bacteriocins. To begin to determine how the intestinal microbiota inhibits in vivo *L. monocytogenes* expansion, we performed ex vivo experiments to assess if commensals, in the absence of host-derived factors, mediate *L. monocytogenes* clearance. Content from small or large intestine was harvested from WT mice, resuspended in reduced PBS, and inoculated with increasing doses of *L. monocytogenes* in either anaerobic or aerobic conditions. Intestinal contents killed *L. monocytogenes* within 24 h, with small intestinal content demonstrating greater activity at reducing *L. monocytogenes* CFUs (Fig. 5 A). Large intestinal contents had more variable inhibitory activity, but in all cases reduced viable CFUs over 24 h. *L. monocytogenes* grew exponentially in intestinal content that had been filtered and cleared of bacteria, indicating that neither lack of nutrients in the initial suspension nor presence of antimicrobial molecules of host origin account for the bactericidal effect of intestinal contents (Fig. 5 B). Exposure of ex vivo cultures to oxygen delayed *L. monocytogenes* clearance from small intestine content, and abolished clearance from large intestine content (Fig. 5 A). These results suggest that obligate anaerobic bacteria inhibit and possibly kill *L. monocytogenes*. Intestinal content from mice that had been previously treated with antibiotics lost the capacity to eliminate *L. monocytogenes*, enabling survival (small intestine) or expansion (large intestine) of the pathogen (Fig. 5 C). Exposure to oxygen synergized with the effect of antibiotic treatment decreasing the capacity of commensals to antagonize *L. monocytogenes* (Fig. 5 C). This corroborated our hypothesis that the enhanced infection and delayed *L. monocytogenes* clearance in antibiotic-treated mice depended on direct effects on microbiota composition, rather than on indirect consequences on the immune system.

To investigate the inhibitory mechanisms operating in the aforementioned assays, contents from small or large intestine were co-cultured with *L. monocytogenes* for 24 h, a time sufficient to allow inhibitory mechanisms to take place, and the resulting culture supernatants were sterile filtered (24 h-sup). The supernatants were then inoculated with *L. monocytogenes* or a laboratory strain of *E. coli* (DH-5α) for an additional 24 h (Fig. 5 D). Although 24 h-sups from large intestinal contents promoted growth of both *L. monocytogenes* and *E. coli*, *L. monocytogenes* was completely eliminated from 8 out of 10 small intestinal 24 h-sups. In contrast, *E. coli* grew exponentially in filtered supernatants, suggesting that different mechanisms interfere with *Listeria* expansion in the small and large intestine, some of which are selective and discriminate among bacteria.

Overall, our results suggest that bacteria inhabiting different intestinal regions efficiently eliminate *L. monocytogenes* by multiple mechanisms; these might include production of antibacterial molecules (Zhu et al., 2000; Corr et al., 2007; Lakshminarayanan et al., 2013; Vijayakumar and Muriana, 2015; Egan et al., 2016; Saraoui et al., 2016), as well as nutrient competition (Maltby et al., 2013) or contact-dependent inhibition (Ruhe et al., 2013).

Identification of intestinal commensal bacteria associated with protection from *L. monocytogenes* infection in vivo

To identify intestinal commensal species that provide colonization resistance against *L. monocytogenes*, we administered streptomycin or MNVC to C57BL/6 mice and challenged them with an oral inoculum of *L. monocytogenes* 1, 5, 16, 21, or 27 d after completion of antibiotic treatment. Mice were euthanized 24 h after infection and *L. monocytogenes* was quantified by culture of intestinal contents, liver, and spleen. Small intestine, cecum, or colon contents were also subjected to 16S rRNA gene sequencing to determine microbiota composition at each time point. High susceptibility to infection persisted for only 1 d after streptomycin treatment and mice fully recovered resistance (i.e., infection levels undistinguishable from PBS-treated animals) within 5 d of streptomycin termination. In contrast, MNVC treatment resulted in susceptibility to *L. monocytogenes* intestinal colonization for up to 27 d after antibiotic cessation, suggesting that some bacterial species crucial for protection were irreversibly ablated (Fig. 6 A). *L. monocytogenes* density in cecum and colon content correlated with that in cecum and colon wall and the liver (Fig. 6 B). PCoA analysis of 16S sequences demonstrated that the microbiota of streptomycin-treated mice returned to pretreatment composition within 5 d, whereas MNVC-treated animals maintained a distinct microbiota composition (Fig. 6 C).

Spearman rank correlation analysis between *L. monocytogenes* susceptibility and bacterial taxa identified by 16S rRNA gene sequencing, as previously described (Ubeda et al., 2013; Buffie et al., 2015), identified several bacterial species significantly associated with protection (Fig. 6 D and Table S1). The majority of taxa belonged to the order Clostridiales (Fig. 6 D and Fig. S4). Although bacteriocin-producing bacteria such as Lactobacilli are known to inhibit *L. monocytogenes* (Zhu et al., 2000; Corr et al., 2007; Delgado et al., 2007; Lakshminarayanan et al., 2013; Donia et al., 2014), our analysis demonstrated a strong negative correlation between multiple Clostridiales strains and *L. monocytogenes*. Clostridiales, however, contribute to protection in various pathological contexts, ranging from infection to allergy (Atarashi et al., 2013; Narushima et al., 2014; Buffie et al., 2015).

Commensal Clostridiales protect from *L. monocytogenes* infection upon in vivo transfer

To identify specific bacterial strains that can inhibit *L. monocytogenes* growth, we screened a panel of Clostridia strains, including human isolates (Atarashi et al., 2013), commercially available strains, and isolates from mouse stool. A commercially available *Lactobacillus gasseri* strain was included as a positive control, as *L. gasseri* produces at least three toxins that target $Gram^+$ bacteria, including *L. monocytogenes* (Zhu et al., 2000; Delgado

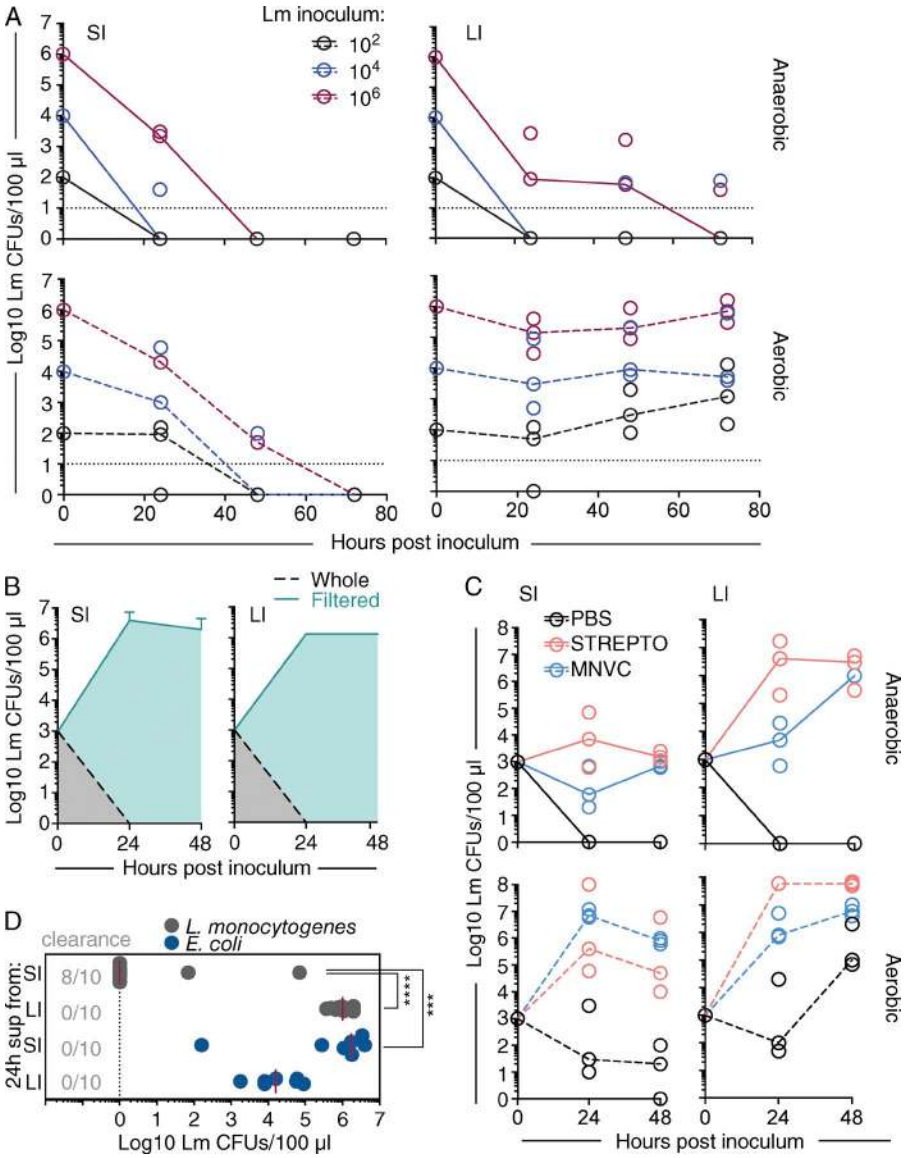

Figure 5. **Intestinal microbes efficiently eliminate *L. monocytogenes* ex vivo, through diverse mechanisms.** (A) Content from small and large intestine was collected from WT mice and resuspended in reduced PBS. *L. monocytogenes* (Lm) was inoculated at the depicted doses in 100 µl of intestinal suspension and cultured in anaerobic (top) or aerobic (bottom) conditions. Lm CFUs were enumerated over time by plating (n = 3 mice per time point; one representative of two experiments shown, circles represent individual values, lines represent medians). (B) 10^3 Lm CFUs were inoculated in intestinal contents prepared as in A or in sterile filtered aliquots of the same intestinal contents and grown anaerobically or aerobically, respectively, for the depicted times (n = 3 mice per time point; one representative of two experiments shown, lines represent medians). (C) Same experimental setup as in A, except that intestinal content was collected from PBS, streptomycin or MNVC-treated mice 1 d after termination of treatment (n = 3 mice per time point; one representative of two experiments shown, circles represent individual values, lines represent medians). (D) Content from small or large intestine was cultured for 24 h in the presence of 10^3 Lm CFUs, then sterile filtered (24 h-sup). 10^3 CFUs of either *L. monocytogenes* or *E. coli* DH5-α were inoculated in sterile-filtered supernatants (24 h-sup) and grown aerobically for 24 h (n = 10 from different mice and three independent experiments, lines represents medians; statistics: Kruskal-Wallis test with Dunn's multiple comparisons). ***, P < 0.001; ****, P < 0.0001.

et al., 2007; Lakshminarayanan et al., 2013; Donia et al., 2014). Co-culture with *L. monocytogenes* under anaerobic conditions demonstrated that several bacterial strains inhibited *L. monocytogenes* growth by over three orders of magnitude (Fig. 7 A). The bacterial strains also inhibited *L. monocytogenes* growth when added to autoclaved cecal content (Fig. 7 B and not depicted). We identified four strains (*C. saccharogumia*, *C. ramosum*, *C. hathewayi*, and *B. producta*) that consistently reduced *L. monocytogenes* growth under varied culture conditions and that provided enhanced antilisterial properties as a consortium (4-Clost mix). We next compared *L. monocytogenes* growth in fecal pellets obtained from GF mice reconstituted with either the 4-Clost mix or microbiota from antibiotic (MNVC)-treated mice (FMT-ABX). Mice reconstituted with FMT-ABX or 4-Clost had similar bacterial densities and overall microbiota diversity (Fig. 7 C and Fig. S5, A and C). Interestingly, only three of the four Clostridiales used for reconstitution appeared to engraft, with *C. saccharogumia* being lost in several animals by day 10 (Fig. S5 C). Culture of *L. monocytogenes* in fecal pellets from the two groups demonstrated reduced growth in fecal cultures derived from 4-Clost mice (Fig. S5B). In vivo challenge studies demonstrated that mice reconstituted with 4-Clost had markedly lower levels of luminal *L. monocytogenes* as compared with ABX-FMT mice (Fig. 7, D and E). Furthermore, whereas dissemination of *L. monocytogenes* to MLNs, spleen, and liver was not detected or moderate in 4-Clost mice, we detected high-level dissemination in FMT-ABX mice (Fig. 7 F). Thus, by reducing *L. monocytogenes* burden in the intestinal lumen, the 4-Clost consortium prevented systemic spread of the pathogen.

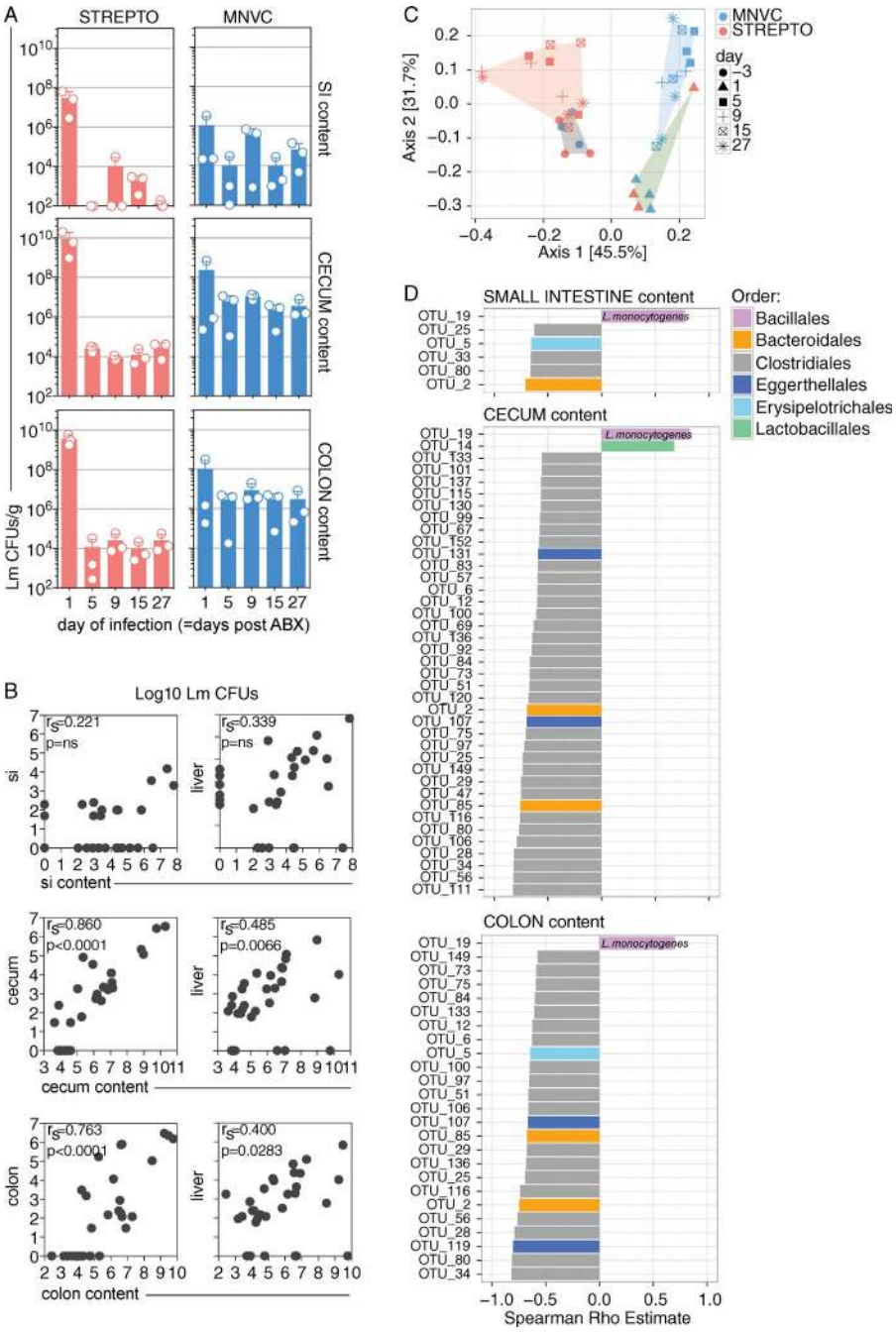

Figure 6. **Correlation of intestinal commensal bacteria with protection from *L. monocytogenes* infection.** (A) WT mice were treated with streptomycin or MNVC. Three mice per group were single-housed and infected with 10^8 Lm CFUs at each of the depicted time points (after antibiotic treatment). 24 h after infection, mice were sacrificed and *L. monocytogenes* CFUs enumerated in their intestinal content and organs. (B) Spearman correlation between Lm CFUs (Log_{10}) in intestinal content, intestinal wall and liver for animals depicted in A (n = 30). Spearman coefficient and significance values are indicated for each correlation. (C) PCoA analysis of microbiota 16S sequences from fecal pellets collected from animals depicted in A on the day of infection. The plotted colored areas indicate: gray = pretreatment; green = d1 after antibiotics (any antibiotics); salmon = d5-27 streptomycin; blue = d5-27 MNVC. (D) Spearman correlation between identified OTUs and Lm CFUs enumerated by plating 1 d after infection, shown separately for small intestine, cecum, and colon contents. Shown are only significant hits, BH corrected. $P < 0.05$.

DISCUSSION

L. monocytogenes has been studied extensively over the past century and has become one of the most widely used model pathogens in experimental immunology. *L. monocytogenes* studies have generally involved intravenous inoculation, a convenient and reproducible model to dissect systemic infection, and immune response mechanisms. Intravenous delivery, however, bypasses the intestinal phase of *L. monocytogenes* infection, which precedes systemic spread and has an important role in the overall pathogenesis of infection (Gahan and Hill, 2005). As a consequence, many aspects of the intestinal phase of *L. monocytogenes* infection remain obscure. Even though ingestion of *L. monocytogenes* from contaminated food is the main cause of infection in humans, there is little clinical information on the early intestinal stages of *L. monocytogenes* infection that might enhance resistance, and preventive interventions target the contaminated food itself (Saraoui et al., 2016). Our results suggest that the microbiota, here shown to be an essential player in defense against *L. monocytogenes*, especially in immunocompromised hosts, might provide a target for intervention in this window.

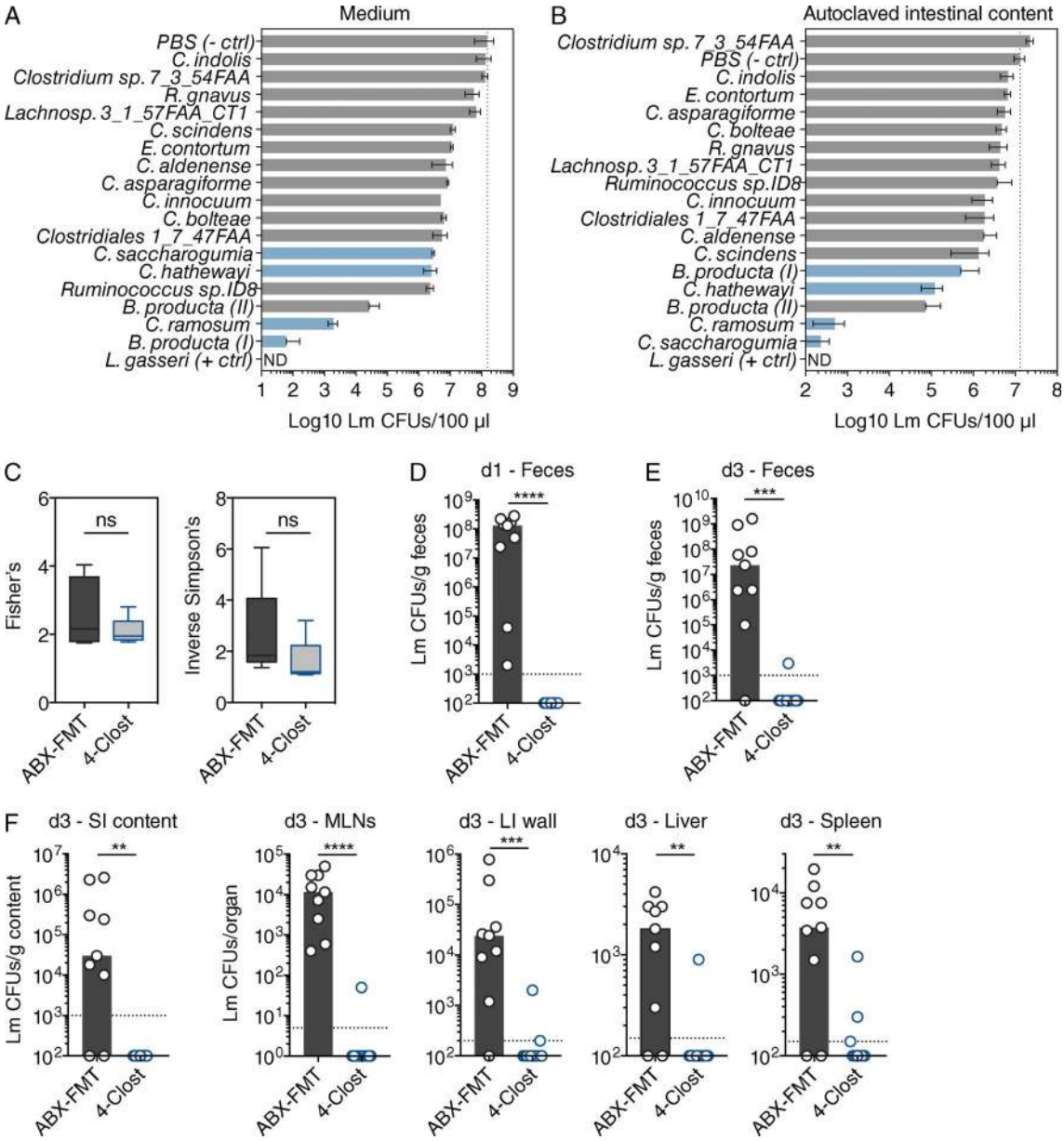

Figure 7. **A rationally designed consortium of four Clostridiales antagonizes *L. monocytogenes* and confers host protection in vivo.** (A and B) Depicted commensal bacteria were grown anaerobically and inoculated in medium (A) or in autoclaved intestinal content (B) at OD = 0.1. 10^3 *L. monocytogenes* (Lm) CFUs were added, and Lm expansion was evaluated after 24 h of anaerobic co-culture ($n = 3$ replicates; shown one representative of two to four experiments per condition). (C–E) GF mice were reconstituted with 4-Clost consortium or fecal pellet from MNVC-treated mice via oral gavage. After 10 d mice were challenged orally with 10^7 Lm CFUs. (C) Diversity index of the microbiota 10 d after reconstitution (=day of *L. monocytogenes* infection) in feces of ex-GF mice of the indicated group, based on OTU composition as assessed by sequencing of 16S rRNA genes. (D) *Listeria* burden in the feces of ex-GF animals was evaluated 24 h after infection. (E and F) Mice described in C and D were euthanized at day 3 after infection and *L. monocytogenes* CFUs quantified in intestinal content and depicted organs. For C–E, circles represent individual mice, bars represent median values ($n = 9$ except in C; $n = 5$, from three independent experiments; Mann-Whitney test). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

Previous studies have suggested, although never conclusively proven, that gut commensals can protect the host from *L. monocytogenes* infection. In the 70s, Zachar and Savage (1979) reported that GF mice, in contrast to SPF mice, were highly susceptible to *L. monocytogenes* infection, and their colon would become rapidly colonized by *L. monocytogenes* after low intra-gastric inocula. The authors hypothesized that the rich microbiota carried by SPF mice might account for their resistance to infection, but were unable to prevent intestinal growth of *L. monocytogenes* by gavage of selected

bacterial species, even though this treatment reduced penetration of *L. monocytogenes* into intestinal tissue, as assessed by microscopy (Zachar and Savage, 1979). Czuprynski and Balish (1981) similarly observed that GF rats, in contrast to conventional rats, were highly permissive for *L. monocytogenes* expansion in the intestine, and proved that intestinal clearance of *L. monocytogenes* could be achieved in these animals by transplantation of a microbiota. Our finding that mice that were stably colonized with *L. monocytogenes* during repeated streptomycin treatments experienced dramatic decreases in intestinal *L. monocytogenes* burden after fecal microbiota transplantation (unpublished data) are consistent with this earlier work. Susceptibility to *L. monocytogenes* infection in mice after antibiotic administration is exacerbated by concurrent corticosteroid administration (Okamoto et al., 1994), suggesting that the immune system and the gut microbiota make distinct contributions to the control of *L. monocytogenes* infection. Consistent with this hypothesis, a recent study demonstrated that cross-transfer of microbiota from C57BL/6 mice and BALB/c mice did not increase the susceptibility to *Listeria* infection in the former strain or reduce it in the latter (Bou Ghanem et al., 2012). As both strains supposedly harbor microbiota with similar complexity and protective activity, genetic and immune factors are likely responsible for the outcome of infection when colonization resistance is intact (Myers-Morales et al., 2013).

The ability of the microbiota to cure patients with recurrent *Clostridium difficile* infections has recently been demonstrated in a randomized, controlled trial of fecal microbiota transplantation (FMT), and FMT is increasingly being tested in clinical trials as a therapeutic approach for a wide range of diseases (van Nood et al., 2013). Our results suggest that *Listeria monocytogenes* should be added to a growing list of intestinal pathogens that might be effectively antagonized by commensal bacteria that colonize the GI tract. Our finding that a diverse, antibiotic-naive microbiota can inhibit *L. monocytogenes*, possibly by multiple, parallel mechanisms, provides at least a partial explanation for why high *L. monocytogenes* inocula are required for laboratory animals and healthy humans to become infected via the oral route, and why luminal clearance occurs rapidly (Dalton et al., 1997). Our study suggests that intestinal survival and expansion of *L. monocytogenes* is an event that precedes severe disease in subjects at risk of infection, and that a compromised microbiota is an important predisposing factor. It was recently proposed that during early phases of intestinal infection, *Listeria* is shed from the tips of infected villi into the lumen, and then penetrate the epithelium at different locations, thus extending tissue invasion (Melton-Witt et al., 2012). In this scenario, the loss of microbiota-mediated colonization resistance might further amplify *L. monocytogenes* expansion.

Consistent with a previous study (Bou Ghanem et al., 2012), but somewhat conflicting with a more established view in the field, our data suggest that large intestine, rather than small intestine, is a major replication site for *Listeria*. Preferential tissue invasion at the level of the small intestine seems at odds with the low local concentration of goblet cells, which have been postulated to be a major portal for InlA-mediated entry in the host tissue, and are instead numerous in cecum and colon (Nikitas et al., 2011). Importantly, our results suggest that carriage of residual amounts of *L. monocytogenes* in the intestinal lumen is relatively long lasting, up to 3 wk or more in a sizable portion of C57BL/6 mice infected orally, in spite of the remarkable antilisterial activity exerted by commensals. Here, we showed that such a residual population can expand upon impairment of the microbiota's protective functions, an observation that is of relevance to immunocompromised patients, in whom systemic spread might be caused by intestinal resident rather than de novo ingested bacteria. In fact, by ablating or perturbing the microbiota, antibiotic treatment makes immunocompromised hosts susceptible to low doses of *L. monocytogenes*, which confirms previous observations on the synergistic effects of antibiotics and corticosteroids in listeriosis (Okamoto et al., 1994). Of note, another important protective contribution of the microbiota was underlined in a recent study demonstrating that long lasting antibiotic treatment reduces hematopoiesis, and enhances susceptibility to listeriosis in a model of retro-orbital *Listeria* infection (Khosravi et al., 2014). However in our system, where antibiotics were administered only once, no major consequence of the treatment on circulating lymphocytes was observed (unpublished data). Instead, using the oral challenge approach, we could clearly demonstrate the consequences on *Listeria* expansion resulting from microbiota perturbation.

We demonstrated the relevance of an intact gut microbiota in two different models of immune-deficiency: congenital deficiency of lymphoid cells (*Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice) and generalized immune deficiency promoted by cancer chemotherapy in otherwise healthy animals. In our model, chemotherapy likely enhanced susceptibility to Listeriosis by reducing immune cells in the circulation, particularly inflammatory monocytes as well as IFN-γ–producing ILCs/NKs, whose defensive role in *Listeria* infection remains a subject of debate, with discrepancies across studies possibly arising from differences in mouse genetic backgrounds or routes of infection (Dunn and North, 1991; Teixeira and Kaufmann, 1994; Ladel et al., 1996; Thäle and Kiderlen, 2005; Viegas et al., 2013; Clark et al., 2016). In the absence of such cell types, we find that the microbiota provides a parallel line of defense, which directly acts on the pathogen, reducing its growth. Antibiotic treatment destroys this defense mechanism, thus exposing the host to infection even with extremely low *L. monocytogenes* doses. Interestingly, chemotherapy administration alone resulted in a significantly higher *L. monocytogenes* burden in the large intestinal lumen 1 d after infection, suggesting that microbiota perturbation or inflammatory conditions induced by anticancer drugs might also contribute to the enhanced susceptibility of cancer patients. Thus, augmenting colonization resistance functions in immunocompromised patients by introducing or supporting protective

bacterial species might represent a novel clinical approach to prevent *L. monocytogenes* infection.

Our results also raise the possibility that in other at-risk categories for listeriosis, such as infants or pregnant women, intestinal dysbiosis might be a contributing factor to susceptibility. Anecdotally, *L. monocytogenes* was found to grow in meconium (Lembet et al., 2003), and pregnant women in their third trimester, the phase of pregnancy in which susceptibility to *Listeria* is known to be highest (Allerberger and Wagner, 2010), present altered microbial profiles, with marked reduction in Clostridiales members (Koren et al., 2012) and increases in Proteobacteria.

Our ex vivo data provide evidence that intestinal microbes have a remarkable ability to rapidly eliminate or outcompete *L. monocytogenes* in culture. Interestingly, multiple mechanisms seems to account for such inhibition, possibly reflecting the different commensal communities represented along the GI tract.

Based on our results, competition for nutrients might be a crucial factor, promoting growth restriction of *L. monocytogenes* in the small intestine (Fig. 5). Furthermore, commensals such as Lactobacilli, which mainly reside in the small intestine, are known to produce antilisterial bacteriocins, and elegant in vivo experiments provided evidence that daily gavage with *L. salivarius* increases resistance to *Listeria* in mice with an intact microbiota (Corr et al., 2007). Commensals such as *L. gasseri*, which is known to produce *L. monocytogenes*–killing bacteriocins, are recurrently identified in our mouse microbiota analyses.

To our knowledge, anaerobes from the large intestine have never been associated before with protection from *L. monocytogenes*. These commensals might be operating through mechanisms that do not involve secretion of toxins or competition for nutrients, as supernatants from cultured large intestine contents still support *Listeria* growth, which is instead prevented when anaerobes are maintained in the culture (Fig. 5). Such alternative mechanisms of inhibition might include contact-dependent inhibition (Aoki et al., 2005, 2010), hypotheses that require further investigation.

Our in vitro, ex vivo, and in vivo assays indicate that colon resident Clostridiales are important and might even be sufficient to protect the host from *Listeria* invasion. Indeed, GF mice reconstituted with a consortium of four Clostridiales before infection, of which 3 consistently engrafted across experiments, showed virtually no intestinal *L. monocytogenes* burden or translocation to the mesenteric lymph nodes, and markedly reduced penetration into intestinal wall and visceral organs (Fig. 7, B and C; and Fig. S5). In contrast, mice reconstituted with a dysbiotic microbiota were highly susceptible to *L. monocytogenes* infection, demonstrating the importance of selected commensal species in exerting colonization resistance. Identifying protective bacteria and the underlying mechanisms might provide new diagnostic, preventive, and possibly curative approaches for listeriosis.

MATERIALS AND METHODS

Mice

C57BL/6 and *Rag1*$^{-/-}$, *Ifng*$^{-/-}$, *Il17*$^{-/-}$ mice were purchased from the Jackson Laboratory. *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ (Raggc) mice were purchased from Taconic Farms, and then bred in-house. *Rag1*$^{-/-}$*Ifng*$^{-/-}$ *and Rag1*$^{-/-}$*Il17*$^{-/-}$ mice were derived on a C57BL/6 background, initially generated by breeding *Rag1*$^{-/-}$ mice with *Ifng*$^{-/-}$ or *Il17a*$^{-/-}$ mice, respectively, and then bred in house. All mice were bred and maintained under specific pathogen–free conditions at the Memorial Sloan-Kettering Research Animal Resource Center. Sex- and age-matched controls were used in all experiments according to institutional guidelines for animal care. Unless otherwise specified, 6–12-wk-old female mice were used for all experiments. For the experiment shown in Fig. 2 F, mice were maintained in wire floor cages to prevent coprophagy. All animal procedures were approved by the Institutional Animal Care and Use Committee of the Memorial Sloan-Kettering Cancer Center.

Antibiotic and chemotherapy treatment

Three different antibiotic treatments were used over the course of this study: (1) a single i.p. injection of clindamycin (200 µg/mouse in PBS); (2) a single dose of streptomycin (Thermo Fisher Scientific; 20 mg/mouse in PBS) administered by oral gavage; (3) a single dose of metronidazole (Sigma-Aldrich), neomycin (Sigma-Aldrich) and vancomycin (NOVAPLUS; 3.5 mg each/mouse in PBS) administered by oral gavage, followed by a single injection of clindamycin 3 d later. For the experiment shown in Fig. 6, 2 mg streptomycin/mouse was administered to facilitate microbiota recovery.

Chemotherapy was composed of cyclophosphamide (Sigma-Aldrich; 1.8 mg/mouse; ∼100 mg/kg) and doxorubicin (Pfizer; 0.27 mg/mouse; ∼15 mg/kg) and administered together via i.p injection in 200 µl total volume. Chemotherapy was administered twice, 1 wk apart; bleeding or infections were performed 1 d after the second administration. For the experiments shown in Fig. 4 F, mice were administered streptomycin via oral gavage on the same day of second chemotherapy treatment, and infected 24 h later.

L. monocytogenes infection, CFU enumeration, and PCR confirmation

L. monocytogenes strain 10403s was used throughout this study. Frozen aliquots of bacteria were freshly inoculated into BHI and grown to OD 0.1–0.4 (OD = 0.1 corresponds to 2×10^8 CFUs/ml), washed once in PBS, and resuspended in PBS for inoculation. Used doses varied depending on the experiment, and are reported in figure legends; *L. monocytogenes* was administered by oral gavage in all in vivo experiments.

To enumerate *L. monocytogenes* growth in mouse tissue, collected organs were resuspended in PBS Triton X-100 (Thermo Fisher Scientific) 0.05%, homogenized for 30 s to 1 min with a Power Gen 125 homogenizer (Thermo Fisher Scientific; power level: 5). Metal probes were washed in between samples through two immersions in ethanol and one

in PBS for 10–15 s each. Serial dilutions of the homogenates were prepared in PBS Triton and plated on BHI plates supplemented with streptomycin (100 µg/ml) and nalidixic acid (50 µg/ml). Colonies were enumerated after 24–36 h of incubation at 37°C.

For CFU enumeration in intestinal wall, small and large intestine were separated after excision, cleared of content by squeezing with forceps, cut longitudinally, and washed vigorously four to six times (10-s vortex or manual shaking) in ice-cold PBS. The washed tissues were then processed as in the previous paragraph.

For CFU enumeration in intestinal content and fecal pellets, starting material was weighed (unless total CFU amount was calculated) and resuspended in PBS to a concentration of 100 mg/ml. Serial dilutions of the original suspension were plated.

Roughly 10% of plates from small and large intestine content and tissue displayed a second type of bacterial colony, clearly distinguishable from *L. monocytogenes* colonies based on size and color. In particular, contaminating colonies were much smaller than *L. monocytogenes* colonies and of a brighter white color. To confirm the identity of *L. monocytogenes* colonies, several colonies of each type from every contaminated plate were picked and subjected to PCR for the *L. monocytogenes p60* gene. A clear band of ∼1300 bp was always detected following PCR of *L. monocytogenes* colonies, but not of contaminating colonies (see Fig. S1), thus confirming *L. monocytogenes* identity as determined by colony size, color, and morphology. Contaminant colonies were not included into CFU counts. Sequence of the p60 PCR primers are: 5′ START: 5′-GCGGTAACAGCATTTGCT GCTCCAACAATC-3′; 3′ END: 5′-GCCATTGTCTTG CGCGTTAATCATTTGAC-3′. Amplification conditions were as follows: 30 cycles (95°C for 1 min; 50°C for 1 min; 65°C for 2.5 min), 72°C for 5 min, hold 4°C.

Pathology score

The pathology score used in this study has been previously published (Abt et al., 2015). After infection, mice were monitored and scored for disease severity by four parameters: weight loss (>95% of initial weight = 0, 95–90% initial weight = 1, 90–80% initial weight = 2, and <80% initial weight = 3), surface body temperature (>32°C = 0, 32°C–30°C = 1, 30°C–28°C = 2, <28°C = 3), diarrhea severity (formed pellets = 0, loose pellets = 1, liquid discharge = 2, no pellets/caked to fur = 3), morbidity (score of 1 for each symptoms with max score of 3; ruffled fur, hunched back, lethargy, ocular discharge).

Blood cell collection and flow cytometry

Blood was obtained by tail bleeding. Red blood cell lysis was performed by three consecutive incubations in RBC lysis buffer (0.15 M NH_4Cl = 1 mM $NaHCO_3$ in dH_2O) for 5′. Lymphocytes were counted and subjected to viability staining (Fixable Aqua Dead Cell staining, Life Technologies, #L34957) and subsequently to receptor Fc blockade (BD). Staining was performed using the following antibodies: CD45 (clone 30-F11; eBioscience), CD8b (clone YTS156.7.7; BioLegend), CD11b (clone RM2817; Thermo Fisher Scientific), Ly6c (clone AL-21; BD), CD3ε (clone 145-2C11; BD), CD19 (clone 1D3; BD), CD90.2 (53–2.1; BD), CD127 (clone A7R34; BD). Samples were fixed (IC Fixation Buffer; eBioscience), washed, resuspended in FACS buffer, and acquired with a LSRII flow cytometer (BD) either immediately or on the next day.

DNA extraction and 16S sequencing

DNA extraction from fecal pellets and intestinal content were performed as previously described (Ubeda et al., 2012). In brief, a frozen aliquot (∼100 mg) of each sample was suspended, while frozen, in a solution containing 500 µl of extraction buffer (200 mM Tris, pH 8.0, 200 mM NaCl, and 20 mM EDTA), 200 µl of 20% SDS, 500 µl of phenol/chloroform/isoamyl alcohol (24:24:1), and 500 µl of 0.1-mm-diam zirconia/silica beads (BioSpec Products). Microbial cells were lysed by mechanical disruption with a bead beater (BioSpec Products) for 2 min, after which two rounds of phenol/chloroform/isoamyl alcohol extraction were performed. DNA was precipitated with ethanol and resuspended in 50 µl of TE buffer with 100 µg ml^{-1} RNase. The isolated DNA was subjected to additional purification with QIAamp Mini Spin Columns (QIAGEN). For each sample, duplicate 50 µl PCR reactions were performed, each containing 50 ng of purified DNA, 0.2 mM dNTPs, 1.5 mM MgCl2, 2.5 U Platinum Taq DNA polymerase, 2.5 µl of 10X PCR buffer, and 0.5 µM of each primer designed to amplify the V4-V5: 563F (5′-nnnnnnnn-NNNNNNNNNNNN-AYTGGGYDTAAAGNG-3′) and 926R (5′-nnnnnnnn-NNNNNNNNNNNN-CCG TCAATTYHTTTRAGT-3′). A unique 12-base Golay barcodes (Ns) precede the primers for sample identification (Caporaso et al., 2012), and 1–8 additional nucleotides were placed in front of the barcode to offset the sequencing of the primers. Cycling conditions were 94°C for 3 min, followed by 27 cycles of 94°C for 50 s, 51°C for 30 s, and 72°C for 1 min. 72°C for 5 min is used for the final elongation step. Replicate PCRs were pooled, and amplicons were purified using the Qiaquick PCR Purification kit (QIAGEN). PCR products were quantified and pooled at equimolar amounts before Illumina barcodes and adaptors were ligated on using the Illumina TruSeq Sample Preparation protocol. The completed library was sequenced on an Illumina Miseq platform following the Illumina recommended procedures with a paired end 250 × 250 bp kit.

The 16S (V4-V5) paired-end reads were merged and demultiplexed. The UPARSE pipeline (Edgar, 2013) was used to: (1) perform error filtering, using maximum expected error (Emax = 1; Edgar and Flyvbjerg, 2015); (2) group sequences into operational taxonomic units (OTUs) of 97% distance-based similarity; and (3) identify and remove potential chimeric sequences, using both de novo and reference-based

methods. Sequencing data were analyzed and processed using the MOTHUR pipeline (Schloss et al., 2009), and operational taxonomical units (OTU) were classified using a modified version of the Greengenes database (DeSantis et al., 2006).

Anaerobic culturing and ex vivo competition experiments

Commensal anaerobes and intestinal content (unless otherwise stated in the text) were cultured using an anaerobic incubator (Coylabs) at 37°C, using reagents that had been reduced for at least 12 h. For assessment of *L. monocytogenes* survival in vitro, intestinal content or fecal pellets were resuspended at 100 mg/ml in reduced PBS, and inoculated with *L. monocytogenes* (inoculum size indicated in figure legends) in 100 µl total using 96-well plates with U bottom. *Listeria* survival was assessed after 24, 48, or 72-h. In some experiment, the intestinal suspension was sterile filtered (0.22 µm) either directly or after a 24-h culture at 37°C.

Bacterial isolates assessed for antilisterial activity included: *L. gasseri* JV-V03, *C. aldenense* WAL-1872 and *R. gnavus* CC55_001C (BEI resources); *C. innocuum* and *B. producta* (I) isolated in house from mouse intestinal content; *C. ramosum*, *C. saccharogumia*, *Clostridiales 1_7_47FAA*, *C. hathewayi*, *C. bolteae*, *C. aldenense*, *C. asparagiforme*, *Ruminococcus sp.ID8*, *R. gnavus*, *C. scindens*, *Lachnospiraceae 3_1_57FAA_CT1*, *Lachnospiraceae 7_1_58_FAA*, *C. indolis*, *Clostridium sp. 7_3_54FAA*, *E. contortum*, *B. producta* (II) isolated from human stool (Atarashi et al., 2013). All bacteria were grown overnight in BHI supplemented with yeast extract 5 g/liter and L-cysteine 1 g/liter (BD), with the exception of *L. gasseri*, which was grown in MRS broth (Difco). Bacterial species present in the original panel of Atarashi et al. (2013) that were not included in the analysis grew poorly in modified BHI.

Autoclaved cecal content used as a medium in Fig. 7 B was prepared by resuspending large intestinal content into PBS at 100 mg/ml and autoclaving the suspension for 21 min; the suspension was then incubated in the anaerobic chamber for at least 24 h before use.

To assess antilisterial activity, commensals of choice were inoculated at OD = 0.1 into either medium or autoclaved cecal content together with 1,000 *L. monocytogenes* CFUs, and residual *Listeria* CFUs were enumerated 24 h later.

Reconstitution of GF mice

GF mice were maintained in isolators until the day of reconstitution. Upon transfer to the SPF facility, mice were reconstituted via oral gavage with comparable amounts of Clostridiales of interest (*C. ramosum, C. saccharogumia, C. hathewayi*, and *B. producta* [I]) of ~1–3 × 10^6/bacterium/mouse, resuspended in 200 µl of reduced PBS. Alternatively, GF mice were reconstituted with a suspension in reduced PBS of a fecal pellet from MNVC-treated mice (pellet collected 1 d after clindamycin treatment, 1 pellet per ml, 200 µl/mouse). Engraftment was confirmed by plating of fecal pellets onto Columbia Agar Plates with 5% Sheep Blood (BD) in anaerobic chamber, 2 d after reconstitution.

Statistical and data analysis

Data are presented as means or medians ± SD. Analyses were performed using GraphPad Prism version 7.0a or R-3.3.2.pkg. Statistical tests used included: Mann-Whitney test for two group comparisons, Kruskall-Wallis test with Dunn's multiple comparisons for three or more group comparisons, two-way ANOVA for time courses, and Log-Rank test for survival. Significance values are indicated as follow: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$. Spearman correlation for the experiment shown in Fig. 6 was calculated using the function 'cor.test' in the R 'stats' package, method="spearman"; significance values were corrected using Benjamini-Hochberg (BH) procedure, FDR<0.05. PCoA was performed using the 'ordinate' function in the R package 'phyloseq', with method="PCoA", distance="JSD".

Human subjects

Patients at MSKCC who developed *L. monocytogenes* infection (bloodstream infection, meningitis, or both) were identified via institutional clinical database query of the last 20 yr. Medical charts of these patients were reviewed; clinical data including extent of infection and medications administered before infection were collected. This retrospective observational review was approved by the MSKCC IRB.

Online supplemental material

Fig. S1 shows examples of colony morphology and PCR results used to confirm the identity of *L. monocytogenes* from plated intestinal content and organs. Fig. S2 shows that *L. monocytogenes* strain 10403s (the strain used in this study) is resistant to streptomycin and metronidazole, but sensitive to vancomycin, neomycin and clindamycin. Fig. S3 shows that most oncologic patients diagnosed with *L. monocytogenes* infection at MSK CC over the past twenty years were treated with chemotherapy, antibiotics, or both before becoming infected. Fig. S4 shows that recovery of clostridia following antibiotic treatment inversely correlates with luminal *L. monocytogenes* expansion in mice infected at different times during microbiota recovery. Fig. S5 shows that reconstitution levels and overall microbiota diversity are similar in GF mice reconstituted with fecal pellets from ABX-treated mice or with the assembled 4-Clost consortium. Nonetheless, fecal pellets from 4-Clost group had significantly higher ex vivo antilisterial potential than FMT-ABX.

ACKNOWLEDGMENTS

We thank all members of the Eric Pamer Lab, Memorial Sloan-Kettering Cancer Center, for their valuable suggestions. Human-derived clostridia were kindly provided by

Kenya Honda (Institute of Physical and Chemical Research, Japan). The following reagents were obtained through BEI Resources, NIAID, National Institutes of Health as part of the Human Microbiome Project: *Clostridium aldenense*, strain WAL-1872, HM-307; *Ruminococcus gnavus*, strain CC55_001C, HM-1056; *Lactobacillus gasseri*, strain JV-V03, HM-104. Lactobacillus gasseri, strain JV-V03 was initially kindly provided by Michael Fischbach (UCSF), while *L. monocytogenes* strain 10403s was a gift of Dan Portnoy (UC Berkeley). We thank Mesruh Turkekul and Ning Fan at the Memorial Sloan-Kettering Molecular Cytology Facility for support with histology slide preparation. We thank the Lucille Castori Center for Microbes, Inflammation and Cancer for assistance with high-throughput sequencing and analysis.

This study was supported by the National Institutes of Health (grant K23 AI095398-01 to Y.T., RO1 AI42135 to E.G.P. and Y.T., U01 AI124275-01 to E.G.P. and Y.T., P30 CA008748), the Lucille Castori Center for Microbes, Inflammation, and Cancer, and the Tow Foundation grant to EGP. SB was supported by a Swiss National Science Foundation Early Postdoc Mobility Fellowship and is currently a Cancer Research Institute Irvington Fellow supported by the Cancer Research Institute.

The authors declare no competing financial interests.

Author contributions SB designed the study, conducted all experiments, acquired and analyzed data, and wrote the manuscript. SB, EL, LL and YT analyzed sequencing data. SM collected patient data. LL, YG and EF prepared 16s amplicon libraries for MySeq analysis. RC, SK and IL assisted with *in vitro* and *in vivo* experiments. EGP designed the study, provided overall guidance and wrote the manuscript.

Submitted: 16 March 2017
Revised: 6 April 2017
Accepted: 19 April 2017

REFERENCES

Abt, M.C., B.B. Lewis, S. Caballero, H. Xiong, R.A. Carter, B. Sušac, L. Ling, I. Leiner, and E.G. Pamer. 2015. Innate Immune Defenses Mediated by Two ILC Subsets Are Critical for Protection against Acute Clostridium difficile Infection. *Cell Host Microbe.* 18:27–37. http://dx.doi.org/10.1016/j.chom.2015.06.011

Allerberger, F., and M. Wagner. 2010. Listeriosis: a resurgent foodborne infection. *Clin. Microbiol. Infect.* 16:16–23. http://dx.doi.org/10.1111/j.1469-0691.2009.03109.x

Alyamkina, E.A., V.P. Nikolin, N.A. Popova, E.V. Dolgova, A.S. Proskurina, K.E. Orishchenko, Y.R. Efremov, E.R. Chernykh, A.A. Ostanin, S.V. Sidorov, et al. 2010. A strategy of tumor treatment in mice with doxorubicin-cyclophosphamide combination based on dendritic cell activation by human double-stranded DNA preparation. *Genet. Vaccines Ther.* 8:7. http://dx.doi.org/10.1186/1479-0556-8-7

Andersson, A., W.J. Dai, J.P. Di Santo, and F. Brombacher. 1998. Early IFN-gamma production and innate immunity during Listeria monocytogenes infection in the absence of NK cells. *J. Immunol.* 161:5600–5606.

Aoki, S.K., R. Pamma, A.D. Hernday, J.E. Bickham, B.A. Braaten, and D.A. Low. 2005. Contact-dependent inhibition of growth in Escherichia coli. *Science.* 309:1245–1248. http://dx.doi.org/10.1126/science.1115109

Aoki, S.K., E.J. Diner, C.T. de Roodenbeke, B.R. Burgess, S.J. Poole, B.A. Braaten, A.M. Jones, J.S. Webb, C.S. Hayes, P.A. Cotter, and D.A. Low. 2010. A widespread family of polymorphic contact-dependent toxin delivery systems in bacteria. *Nature.* 468:439–442. http://dx.doi.org/10.1038/nature09490

Atarashi, K., T. Tanoue, K. Oshima, W. Suda, Y. Nagano, H. Nishikawa, S. Fukuda, T. Saito, S. Narushima, K. Hase, et al. 2013. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature.* 500:232–236. http://dx.doi.org/10.1038/nature12331

Aureli, P., G.C. Fiorucci, D. Caroli, G. Marchiaro, O. Novara, L. Leone, and S. Salmaso. 2000. An outbreak of febrile gastroenteritis associated with corn contaminated by Listeria monocytogenes. *N. Engl. J. Med.* 342:1236–1241. http://dx.doi.org/10.1056/NEJM200004273421702

Becattini, S., Y. Taur, and E.G. Pamer. 2016. Antibiotic-Induced Changes in the Intestinal Microbiota and Disease. *Trends Mol. Med.* 22:458–478. http://dx.doi.org/10.1016/j.molmed.2016.04.003

Bohnhoff, M., and C.P. Miller. 1962. Enhanced susceptibility to Salmonella infection in streptomycin-treated mice. *J. Infect. Dis.* 111:117–127. http://dx.doi.org/10.1093/infdis/111.2.117

Bojsen-Moller, J. 1964. Occurrence of Listeria monocytogenes in feces from healthy and sick persons. *Proc. of the XIV Scandinavian Congr. of Pathol., and Microbiol., Oslo-Norwegian Universities Press.*

Bou Ghanem, E.N., G.S. Jones, T. Myers-Morales, P.D. Patil, A.N. Hidayatullah, and S.E. D'Orazio. 2012. InlA promotes dissemination of Listeria monocytogenes to the mesenteric lymph nodes during food borne infection of mice. *PLoS Pathog.* 8:e1003015. http://dx.doi.org/10.1371/journal.ppat.1003015

Brandl, K., G. Plitas, B. Schnabl, R.P. DeMatteo, and E.G. Pamer. 2007. MyD88-mediated signals induce the bactericidal lectin RegIII gamma and protect mice against intestinal Listeria monocytogenes infection. *J. Exp. Med.* 204:1891–1900. http://dx.doi.org/10.1084/jem.20070563

Brandl, K., G. Plitas, C.N. Mihu, C. Ubeda, T. Jia, M. Fleisher, B. Schnabl, R.P. DeMatteo, and E.G. Pamer. 2008. Vancomycin-resistant enterococci exploit antibiotic-induced innate immune deficits. *Nature.* 455:804–807. http://dx.doi.org/10.1038/nature07250

Bregenholt, S., P. Berche, F. Brombacher, and J.P. Di Santo. 2001. Conventional alpha beta T cells are sufficient for innate and adaptive immunity against enteric Listeria monocytogenes. *J. Immunol.* 166:1871–1876. http://dx.doi.org/10.4049/jimmunol.166.3.1871

Buffie, C.G., and E.G. Pamer. 2013. Microbiota-mediated colonization resistance against intestinal pathogens. *Nat. Rev. Immunol.* 13:790–801. http://dx.doi.org/10.1038/nri3535

Buffie, C.G., V. Bucci, R.R. Stein, P.T. McKenney, L. Ling, A. Gobourne, D. No, H. Liu, M. Kinnebrew, A. Viale, et al. 2015. Precision microbiome reconstitution restores bile acid mediated resistance to Clostridium difficile. *Nature.* 517:205–208. http://dx.doi.org/10.1038/nature13828

Caballero, S., and E.G. Pamer. 2015. Microbiota-mediated inflammation and antimicrobial defense in the intestine. *Annu. Rev. Immunol.* 33:227–256. http://dx.doi.org/10.1146/annurev-immunol-032713-120238

Caporaso, J.G., C.L. Lauber, W.A. Walters, D. Berg-Lyons, J. Huntley, N. Fierer, S.M. Owens, J. Betley, L. Fraser, M. Bauer, et al. 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J.* 6:1621–1624. http://dx.doi.org/10.1038/ismej.2012.8

CENTERS FOR DISEASE CONTROL AND PREVENTION (CDC). 2016. *Listeria Outbreaks* [Online]. Available: http://www.cdc.gov/listeria/outbreaks/index.html.

Chiba, S., T. Nagai, T. Hayashi, Y. Baba, S. Nagai, and S. Koyasu. 2011. Listerial invasion protein internalin B promotes entry into ileal Peyer's patches in vivo. *Microbiol. Immunol.* 55:123–129. http://dx.doi.org/10.1111/j.1348-0421.2010.00292.x

Clark, S.E., H.C. Filak, B.S. Guthrie, R.L. Schmidt, A. Jamieson, P. Merkel, V. Knight, C.M. Cole, D.H. Raulet, and L.L. Lenz. 2016. Bacterial Manipulation of NK Cell Regulatory Activity Increases Susceptibility to Listeria monocytogenes Infection. *PLoS Pathog.* 12:e1005708. http://dx.doi.org/10.1371/journal.ppat.1005708

Cobb, C.A., G.D. Curtis, D.S. Bansi, E. Slade, W. Mehal, R.G. Mitchell, and R.W. Chapman. 1996. Increased prevalence of Listeria monocytogenes in the faeces of patients receiving long-term H2-antagonists. *Eur. J. Gastroenterol. Hepatol.* 8:1071–1074. http://dx.doi.org/10.1097/00042737-199611000-00008

Corr, S., C. Hill, and C.G. Gahan. 2006. An in vitro cell-culture model demonstrates internalin- and hemolysin-independent translocation of Listeria monocytogenes across M cells. *Microb. Pathog.* 41:241–250. http://dx.doi.org/10.1016/j.micpath.2006.08.003

Corr, S.C., Y. Li, C.U. Riedel, P.W. O'Toole, C. Hill, and C.G. Gahan. 2007. Bacteriocin production as a mechanism for the antiinfective activity of Lactobacillus salivarius UCC118. *Proc. Natl. Acad. Sci. USA.* 104:7617–7621. http://dx.doi.org/10.1073/pnas.0700440104

Czuprynski, C.J., and E. Balish. 1981. Pathogenesis of Listeria monocytogenes for gnotobiotic rats. *Infect. Immun.* 32:323–331.

Dalton, C.B., C.C. Austin, J. Sobel, P.S. Hayes, W.F. Bibb, L.M. Graves, B. Swaminathan, M.E. Proctor, and P.M. Griffin. 1997. An outbreak of gastroenteritis and fever due to Listeria monocytogenes in milk. *N. Engl. J. Med.* 336:100–105. http://dx.doi.org/10.1056/NEJM199701093360204

Delgado, S., E. O'Sullivan, G. Fitzgerald, and B. Mayo. 2007. Subtractive screening for probiotic properties of lactobacillus species from the human gastrointestinal tract in the search for new probiotics. *J. Food Sci.* 72:M310–M315. http://dx.doi.org/10.1111/j.1750-3841.2007.00479.x

DeSantis, T.Z., P. Hugenholtz, N. Larsen, M. Rojas, E.L. Brodie, K. Keller, T. Huber, D. Dalevi, P. Hu, and G.L. Andersen. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl. Environ. Microbiol.* 72:5069–5072. http://dx.doi.org/10.1128/AEM.03006-05

Donia, M.S., P. Cimermancic, C.J. Schulze, L.C. Wieland Brown, J. Martin, M. Mitreva, J. Clardy, R.G. Linington, and M.A. Fischbach. 2014. A systematic analysis of biosynthetic gene clusters in the human microbiome reveals a common family of antibiotics. *Cell.* 158:1402–1414. http://dx.doi.org/10.1016/j.cell.2014.08.032

Dunn, P.L., and R.J. North. 1991. Early gamma interferon production by natural killer cells is important in defense against murine listeriosis. *Infect. Immun.* 59:2892–2900.

Edgar, R.C. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods.* 10:996–998. http://dx.doi.org/10.1038/nmeth.2604

Edgar, R.C., and H. Flyvbjerg. 2015. Error filtering, pair assembly and error correction for next-generation sequencing reads. *Bioinformatics.* 31:3476–3482. http://dx.doi.org/10.1093/bioinformatics/btv401

Egan, K., D. Field, M.C. Rea, R.P. Ross, C. Hill, and P.D. Cotter. 2016. Bacteriocins: Novel Solutions to Age Old Spore-Related Problems? *Front. Microbiol.* 7:461. http://dx.doi.org/10.3389/fmicb.2016.00461

Fernández Guerrero, M.L., R. Torres, B. Mancebo, J.J. González-López, M. Górgolas, J.J. Jusdado, and R.F. Roblas. 2012. Antimicrobial treatment of invasive non-perinatal human listeriosis and the impact of the underlying disease on prognosis. *Clin. Microbiol. Infect.* 18:690–695. http://dx.doi.org/10.1111/j.1469-0691.2011.03616.x

Gahan, C.G., and C. Hill. 2005. Gastrointestinal phase of Listeria monocytogenes infection. *J. Appl. Microbiol.* 98:1345–1353. http://dx.doi.org/10.1111/j.1365-2672.2005.02559.x

Gessain, G., Y.H. Tsai, L. Travier, M. Bonazzi, S. Grayo, P. Cossart, C. Charlier, O. Disson, and M. Lecuit. 2015. PI3-kinase activation is critical for host barrier permissiveness to Listeria monocytogenes. *J. Exp. Med.* 212:165–183. http://dx.doi.org/10.1084/jem.20141406

Gillespie, I.A., J. McLauchlin, C.L. Little, C. Penman, P. Mook, K. Grant, and S.J. O'Brien. 2009. Disease presentation in relation to infection foci for non-pregnancy-associated human listeriosis in England and Wales, 2001 to 2007. *J. Clin. Microbiol.* 47:3301–3307. http://dx.doi.org/10.1128/JCM.00969-09

Goulet, V., M. Hebert, C. Hedberg, E. Laurent, V. Vaillant, H. De Valk, and J.C. Desenclos. 2012. Incidence of listeriosis and related mortality among groups at risk of acquiring listeriosis. *Clin. Infect. Dis.* 54:652–660. http://dx.doi.org/10.1093/cid/cir902

Grif, K., G. Patscheider, M.P. Dierich, and F. Allerberger. 2003. Incidence of fecal carriage of Listeria monocytogenes in three healthy volunteers: a one-year prospective stool survey. *Eur. J. Clin. Microbiol. Infect. Dis.* 22:16–20.

Hardy, J., K.P. Francis, M. DeBoer, P. Chu, K. Gibbs, and C.H. Contag. 2004. Extracellular replication of Listeria monocytogenes in the murine gall bladder. *Science.* 303:851–853. http://dx.doi.org/10.1126/science.1092712

Hoelzer, K., R. Pouillot, and S. Dennis. 2012. Animal models of listeriosis: a comparative review of the current state of the art and lessons learned. *Vet. Res. (Faisalabad).* 43:18. http://dx.doi.org/10.1186/1297-9716-43-18

Huleatt, J.W., I. Pilip, K. Kerksiek, and E.G. Pamer. 2001. Intestinal and splenic T cell responses to enteric Listeria monocytogenes infection: distinct repertoires of responding CD8 T lymphocytes. *J. Immunol.* 166:4065–4073. http://dx.doi.org/10.4049/jimmunol.166.6.4065

Kampelmacher, E.H., and L.M. van Noorle Jansen. 1969. Isolation of Listeria monocytogenes from faeces of clinically healthy humans and animals. *Zentralbl. Bakteriol. [Orig].* 211:353–359.

Khosravi, A., A. Yáñez, J.G. Price, A. Chow, M. Merad, H.S. Goodridge, and S.K. Mazmanian. 2014. Gut microbiota promote hematopoiesis to control bacterial infection. *Cell Host Microbe.* 15:374–381. http://dx.doi.org/10.1016/j.chom.2014.02.006

Koren, O., J.K. Goodrich, T.C. Cullender, A. Spor, K. Laitinen, H.K. Bäckhed, A. Gonzalez, J.J. Werner, L.T. Angenent, R. Knight, et al. 2012. Host remodeling of the gut microbiome and metabolic changes during pregnancy. *Cell.* 150:470–480. http://dx.doi.org/10.1016/j.cell.2012.07.008

Ladel, C.H., C. Blum, and S.H. Kaufmann. 1996. Control of natural killer cell-mediated innate resistance against the intracellular pathogen Listeria monocytogenes by gamma/delta T lymphocytes. *Infect. Immun.* 64:1744–1749.

Lakshminarayanan, B., C.M. Guinane, P.M. O'Connor, M. Coakley, C. Hill, C. Stanton, P.W. O'Toole, and R.P. Ross. 2013. Isolation and characterization of bacteriocin-producing bacteria from the intestinal microbiota of elderly Irish subjects. *J. Appl. Microbiol.* 114:886–898. http://dx.doi.org/10.1111/jam.12085

Lecuit, M. 2005. Understanding how Listeria monocytogenes targets and crosses host barriers. *Clin. Microbiol. Infect.* 11:430–436. http://dx.doi.org/10.1111/j.1469-0691.2005.01146.x

Lecuit, M., S. Dramsi, C. Gottardi, M. Fedor-Chaiken, B. Gumbiner, and P. Cossart. 1999. A single amino acid in E-cadherin responsible for host specificity towards the human pathogen Listeria monocytogenes. *EMBO J.* 18:3956–3963. http://dx.doi.org/10.1093/emboj/18.14.3956

Lecuit, M., S. Vandormael-Pournin, J. Lefort, M. Huerre, P. Gounon, C. Dupuy, C. Babinet, and P. Cossart. 2001. A transgenic model for listeriosis: role of internalin in crossing the intestinal barrier. *Science.* 292:1722–1725. http://dx.doi.org/10.1126/science.1059852

Lembet, A., S. Gaddipati, I.R. Holzman, R.L. Berkowitz, and E.J. Bottone. 2003. Meconium enhances the growth of perinatal bacterial pathogens. *Mt. Sinai J. Med.* 70:126–129.

MacDonald, T.T., and P.B. Carter. 1980. Cell-mediated immunity to intestinal infection. *Infect. Immun.* 28:516–523.

MacGowan, A.P., K. Bowker, J. McLauchlin, P.M. Bennett, and D.S. Reeves. 1994. The occurrence and seasonal changes in the isolation of Listeria spp. in shop bought food stuffs, human faeces, sewage and soil from urban sources. *Int. J. Food Microbiol.* 21:325–334. http://dx.doi.org/10.1016/0168-1605(94)90062-0

Mainetti, L.E., M.J. Rico, M.V. Fernández-Zenobi, H.A. Perroud, E.A. Roggero, V.R. Rozados, and O.G. Scharovsky. 2013. Therapeutic efficacy of metronomic chemotherapy with cyclophosphamide and doxorubicin on murine mammary adenocarcinomas. *Ann. Oncol.* 24:2310–2316. http://dx.doi.org/10.1093/annonc/mdt164

Maltby, R., M.P. Leatham-Jensen, T. Gibson, P.S. Cohen, and T. Conway. 2013. Nutritional basis for colonization resistance by human commensal Escherichia coli strains HS and Nissle 1917 against E. coli O157:H7 in the mouse intestine. *PLoS One.* 8:e53957. http://dx.doi.org/10.1371/journal.pone.0053957

Marco, A.J., J. Altimira, N. Prats, S. López, L. Dominguez, M. Domingo, and V. Briones. 1997. Penetration of Listeria monocytogenes in mice infected by the oral route. *Microb. Pathog.* 23:255–263. http://dx.doi.org/10.1006/mpat.1997.0144

Melton-Witt, J.A., S.M. Rafelski, D.A. Portnoy, and A.I. Bakardjiev. 2012. Oral infection with signature-tagged Listeria monocytogenes reveals organ-specific growth and dissemination routes in guinea pigs. *Infect. Immun.* 80:720–732. http://dx.doi.org/10.1128/IAI.05958-11

Mook, P., S.J. O'Brien, and I.A. Gillespie. 2011. Concurrent conditions and human listeriosis, England, 1999-2009. *Emerg. Infect. Dis.* 17:38–43. http://dx.doi.org/10.3201/eid1701.101174

Myers-Morales, T., K.M. Bussell, and S.E. D'Orazio. 2013. Fecal transplantation does not transfer either susceptibility or resistance to food borne listeriosis in C57BL/6 and BALB/c/By mice. *F1000 Res.* 2:177.

Narushima, S., Y. Sugiura, K. Oshima, K. Atarashi, M. Hattori, M. Suematsu, and K. Honda. 2014. Characterization of the 17 strains of regulatory T cell-inducing human-derived Clostridia. *Gut Microbes.* 5:333–339. http://dx.doi.org/10.4161/gmic.28572

Nikitas, G., C. Deschamps, O. Disson, T. Niault, P. Cossart, and M. Lecuit. 2011. Transcytosis of Listeria monocytogenes across the intestinal barrier upon specific targeting of goblet cell accessible E-cadherin. *J. Exp. Med.* 208:2263–2277. http://dx.doi.org/10.1084/jem.20110560

Okamoto, M., A. Nakane, and T. Minagawa. 1994. Host resistance to an intragastric infection with Listeria monocytogenes in mice depends on cellular immunity and intestinal bacterial flora. *Infect. Immun.* 62:3080–3085.

Pamer, E.G. 2004. Immune responses to Listeria monocytogenes. *Nat. Rev. Immunol.* 4:812–823. http://dx.doi.org/10.1038/nri1461

Pinner, R.W., A. Schuchat, B. Swaminathan, P.S. Hayes, K.A. Deaver, R.E. Weaver, B.D. Plikaytis, M. Reeves, C.V. Broome, and J.D. Wenger. The Listeria Study Group. 1992. Role of foods in sporadic listeriosis. II. Microbiologic and epidemiologic investigation. *JAMA.* 267:2046–2050. http://dx.doi.org/10.1001/jama.1992.03480150052036

Pron, B., C. Boumaila, F. Jaubert, S. Sarnacki, J.P. Monnet, P. Berche, and J.L. Gaillard. 1998. Comprehensive study of the intestinal stage of listeriosis in a rat ligated ileal loop system. *Infect. Immun.* 66:747–755.

Ruhe, Z.C., D.A. Low, and C.S. Hayes. 2013. Bacterial contact-dependent growth inhibition. *Trends Microbiol.* 21:230–237. http://dx.doi.org/10.1016/j.tim.2013.02.003

Saraoui, T., P.A. Fall, F. Leroi, J.P. Antignac, S. Chéreau, and M.F. Pilet. 2016. Inhibition mechanism of Listeria monocytogenes by a bioprotective bacteria Lactococcus piscium CNCM I-4031. *Food Microbiol.* 53(Pt A):70–78. http://dx.doi.org/10.1016/j.fm.2015.01.002

Schloss, P.D., S.L. Westcott, T. Ryabin, J.R. Hall, M. Hartmann, E.B. Hollister, R.A. Lesniewski, B.B. Oakley, D.H. Parks, C.J. Robinson, et al. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* 75:7537–7541. http://dx.doi.org/10.1128/AEM.01541-09

Schuchat, A., K.A. Deaver, J.D. Wenger, B.D. Plikaytis, L. Mascola, R.W. Pinner, A.L. Reingold, and C.V. Broome. The Listeria Study Group. 1992. Role of foods in sporadic listeriosis. I. Case-control study of dietary risk factors. *JAMA.* 267:2041–2045. http://dx.doi.org/10.1001/jama.1992.03480150047035

Swaminathan, B., and P. Gerner-Smidt. 2007. The epidemiology of human listeriosis. *Microbes Infect.* 9:1236–1243. http://dx.doi.org/10.1016/j.micinf.2007.05.011

Teixeira, H.C., and S.H. Kaufmann. 1994. Role of NK1.1+ cells in experimental listeriosis. NK1+ cells are early IFN-gamma producers but impair resistance to Listeria monocytogenes infection. *J. Immunol.* 152:1873–1882.

Thäle, C., and A.F. Kiderlen. 2005. Sources of interferon-gamma (IFN-gamma) in early immune response to Listeria monocytogenes. *Immunobiology.* 210:673–683. http://dx.doi.org/10.1016/j.imbio.2005.07.003

Ubeda, C., L. Lipuma, A. Gobourne, A. Viale, I. Leiner, M. Equinda, R. Khanin, and E.G. Pamer. 2012. Familial transmission rather than defective innate immunity shapes the distinct intestinal microbiota of TLR-deficient mice. *J. Exp. Med.* 209:1445–1456. http://dx.doi.org/10.1084/jem.20120504

Ubeda, C., V. Bucci, S. Caballero, A. Djukovic, N.C. Toussaint, M. Equinda, L. Lipuma, L. Ling, A. Gobourne, D. No, et al. 2013. Intestinal microbiota containing Barnesiella species cures vancomycin-resistant Enterococcus faecium colonization. *Infect. Immun.* 81:965–973. http://dx.doi.org/10.1128/IAI.01197-12

van Nood, E., A. Vrieze, M. Nieuwdorp, S. Fuentes, E.G. Zoetendal, W.M. de Vos, C.E. Visser, E.J. Kuijper, J.F. Bartelsman, J.G. Tijssen, et al. 2013. Duodenal infusion of donor feces for recurrent Clostridium difficile. *N. Engl. J. Med.* 368:407–415. http://dx.doi.org/10.1056/NEJMoa1205037

Vázquez-Boland, J.A., M. Kuhn, P. Berche, T. Chakraborty, G. Domínguez-Bernal, W. Goebel, B. González-Zorn, J. Wehland, and J. Kreft. 2001. Listeria pathogenesis and molecular virulence determinants. *Clin. Microbiol. Rev.* 14:584–640. http://dx.doi.org/10.1128/CMR.14.3.584-640.2001

Viaud, S., F. Saccheri, G. Mignot, T. Yamazaki, R. Daillère, D. Hannani, D.P. Enot, C. Pfirschke, C. Engblom, M.J. Pittet, et al. 2013. The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. *Science.* 342:971–976. http://dx.doi.org/10.1126/science.1240537

Viegas, N., L. Andzinski, C.F. Wu, R.M. Komoll, N. Gekara, K.E. Dittmar, S. Weiss, and J. Jablonska. 2013. IFN-γ production by $CD27^+$ NK cells exacerbates Listeria monocytogenes infection in mice by inhibiting granulocyte mobilization. *Eur. J. Immunol.* 43:2626–2637. http://dx.doi.org/10.1002/eji.201242937

Vijayakumar, P.P., and P.M. Muriana. 2015. A Microplate Growth Inhibition Assay for Screening Bacteriocins against Listeria monocytogenes to Differentiate Their Mode-of-Action. *Biomolecules.* 5:1178–1194. http://dx.doi.org/10.3390/biom5021178

Wollert, T., B. Pasche, M. Rochon, S. Deppenmeier, J. van den Heuvel, A.D. Gruber, D.W. Heinz, A. Lengeling, and W.D. Schubert. 2007. Extending the host range of Listeria monocytogenes by rational protein design. *Cell.* 129:891–902. http://dx.doi.org/10.1016/j.cell.2007.03.049

Zachar, Z., and D.C. Savage. 1979. Microbial interference and colonization of the murine gastrointestinal tract by Listeria monocytogenes. *Infect. Immun.* 23:168–174.

Zhu, W.M., W. Liu, and D.Q. Wu. 2000. Isolation and characterization of a new bacteriocin from Lactobacillus gasseri KT7. *J. Appl. Microbiol.* 88:877–886. http://dx.doi.org/10.1046/j.1365-2672.2000.01027.x