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Fecal microbiota transplant rescues mice from human pathogen mediated sepsis by restoring systemic immunity

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Death due to sepsis remains a persistent threat to critically ill patients confined to the intensive care unit and is characterized by colonization with multi-drug-resistant healthcare-associated pathogens. Here we report that sepsis in mice caused by a defined four-member pathogen community isolated from a patient with lethal sepsis is associated with the systemic suppression of key elements of the host transcriptome required for pathogen clearance and decreased butyrate expression. More specifically, these pathogens directly suppress interferon regulatory factor 3. Fecal microbiota transplant (FMT) reverses the course of otherwise lethal sepsis by enhancing pathogen clearance via the restoration of host immunity in an interferon regulatory factor 3-dependent manner. Taken together these results suggest that fecal microbiota transplantation may be a treatment option in sepsis associated with immunosuppression.
Since the discovery of penicillin in 1928, antibiotics have saved millions of lives, however, their promiscuous use in medicine and agriculture has borne witness to the emergence of multidrug resistant (MDR) pathogens which has become one of the most pressing issues in public health today\(^1\). The most common cause of death in modern intensive care units is “late-onset sepsis,” a disorder characterized by colonization with highly virulent MDR pathogens\(^3\). Colonization of critically ill patients with MDR pathogens is likely a result of the unusual selective pressures to which these patients and their microbiomes are exposed, including life support measures, prolonged antibiotic use, polypharmacy, and artificial feeding. These well-intentioned interventions can result in collapse of the microbiome, the dysregulation of which is increasingly being demonstrated to have a major adverse effect on the immune system. Here we demonstrate that a fecal microbiota transplant (FMT) can rescue mice from lethal sepsis due to a defined four-member pathogen community (PC) isolated from a critically ill patient by reversing the immunosuppressive effect of this PC.

Results

FMT rescues mice from gut-derived sepsis. We infected mice with a PC isolated from the stool of a surgical patient who died of late-onset sepsis, consisting of three species of bacteria (tetracycline resistant Enterococcus faecalis, MDR Klebsiella oxytoca, and MDR Serratia marcescens) and one species of yeast (Candida albicans)\(^4\) (Supplementary Table 1). With advances in sequencing, it is now known that the gut is the primary site of colonization and emergence of the “antibiotic resistome” consisting of MDR pathogens and is a major source of lethal systemic infections\(^5\). Therefore, we first inoculated this four-member PC into the gut using a model that exposes the PC to the selective pressures of surgical injury and its treatment. In this model, mice were subjected to 30% hepatectomy, with preoperative food-squeeze of surgical injury and its treatment. In this model, mice (Mann–Whitney test, \(P<1\times 10^{-15}\)) (Fig. 1d), suggesting that FMT treatment restores a dysregulated transcriptional program associated with PC dissemination. Pathway and database analysis of the PC-induced dysregulated transcriptional program (Supplementary Fig. 1, Supplementary Data 1, and 2) suggested that the systemic dissemination of pathogens was accompanied by a coordinated abrogation of signaling downstream of pattern recognition receptors (PRRs), a pathway that is commonly targeted by microbial pathogens and required for their clearance\(^13\). Both interferon regulatory factor 3 (IRF3) and the nuclear factor-kappa B (NF-kB) signaling pathways were dysregulated in AC-FMT-treated septic mice, while FMT treatment restored these pathways back to homeostatic levels in three different organs, two of which are remote from the gut (Supplementary Fig. 1a, Supplementary Data 1, and 2).

Intriguingly, although the translocation of the PC began before treatment with FMT on POD1 (Fig. 1b), FMT was still able to drive a reduction in PC burden in systemic organs (Fig. 1d) suggesting two possible, but non-mutually exclusive mechanisms. First, FMT could promote colonization resistance and the competitive exclusion of the PC within the gut environment in a manner similar to the mechanism by which FMT clears recurrent Clostridium difficile infection\(^10\–\12\) thereby preventing further translocation and facilitating systemic clearance of the PC. Indeed, temporal sequencing of 16S rRNA of cecal contents demonstrated that FMT, but not AC-FMT treatment, restored intestinal microbial diversity (Supplementary Fig. 2a–d and Supplementary Table 3) in association with a marked reduction of all three inoculated bacterial pathogens over time (Supplementary Fig. 2e–g). By POD7, the inoculated pathogens were completely absent from the ceca of FMT-treated mice (Supplementary Fig. 2e–g). A second mechanism might involve the ability of FMT to drive a recovery-directed immune response at the systemic level, as suggested by our transcriptional analysis (Fig. 1e,f, Supplementary Fig. 1, Supplementary Data 1 and 2), thereby enhancing bacterial clearance in peripheral organs and preventing the progression to lethal infection.

FMT drives the clearance of systemically disseminated PC. To test the latter possibility that FMT drives a systemic recovery-directed immune response in the above model, we injected the PC directly into the peritoneal cavity (intraperitoneal-i.p.) which caused immediate systemic dissemination of pathogens; in this series of experiments there was no fasting, surgery, or antibiotics (IP model) (Fig. 2a). Therefore, this model separated the FMT treatment and PC inoculation into physically isolated compartments (i.e., FMT via enema, PC via i.p. injection) thus allowing assessment of the direct role of FMT on systemic PC clearance. In this model, PC inoculation resulted in immediate systemic sepsis and death in more than 60% of mice in less than 72 h (PC) (Fig. 2b). To assess the ability of an FMT enema to reverse the mortality from i.p. administered PC, mice were treated with gene expression in the cecum, liver, and spleen on POD2, the time point where we observed FMT-driven reduction of systemic pathogen burden (Fig. 1d). We looked at genes that were coordinately regulated across all three organs and observed significant changes in gene expression in both FMT and AC-FMT-treated groups compared with non-manipulated control mice (referred to as “Untreated” hereafter) (Fig. 1e and Supplementary Data 1). However, the number of differently expressed genes was approximately tenfold less (349 vs. 3242 at an FDR ≤ 0.01) in FMT-treated mice as compared with AC-FMT-treated septic mice (Fig. 1e and Supplementary Data 1). Among differently expressed genes, the magnitude of the gene expression changes was also consistently lower in FMT-treated mice (Mann–Whitney test, \(P<1\times 10^{-15}\)) (Fig. 1d), suggesting that FMT treatment restores a dysregulated transcriptional program associated with PC dissemination. Pathway and database analysis of the PC-induced dysregulated transcriptional program (Supplementary Fig. 1, Supplementary Data 1, and 2) suggested that the systemic dissemination of pathogens was accompanied by a coordinated abrogation of signaling downstream of pattern recognition receptors (PRRs), a pathway that is commonly targeted by microbial pathogens and required for their clearance\(^13\). Both interferon regulatory factor 3 (IRF3) and the nuclear factor-kappa B (NF-kB) signaling pathways were dysregulated in AC-FMT-treated septic mice, while FMT treatment restored these pathways back to homeostatic levels in three different organs, two of which are remote from the gut (Supplementary Fig. 1a, Supplementary Data 1, and 2).

Intriguingly, although the translocation of the PC began before treatment with FMT on POD1 (Fig. 1b), FMT was still able to drive a reduction in PC burden in systemic organs (Fig. 1d) suggesting two possible, but non-mutually exclusive mechanisms. First, FMT could promote colonization resistance and the competitive exclusion of the PC within the gut environment in a manner similar to the mechanism by which FMT clears recurrent Clostridium difficile infection\(^10\–\12\) thereby preventing further translocation and facilitating systemic clearance of the PC. Indeed, temporal sequencing of 16S rRNA of cecal contents demonstrated that FMT, but not AC-FMT treatment, restored intestinal microbial diversity (Supplementary Fig. 2a–d and Supplementary Table 3) in association with a marked reduction of all three inoculated bacterial pathogens over time (Supplementary Fig. 2e–g). By POD7, the inoculated pathogens were completely absent from the ceca of FMT-treated mice (Supplementary Fig. 2e–g). A second mechanism might involve the ability of FMT to drive a recovery-directed immune response at the systemic level, as suggested by our transcriptional analysis (Fig. 1e,f, Supplementary Fig. 1, Supplementary Data 1 and 2), thereby enhancing bacterial clearance in peripheral organs and preventing the progression to lethal infection.
Fig. 1 FMT rescues mice from lethal infection due to a four-member pathogen community isolated from a patient with lethal sepsis. a–f To mimic the development of sepsis following major surgery, C57BL/6 mice were starved and received a single-intramuscular injection of cefoxitin, after which they were opened, subjected to 30% hepatectomy, and inoculated with the PC directly into their cecum. To test the protective effects of FMT, 24 h (POD1) and 48 h (POD2) after surgery, FMT or AC-FMT was administered via enema. 

a Sketch and timeline of the gut-derived sepsis model. 

b The percentage of mice with microbes present on POD1 in the indicated sites was measured by homogenizing organs and culturing them on TSB agar plates. The percentage of mice that had positive cultures out of all of the mice assessed are graphed for the groups indicated (n = 5, 7, and 8 mice for untreated, POD1 blood and spleen, and POD1 liver and lung, respectively). 

c Kaplan–Meier survival curves for indicated groups. “Surgery” are control mice that had starvation, antibiotics, and surgery without pathogen inoculation (n = 15 mice/group; Log-rank (Mantel–Cox) test; P < 0.001 between Surgery + PC/Surgery + PC + FMT, P = 0.9554 between Surgery + PC/Surgery + PC + AC-FMT, P < 0.0001 between Surgery + PC + FMT/Surgery + PC + AC-FMT). 

d Quantitative culture results of indicated sites comparing the PC burden of Surgery + PC + FMT and Surgery + PC + AC-FMT-treated mice at POD2. Each of the four PC members were assessed separately using selective culture plates. On radar plot, each dot along the axes represents the CFU/mL of blood or CFU/mg of liver and spleen of one mouse, the further the distance from the center of the plot, the higher the pathogen burden. The third and first quartiles and the median are indicated with the edges of the colored boxes and the thick black line respectively (n = 5 mice/group; Mann–Whitney test; PC burden significantly different between AC-FMT/FMT in the blood [SM*], liver [CA*], spleen [CA*], *P ≤ 0.05). 

f Distribution of the absolute log2-fold change in FMT and AC-FMT-treated mice on POD2 among genes differently expressed in either condition (Mann–Whitney test; P < 1 × 10^{-15}).
either FMT or AC-FMT via enema two times; at the time of PC inoculation and 14 h post inoculation (Fig. 2a). Remarkably, administration of FMT resulted in a greater than 40% increase in survival in the i.p. injected mice compared with no improvement in survival in AC-FMT-treated mice (Fig. 2b). Treatment with FMT resulted in significant clearance of all the PC members from peripheral organs compared with AC-FMT-treated mice (Fig. 2c). Taken together these data suggest that FMT can modulate systemic host physiology in a way that drives pathogen clearance at remote sites.

The rescue effect of FMT occurs in an IRF3-dependent manner. Microbial pathogens are sensed by the wide array of PRRs that are found on the surface and inside of cells that lead to the activation of the NF-κB and/or IRF3 signaling pathways. In the case of Gram-negative bacteria, recognition by TLR4 causes the concomitant activation of both pathways18,19. Many successful pathogens have evolved methods to avoid detection by these PRRs through a variety of molecular mechanisms13–17, including the transcriptional modulation of host signaling pathways17,20,21. Consistent with our findings in the gut-derived lethal infection model (Fig. 3a, Supplementary Fig. 1, Supplementary Data 1 and 2), i.p. injection of the PC resulted in downregulation of IRF3 and the upregulation of NF-kB Inhibitor Alpha (NFKBIA), NF-kB Inhibitor Beta (NFKBIB), and TNF Alpha Induced Protein 3 (TNFAIP3), known NF-kB pathway inhibitors22,23, in the cecum.
liver, and spleen, 20 h post PC injection in AC-FMT mice (Fig. 3b, Supplementary Fig. 3a, and Supplementary Table 4). Intriguingly, we observed the same gene expression changes in vitro when we cultured mouse embryonic fibroblasts (MEFs) with individual members of the PC that were either live (Supplementary Fig. 3b) or lysed and filtered (Fig. 3c and Supplementary Fig. 3c), with specific members driving more dramatic levels of transcriptional modulation. In particular, both live S. marcescens and its lysates drove the most dramatic down-regulation of IRF3, while live K. oxytoca drove the most dramatic upregulation of NF-κB inhibitors (Fig. 3c and Supplementary Fig. 3b, c). Bacterial lysates prepared from cecal contents of untreated mice (Microbiota) did not lead to a reduction in IRF3, although it did upregulate NFKBIA and TNFAIP3 at the highest dose tested (Fig. 3c and Supplementary Fig. 3c). Taken together, these results suggest that individual members of the PC have both distinct and complementary abilities to directly modulate NF-κB or IRF3 signaling with the net result being blockade of NF-κB interferons and in protecting these mice through restoration of immune homeostasis in Fig. 3b, and Supplementary Fig. 3a) suggesting that FMT may demonstrate an increase in survival upon treatment with FMT required intact IRF3 to clear the PC and rescue mice from liver, and spleen, 20 h post PC injection as compared with IRF3-suf cient patients are resistant to most common bacterial infections27-29. Finally, in the IP model with IRF3-suf cient mice had significantly increased mortality upon PC infection (Fig. 4a). Consistent with this observation, selective downmodulation of butyrate production has been reported in other models of inflammation34. These findings led us to investigate whether reduction and restoration in butyrate levels could be linked to changes in specific bacterial populations (OTUs).

Interestingly, in PC infected mice, a significant reduction in the abundance of five OTUs from the phylum Firmicutes are members of the Clostridium cluster XIVa (based on RDP SeqMatch searches36; Supplementary Fig. 6c), which is a cluster that contains many taxa that are known butyrate producers37. Butyrate kinase (buk) and butyrate transferase (but) are enzymes catalyzing the last step in butyrate production by bacteria37. More specific analysis, however, found only one of the OTUs proportionally decreased in PC infected mice to match organisms containing the buk or but genes in the RDP FunGene database36. Hence, it is possible that in addition to changes in OTUs, there is a defect in the transcription of butyrate processing enzymes, and/or an increased uptake of butyrate that contribute to the observed decreased expression in butyrate. Whatever caused the decrease in butyrate, FMT treatment of septic mice led to a restoration of butyrate levels (Fig. 4a) that was associated with a significant increase in OTUs that belonged to the phylum Bacteroidetes, including a large number of S24-7 family members. Based on RDP FunGene searches most of these OTUs have buk and/or but (Fig. 4b). Although there was some overlap between untreated mice and septic mice receiving FMT (Supplementary Fig. 6d and Supplementary Data 3), the OTUs from Bacteroidetes are specifically enriched in PC + FMT mice (Supplementary Fig. 6d). There are indications that members of Bacteroidetes actively alter the gut environment to their benefit38 and are more resilient colonizers than other phyla in the presence of acute inflammation39. The rapid colonization of septic mice by “non-traditional” butyrate-producing members of Bacteroidetes may hence account for the restoration of butyrate levels in FMT-treated mice.

Butyrate and HDACi normalize IRF3 levels in vitro. Given that butyrate has histone deacetylase inhibitory (HDACi) activity that can positively or negatively modulate transcription, we hypothesized that FMT may mediate its beneficial effects via the HDACi properties of butyrate. To test this hypothesis, we determined whether butyrate could restore normal levels of IRF3 in vitro. When MEFs were treated with an equal concentration of lysates from all four members of the PC, IRF3 transcript levels were reduced to ~50%; however, culturing these cells in the presence of butyrate led to a restoration of IRF3, in a

PC infection drives a reduction in fecal butyrate levels. We next sought to clarify how FMT might protect mice in the IP model, given that, unlike in the gut-derived sepsis model, it is presumed that IP injected mice will have maintained an intact microbiota at the time of FMT treatment. However, others have shown that any sudden physiologic insult can rapidly (within 6 h) deplete microbiome composition and function31. I.p. injected mice demonstrated no significant change in either total bacterial load (Supplementary Fig. 5a) or microbiota composition (Supplementary Fig. 5b, c) at 14 h post PC injection. This lack of compositional change by 16S rRNA analysis was also observed in mice that received AC-FMT or FMT treatment (Supplementary Fig. 5a–c). This finding further indicates that FMT in this model does not mediate survival by promoting colonization resistance and the enhanced exclusion of the PC.

Healthy microbiota can affect systemic immune homeostasis and enhance pathogen clearance via the production of metabolites such as short chain fatty acids (SCFA)32-34. To further define through which mechanisms FMT may mediate its effect, we investigated whether there were any alterations in SCFA expression in PC injected septic mice. Strikingly, cecal butyrate levels, but not acetate and propionate levels (Supplementary Fig. 6a, b), were significantly diminished in mice following i.p. PC injection (Fig. 4a). Consistent with this observation, selective downmodulation of butyrate production has been reported in other models of inflammation34. To further de...
Fig. 3 Clearance of systemically disseminated pathogens by FMT requires intact IRF3. a Summary of transcriptional changes in the NF-κB/IRF3 signaling pathway downstream of various pattern recognition receptors in the cecum, liver, and spleen of PC + Surgery + AC-FMT mice on POD2. Red shaded circles are genes that are significantly upregulated, blue shaded circles are genes that are significantly downregulated. b Mice were injected i.p. with PC and treated with FMT or AC-FMT as before. RNA was isolated from indicated organs ~20 h post injection of PC and qPCR for IRF3 was performed. Fold change compared with the mean of untreated group IRF3 relative expression for each organ; n = 7, 7, and 8 for untreated, PC + AC-FMT, and PC + FMT-treated mice, respectively; center is mean; one-way ANOVA/Tukey’s multiple comparison; *P ≤ 0.05, **P ≤ 0.01. c MEFs were cultured for 12 h in culture media containing filtered lysates made from cultured individual PC members, or from cecal contents of untreated mice at the indicated concentrations. MEF RNA was isolated and IRF3 expression was measured by qPCR. (Fold change compared with the baseline of untreated MEF IRF3 relative expression [highlighted with the dotted line at y = 1] shown; 3 independent experiments; n ≥ 3 for all conditions; mean ± SD). d, e To assess the requirement for IRF3 in FMT-mediated protection, IRF3+/+ and IRF3−/− littermates were injected i.p. with PC and treated with FMT or AC-FMT as before. d Kaplan–Meier survival curves for genotypes and treatments indicated (n = 10, 7, 13, and 11 for IRF3+/+ mice with PC + FMT, IRF3+/+ mice with PC + AC-FMT, IRF3−/− mice with PC + FMT, and IRF3−/− mice with PC + AC-FMT, respectively; Log-rank (Mantel-Cox) test; P = 0.0469 between IRF3+/+ PC + FMT/IRF3+/+ PC + AC-FMT, P = 0.5440 between IRF3+/+ PC + FMT/IRF3−/− PC + AC-FMT). e Quantitative culture results of indicated sites comparing the PC burden of PC + FMT-treated IRF3+/+ and IRF3−/− mice ~20 h post injection of PC. On radar plot, each dot along the axes represents the CFU/mL of blood and peritoneal fluid or CFU/mg of liver and spleen of one mouse, the further the distance from the center of the plot, the higher the pathogen burden. The third and first quartiles and the median are indicated with the edges of the colored boxes and the thick black line respectively (n = 5 mice/group; Mann–Whitney test; PC burden significantly different between IRF3+/+ PC + FMT/IRF3+/+ PC + FMT in the blood [EF+, SM+], liver [EF+, KO+, SM+], peritoneum [CA+, KO+, SM+], and spleen [EF+, KO+]. *P ≤ 0.05, **P ≤ 0.01).
intestinal butyrate. FMT, by providing OTUs such as Bacteroidetes, that can expand under inflammatory conditions and produce butyrate, is able to protect against systemic PC infection.

**Discussion**

Here we present evidence that a PC expressing varying degrees of antibiotic resistance isolated from the stool of a terminally septic patient dysregulates, in a concerted manner, the transcriptional program associated with host defenses against infections. By blocking key parts of both the NF-κB and IRF3 signaling pathways, these pathogens prevent the host from mounting a protective immune response, which likely accounts for the high systemic pathogen burden, host deterioration, and death seen in this model. Unexpectedly, we found that FMT was able to counter the immunosuppressive effects of these pathogens in part through its ability to restore normal levels of butyrate. The ability of FMT to provide protection in the IP model of lethal infection indicates that FMT can act systemically, beyond its local effect on the gastrointestinal microbiota or pathobiota, and restore protective immunity against highly virulent and MDR pathogens. Understanding how FMT drives a recovery-directed immune response at the systemic level may have important implications for the management of critically ill patients who are regularly treated with antibiotics and colonized by healthcare-associated pathogens. Given that the pathogen communities that colonize critically ill patients each carry their own unique life histories, results from this study underscore the importance of humanizing the
mouse gut with clinically relevant human pathogens when assessing the immune response. The immunosuppressive nature of the human PC herein described may explain, in part, why mouse models of endotoxin administration or sepsis due to rodent flora have failed to translate into effective therapies in clinical trials.\(^8\)\(^9\) Whether the current approach will be useful in the design of future human trials will require further study.

**Methods**

**Mice.** Seven-to-nine week-old male C57BL/6 mice weighing 18–22 g were used for experiments. WT C57BL/6 were purchased from Charles River Laboratories. IRF3\(^−/−\) mice on a C57BL/6 background were kindly provided by Dr Tatyania V. Golovkina (University of Chicago) and mice used in comparative studies were the progeny of IRF3\(^+/−\) mice resulting in IRF3\(^+/+\) and IRF3\(^−/−\) littersmates. IRF3\(^+/−\) and IRF3\(^−/−\) litters were cohoused until the day of the experiment to prevent genotypic differences between genotypes affecting results.

**PC preparation.** The PC consisted of four members including *C. albicans*, *E. faecalis*, multidrug-resistant *K. oxytoca*, and multidrug-resistant *S. marcescens* and was isolated from the stool of a critically ill patient (ICU1-2) who was exposed to numerous antibiotics during the course of their sepsis.\(^4\) The individual pathogens were plated on TSB agar from individual frozen stocks and grown overnight at 37 °C. Colonies were suspended in liquid TSB medium, subgrown for 1 h and then adjusted to an optical density measured at wavelength of 600 nm (OD600) of 0.2. All four species were then combined together in equal volumes. The microbial suspension was centrifuged at 6000 rpm for 10 min, the excess TSB was removed and the remaining pellet was resuspended in the same volume of 10% glycerol. The resulting microbial suspension was administered in the two sepsis mouse models as described below.

**Gut-derived sepsis mouse model.** All experiments were approved by the Institutional Animal Care and Use Committee at the University of Chicago (IACUC protocol 71744). Mice were routinely fed tap water and Harland Teklad feed (Madison, WI) under 12-h light/dark cycles and were allowed to acclimate for at least 1 week in order to minimize endogenous stress.\(^4\) The individual pathogens were plated on TSB agar from individual frozen stocks and grown overnight at 37 °C. Colonies were suspended in liquid TSB medium, subgrown for 1 h and then adjusted to an optical density measured at wavelength of 600 nm (OD600) of 0.2. All four species were then combined together in equal volumes. The microbial suspension was centrifuged at 6000 rpm for 10 min, the excess TSB was removed and the remaining pellet was resuspended in the same volume of 10% glycerol. The resulting microbial suspension was administered in the two sepsis mouse models as described below.

**Nonquantitative culture of PC.** The blood, liver, lung, and spleen of mice were harvested on POD1 to determine the percentage of mice that had microbes present in these compartments. Blood was collected via direct cardiac puncture, mixed with glycerol to a final concentration of 10%, and stored at −80 °C. Ten milligrams of liver, lung, and spleen were homogenized in 500 µl of 10% glycerol and stored at −80 °C. Each sample was serially diluted and plated on TSA agar plates. All the plates were incubated in 37 °C incubator for 24 h and then assessed for the presence of colonies.

**Quantitative culture of PC.** The blood, spleen and liver of mice were assessed for colonization with PC species using selective plates: for *C. albicans*, CHROMagar Candida plates (BBL); for *K. oxytoca*, MacConkey supplemented with ciprofloxacin (10 µg/ml); for *S. marcescens*, Trypticase Soy Agar (BBL) supplemented with 100 µg/ml of tetracycline. All the plates were incubated in 37 °C incubator for 24 h. CFU counts were normalized to tissue weight for spleen and liver and volume for blood and peritoneal fluid.

**Mouse genome transcription analysis.** As indicated in the original model description, mice underwent the full protocol to allow for the development of gut-derived sepsis (i.e., intestinal inoculation of the PC and surgical hepatectomy) and were then treated with either a live FMT or AC-FMT (n = 3/group). We performed gene expression analysis on POD2, because this was the time point where we observed FMT-driven reduction of systemic pathogen burden. In accordance with this reduction in systemic pathogen burden, the FMT-treated mice had a clinical score of 1, while the AC-FMT-treated mice had a clinical score of 4 at the time of sampling (scoring system described in Supplementary Table 2). The tissues were excised andsnap-frozen in liquid nitrogen (Qiagen). Tissue was homogenized using Tissue Tearor (Biospec Products) and RNA extraction was performed using the RNeasy Plus Mini Kit (Qiagen) according to the manufacturer’s instructions. Whole-genome transcriptional profiling was performed using Illumina MouseRef-8 v2 arrays (Illumina) by the Functional Genomics Facility of the University of Chicago.

Low-level microarray analyses were performed in R, using the Bioconductor software package lumi.\(^3\) We first applied a variance stabilizing transformation to all arrays\(^3\) and then quantile normalized the data. After normalization, we removed probes with intensities indistinguishable from background noise (as measured by the negative controls present on each array). After these preprocessing steps, data from 13,312 genes were available for differential expression analysis.

To identify genes whose expression levels were altered at POD2 after AC-FMT or FMT treatment (as compared with untreated control mice), we used a linear modeling-based approach. Specifically, we used the Bioconductor limma package to fit, for each gene, a linear model with individual treatment (i.e., AC-FMT or FMT) and tissue as fixed effects. To identify changes in gene expression within each tissue we ran a nested linear model where treatment was nested within tissue type.
assembly was performed using the Spades version 3.5.0 for each species. Reads CAV1492 (NZ_CP011642.1), or the Illumina HiSeq system at Argonne National Laboratory (Lemont, IL). generated using Nextera XT protocol according to manufacturer from exponential phase during growth in liquid TSB. Sequence libraries were Sequencing of bacterial pathogen genomes NATURE COMMUNICATIONS | https://doi.org/10.1038/s41467-020-15545-w ARTICLE

For each gene, we subsequently used the empirical Bayes approach of Smyth to calculate a moderated t statistic and P value. We corrected for multiple testing using the false discovery rate (FDR) method of Benjamini and Hochberg. Gene Ontology enrichment analysis was done using GORILLA using all expressed genes (i.e., 13,312 genes) as a background set.

16S rRNA analysis. Microbial DNA was extracted from cecal luminal contents and cecal tissues using Bacteremia DNA Isolation Kit (BiOstic, 12240-50), and the 16S rRNA analysis was performed at Argonne National Laboratories (Lemont, IL). Samples were amplified by 16S rRNA V4 ITAG amplicon sequencing analysis. Paired end reads were quality trimmed and processed for OTU (operational taxonomic unit) clustering using UPARSE pipeline, set at 0.97% identity cutoff. Taxonomic status was assigned to the high quality (<1% incorrect bases) candidate OTUs using the “parallel_assign_taxonomy_rdp.py” script of QIME software. Multiple sequence alignment and phylogenetic reconstruction was performed using PyNast and FastTree. OTU matrix was processed to remove OTUs containing less than five reads in order to reduce the PCR and sequencing based bias; then the OTU table was rarified to the minimum numbers of reads present in the smallest library. We used the oligotyping pipeline to identify the sub-OTU level differences in the bacterial strains of K. oxytoca ICU-2 S. marcescens and E. faecalis using Blastn. Megablast (minimum identity cutoff = 100%) was used to confirm the strain identification between full length 16S rRNA gene sequences and oligotype representative sequences.

Sequencing of bacterial pathogen genomes. For isolation of DNA, strains of the original stock consisting of K. oxytoca, S. marcescens, and E. faecalis were collected from exponential phase during growth in liquid TSB. Sequence libraries were generated using Nextera XT protocol according to manufacturer’s instructions (Illumina). Libraries were sequenced by whole-genome shotgun sequencing using the Illumina HiSeq system at Argonne National Laboratory (Lemont, IL). Approximately 100-fold coverage of each genome was generated. Reference-guided genome assembly was conducted using a combination of de novo assembly and read alignment to the K. oxytoca CAV1374 (NZ_CP011636.1), S. marcescens CAV1492 (NZ_CP011612.1), or E. faecalis V583 (NC_004668.1) genome. De novo assembly was performed using the Spades version 3.5.0 for each species. Reads were aligned to the appropriate species-specific reference genome using Bowtie2 version 2.2.5. Contigs from de novo assembly, greater than 1000 bp in length, were placed onto the reference genome using BLAST version 2.2.30, and merged into longer scaffolds with reference-aligned sequence using custom Perl scripts. The draft genome sequence of K. oxytoca ICU-1b is 5,852,736 bp in length across 12 scaffolds. S. marcescens ICU-2a is 5,115,171 bp in length across 20 scaffolds and E. faecalis ICU-1c is 2,929,980 bp in length across 14 scaffolds. Accession numbers in NCBI are LQAM000000000 Enterococcus, LQAL000000000 Klebsiella, and LQAK000000000 Serratia in the BioProject PRJNA307050.

Intraperitoneal (IP) sepsis mouse model. In order to determine if the rescue effect of FMT functions at the level systemic infection, we directly injected mice with the bacteria into the peritoneum (i.p.). In this IP model, mice were not subjected to starvation, antibiotic treatment, or hepatectomy. The suspension was prepared using the identical strains (S. marcescens, C. albicans, K. oxytoca, and E. faecalis) and methods above. One milliliter of PC was injected intraperitoneally using a 1 mL insulin syringe (BD). The optimal and lethal dose of PC was determined by preliminary experiments. FMT and AC-FMT were prepared as described above. Each mouse received two doses of either FMT, AC-FMT immediately after i.p. injection and 14 later. Survival and health status were monitored and documented for 72 hours using the clinical scoring system shown in Supplementary Table 2 (mice that survive until this point, regardless of treatment group return to full health and survive indefinitely). All mice that were moribund were sacrificed and considered to be a mortality.

Quantitative polymerase chain reaction (qPCR). RNA was isolated from cecum, liver, and spleen as before and converted to cDNA using reverse transcriptase (Promega) according to the manufacturer’s instructions. Expression analysis was performed in duplicate via qPCR on a Roche LightCycler 480 using SYBR Advantage qPCR Premix (Clontech). Expression levels were quantified and normalized to housekeeping genes HPRT (for qPCR on organs in both mouse models), GAPDH (for in vitro MEF experiments), or ASL (for 16S relative expression). For Fig. 3b, Supplementary Fig. 3a, and Fig. 4d, relative expression values were normalized to the mean of the untreated controls for each organ and displayed as fold change to allow for display on the same axis. For Fig. 3c, Supplementary Fig. 3b, c, and Fig. 4b, relative expression values were normalized to untreated MEF baseline relative expression for each gene (indicated with a dotted line at 1) and displayed as fold change. For Supplementary Fig. 4a, the number of 16S copies was calculated using a purified Rrn6301 pre-PCR standard and normalized to the relative expression of host ASL for each mouse assessed. The qPCR primers shown in Supplementary Table 4 were custom ordered from Integrated DNA Technologies.

MEF and PC co-culture. C57Bl/6 primary MEFs were obtained from 12.5 to 14.5 days post coitus embryos and cultured in Dulbecco’s Modified Eagle’s Medium (Gibco) supplemented with 10% fetal bovine serum (Gibco), and 1% non-essential amino acids (Gibco). MEFs were plated at a density of 2.5×10^4/mL in 24- well plates (Costar) overnight. Live PC members were prepared as before to an OD600 of 0.2, and diluted (1/10^3) by volume in MEF containing culture media. PC lysates were prepared by taking live PC culture, bead beating with 0.1-mm diameter glass beads (BioSpec) for 5 min and filtering using Millipore 0.22 µm filter (Millipore). Plates were incubated at 37°C, 5% CO2 for 1, 3, 6, and 14 hours for live PC, or 12 hours for lysates in the presence of acetic acid (Fisher), butyric acid (Sigma), propionic acid (Sigma), nicotinic acid (Sigma), or valproic acid sodium salt (Sigma) at the indicated concentrations. After incubation, culture media was removed and cells were harvested in Buffer RLT Plus (Qiagen) with 1% 2-Mercaptoethanol (Sigma) and RNA extraction was performed using the RNeasy Plus Mini Kit (Qiagen) according to the manufacturer’s instructions. All MEF experiments were ≥2 independent experiments, with at least two experimental replicates/experiment. The mean fold change for each experiment was plotted and represented as bar graphs.

Gas chromatography-mass spectrometry. SCFA were extracted from mouse cecal contents using diethyl ether (Fisher Scientific), derivatized using N-tet-Butyldimethylsilyl-N-methyl trifluoroacetamide with 1% tert-Butyldimethylchlorosilane (Sigma) and run on an Agilent Single Quad GC–MS (P/N 7890A, Quad and 7890B GC). All values are normalized to focal sample mass. Raw spectral data were uploaded to the MassIVE repository (https://doi.org/10.25345/C5IJQYV).

Statistical analysis. Statistical analysis was performed using GraphPad Prism software. Unpaired t-test (two-tailed) was used when analyzing the differences between two means, whereas one-way ANOVA/Tukey’s multiple comparisons test was used when more than two means were compared. To determine the statistical significance of quantitative culture analyses, we used nonparametric tests because of the distribution of data: Mann–Whitney test (two-tailed) was used when two groups were compared, Kruskal–Wallis test/Dunn’s multiple correction test was used for three groups. Log-rank (Mantel–Cox) test was used to determine statistical significance between Kaplan–Meier survival curves.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The datasets generated during and/or analyzed during the current study are available in the Article. Supplementary Information files, or available from the corresponding author on reasonable request. Microarray data was uploaded to the NCBI Gene Expression Omnibus database; GEO accession numbers are GSE71530. Genomic sequences of the ICU 2 pathogen community members were uploaded to NCBI in BioProject PRJNA307050. Raw spectral data for measurement of SCFA by GC-MS were uploaded to the MassIVE repository (https://doi.org/10.25345/C5IJQYV).

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performed transcriptional data analysis. NS, SC, DAA, and JAG performed 16S analysis. DRR and RRW provided essential reagents. SMK, BJ, and JCA wrote the paper.

**Competing interests**
The authors declare no competing interests.

**Additional information**
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