Neonatal intestinal immune regulation by the commensal bacterium, P. UF1

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Newborns are highly susceptible to pathogenic infections with significant worldwide morbidity possibly due to an immature immune system. Recently, we reported that Propionibacterium strain, P. UF1, isolated from the gut microbiota of preterm infants, induced the differentiation of bacteria-specific Th17 cells. Here, we demonstrate that P. UF1 significantly increased the number of protective Th17 cells and maintained IL-10+ regulatory T cells (Tregs) in newborn mice. In addition, P. UF1 protected mice from intestinal Listeria monocytogenes (L. m) infection. P. UF1 also functionally sustained the gut microbiota and induced critical B vitamin metabolites implicated in the regulation of T cell immunity during L. m intestinal infection. Transcriptomic analysis of P. UF1-induced Th17 cells revealed genes involved in the differentiation and regulation of these cells. These results illustrate the potency of P. UF1 in the enhancement of neonatal host defense against intestinal pathogen infection.

ARTICLE

INTRODUCTION

Despite advances, approximately one million neonates under 6 months of age succumb to infections annually. Studies demonstrate that the susceptibility of newborns to infections may be due to immature adaptive immune responses to pathogenic challenges. Available vaccines provide insufficient protection against pathogenic microorganisms, or require multiple injections to sustain protective immunity. The mechanisms for this lack of protection remain elusive. However, there is some evidence that the lack of protection may partially be due to low numbers of protective T cells in newborns compared to adults. Thus, mounted T cell immunity may be insufficient to clear the infection leading to tissue injury. Moreover, the development of protective immunity is naturally slow and requires a myriad of additional stimulatory factors, including innocuous dietary antigens and a repertoire of critical microbial metabolites to induce a protective immunity. This gradual process of immune development may allow for deterioration due to invading pathogens inducing inflammation that can shut down functional T cell homeostasis against infections. Data demonstrate the critical role of a stable microbial consortium in enhancing immune resistance to various enteric pathogens in infants compared to neonates. This may account in part for the increased susceptibility of neonates to particular foodborne pathogens if the commensal flora is not well established. Understanding how immunity evolves to protect newborns against life-threatening infections is key to developing novel approaches, including the administration of particular bacteria with bifidogenic properties such as P. UF1 to potentially shorten the slow period of neonatal adaptive immunological development, resulting in the acceleration of protective T cell differentiation. This may help control and prevent pathological proinflammatory conditions in neonates, including necrotizing enterocolitis (NEC). With this in mind, we demonstrate that the administration of our newly discovered P. UF1 bacterium to newborn mice accelerates the neonatal protective CD4+ T cell frequency against intestinal infection.

RESULTS

Acceleration of neonatal T cell immunity by P. UF1

Recently, we reported the discovery of a novel Propionibacterium strain, dubbed P. UF1, capable of inducing the differentiation of dihydrolipoamide acetyltransferase (DlaT)-specific Th17 cells. DlaT protein was isolated from bacterial surface layer. As an enzyme, DlaT is implicated in bacterial pyruvate decarboxylation that links glycolysis to the citric acid cycle. However, DlaT can also be synthesized by other yet-to-be identified mechanism(s) on the S-layer to activate antigen-specific Th17 cells. Induced protective DlaT-specific Th17 cells were highly regulated by P. UF1-induced IL-10+ Tregs to resolve L. m infection in adult mice. P. UF1 also significantly controlled deleterious signals (e.g., iNOS, IL-1β) involved in necrotizing enterocolitis (NEC)-like injury by regulating Th17 cells and sustaining IL-10+ Tregs, which protected the newborn mice, whose mothers were orally pretreated with P. UF1, against NEC manifestation. To investigate the effects of gavaging pregnant dams with P. UF1, which initially resulted in an increased number of Th17 cells and Tregs in newborn mice, we first constructed an antibiotic-resistant strain (CmR P. UF1) by insertion of chloramphenicol resistant gene (CmR)
into the P. UF1 chromosome (Supplementary Fig. 1a). The possibility of P. UF1-transfer into newborn mice was then discerned by gavaging pregnant mice with CmR P. UF1 (10⁹ CFU/mouse/twice a week). Obtained data demonstrated the presence of P. UF1 in the fecal contents of the newborn mice as early as 5 days after they were delivered by their mothers (Supplementary Fig. 1b). These data may explain the regulated immune protection of newborn mice induced by P. UF1 previously subjected to experimental NEC-like injury. Furthermore, to demonstrate any maternal effects on the newborn mouse immunity against pathogen infection, newborn mice without any P. UF1, or PBS oral treatment were kept with their mothers that were gavaged with P. UF1, or PBS for 3 weeks. To limit L. m infection to the gut, these groups of mice were orally infected with ΔactA L. m (10⁸ CFU/mouse), ΔactA L. m lacking the ActA polypeptide cannot interact with actin filaments and is, consequently, nonmotile to spread in the periphery of the mice. Interestingly, enhanced Th17 cells and Tregs were induced against ΔactA L. m in mice that were initially delivered by dams gavaged with P. UF1 when compared to the other group (Supplementary Fig. 1c, d). Additionally, the clearance of ΔactA L. m after 4 days demonstrated a significant trend of ΔactA L. m clearance in this group compared to control group (Supplementary Fig. 1e). These data indicate that it may be of significance if mothers and newborn mice would be continuously gavaged with P. UF1 to increase the enhancement of T cell immunity in newborn mice. To expand on this notion and our initial observation, we hypothesized that the slow process of T cell differentiation in the neonatal host may be accelerated by P. UF1 to fortify regulated-intestinal T cell response in steady state and during intestinal L. m infection. Thus, we first gavaged groups of pregnant C57BL/6 mice with P. UF1, ΔflaT P. UF1 (10⁹ CFU/mouse/weekly), or PBS. After birth, the same regimen was used to gavage newborn mice of the respective dams for 8 weeks. Newborn mice were then euthanized at 1, 2, 4, 6, or 8 weeks to study CD4⁺ T cell response over time (Fig. 1a). Data demonstrated a significant increase in regulated CD4⁺ T cells, particularly IL-10⁺ Th17 cells induced by P. UF1 compared to other groups (Fig. 1b, c). Notably, the increased frequency of IL-10⁺ Th17 cells was followed by IL-10⁺ IFNγ⁺ T1 cells (Fig. 1c). Although the frequency of FoxP3⁺ Tregs was not increased, the numbers of functional IL-10⁺ TGFβ⁺ Tregs were significantly enhanced in newborn mice gavaged with P. UF1 for 8 weeks (Fig. 1c). These data may highlight the potency of P. UF1 to increase the numbers of regulated functional colonic CD4⁺ T cells in the steady state.

Regulation of gut homeostasis by P. UF1 during intestinal Listeria infection

To limit tissue damage, activation of protective T cell responses requires regulatory signals to suppress pathogenic inflammation. To once again demonstrate the ability of P. UF1 in the regulation of Th17 cells, along with the maintenance of IL-10⁺ Tregs in a pathogen-induced inflammatory condition, pregnant C57BL/6 were gavaged with P. UF1, ΔflaT P. UF1 (10⁹ CFU/mouse), or PBS. Once these dams had delivered their newborns, groups of the newborn mice were then gavaged with P. UF1, ΔflaT P. UF1, or PBS. These groups of mice were orally infected with ΔactA L. m (10⁸ CFU/mouse 3 weeks of age, or 10⁹ CFU/mouse 4–8 weeks of age) at 3, 4, 6, and 8 weeks of age (Fig. 2a). Data revealed that the protective regulatory Th17 cell response was induced by P. UF1 against ΔactA L. m as early as 3 weeks (Fig. 2b, c and Supplementary Fig. 2a). Protective Th17 cells, along with Th1 cells, both potentially controlled by IL-10⁺ Tregs (Fig. 2b, c and Supplementary Fig. 2a), significantly decreased ΔactA L. m infection within 3, or 4 days, while the other groups of mice showed higher burdens of ΔactA L. m (Fig. 2d and Supplementary Fig. 2b). These results demonstrate P. UF1’s ability to enhance protective T cell response against L. m infection.

Recently, we and others demonstrated the implication of Th17 cells in the clearance of pathogen infection. To further demonstrate the role of P. UF1-induced Th17 cells against ΔactA L. m, we neutralized IL-17A by intraperitoneal injection (ip) of anti-IL-17A antibodies (200 μg/mouse) in mice that were initially gavaged with P. UF1, ΔflaT P. UF1 (10⁹ CFU/mouse), or PBS for 8 weeks. These groups of mice were then infected with ΔactA L. m (10⁶ CFU/mouse). Data demonstrated that neutralizing IL-17A resulted in the persistence of ΔactA L. m infection in all groups of mice illuminating an important role of IL-17A in the clearance of ΔactA L. m (Fig. 2e). Notably, such pathogen persistence was significantly higher on days 2 and 3 in mice gavaged with P. UF1 that received anti-IL-17A neutralizing antibodies, potentially indicating the prominent role of Th17 cells induced by this bacterium controlling ΔactA L. m infection (Fig. 2e). To further emphasize the role of T cell responses against pathogen infection, we then employed Rag1⁻/⁻ mice exhibiting no mature T and B cells. Here, groups of Rag1⁻/⁻ mice were gavaged either with P. UF1(10⁹ CFU/mouse/ every other day), or PBS four times. Subsequently, mice were orally infected with ΔactA L. m (10⁹ CFU/mouse). Data demonstrated that in both groups of Rag1⁻/⁻ mice, ΔactA L. m clearance was not obvious and ΔactA L. m infection persisted even more in P. UF1-gavaged mice, once again highlighting the protective role of T cells, particularly P. UF1-induced Th17 cells, against ΔactA L. m infection (Fig. 2f).

Impact of P. UF1 on gut microbiota and the induced metabolites during Listeria infection

Intestinal infection may manifest in gut dysbiosis and pathogen growth that can induce inflammatory changes leading to deterioration of intestinal homeostasis and eventual tissue damage. Accordingly, we investigated the status of gut microbiota before and after ΔactA L. m infection in newborn mice gavaged with P. UF1 for 8 weeks compared with other groups. Here, the principal coordinate analysis (PcoA) of mice gavaged with P. UF1, ΔflaT P. UF1, or PBS and subsequently infected with ΔactA L. m showed significant separation in their gut microbiota (PERMANOVA p = 0.036), which was not evident before infection (PERMANOVA p = 0.141) (Supplementary Fig. 3a, b). To identify intestinal commensal species contributing to such PcoA-differences, we then performed linear discriminant analysis effect size (LEfSe) among the infected groups with ΔactA L. m. Analyzed data demonstrated that the family of Lactobacillaceae, particularly Lactobacillus genera, and Clostridiaceae producing short-chain fatty acids (SCFA), were highly enriched in mice gavaged with P. UF1 while Prevotella was dominantly represented in the microbiota of PBS-gavaged group after infection (Fig. 3c and Supplementary Fig. 3b). Thus, we focused on the significant species identified through LEfSe analyses of mice infected with ΔactA L. m, and compared the relative abundance of those species before and after infection. Data revealed that P. UF1 sustained beneficial microbes (e.g., Lactobacillus and Peptostreptococcaceae) and even propagated Clostridiaceae after infection (Fig. 3a, b, and Supplementary Fig. 3c). Accordingly, lowering pH and increasing propionic acid level in the proximity of beneficial bacteria could potentially alter the growth of pathogenic bacteria. This aspect may require further investigation, as data illuminated the effects of diverse gut bacteria (e.g., Clostridium species) reducing L. m infectivity in mice. This indicates that gut microbes may function as the host-specific signaling that initiates functional gut homeostasis.

A myriad of microbiota-associated metabolites may affect the host-specific signaling that initiates functional gut homeostasis. Notably, analyzing the induced metabolites in fecal samples of newborn mice gavaged with P. UF1 for 8 weeks and then infected with ΔactA L. m when compared with other groups revealed unique features of induced metabolites. Here, the principal component analysis (PCA) showed a distinct clustering of fecal metabolomes from P. UF1, ΔflaT P. UF1, and PBS-gavaged mice.
Vitamin B2 metabolism, CoA catabolism/biosynthesis, prostaglandin formation, arachidonic acid metabolism and leukotriene metabolism were exclusively altered in P. UF1-gavaged mice in comparison to other groups (Fig. 4b). Significantly increased intensities of metabolite features, particularly riboflavin (vitamin B2), pantetheine (vitamin B5), and 5-methyltetrahydrofolate (vitamin B9), were observed in fecal samples of P. UF1-gavaged mice potentially regulating T cells, including Th17 cells, against pathogen infection (Fig. 4c). By contrast, proinflammatory metabolites, including prostaglandin E1, and 20-Hydroxyleukotriene E4, exhibited lower intensities in P. UF1-gavaged mice compared to other groups (Fig. 4c). These data may highlight the role of metabolite-associated signals that may regulate induced protective host immunity against pathogen invasion and the induced inflammation.

Transcriptomic regulation of Th17 cells by P. UF1 during intestinal infection
To investigate the transcriptomic program(s) potentially involved in the regulation of P. UF1-dependent Th17 cells, IL-17A^GFP mice receiving P. UF1 or ΔdlaT P. UF1 for 8 weeks were infected with ΔactA L. m, and CD4^+ EGFP^+ Th17 cells were sorted for transcriptomic analysis. Here, the PcoA demonstrated the differential transcriptomes of P. UF1- and ΔdlaT P. UF1-induced Th17 cells (Fig. 5a). Furthermore, among the 248 identified differentially expressed transcripts (FDR p < 0.05), a signature of key Th17 cell-related transcripts with significantly higher expression was observed in the P. UF1 group (e.g., lckf3, Nr1d1, Cxcr6, and ll22) compared to the other group (Fig. 5b, c, and Supplementary Fig. 4a). To illuminate potential pathways enriched in either Th17 cells induced by P. UF1, or ΔdlaT P. UF1, Gene Set Enrichment Analysis (GSEA) was performed. Data demonstrated pathways related to extracellular matrix involved in the regulation of tissue homeostasis and healing were significantly enriched in P. UF1-induced Th17 cells (Fig. 5d). Additionally, genes implicated in TCR signaling and T cell activation (e.g., Lck, Cd69, Prkcq, and Zap70) were also upregulated in P. UF1-induced Th17 cells and supported by GSEA analysis demonstrating a gene set for a TCR signaling pathway (p < 0.005) (Supplementary Fig. 4b). In contrast, gene sets involved in inflammation and chemokine activity (e.g., Alox5, Cybb, etc.)
Ccl3 and Ccl4) were highly enriched in ΔdlaT P. UF1-induced Th17 cells, suggesting the proinflammatory property of these cells during ΔactA L. m infection (Fig. 5c–e). Finally, we computed overlaps of the significantly upregulated genes in either P. UF1-, or ΔdlaT P. UF1-induced Th17 cells with the gene ontology (GO) term in the molecular signature database (MSigDB). Here, gene sets involved in immune regulation were observed in P. UF1-induced Th17 cells (Supplementary Fig. 4c, d). Furthermore, genes (e.g., Pias2 and Nr4a1) involved in the modulation of P. UF1-induced Th17 cell signaling were significantly increased in these cells compared to their counterparts (Fig. 5b). Together, these data may further demonstrate the critical role of P. UF1 in regulating DlaT-specific Th17 cells during L. m infection to clear this pathogen.
Fig. 2 T helper cells induced by P. UF1 protect neonatal mice against L. m infection. a Schematic depiction of the feeding schedule of newborn mice. Pregnant C57BL/6 dams were gavaged with P. UF1, ΔdlaT P. UF1 (10^9 CFU/dam) or PBS once a week. After birth, newborn mice of the respective dams were gavaged once a week either with P. UF1, ΔdlaT P. UF1 (10^9 CFU/mouse), or PBS. After weaning, mice continued to receive P. UF1, ΔdlaT P. UF1 (10^9 CFU/mouse), or PBS once a week. Mice were then infected with ΔactA L. m (10^9 CFU/mouse) at 4, 6, or 8 weeks old and euthanized 1 week after infection to analyze colonic CD4^+ T cell responses by FACS. b Representative flow plots for IL-10^+ Th17, IL-10^+ Treg and IL-10^+ IFNγ^+ Th1 cells analyzed from fecal samples (bottom) of colonic Th17, IL-10^+ Treg, and IL-10^+ IFNγ^+ Th1 cells analyzed from fecal samples (top) of colonic Th17, IL-10^+ Treg, and IL-10^+ IFNγ^+ Th1 cells. c Th1 cells analyzed from 4- (ΔactA L. m, n = 5; ΔactA L. m + P. UF1, n = 7; ΔactA L. m + ΔdlaT P. UF1, n = 9), 6- (ΔactA L. m, n = 4; ΔactA L. m + P. UF1, n = 8; ΔactA L. m + ΔdlaT P. UF1, n = 5), and 8- (ΔactA L. m, n = 5; ΔactA L. m + P. UF1, n = 8; ΔactA L. m + ΔdlaT P. UF1, n = 10) week-old mice. d Pathogen burdens in the feces of mice infected with ΔactA L. m. e 8-week-old mice (n = 4-5) gavaged with P. UF1, ΔdlaT P. UF1 or PBS were intraperitoneally injected with anti-IL-17A neutralizing antibody (100 μg/mouse/time, days −6 and −2) and then infected with ΔactA L. m (10^9 CFU/mouse, day 0). Microbial burdens were monitored on days 1-5 post infection. Dashi lines indicate the limit of pathogen detection. f Groups of 8-week-old mice were gavaged with P. UF1 (4 gavages, 10^9 CFU/mouse) or PBS, and then orally infected with ΔactA L. m (10^9 CFU/mouse). ΔactA L. m clearance was determined on days 1-5 post infection. Data are from 1 experiment (e, f), or representative of 3 (a-d) independent experiments. Data are presented as mean ± SEM; * p < 0.05, ** p < 0.01, *** p < 0.001, ANOVA plus Tukey post-test (c, d) or Kruskal–Wallis test (d-f).
infection. Additionally, the relevance of the protective property of T cells, particularly Th17, against pathogen infection was also shown by neutralizing IL-17A in mice in the present report. Our obtained data from Rag1-/- mice with no T cell transfer supported this notion, once again highlighting the protective role of T cells against pathogen infection. Yet, would other critical factors, including functional microbiota and their induced metabolites, also contribute to the rigid protection of mice against pathogen infection? To elucidate these significant points of interest, further experiments are warranted in the near future using relevant mouse models, including Rag1-/- germfree mice. Nonetheless, the concurrent data clearly demonstrate not only the important contribution of gut microbiota and their induced metabolites against pathogen infection (e.g., Listeria), but also that these two factors may be critically involved in the functional improvement of protective T cells in a pathogen-induced condition, as demonstrated in this report.

Collectively, our data demonstrate that P. UF1 significantly increases the frequencies of T cells (e.g., Th17 cells) and influences the commensal phylum and the induced metabolic profile. This beneficial bacterium also fortifies the transcripts in Th17 cell regulation during intestinal pathogen infection potentially contributing to immune regulation that may prevent tissue damage. Further mechanistic analyses are warranted to understand the molecular cues exerted by P. UF1, including its metabolic machinery and gene products, which includes surface layer proteins (S-layers) involved in immune regulation resisting intestinal infection inducing-pathogenic-inflammation that may result in intestinal tissue damage, and to implement novel bacteria with bifidogenic properties to possibly prevent intestinal inflammatory diseases and neonatal pathogenic infections.

Fig. 3  P. UF1 modulates the microbiota composition during ΔactA L. m infection. Pregnant C57BL/6 dams were gavaged weekly with P. UF1, ΔdlaT P. UF1 (10^9 CFU/dam) or PBS. After birth, newborn mice of the respective dams continued to receive PBS, P. UF1, or ΔdlaT P. UF1 (10^7 CFU/mouse during breastfeeding, 10^8 CFU/mouse after weaning, once a week). Mice (n = 10) were then infected with ΔactA L. m (10^9 CFU/mouse) at 8 weeks of age. Seven days after infection, fecal samples were collected and microbiota composition was analyzed by 16S rDNA sequencing. a Histogram of linear discriminant analysis (LDA) scores reveals the most differentially abundant taxa among the indicated treatment groups. Only taxa meeting an LDA significant threshold > 2 are shown. b Taxonomic cladogram created using LDA effect size (LEfSe) method showing the phylogenetic distribution of differentially enriched taxa was compared among the indicated treatment groups. P. UF1-enriched taxa are indicated in green, ΔdlaT P. UF1-enriched taxa are indicated in blue and taxa enriched in PBS control are shown in red.
METHODS

Animals
C57BL/6 and C57BL/6-Il17atm1Bcgen/J (IL-17AeGFP) and Rag1-/- mice were obtained from Jackson Laboratory and maintained under specific pathogen-free, Helicobacter-free conditions, in accordance with the Animal Welfare Act and the Public Health Policy on Humane Care. Procedures were approved by UF’s Institutional Animal Care and Use Committee (IACUC), protocol numbers: 201708484 and 201609388.

Bacterial cultures
Propionibacterium University of Florida 1 (P. UF1) and its derivative strains, were grown anaerobically at 30 °C in MRS medium (Difco Laboratories, Detroit, MI) supplemented with 1% (w/v) sodium lactate (Thermo Fisher Scientific, Rockford, IL), as previously described.11 ΔactA Listeria monocytogenes (L. m) was grown in Brain Heart Infusion (BHI) medium (Difco Laboratories, Detroit, MI) at 37 °C. Antibiotics were added at the following final concentrations: 5 μg/ml chloramphenicol for isogenic P. UF1 strains; 200 μg/ml streptomycin and 50 μg/ml kanamycin for ΔactA L. m.

Bacterial administration
Female mice (dam) were gavaged with PBS, P. UF1, ΔdlaT P. UF1 (10⁹ CFU/dam, once a week) during pregnancy. After birth, newborn mice of the respective dams were gavaged with P. UF1, ΔdlaT P. UF1 (10⁷ CFU/mouse during breastfeeding, 10⁸ CFU/mouse after weaning, once a week), or PBS. Mice (n = 10) were then orally infected with ΔactA L. m (10⁹ CFU/mouse) at 8 weeks of age. Seven days after infection, fecal samples were collected and metabolome was analyzed by mass spectrometry. a PCA plot showing distinct clustering of fecal metabolomes from P. UF1-, ΔdlaT P. UF1- and PBS-gavaged mice. b Metabolic pathway analysis of metabolites with intensities significantly altered both in comparisons between P. UF1- versus PBS-gavaged group and P. UF1- versus ΔdlaT P. UF1-gavaged group. Red dashed line shows the permutation p value of 0.05 using Mummichog. c Scatter plots for selected metabolite features, with putative annotation, in the significant pathways identified by Mummichog. The m/z, retention time (in seconds) and adduct ion were labeled for each metabolite. Riboflavin, pantetheine, 5-methyltetrahydrofolate 2-arachidonoyl-glycerophosphocholine were identified in AE column; 20-hydroxyeikosatriene E4 and prostaglandin E1 were identified in C18 column. Level of significance is shown as * p < 0.05, ** p < 0.01, *** p < 0.001

Fig. 4 P. UF1 induces metabolite changes during ΔactA L. m infection. Pregnant C57BL/6 dams were gavaged weekly with P. UF1, ΔdlaT P. UF1 (10⁹ CFU/dam), or PBS. After birth, newborn mice of the respective dams were gavaged with P. UF1, ΔdlaT P. UF1 (10⁷ CFU/mouse during breastfeeding, 10⁸ CFU/mouse after weaning, once a week), or PBS. Mice (n = 10) were then orally infected with ΔactA L. m (10⁹ CFU/mouse) at 8 weeks of age. Seven days after infection, fecal samples were collected and metabolome was analyzed by mass spectrometry. a PCA plot showing distinct clustering of fecal metabolomes from P. UF1-, ΔdlaT P. UF1- and PBS-gavaged mice. b Metabolic pathway analysis of metabolites with intensities significantly altered both in comparisons between P. UF1- versus PBS-gavaged group and P. UF1- versus ΔdlaT P. UF1-gavaged group. Red dashed line shows the permutation p value of 0.05 using Mummichog. c Scatter plots for selected metabolite features, with putative annotation, in the significant pathways identified by Mummichog. The m/z, retention time (in seconds) and adduct ion were labeled for each metabolite. Riboflavin, pantetheine, 5-methyltetrahydrofolate 2-arachidonoyl-glycerophosphocholine were identified in AE column; 20-hydroxyeikosatriene E4 and prostaglandin E1 were identified in C18 column. Level of significance is shown as * p < 0.05, ** p < 0.01, *** p < 0.001
Maternal effects of P. UF1

To investigate P. UF1 colonization in the mouse intestine, an antibiotic-resistant P. UF1 strain was constructed by integration of chloramphenicol resistant gene (\( \text{cmR} \)) into the P. UF1 chromosome at the alpha-amylase (\( \text{amyE} \)) locus. Briefly, a 689-bp downstream fragment of \( \text{amyE} \) gene was amplified from P. UF1 genome using primers \( \text{amyE}-\text{F} (5'\text{′}-\text{CCCAAGCTTGTATGGCTCAGCTGCACTCG-3'}\)′) and \( \text{amyE}-\text{R} (5'\text{′}-\text{CCCGAATTCCGTGACCGGCCTXAGTCCTT-3'}\)′), and the \( \text{cmR} \) gene amplified from pUCC-dlaT9 using \( \text{cmR}-\text{F} (5'\text{′}-\text{CCCGGATCCCGTCACACCCGAACATGTCG-3'}\)′) and \( \text{cmR}-\text{R} (5'\text{′}-\text{CCCGGTACCGATGGGTCATCAATTGGCCTC-3'}\)′). The purified fragments \( \text{amyE} \) and \( \text{CmR} \) were cloned into pUC19 plasmid to construct suicide plasmid pUCCmR. Following electroporation into P. UF1, chloramphenicol-resistant colonies were selected and the \( \text{CmR} \) P. UF1 strain was identified by PCR using primers P1 (5′-CGGTGGGATGGAAGAGCC-3′) and P2 (5′-GCGGTGGACCCGGAATCTTGCTC-3′). For demonstrating the bacterial transfer from dams to their pups, C57BL/6 dams were gavaged with CmR P. UF1 (10^9 CFU/dam, twice a week) during pregnancy and breastfeeding. After birth, newborn mice were sacrificed at 5–14 days of age, and colonic luminal contents were collected, weighed, and resuspended in PBS. The presence of CmR P. UF1 was determined by plating the fecal samples on chloramphenicol-resistant MRS-lactate agar plates. For investigating the maternal effect, C57BL/6 dams were gavaged with P. UF1 (10^9 CFU/dam, twice a week) or PBS during pregnancy and breastfeeding. After birth, newborn mice did not receive any gavages with P. UF1 or PBS. On day 21,
the newborn mice were infected with \( \Delta actA \) \( L. \) m (10⁸ CFU/mouse), and fecal samples were collected on days 1–4 post infection for monitoring pathogen burdens. Seven days post infection, mice were sacrificed to analyze colonic CD4⁺ T cell response.

IL-17A neutralization

C57BL/6 mice were continuously gavaged with PBS, P. UF1, or \( \Delta actA \) P. UF1 as described above until 8 weeks of age, and then intraperitoneally injected with purified anti-mouse IL-17A monoclonal antibody (Biolegend, San Diego, CA) on day –6 and –2 relative to infection (100 μg/mouse/injection). On day 0, mice were orally infected with 10⁷ CFU of \( \Delta actA \) \( L. \) m and fecal Listeria burdens were determined from days 1 to 4 post infection.

Flow cytometry

Colonies were isolated as previously described.² For Supplementary Figs. 1 and 2, an improved protocol for T cell isolation was used. Briefly, after shaking in PBS containing 20 mM hepes and 10 mM EDTA for 30 min at 37 °C, colonic tissues were cut into small pieces and incubated with digestion solution (RPMI 1640 containing 10% FBS (Thermo Fisher Scientific, Waltham, MA), 0.4% β-mercaptoethanol, 400 U/ml collagenase VIII (Sigma Aldrich, St. Louis, MO) and 100 μg/ml DNase I (Sigma Aldrich, St. Louis, MO)) for 1.5 h at 37 °C. Digested tissues were filtered through 100 μm cell strainer (Genesee Scientific, San Diego, CA), and cells were resuspended in 5 ml of 40% Percoll (Sigma Aldrich, St. Louis, MO) and overlaid on 5 ml of 80% Percoll. Percoll gradient separation was performed by centrifugation at 700 × g for 1.5 h at 37 °C. Leukocytes at the interface were collected, and used for flow cytometry analysis.⁶ Briefly, after stimulation with phorbol 12-myristate 13-acetate (PMA) (50 ng/ml) and ionomycin (2.5 μM) in the presence of brefeldin A (Sigma-Aldrich, St. Louis, MO), colonic cells were stained with LIVE/DEAD® Fixable Blue Dead Cell Stain Kit (Thermo Fisher Scientific, Waltham, MA). Cells were then stained for cell-surface markers and resuspended in FOXP3 Fix/Perm buffer set (Biolegend, San Diego, CA) for intracellular staining. Antibodies and isotypes were purchased from Biolegend, eBioscience (San Diego, CA). The following antibodies and their corresponding isotypes were used: CD45 (30-F11), CD4 (RM4-5), CD8α (53-67), IFNγ (XMG1.2)/Rat IgG1, κ, IL-17A (TC11-18H10.1)/Rat IgG1, κ, IL-10 (JES5-16E3)/Rat IgG2b, κ, FoxP3 (FJK-16A)/Rat IgG2a, κ, TGFβ (TW7-16B4)/Rat IgG1. Flow cytometric data were collected and analyzed with FlowJo software (TreeStar, Ashland, OR). After dead and doublet cell exclusion and the subsequent CD45⁺ and FCS/SSC selection, T cells were defined as CD3⁺ CD4⁺ CD8⁻. IL-10⁺ IL-17A⁺ CD4⁺, IL-10⁺ IFNγ⁺ CD4⁺, IL-10⁺ FoxP3⁺ CD4⁺, and IL-10⁺ TGFβ⁺ FoxP3⁺ CD4⁺ cells were then plotted (Supplementary Fig. 5).

Sorting Th17 cells

IL-17AGFP dams and their newborns were gavaged with P. UF1 or \( \Delta actA \) P. UF1 for 8 weeks, as described above. Mouse were then orally infected with \( \Delta actA \) \( L. \) m, and colonic cells were isolated 7 days post infection. Th17 cells were sorted with a Sony SH800S Cell Sorter (Sony, Tokyo, Japan) after being stained with anti-CD45, anti-CD3, anti-CD4 antibodies following manufacturers’ suggested protocol.

RNA-sequencing

Total RNA was isolated from sorted Th17 cells with an RNeasy Plus Micro kit (Qiagen, Germantown, MD), and cDNA was generated with a SMART-Seq v4 Ultra-Low Input RNA Kit (Clontech, Mountain View, CA). Following fragmentation, cDNA library was constructed using a Nextera XT DNA Library Preparation Kit (Illumina, Inc., San Diego, CA) and sequenced on an Illumina HiSeq instrument (Illumina, Inc., San Diego, CA) at the University of Florida ICBR NextGen DNA Sequencing Core Facility. FastQ files were aligned to the mouse genome (NCBI GRCm38/mm10) using STAR aligner (v2.6.0),⁴⁸ and count table was generated using SubReads featureCounts (v1.6.0).⁴⁹ Genes with lower expressed transcripts (mean expression value less than one RPKM throughout the dataset) were excluded. Subsequently, DESeq2 was used to determine differential expression.³⁰ Stringent criteria were set to determine significantly dysregulated genes: FDR p-value less than 0.05 and fold-change (FC) greater than 1.5. Selected genes were mapped on the Volcano plot. Regularized-log-transformation of count data was performed before principal component analysis (PCA) and heatmap plotting. Gene set enrichment analysis (GSEA) was performed in the javaGSEA (v3.0) using the GseaPreranked method, with genes ranked according to their DESeq2 Wald test statistics. GSEA’s “weighted” scoring scheme was used to calculate enrichment scores for all gene sets in database, including GO, Hallmark, KEGG and REACTOME. Nominal enrichment p values were based on 1000 permutations. GSEA enrichment plots were made using code adapted from Rtoolbox. Over-representative test of genes significantly upregulated in either condition (FDR p < 0.05) was performed in GSEA website (http://software.broadinstitute.org/gsea/msigdb/annotate.jsp).

Quantitative reverse-transcription PCR (qRT-PCR)

Transcripts for genes of interest were quantified by qRT-PCR using cDNA generated for RNA-seq. PCR was performed on a CFX96 real-time PCR system (Bio-Rad, Hercules, CA) using SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, Hercules, CA) with following primers: Gapdh, 5′-GGTTAAGGGTCGGTGAAGC-3′ and 5′-CTCGCTCTGGAAGATGTGG-3′; Cxcl6, 5′-ACCAGCTTTAAGTATG CGATGCTGA-3′ and 5′-TCCGGACCTTTAGCACAATA-3′; Prcn, 5′-TGGCGGACAGAAATATGGT-3′ and 5′-TCTCTTATTTCTCAAGCTA CTCATGT-3′; Neu3, 5′-CTCTATCGAGTAGTATGC-3′ and 5′-GTGAGACATAGTGGCATAGG-3′; Alox5, 5′-CTTTTCAAGCTGA GTGC-3′ and 5′-TGATGTACCTAGGATGAG-3′; Cxcl1, 5′-TGGCTGGGCTTCACCTCAAG-3′ and 5′-CAACGGCTCGCACATT CT-3′; Cxcl2, 5′-CGCTGTAATGCTCGGAC-3′ and 5′-CACTCAGGGTCGTGGATGTCTGTG-3′; Ccld, 5′-TCTGATCCAGGGAGA ATTT-3′ and 5′-TCTTCTCTGTGGTGTCAGCACACTT-3′; Cybb, 5′-CATCGGTGA CAATGTGAAGAC-3′ and 5′-AAGGCCGATGAAGAAGATCA-3′. Measurements were made in duplicate wells, and results were normalized to those obtained with Gapdh and are presented as fold change over P. UF1.

Ultra-high-resolution metabolomics analysis

Mouse fecal samples were collected and analyzed on an LTQ-FTICR mass spectrometer coupled with amino acid exchange (AE) and reversed-phase (C18) liquid chromatography, as previously described.³ We used apLCMS to perform peak detection, noise filtering, mass to charge (m/z) ratio and retention time alignment and feature quantification.⁵¹ An in-house informatics pipeline was used to quality control and clean up the feature table, as previously described.³⁰ PCA was performed on log2-transformed intensity values of top 500 metabolite features with highest CV across all samples. Pairwise student’s t-tests were performed among treatment groups. Subsequently, metabolic pathway analysis was performed by Mummichog (v2.0), a software specifically designed for targeted metabolomics.⁵₂ using default parameters. 205 metabolite features (C18 run) and 203 metabolite features (AE run), which are either significantly higher or lower in P. UF1 group compared to PBS or \( \Delta actA \) P. UF1 groups of mice (p < 0.05, Student’s t-test) were used as input to Mummichog. The total list of features was used as a reference. Pathway enrichment p-values were calculated in Mummichog based on permutation. The pathways were represented by at least two significant metabolites and enriched at p < 0.05 in either chromatographic column.

Microbiota analysis

Fecal DNA was isolated and DNA library constructed.⁹ The 16S rDNA library was sequenced on an Illumina Miseq (Illumina, Inc.,
San Diego, CA), and sequence analysis was performed as previously described.9 Briefly, FastQ files were jointed, de-multiplexed and analyzed by QIIME (v1.9.1). PcoA and the Permanova analyses were also performed using QIIME (v1.9.1) and replotted using Python. Significant taxons were filtered by LDA Effect Size (LEfSe) analyses with default criteria (p < 0.05 by Kruskal–Wallis test; LDA score > 2).

Statistical analysis
Statistical analyses were performed using GraphPad Prism v7.0. Data are expressed as mean ± SEM of number (n) of mice or samples per group. Prior to statistical analysis, normality was tested using the Shapiro–Wilk normality test. Where the groups follow a Gaussian distribution, two-tailed unpaired t-test (for two variables) or one-way ANOVA followed by Tukey’s post-test (for three variables) was performed. Where the groups did not follow a Gaussian distribution, Mann–Whitney U test (for two variables) or Kruskal–Wallis test followed by Dunn’s post-test (for three variables) was performed. P values lower than 0.05 were considered as significant: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

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AUTHOR CONTRIBUTIONS
M.M. directed and designed the cellular and molecular experiments, which were executed by N.C., Y.G., M.G., J.L., and M.Z. N.C., Y.G. and M.Z. performed the mouse work and flow cytometry analysis. Y.G. and J.L. performed the molecular studies, including transcriptomic studies. M.G., S.L. and D.J. performed, analyzed, and directed metabolic studies. M.G. conducted all bioinformatic work for microbiota, metabolic, and transcriptomic data analyses. Y.G., M.C., M.G., J.L., S.L., D.P.J., and M.M. analyzed and interpreted the data. M.M. wrote the manuscript.

ADDITIONAL INFORMATION
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