Supporting Information

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SI Materials and Methods

Bacterial Strains. *Listeria monocytogenes* EGDe strain was grown in brain heart infusion (BHI) medium (Difco) at 37 °C. *Lactobacillus paracasei* CNCM I-3689 and *Lactobacillus casei* BL23 strains were grown in de Man, Rogosa, and Sharpe (MRS) medium (Oxoid) at 37 °C. The complete genome sequence of the *L. casei* strain BL23 has been published in 2010 (1).

Animals and Generation of a Germ-Free Mouse Line. All experiments involving mice were handled in accordance with the Pasteur Institute guidelines for animal welfare. We derived the knock-in E16P mouse line (2) as germ-free mice (Taconic) and compared the growth rates, adult weights, and fertility of germ-free and wild-type E16P mice. They were indistinguishable. Germ-free E16P mice were housed in plastic gnotobiotic isolators. Only 9- to 12-wk-old female mice were used for experiments.

Monoassociation and Treatment with *Lactobacillus*. Lactobacilli overnight cultures were collected and centrifuged at 3,500 × *g* for 15 min. After three washes in PBS, the pellets were resuspended in PBS at a final concentration of 1×10^{10} bacteria/mL. For each *Lactobacillus*, mice were inoculated orally with 2×10^9 bacteria diluted in 200 μL of PBS. Mice ($n \geq 3$ per group) were either (*i*) monoassociated with each *Lactobacillus* for 3 consecutive days (3 d) (or for 24 h when required) and killed 6 d after the first inoculation or (*ii*) monoassociated with the lactobacilli for 3 consecutive days and infected 3 d later with *L. monocytogenes* for 24 h. Serial dilutions of the inoculums were plated to control the number of lactobacilli that were inoculated in mice. To examine the persistence of the two lactobacilli in gnotobiotic mice, we determined the numbers of each *Lactobacillus* in the feces of the monoassociated mice by plating serial dilutions of the homogenates on MRS agar plates for 3 wk. In conventional mice, serial dilutions of the feces homogenates were plated on Rogosa medium (Oxoid) allowing the isolation and enumeration of lactobacilli among other bacteria from the intestinal flora. Untreated mice were used as a control for the basal level of endogenous lactobacilli present in the feces.

Infection. *L. monocytogenes* overnight cultures were diluted in BHI and bacteria were grown until OD = 1. Bacterial cultures were centrifuged at 3,500 × *g* for 15 min. After three washes in PBS, *L. monocytogenes* pellet was resuspended in PBS at a final concentration of 2.5×10^{10} bacteria/mL. Mice ($n \geq 3$ per condition) were infected orally with 5×10^9 bacteria diluted in 200 μL of PBS supplemented with 300 μL of $CaCO_3$ (50 mg/mL) for 24 h. Serial dilutions of the inoculum were plated to control the number of *L. monocytogenes* inoculated in mice.

Bacterial Counts in Organ. The small intestine, cecum, mesenteric lymph nodes, liver, and spleen were removed. Mesenteric lymph nodes, liver, and spleen were directly disrupted in PBS. The small intestine was cut into 16 equal-sized segments (numbered 1–16; proximal to distal). Intestinal fragments (3-7-11-15) and cecum were washed five times in DMEM and incubated 2 h in DMEM containing 100 μg/mL gentamicin. After five washes in DMEM, intestinal segments and cecum were disrupted in PBS. Serial dilutions of all organ homogenates were plated on BHI plates and incubated for 2 d at 37 °C. Intestinal and cecal luminal contents were harvested, weighed, and resuspended in PBS. Serial dilutions of luminal contents were plated on *Listeria* selective Oxford plates (Oxoid) for *Listeria* counts and on MRS plates for lactobacilli counts. Statistical tests were performed using a Mann–Whitney test on at least three different experiments.

Antimicrobial Activity. The antimicrobial activity was tested as previously described (3). Briefly, the lactobacilli were grown on MRS agar plates for 24 h at 37 °C. BHI soft agar inoculated with *L. monocytogenes* was then poured on the MRS plates and incubated for 24 h at 37 °C. pH-dependent effect has been assessed on MRS agar plates supplemented with bromocresol purple as a pH indicator.

Mouse Gene Chip Analysis. RNAs from the ileal tissue (segment 12) were extracted and purified using classical TRIzol/chloroform protocol. All samples were treated with Turbo DNase (Ambion). RNA quality was determined using Experion Automated Electrophoresis Station (Bio-Rad). Labeled cDNA was synthesized from 200 ng total RNAs using NuGEN Applause WT-Amp Plus ST systems (NuGEN Technologies). Labeled samples were hybridized to Affymetrix MoGene 1.0 ST GeneChips and scanned with an Affymetrix Genechip Scanner 3000, generating CEL files for each array. At least three biological replicates were run for each condition. Gene-level expression values were derived from the CEL file probe-level hybridization intensities using the model-based robust multichip average algorithm (4). Statistical analysis has been performed using the Local-Pooled-Error test (5). The estimated false discovery rate was calculated using the Benjamini and Hochberg approach (6). Canonical pathways have been identified using the Ingenuity IPA application using a *P* value calculated by Fisher's exact test, right tailed.

Quantitative RT-PCR (qRT-PCR). For gene expression analysis, total eukaryotic RNAs (1 μg) or *L. monocytogenes* RNAs (100 ng) were reverse transcribed using iScript cDNA synthesis (Biorad). The cDNAs were used as templates for PCR using SYBR Green PCR Master mix (Applied Biosystems) and detected using Real-Time PCR system ABI PRISM 7900HT (Applied Biosystems). Expression of eukaryotic genes from individual mice ($n \geq 3$ per group) was normalized to expression of the GADPH gene. Expression of *L. monocytogenes* genes and sRNAs extracted from individual mice ($n \geq 3$ per group) was normalized to expression of the *rpoB* gene. For miR expression analysis, total eukaryotic RNAs (1 μg) were reverse transcribed using miScript Reverse Transcription kit (Qiagen). The cDNAs were used as templates for PCR using miScript SYBR Green PCR kit (Qiagen) and detected using Real-Time PCR system ABI PRISM 7900HT (Applied Biosystems). Statistical tests were performed using a two-tailed Student *t* test on at least two independent experiments. Each experiment has been performed using at least three mice per condition.

***Listeria* Tiling Array Analysis.** Total bacterial RNAs from the ileocecal content were extracted and treated as previously described; extracted RNAs from each mouse ($n = 3$ for each condition) were treated separately (7). Total RNAs (200 ng) were amplified using the MessageAmpII-Bacteria kit (Ambion) before fragmentation using the 5× fragmentation buffer. A total of 7.5 μg of amplified and fragmented RNA were used per chip. Sample preparation for each chip was then processed following the Affymetrix GeneChip Expression Analysis Technical Manual (P/N 702232 Rev. 2) as previously described (7). Tiling arrays were normalized using DNA reference normalization (8) against *L. monocytogenes* genomic DNA grown in BHI at 37 °C generating log-transformed expression values. Median expression value of tiling data has been adjusted to zero. Normalization on gene and sRNA expression using *L. monocytogenes* housekeeping gene expressions (*dnaA*, *dnaN*,

gyrA, *gyrB*, and *rpoB*) was applied. Statistical deviation of each gene and sRNA was calculated using all corresponding tiling probes, a common independent two sample *t* test was derived from it for assessing differentially expressed genes or sRNAs.

Cytokine dosage by ELISA. Cytokine levels from cell culture supernatants were analyzed by classical ELISA. The IFNγ, IL-2, IL-10, and IL-22 levels were determined by using the mouse ELISA Ready-SET-Go! kits (eBioscience). Cytokine level was then measured using a Tristar LB491 luminometer (Berthold Technologies). Statistical tests were performed using a Mann–Whitney test in three independent experiments. Each experiment has been performed using at least three mice per condition.

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3. Patent Application Publication (2011) Novel strain of *Lactobacillus paracasei* subspecies *paracasei* having antimicrobial and immunomodulary properties. US 2011/0150852 A1.
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Fig. S1. Infection with *Lm* after *Lactobacillus* treatment. (*A*) *Lactobacillus* counts in the ileal or cecal content (cfu/g) in mice monoassociated for 3 consecutive days (3 d) with *L. paracasei* or *L. casei* or in mice that were monoassociated with the lactobacilli for 3 d and infected 3 days later with *Lm* for 24 h. (*B*) *Lactobacillus* counts in the feces (cfu/g) of gnotobiotic E16P mice after *L. paracasei* and *L. casei* monoassociation (*Left*) and total *Lactobacillus* counts in the feces (cfu/g) of conventional E16P mice (*Right*). The first 3 d correspond to the three consecutive *Lactobacillus* administrations. In conventional mice, the treatment was not detectable, suggesting that the monoassociation does not lead to drastic changes in *Lactobacillus* population. (*C*) *Listeria* counts in the ileal and cecal content, the mesenteric lymph nodes, cecum tissue, and liver of the gnotobiotic E16P mice infected for 24 h with *Lm*, 3 d after treatment or not with each *Lactobacillus* for 3 d. (*D*) *Lm* counts in the spleen and in the liver of conventional E16P mice that were monoassociated or not with the lactobacilli for 3 d and infected 3 d later with *Lm* for 24 h. Each dot represents one organ of mouse. Horizontal bars represent the mean for each condition. Statistical tests were performed using a Mann–Whitney test. NS, nonsignificant difference.

Fig. S1

Fig. S2. Antimicrobial activity. (*A*) Antimicrobial activity was tested as previously described (3). Briefly, the lactobacilli were grown on MRS agar plate for 24 h at 37 °C. BHI soft agar inoculated with *Lm* was then poured on the MRS plate and incubated for 24 h at 37 °C. (*B*) pH-dependent effect has been assessed on MRS agar plate supplemented with bromocresol purple as a pH indicator. The area of inhibition observed for the two different strains correlates with the transition of the pH indicator.

Fig. S2

3. Chambaud I, et al. (2011) Patent application publication. Novel strain of *Lactobacillus paracasei* subspecies *paracasei* having antimicrobial and immunomodulary properties. US 2011/0150852 A1 (June 23, 2011).

Fig. S3. Whole host genome transcriptomic analysis. Heatmaps present host genes whose expression was significantly affected (FDR-BH $P < 0.05$) after monoassociation with each *Lactobacillus* (*A*), upon *Lm* infection (*B*), and in mice that were monoassociated with the lactobacilli for 3 d and infected 3 d - later with *Lm* for 24 h (*C*–*E*). (*A* and *B*) Values correspond to the fold change (FC) of gene relative expression in *L. paracasei* or *L. casei* monoassociated mice (*A*), in *Lm*-infected mice (*B*) compared with control mice (FC/control mice). (*C*–*E*) *Left*, the three columns show values corresponding to the FC of gene relative expression in *Lm*-infected mice (*Lm*), in mice treated with *L. paracasei* and infected by *Lm* (*L. paracasei* + *Lm*) and in mice treated with *L. casei* and infected by *Lm* (*L. casei* + *Lm*) compared with control mice (FC/control mice). *Right*, the last two columns show FC of gene relative expression in (*L. paracasei* + *Lm*) and (*L. casei* + *Lm*) mice compared with *Lm*-infected mice (FC/*Lm* infected mice). In contrast, a white square in the heatmap indicates a gene whose expression was not significantly affected by the treatment with the lactobacilli.

Fig. S3

Fig. S4. Identification of signaling or metabolic pathways using Ingenuity IPA software. Pathways that are significantly regulated after 3 d (3 d) *L. paracasei* (*A*) and *L. casei* (*B*) monoassociation or after 24 h *Lm* infection (*C*) have been identified. The *P* value has been calculated by Fisher's exact test, right tailed. Molecules belonging to the corresponding pathways are indicated in the *Left* column.

Fig. S4

Fig. S5. qRT-PCR validation. (*A*) qRT-PCR analysis of *Ifitl* and *Oas2* gene expression in mice monoassociated for 24 h or 3 consecutive days (3 d) with *L. paracasei* or *L. casei,* or in mice that were monoassociated or not monoassociated with the lactobacilli for 3 d and infected 3 d later with *Lm* for 24 h. FC is presented after standardization to the GADPH as an internal control and corresponds to the comparison of each condition with the control mice normalized to 1. (*B* and *C*) qRT-PCR analysis of *lmo1153* and *lmo1174* gene and Rli47 and Rli116 sRNA expression in mice that were monoassociated or not monoassociated with the lactobacilli for 3 d and infected 3 d later with *Lm* for 24 h. FC is presented after standardization to *rpoB* and using *Lm* grown in broth medium that have been normalized to 1 as reference. Data are represented as mean with SEM. Statistical tests were performed using a two-tailed Student *t* test. Asterisks indicate a value considered statistically significant (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$); NS, nonsignificant difference.

Fig. S5

Fig. S6. Cytokine production. Cytokine dosage of IFNγ in the intestinal tissue or spleen (*A*) and of the interleukins IL-2, IL-10, and IL-22 in the intestinal tissue (*B*) of mice monoassociated for 24 h or 3 consecutive days (3 d) with *L. paracasei* or *L. casei,* or in mice that were monoassociated or not with the lactobacilli for 3 d and infected 3 d later with *Lm* for 24 h. Data are represented as mean with SEM. Statistical tests were performed using a Mann–Whitney test. Asterisks indicate a value considered statistically significant (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$); NS, nonsignificant difference.

Fig. S6

Fig. S7. Impact of the lactobacilli on the whole *Lm* gene expression profile. Mice were monoassociated or not monoassociated for 3 consecutive days (3 d) and infected 3 d later with *Lm* for 24 h. Heatmap presents *Lm* genes whose expression was significantly affected by the lactobacilli during *Lm* infection (*t* test $P < 0.05$). *Left*, the three columns show values corresponding to the fold change (FC) of gene relative expression in ileal-cecal content from *Lm*-infected mice (*Lm*), in mice treated with *L. paracasei* and infected by *Lm* (*L. paracasei* + *Lm*) and in mice treated with *L. casei* and infected by *Lm* (*L. casei* + *Lm*) compared with *Lm* growing in broth medium (FC/*Lm* BHI). *Right*, the last two columns show FC of gene relative expression in (*L. paracasei* + *Lm*) and (*L. casei* + *Lm*) mice compared with *Lm*-infected mice (FC/*Lm* intestinal lumen). White square in the heatmap indicates a gene whose expression was not significantly affected by treatment with the lactobacilli.

Fig. S7

Fig. S8. sRNAs regulated upon *Lm* infection and not affected by the two lactobacilli. Heatmap presents *Lm* sRNAs whose expression was significantly affected by *Lm* and not affected by treatment with the lactobacilli (*t* test $P < 0.05$). Absence (–) or presence (+) on the sRNAs in *L. innocua* (*Lin*) is indicated.

Fig. S8

Fig. S9. miR network. Genes regulated by *Lm* infection in our transcriptomic analysis have been overlapped with genes predicted to be targeted by the miR-181b, mir-192, mir-200b, and mir-215 (TargetScanMouse 6.0). Node represents genes linked to the miR, which potentially targets them. They are colorized according to the fold change of gene relative expression in *Lm*-infected mice compared with control mice.

Fig. S9