Mice Lacking Fatty Acid-Binding Protein 5 Are Resistant to *Listeria monocytogenes*

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Abstract

To investigate the role of fatty acid-binding protein 5 (FABP5) in infectious diseases, FABP5-deficient mice were challenged with *Listeria monocytogenes*, a facultative intracellular bacterial pathogen. Interestingly, FABP5-deficient animals were able to clear the infection within 3 days whereas control wild-type (WT) animals showed comparatively higher bacterial burdens in the liver and spleen. Sections of infected tissues showed an increase in inflammatory foci in WT mice compared to FABP5-deficient mice. FABP5-deficient mice had lower circulating inflammatory cytokines and increased inducible nitric oxide synthase production. FABP5-deficient mouse bone marrow-derived macrophages produced higher levels of nitrite anion than their WT counterparts in response to various stimuli. Additionally, in contrast to FABP5^{-/-} mice, transgenic mice overexpressing FABP5 in myeloid cells (LysM-Cre driven) showed decreased survival rates and increased bacterial burden and inflammatory cytokines. Overall, these findings suggest that increased FABP5 levels correlate with a higher *L. monocytogenes* bacterial burden and elevated subsequent inflammation.

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Introduction

Listeria monocytogenes is a facultative intracellular Gram-positive bacterium responsible for listeriosis. This bacterium is one of the most virulent food-borne pathogens with fatality rates as high as 20–30% in high-risk individuals, and the third-most common cause of meningitis in newborns. Host cell invasion by this bacterium occurs via phagocytosis or pathogen-induced endocytosis in hepatocytes, neurons, epithelial cells, endothelial cells and fibroblasts [1]. Mouse models of *L. monocytogenes* infection have been critical for our understanding of the immune system's response to intracellular bacterial infections [2]. These models have been instrumental in showing that macrophages are critical in the early stages of infection [3] by producing reactive nitrogen species, reactive oxygen species (ROS), and inflammatory cytokines such as TNF α , IL-12, and IFN γ [3, 4].

Fatty acids have been shown to alter the oxidative environment of cellular lipids [5] and, by doing so, potentially affect macrophage functions [6]. Fatty acid-binding proteins (FABPs) are a family of small, highly conserved, cytoplasmic proteins that bind long chain fatty acids and other hydrophobic ligands, and are involved in fatty acid uptake, transport, and metabolism [7]. FABP5, the epidermal fatty acid-binding protein [8], has been shown to

be upregulated in alveolar macrophages during acute lung rejection [9]. We have previously demonstrated an unknown contribution of FABP5 to *Influenza A virus* pathogenesis, where it controls excessive oxidative damage and inflammation [10]. Recently, however, FABP5 has been shown to limit the anti-inflammatory response in murine macrophages following intraperitoneal lipopolysaccharide (LPS) treatment [11]. This apparent contradiction prompted us to identify the role of FABP5 in the outcome of *L. monocytogenes* infection and to explore the immune response elicited by this intracellular pathogen in FABP5-deficient macrophages. Here, we clearly demonstrate that FABP5 increases susceptibility to *L. monocytogenes* infection as FABP5^{-/-} animals exhibited heightened resistance to bacterial infection, whereas increased mortality and bacterial burden were observed in mice overexpressing FABP5 in their myeloid cells.

Materials and Methods

Construction of the Rosa26-STOPflox-Fabp5-2A-YFP Mouse

Rosa26-STOPflox-Fabp5-2A-YFP mice were generated by the Mouse Genetics Core Facility at National Jewish Health. In these mice, the ROSA26 locus contains a STOP-flox cassette upstream of mouse Fabp5 cDNA linked by a 2A peptide to YFP. Upon exposure to Cre recombinase (LysM-Cre), the stop cassette is deleted and the Fabp5-2A-YFP transcript undergoes cotranslational cleavage due to the 2A peptide, generating 2 separate Fabp5 and YFP proteins in myeloid cells.

Briefly, the targeting construct was made in a pBSKS-DTA vector. The Rosa26 genomic regions were isolated from BAC clone RP23-324O18, mouse Fabp5 was amplified from a plasmid containing its cDNA (clone ID 301376900, Dharmacon), the STOP^{flox} cassette (containing a positive selection marker Neo flanked by loxP sequences) was cloned from pBigT (Addgene, #21270), and the 2A peptide was synthesized in the primers to amplify YFP. The linearized construct was electroporated into JM8.A3 cells and cells were selected with G418 (positive selection) for targeted insertion of the construct. Selected clones were picked and frozen. Successfully targeted homologous recombinant clones were determined by screening for the loss of the Rosa26 allele by qPCR, followed by PCR using a primer pair nested in Rosa26 and YFP [12]. Functionality of the construct in the ROSA26 locus was verified on homologous recombinant embryonic stem (ES) cell clones by expansion and electroporation of these clones with a plasmid expressing Cre recombinase expression under the control of the CMV promoter. Four days after electroporation, these ES cell clones were analyzed by flow cytometry to assess the expression of YFP in the absence or presence of Cre recombinase. Two clones were selected following chromosome counting for injection into C57BL/6J blastocysts, which were transferred into pseudopregnant ICR females. Chimeric offspring were bred for germline transmission, and germline transmission was achieved for both clones.

Animals

FABP5^{-/-} mice and littermate wild-type (WT) controls, on a C57BL/6J background, were kindly provided by Dr. Gokhan Hotalmisilgil at Harvard University (Boston, MA, USA), and bred in our Biological Resources Center, along with the transgenic mice described above. Mice were kept on a 12-h light-dark cycle with ad libitum access to food and water.

L. monocytogenes and Mouse Infection

The WT virulent strain 10403S of *L. monocytogenes* was used for all animal studies. Mice were injected i.v. with 200 μ L of *L. monocytogenes* diluted in PBS. The actual *L. monocytogenes* infectivity dose was determined by plating the injection input on TSB agar plates. Liver and spleen bacterial burden were determined by homogenization in 0.1% Nonidet P-40 (USB) using a T25 digital Ultra Turrax tissue grinder (IKA Works), and lysed at room temperature for 30 min. Serial dilutions of homogenates were plated onto TSB agar plates and counted after overnight incubation at 37 °C. To determine survival, mice were monitored every 12 h postinfection for signs of morbidity.

Alanine Aminotransferase Assay

Serum alanine aminotransferase (ALT) was measured using an ALT colorimetric reagent kit (Teco Diagnostics) and adapted to a 96-well plate format. Briefly, 10 μ L of fresh serum was placed in each well and incubated at 37 °C for 30 min with 50 μ L ALT substrate, 10 min with 50 μ L color reagent, and 5 min with 200 μ L color developer in accordance with the manufacturer's instructions. Absorbance was measured at 505 nm using a SynergyHT plate reader with Gen5 software (BioTek) and the serum ALT concentrations were determined using a standard curve of serially diluted ALT calibrator.

Liver Histological Evaluation

Whole livers were removed from naïve or infected mice, fixed in 10% buffered formalin, paraffin-embedded, and stained with hematoxylin and eosin. Color images of liver sections were acquired on a Nikon Eclipse 80i microscope equipped with a Nikon DS-U/L2-Ri1 camera using NIS-Elements AR software.

Intracellular Inducible Nitric Oxide Synthase Determination

All staining procedures were done in PBS supplemented with 0.1% BSA and 0.05% sodium azide at 4 °C. Single-cell suspensions from spleens were prepared by mechanical disruption using 100- μ m nylon mesh (Fisherbrand). After red blood cell lysis with ACK buffer, cells were counted using trypan blue exclusion, and stained using the following antibodies: CD3 ϵ -APC (145-2C11), CD8-APC (53-6.7), CD4-APC (L3T4), NK1.1-APC (PK136), CD19-APC (MB19-1), CD11b-Pacific Blue (M1/70), CD11c-APC Cy7 (N418) and Ly6G PerCP (1A8) from eBiosciences; and Ly6C-FITC (AL-21) from BD Pharmingen. Intracellular inducible nitric oxide synthase (iNOS) proteins were detected using NOS2-PE (CXNFT, eBiosciences). To identify iNOS-producing monocytes, we first gated on live cells and excluded doublets, lymphocytes APC⁺ cells, and Ly6G⁺ neutrophils. Second, we gated on CD11b⁺/Ly6C⁺ monocytes expressing iNOS. For the detection of intracellular iNOS, the BD Cytofix/Cytoperm plus kit (BD BioSciences) was used according to the manufacturer's instructions. Samples were acquired using a CyAnTM Flow Cytometer (Dako) and data were analyzed using FlowJo v10.2 software (Tree Star, Inc.).

Cytokine Determination

Serum was separated by centrifugation using Z-Gel microtubes (Sarstedt). Cytokine concentrations in mouse serum were measured using the MSD Mouse Th1/Th2 9-plex ultrasensitive kit (Meso Scale Discovery, Gaithersburg, MD, USA) per manufacturer's instructions.

Bone Marrow-Derived Macrophages

Bone marrow-derived macrophages (BMDMs) were generated *in vitro* by flushing bone marrow from mouse tibias and femurs. Progenitor cell suspensions were cultured for 5 days in DMEM containing 10% FBS, 100 U/mL penicillin, 100 µg/mL streptomycin, and 10% L929 cell-conditioned medium as a source of CSF-1. Macrophage differentiation was confirmed by flow cytometry (>95% positive for F4/80 and CD11b).

Griess Reaction

Nitrate anion (NO₂⁻) production by BMDM was measured using the Griess reagent system (Promega, Madison, WI, USA) following the manufacturer's instructions. Briefly, cells were stimulated with recombinant mouse TNFα (10 ng/mL; eBiosciences), mouse IFNγ (10 U/mL; eBiosciences), or LPS (100 ng/mL; Sigma) diluted in BMDM media, and added alone or in combination to adherent BMDM cultures for up to 48 h. Fifty microliters of supernatant was used to determine the micromolar amount of nitrite by measuring the absorbance at 540 nm using a SynergyHT plate reader with Gen5 software (Bio-Tek).

Macrophage *Listeria*-Killing Assay

Exponential-phase *L. monocytogenes* (10403S) was added to BMDM at a multiplicity of infection (MOI) of 10, incubated for 30 min at 37°C to allow phagocytosis, removed, and replaced with antibiotic-free media for another 30 min at 37°C to complete bacterial uptake. Gentamycin (200 µg/mL; Sigma) was added to kill any remaining extracellular bacteria so that only intracellular bacteria were measured. Colony-forming units were determined by plating serial dilutions of lysates on TSB agar plates after overnight incubation at 37°C.

Bone Marrow Chimeras

Bone marrow cells were flushed from the femurs and tibias of donor mice. Red blood cells were lysed with ACK buffer (Quality Biological, Inc.) and washed twice and resuspended in PBS. Recipient mice were irradiated with 900 rad and received a total of 5 × 10⁶ bone marrow cells through tail vein injection. Mice were allowed to recover for 8 weeks before being used in experiments.

Statistical Analysis

All data are expressed as means ± SEM. Statistical significance was evaluated using Prism 5 for Mac OS X software to perform Student's *t* test (unpaired, two-tailed) for comparisons between 2 groups. One-way analysis of variance was used for multiple comparisons, and Tukey's post hoc test was applied where appropriate. For Kaplan-Meier survival data, median survival times and significance were compared between groups using a log-rank (Mantel-Cox) test.

Results

FABP5-Deficient Mice Are Resistant to Lethal *Listeria* Infection

A recent study reported that LPS injury of *FABP5*^{-/-} mice resulted in less liver injury despite increased macrophage infiltration [11]. Thus, we hypothesized that *FABP5*^{-/-} mice may be protected against bacteria-induced liver injury. To test this hypothesis, WT and *FABP5*^{-/-} mice were injected *i.v.* with a lethal dose of *L. monocytogenes*. Confirming our hypothesis, *FABP5*^{-/-} mice survived a dose of 1.7 × 10⁵ CFUs and showed no outward signs of illness (Fig. 1a), while half of the WT mice succumbed to *L. monocytogenes* infection. In addition, *FABP5*^{-/-} mice experienced less weight loss during infection in comparison to WT mice (Fig. 1b). Next, we examined the bacterial load in the liver and spleen 48 h after infection, and found it reduced in *FABP5*^{-/-} mouse organs (Fig. 1c). We assessed liver damage by not only measuring serum levels of ALT (Fig. 1d), but also by quantifying infiltrations of inflammatory cells in the whole liver (Fig. 1e, f). While the ALT levels were not significantly different between WT and *FABP5*^{-/-} mice, WT mice had more inflammatory infiltrates in the liver than *FABP5*^{-/-} mice.

FABP5-Deficient Mice Have Diminished *Listeria*-Induced Cytokine Production

The transactivation of macrophage bactericidal activity and survival to *L. monocytogenes* infection requires IL12-dependent production of IFNγ by NK and CD8⁺ T cells in an antigen-independent manner [13, 14]. Thus, we quantified the level of IFNγ and IL-12p70 in the serum following infection. Both IFNγ (Fig. 2a) and IL-12p70 (Fig. 2b) were reduced by 50% in *FABP5*^{-/-} mice compared to WT mice. Similarly, other inflammatory cytokines were significantly reduced in the serum of *FABP5*^{-/-} mice, including IL-1β (Fig. 2c), IL-6 (Fig. 2d), KC (Fig. 2e), and TNFα (Fig. 2f). Furthermore, we measured the level of anti-inflammatory cytokine IL-10 (Fig. 2h) and showed that it was also significantly reduced in *FABP5*^{-/-} mice. Together, these data suggest that *FABP5* deficiency in mice results in a lower *L. monocytogenes* burden and reduced tissue damage, as well as dampened inflammatory responses.

FABP5-Deficient Macrophages Are Better Producers of ROS

Macrophages are central to the control of *L. monocytogenes* infection and replication via the inhibition of vac-

Fig. 1. Enhanced resistance of FABP5^{-/-} mice to lethal infection with *L. monocytogenes*. **a** Kaplan-Meier survival plot of mice i.v. infected with *L. monocytogenes*. Six WT (dashed line) and 10 FABP5^{-/-} (continuous line) mice were infected with 1.7×10^5 CFUs. **b** Changes in body weight loss following infection from mice described in **a**. **c** *L. monocytogenes* burden in the liver and spleen of WT (open circles) and FABP5^{-/-} (black squares) mice 48 h postinfection (7 mice per group) infected with 2.4×10^4 CFUs.

d Serum was collected from the same WT (open circles) and FABP5^{-/-} (black squares) mice described in **c** and ALT was quantified using a modified Reitman-Frankel colorimetric end-point reaction. **e** Stained WT and FABP5^{-/-} livers 48 h after *L. monocytogenes* (*Lm*) infection. Cellular infiltrates are surrounded by black lines. HE. $\times 20$. **f** Quantification of cellular infiltrates in WT (open circles) and FABP5^{-/-} (black squares) livers (8 mice per group). Data are representative of 4 independent experiments.

Fig. 2. Decreased levels of multiple cytokines in *L. monocytogenes*-infected FABP5^{-/-} mice in comparison to WT mice. **a** IFN γ . **b** IL-12p70. **c** IL-1 β . **d** IL-6. **e** KC. **f** TNF α . **g** IL-10. Cytokines were measured in the serum of WT (open circles) or FABP5^{-/-} (black

squares) mice infected with 4.5×10^4 CFUs 48 h postinfection using the multiplex MSD plate ($n = 7$ mice/group). Data are representative of 4 independent experiments.

Fig. 3. Increased in vivo iNOS activation by Ly6C⁺ monocytes in FABP5^{-/-} mice and increased in vitro NO₂⁻ production by FABP5^{-/-} macrophages. **a** Representative density plots of iNOS expression in CD11b⁺/Ly6G⁻/Ly6C⁺ gated spleen monocytes ($n = 5-6$ mice/group) *Lm*, *L. monocytogenes*. **b** Absolute numbers of iNOS⁺ monocytes in WT (open circles) and FABP5^{-/-} (black squares) spleen 48 h postinfection ($n = 5-6$ mice/group). **c** Proportion of neutrophils (Ly6G⁺) in WT (open circles) and FABP5^{-/-} (black squares) spleen 48 h postinfection ($n = 5-6$ mice/group). **d** Abso-

lute numbers of iNOS⁺ neutrophils in WT (open circles) and FABP5^{-/-} (black squares) spleen 48 h postinfection ($n = 5-6$ mice/group). **e** FABP5^{-/-} BMDMs secrete more NO₂⁻ than WT BMDMs in response to inflammatory stimuli. WT and FABP5^{-/-} BMDMs were left untreated or stimulated with 10 ng/mL TNF α , 100 ng/mL LPS, and 10 U/mL IFN γ in combination as indicated. Supernatants were obtained 24 h after stimulation and NO₂⁻ production was measured by Griess reaction. Bars represent mean nitrite (NO₂⁻, μ M) $\pm$ SEM. Data are representative of 3 independent experiments.

uole and cell-to-cell escape of the bacterium. Therefore, we hypothesized that the resistance of FABP5^{-/-} mice to *L. monocytogenes* may be due to more efficient macrophage activation in the absence of FABP5. To test this hypothesis, we analyzed the intracellular expression of iNOS in splenic monocytes by flow cytometry (Fig. 3a)

and observed twice as many iNOS⁺ monocytes in the FABP5^{-/-} spleens when compared to WT mice (Fig. 3b). In contrast, neutrophil numbers and iNOS-producing neutrophils, assessed by flow cytometry, were not significantly different between WT and FABP5^{-/-} mice (Fig. 3c, d). To understand whether FABP5^{-/-} macrophages have

Fig. 4. Bone marrow chimeras confirm the resistance to *L. monocytogenes* infection in FABP5^{-/-} mice. *L. monocytogenes* burden in the liver (a) and spleen (b) of bone marrow chimeric mice 48 h postinfection (5 mice per group) infected with 3.8×10^4 *L. monocytogenes*. IFN γ (c) and KC (d) were measured in the serum of bone marrow chimeric mice infected with 3.8×10^4 CFUs 48 h postinfection using the multiplex MSD plate. Data are representative of 2 independent experiments.

enhanced activation, we measured the secretion of NO₂⁻ by BMDMs in response to inflammatory stimuli. LPS alone, or in combination with TNF α , resulted in significantly increased levels of NO₂⁻ in the supernatant of FABP5^{-/-} BMDM compared to WT BMDM (Fig. 3e).

FABP5 Deficiency in the Hematopoietic Compartment Confers Resistance to *Listeria* Infection-Induced Inflammation

To test whether FABP5-deficient hematopoietic cells are responsible for resistance to *L. monocytogenes* infection, we created bone marrow chimeric mice, using WT and FABP5^{-/-} irradiated mice as both recipients and

donors. The following chimeras were generated: WT recipient with WT bone marrow (WT > WT), WT recipient with FABP5^{-/-} bone marrow (FABP5^{-/-} > WT), FABP5^{-/-} recipient with WT bone marrow (WT > FABP5^{-/-}), and FABP5^{-/-} recipient with FABP5^{-/-} bone marrow (FABP5^{-/-} > FABP5^{-/-}). Eight weeks after radiation and bone marrow reconstitution, recipient mice were infected with *L. monocytogenes*, and tissues were harvested 48 h postinfection. As shown in Figure 4(a, b), any mouse that already had or received FABP5-deficient immune cells had a lower bacterial burden. However, mice that received FABP5^{-/-} bone marrow had lower inflammatory cytokines (e.g., IFN γ and KC) (Fig. 4c, d).

Fig. 5. Increased susceptibility of myeloid cell-specific FABP5 transgenic mice to lethal infection with *L. monocytogenes*. **a** Kaplan-Meier survival plot of mice i.v. infected with *L. monocytogenes*. Five LysM-FABP5-Tg⁻ (continuous line) and 7 LysM-FABP5-Tg⁺ (dashed line) mice were infected with 5.55×10^4 CFUs. **b** Changes in body weight loss following infection from mice described in **a**. **c** *L. monocytogenes* burden in liver and spleen of LysM-FABP5-Tg⁻ (open circles) and LysM-FABP5-Tg⁺ (black squares) mice 48 h postinfection (5–8 mice per group) infected with 4.2×10^4

L. monocytogenes. **d** Serum was collected from the same LysM-FABP5-Tg⁻ (open circles) and LysM-FABP5-Tg⁺ (black squares) mice as described in **c** and ALT was quantified using a modified Reitman-Frankel colorimetric end-point reaction. **e** IFN γ . **f** IL-12p70. **g** KC. **h** IL-1 β . Cytokines were measured in the serum of LysM-FABP5-Tg⁻ (open circles) or LysM-FABP5-Tg⁺ (black squares) mice infected with 4.2×10^4 *Lm* 48 h postinfection using the multiplex MSD plate. Data are representative of 3 independent experiments.

Fig. 6. FABP5^{-/-} bone marrow-derived macrophages (BMDMs) have increased bactericidal activity and kill intracellular *L. monocytogenes* more efficiently than WT or LysM-FABP5-Tg⁺ cells. BMDMs from WT, FABP5^{-/-}, and LysM-FABP5-Tg⁺ mice were plated on glass coverslips ($n = 3$ per group), allowed to adhere, and treated with 100 ng/mL LPS $\pm$ 10 U/mL IFN γ for 18 h. Cells were infected for 4 h with *L. monocytogenes* at a MOI = 10 and the coverslips were lysed for enumeration of the bacterial load by plating serial dilutions of lysate. Bars represent mean intracellular *L. monocytogenes* CFUs $\pm$ SEM from 1 of 3 independent experiments.

These data suggest that FABP5 deficiency in immune cells confers heightened protection from *L. monocytogenes* infection. In addition, because tissue-resident macrophages are radio-resistant, they might still be present in irradiated FABP5^{-/-} recipient mice, thus explaining the protection in WT > FABP5^{-/-} chimeric mice.

Myeloid Cell-Specific FABP5 Overexpression Further Confirms the Importance of FABP5 Deficiency in Macrophages for Resistance to *Listeria* Infection

To test whether FABP5^{-/-} myeloid cells are responsible for the resistance to *L. monocytogenes* infection, we generated myeloid cell-specific FABP5-overexpressing mice by crossing LysM-Cre mice with mice carrying Rosa26-STOPflox-Fabp5-2A-YFP (FABP5-Tg) (online suppl. Fig. S1a, b; see www.karger.com/doi/10.1159/000496405 for all online suppl. material). We then challenged LysM-FABP5-Tg⁺ and LysM-FABP5-Tg⁻ mice with a lethal dose of *L. monocytogenes*. LysM-FABP5-Tg⁺ mice exhibited worse survival than LysM-FABP5-Tg⁻ mice (Fig. 5a), indicating that overexpression of FABP5 in the myeloid cells decreases the innate immune defense against *L. monocytogenes* infection. Of note, we did not see any difference in weight loss between LysM-FABP5-Tg⁺ and LysM-FABP5-Tg⁻ mice during infection (Fig. 5b).

Next, we examined the bacterial load in the liver and spleen 48 h after infection and found increased bacterial load in LysM-FABP5-Tg⁺ mouse organs compared to in LysM-FABP5-Tg⁻ mice (Fig. 5c). We assessed liver damage by measuring serum levels of ALT but saw no difference between LysM-FABP5-Tg⁺ and LysM-FABP5-Tg⁻ mice (Fig. 5d). We also quantified the level of IFN γ and IL-12p70 in the serum following infection. Both IFN γ (Fig. 5e) and IL-12p70 (Fig. 5f) were increased in LysM-FABP5-Tg⁺ mice compared to in LysM-FABP5-Tg⁻ mice. Similarly, other inflammatory cytokines were significantly increased in the serum of LysM-FABP5-Tg⁺ mice, including KC (Fig. 5g), and IL-1 β (Fig. 5h). Together, these data suggest that FABP5 overexpression in myeloid cells contributes to a higher bacterial burden and increased inflammatory response.

Increased Bactericidal Activity of FABP5-Deficient Macrophages

To demonstrate whether FABP5 expression decreases the bactericidal activity of macrophages, we examined macrophage killing abilities in vitro using BMDMs of WT, FABP5^{-/-}, and LysM-FABP5-Tg⁺ mice. We found that cultured FABP5^{-/-} BMDM have an increased ability to kill intracellular *L. monocytogenes* compared to WT or LysM-FABP5-Tg⁺ cells (Fig. 6). Together, our data show that in comparison to WT mice, FABP5^{-/-} mice exhibit significantly elevated monocyte/macrophage activation in response to *L. monocytogenes* infection in vitro, likely resulting in improved protection against *L. monocytogenes* infection in vivo.

Discussion

Myeloid cells orchestrate the innate immune response against *L. monocytogenes* bacterial infection [15]. As previous data showed that FABP5 deletion attenuated LPS-induced hepatic injury [11], we hypothesized that FABP5 expression would limit the early innate immune response of myeloid cells in response to *L. monocytogenes* infection. Here, we showed that FABP5^{-/-} mice have improved viability compared to WT mice, as well as a decreased bacterial burden in the liver and spleen, and reduced hepatic inflammation, revealing a previously unknown key role of FABP5 in controlling the innate immune response to *L. monocytogenes* infection, specifically in myeloid cells.

Previous studies have shown that the inflammatory cytokines IFN γ [16, 17], TNF α [18, 19], and IL-6 [20] play an important role in the host's resistance to *L. monocyto-*

genes infection, and that the peak of these cytokines in the bloodstream corresponds with the highest bacterial titers [21]. However, these cytokines did not appear to play an active role in lethality from infection with a high dose of *L. monocytogenes* [22]. Our observations of reduced bacterial burden and reduced inflammation in FABP5^{-/-} mice correlate with decreased systemic levels of cytokines that are usually induced by *L. monocytogenes* invasion [23, 24]. Deficiency of the anti-inflammatory cytokine IL-10 has been shown to improve resistance to *L. monocytogenes* infection [25, 26], yet it is also required for memory T cells [27]. Since IL-10 was reduced in FABP5^{-/-} mice compared to WT mice, it would be intriguing to determine whether FABP5^{-/-} mice have an increased sensitivity to a secondary *L. monocytogenes* infection, given that FABP5 has been shown to be essential for the maintenance, longevity, and function of tissue-resident memory T cells [28].

We also showed that FABP5-deficient inflammatory macrophages had increased amounts of iNOS while FABP5-deficient BMDMs produced higher amounts of NO₂⁻ when stimulated with TNFα, IFNγ, or LPS. This suggests that FABP5-deficient macrophages are better equipped at killing *L. monocytogenes* than their WT counterparts. As neutrophils are the most potent ROS producers, we also considered that they might play a role in bacterial killing. However, FABP5 is poorly expressed in neutrophils, and we did not see a difference in neutrophil proportions or in iNOS⁺ neutrophil numbers between WT and FABP5^{-/-} mice.

To test whether FABP5-deficient immune cells, in particular macrophages, confer resistance to *L. monocytogenes* infection, we generated bone marrow chimeric mice. Interestingly, any chimera that already had or received FABP5-deficient cells showed a lower bacterial burden. This result corroborated our hypothesis that FABP5-deficient macrophages decrease the *L. monocytogenes* bacterial burden, since tissue-resident macrophages are known to be resistant to radiation [29, 30], and thus would still be present in the irradiated recipient mouse. Of note, the cytokines in the bloodstream of the chimeric mice followed the phenotype of the bone marrow donor. We found that WT recipients that received FABP5-deficient bone marrow (FABP5^{-/-} > WT) had similar levels of cytokines than FABP5^{-/-} > FABP5^{-/-} mice, while FABP5-deficient recipient that received WT bone marrow (WT > FABP5^{-/-}) had cytokine levels comparable to WT > WT mice. This could be due to the fact that, in the mouse liver, 2 different subsets of F4/80⁺ macrophages have been identified: the radio-resistant resident CD68⁺ subset (Kupffer cell) and the radio-sensitive recruited CD11b⁺

subset. The CD11b⁺ subset, which is involved in antitumor immunity and cytokine production [31, 32], is derived from bone marrow and originates from the donor mouse while the radio-resistant Kupffer cells, which produce ROS and have bactericidal activity, are resident cells from the recipient mouse, explaining the cytokine results obtained with our bone marrow chimeric animals.

We then created transgenic mice that overexpressed FABP5 specifically in myeloid cells by crossing them with LysM-Cre mice, and we found that overexpression of FABP5 decreased survival and increased bacterial burden and inflammation, suggesting that FABP5 overexpression in myeloid cells increases the *L. monocytogenes* burden and subsequent inflammation. Finally, using an in vitro assay, we found that FABP5-deficient macrophages were more efficient at killing *L. monocytogenes* than WT or LysM-FABP5-Tg⁺ macrophages.

Previously, we showed that FABP5^{-/-} mice have increased viral-induced lung inflammation compared to WT mice [10], which contradicts the decreased inflammation seen in the livers of the FABP5^{-/-} mice. The disparity in these results may be due to the difference in cell types found in these 2 organs as well as inflammatory mediators (*L. monocytogenes* versus *Influenza A virus*). Our study demonstrates an increase in the bactericidal activity of FABP5-deficient macrophages, suggesting a proinflammatory programming or “M1 phenotype,” while the persistence of lung inflammation suggests a deficit in a proresolving programming or “M2 phenotype.” Both studies suggest that FABP5 deficiency prevents macrophage polarization towards a proresolving phenotype, favoring a proinflammatory phenotype instead.

This increased proinflammatory programming is in direct contradiction with the findings of Moore et al. [11], who demonstrated that FABP5^{-/-} mice were protected from intraperitoneal LPS induced hepatic injury, shown by increased F4/80⁺ macrophage numbers and “M2 macrophage profiling.” These contradictions could be due to the different nature of injury (LPS vs. *L. monocytogenes*), the activation of different pathways, or the time after injury at which the tissues were observed. Furthermore, as discussed earlier, 2 different types of F4/80⁺ macrophages are present in the mouse liver. It is possible that our study underlines the function of the resident subset while Moore et al. [11] describe the recruited macrophage subset. Interestingly, in both studies, the FABP5-deficient livers were protected from injury.

It has been proposed that FABPs promote anti-inflammatory functions by transporting anti-inflammatory mediators, among them ligands of the nuclear receptor per-

oxisome proliferator-activated receptor γ (PPAR γ) [33–35]. However, other studies suggest that FABP5 sequesters these mediators from their target, creating a proinflammatory environment [36–38]. PPAR γ is a ligand-dependent transcription factor and a member of the nuclear receptor superfamily [39] that has been shown to have proresolving capabilities [40, 41]. Interestingly, similarly to FABP5 deficiency, a lack of PPAR γ in myeloid cells confers resistance to *L. monocytogenes* infection [42], suggesting that, at least in the case of *L. monocytogenes* infection, PPAR γ and FABP5 act in concert to decrease the innate immune response to bacterial infection.

Taken together, our data suggest that FABP5 may represent a valuable target for pharmacological intervention to suppress intracellular bacterial infections such as *L. monocytogenes*. The question that remains is whether inhibiting FABP5 can increase the immunopathology of infected organs. Our novel transgenic mouse model, when crossed onto the FABP5 knockout background, will be an invaluable tool for answering this question.

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Statement of Ethics

All experimental animals used in this study were covered under protocols approved by the Institutional Animal Care and Use Committee of National Jewish Health.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

F.G. designed the research studies; D.M.R., D.T.P., M.J.C., A.L.O., and F.G. conducted the experiments; D.M.R. and F.G. acquired the data, D.M.R. and F.G. analyzed the data, A.-L.P. provided the reagents, and F.G. wrote the manuscript.

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