STING/MPYS Mediates Host Defense against *Listeria monocytogenes* Infection by Regulating Ly6C hi Monocyte Migration


*J Immunol* 2013; 190:2835-2843; Prepublished online 1 February 2013;
doi: 10.4049/jimmunol.1201788
http://www.jimmunol.org/content/190/6/2835

Supplementary Material
http://www.jimmunol.org/content/suppl/2013/02/01/jimmunol.1201788.DC1

References
This article cites 38 articles, 17 of which you can access for free at:
http://www.jimmunol.org/content/190/6/2835.full#ref-list-1

Subscription
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
STING/MPYS Mediates Host Defense against Listeria monocytogenes Infection by Regulating Ly6C<sup>hi</sup> Monocyte Migration


MPYS (also known as STING, MITA, and TMEM173) is a type I IFN stimulator that is essential for host defense against DNA virus infection and appears important in defense against certain bacteria. The in vivo significance and mechanisms by which MPYS mediates host defense against nonviral pathogens are unknown. Using an MPYS-deficient mouse (Tmem173<sup>tm1Camb</sup>), we determined that, distinct from the IFNAR<sup>−/−</sup> mice, MPYS deficiency leads to increased bacterial burden in the liver upon Listeria monocytogenes infection. The increase was correlated with the diminished MCP-1 and MCP-3 chemokine production and decreased blood and liver Ly6C<sup>hi</sup> monocyte frequency. We further demonstrate that MPYS-deficient Ly6C<sup>hi</sup> monocytes are intrinsically defective in migration to the liver. Lastly, adoptive transfer of wild-type Ly6C<sup>hi</sup> monocyte into MPYS-deficient mice decreases their liver bacterial burden. Our findings reveal a novel in vivo function of MPYS that is distinct from its role in activating type I IFN production. The Journal of Immunology, 2013, 190: 2835–2843.

Listeria monocytogenes is a Gram-positive intracellular bacterium. Infection by L. monocytogenes is the cause of listeriosis, which primarily affects the elderly, pregnant women, newborns, and people with weakened immune systems. In the U.S., an estimated 1,600 people suffer serious illness from L. monocytogenes each year; ~16% of these illnesses result in death (1). The murine model of listeriosis has been used to investigate immune responses to human infection. These studies have shown that innate immunity controls the early response of the infection and is critical for survival (2).

Neutrophils were thought to be the primary cells responsible for killing L. monocytogenes during the innate immune response (3). This was supported by the observation that the depletion of neutrophils with Gr-1 mAb greatly enhances susceptibility of mice to infection with L. monocytogenes (3). However, it has since been recognized that Gr-1 mAb reacts with both Ly6G on neutrophils and Ly6C on monocytes and CD8<sup>+</sup> memory T cells. Recent reports using mAb specific for Ly6G showed that neutrophils are dispensable for host defense against L. monocytogenes (4, 5).

Rather, Ly6C<sup>hi</sup> monocytes are essential for the control of bacterial infection (4, 5). Ly6C<sup>hi</sup> monocytes are mononuclear phagocytes that are generated in the bone marrow (BM) and circulate in the bloodstream (6). It has been shown that recruitment of Ly6C<sup>hi</sup> monocytes to the foci of infection is essential for the eradication of L. monocytogenes (7). Emigration of Ly6C<sup>hi</sup> monocytes from BM to the bloodstream is controlled by the interaction of chemokine receptor CCR2 and its ligands, MCP-1 and MCP-3, on monocytes (8, 9). Mice deficient in CCR2, MCP-1, or MCP-3 are susceptible to L. monocytogenes infection (8, 9). Interestingly, once monocytes are in the bloodstream, their migration to the liver is CCR2 and chemokine independent, but is dependent on the interaction between CD11b on Ly6C<sup>hi</sup> monocytes and ICAM-1 on endothelial cells (10).

MPYS, also known as STING, MITA, and TMEM173, is a four-transmembrane protein essential for L. monocytogenes induced type I IFN (IFN-I) production in vitro (11). MPYS mediates IFN-I production by sensing cytosolic DNA released from bacteria (12). Studies done in STING<sup>−/−</sup> mice (Tmem173<sup>−/−</sup>) have demonstrated that MPYS/STING is critical for host defense against DNA virus infections (12). However, increasing evidence suggests that MPYS/STING may also be important for host defense against nonviral pathogens such as Francisella tularensis, Brucella abortus, Streptococcus pneumoniae, Chlamydia muridarum, and Plasmodium falciparum (13–17). The in vivo significance of MPYS/STING function in host defense against these nonviral infections has not been demonstrated. Using MPYS-deficient mice (Tmem173<sup>−/−</sup>)<sup>(tm1Gnb)</sup>, we show in this study that MPYS mediates host defense against L. monocytogenes infection in vivo by regulation of Ly6C<sup>hi</sup> monocyte recruitment.

Materials and Methods

Mice

Six- to 12-wk-old mice were used for all experiments. IFNAR<sup>−/−</sup> (B6.129PF2/fnab) have been described previously. Generation of MPYS-deficient mice (Tmem173<sup>−/−</sup>)<sup>(tm1Camb)</sup> has been described in detail previously (18). Briefly, exons 3–5 are flanked by loxp sites, and a neo gene is...
inserted into intron 5, which is flanked by Flt elements. The presence of the neo gene disrupts expression of MPYS, causing null MPYS protein deficiency. Mice were housed and bred in the Biologic Resource Center at National Jewish Health (Denver, CO). All in vivo experiments were performed in accordance with regulations and the approval of National Jewish Health and the Institutional Animal Care and Use Committee.

**In vivo L. monocytogenes infections**

Mice were infected (tail vein) with 4–7 × 10⁸ CFU log-phase *L. monocytogenes* (10403S). Spleens and livers were harvested at days 2, 3, and 4 postinfection. Bacterial CFUs in infected organs were determined by dilution plating, as previously described (18).

**Cytokine and chemokine ELISAs**

Serum from infected mice was collected at indicated time points and concentrations of MCP-1 (88-7391; E Bioscience), MCP-3 (BMS60601NST; E Bioscience), IL-12p70 (89-7921; E Bioscience), TNF (88-7352; E Bioscience), IL-4 (88-7013; E Bioscience), IFN-γ (551866; BD Biosciences), and IFN-β (42400; PBI InterferonSource) were measured using commercial ELISAs.

**Calcium mobilization**

Intracellular calcium concentration was measured, as previously described (18). Briefly, BM cells were loaded with Indo-1 AM (Molecular Probes, Eugene, OR) together with Ly6C-allophycocyanin (clone AL-21; BD Biosciences) for 30 min at 37°C. Cells were then washed and resuspended in IMDM supplemented with 2% FCS at 10⁶ cells/ml. Cells were stimulated with MCP-1. Data were collected on an LSR II (BD Biosciences) and analyzed using FlowJo software (Tree Star, San Carlos, CA).

**Flow cytometric assay of immunofluorescence**

Cells were stained with FITC- or PerCP-CD45 (30-F11; BD Pharmingen), allophycocyanin-F4-80 (CL-A3-1; AbD Serotec), PE-F4/80 (BM8; Caltag Laboratories), FITC- or allophycocyanin-Ly6C (AL-21; BD Pharmingen), PE-Ly6G (1A8; BD Pharmingen), and allophycocyanin–CY7–CD11b (M1/70; BD Pharmingen). FACS was performed using a FACScan flow cytometer (BD Biosciences) and analyzed by FlowJo software (Tree Star, San Carlos, CA).

**Liver lymphocytes isolation**

Mice were euthanized at the indicated time. Livers were perfused through the portal vein, and digested in collagenase IV (0.05%, #15140; Life Technologies), and 50 μg/ml penicillin plus 100 units/ml streptomycin (catalog number 15140; Life Technologies), and 50 μM 2-ME. The medium was exchanged after 3 d, and cells were stained for the macrophage marker F4/80 at day 7.

**In vitro L. monocytogenes infection**

Cells were seeded in 12-well plates at 1 × 10⁵ cells/well. The following day, the medium was exchanged with fresh antibiotic-free medium. GFP-expressing *L. monocytogenes* (*Listeria-GFP*) (strain 10403S) were grown to log phase and added to the cell cultures. After 30 min, gentamicin (50 μg/ml; catalog number 15750-060; Life Technologies) was added to the medium to kill all extracellular bacteria. At 1 h postinfection, the medium was exchanged. At indicated time points, supernatants were collected and analyzed for cytokines by ELISA. For CFU measurement, cells were harvested, washed in PBS, and then lysed in 0.2% Nonidet P-40 buffer. Serial dilutions of lysates were plated onto tryptic soy broth agar plates.

**RT-PCR detection of MPYS transcript**

Cells were sorted based on the indicated surface markers. Total RNA was isolated using the Qiagen RNeasy Plus mini kit (Qiagen) and reverse-transcribed using oligo deoxythymidyldite primers and the IMPROM II reverse transcriptase. For the detection of *Ly6C* mRNA, the following primer sequence and conditions were used: forward, 5′-GAGAAGCCCATCACCATCTT-3′; and reverse, 5′-AAACATCCAACTGAGGT-3′. RNA (0.5 μg) was transcribed using oligodeoxythymidylate primers and the IMPROM II reverse transcriptase. The resulting cDNA was diluted 1:20 in water and used for the PCR amplification of *Ly6C* gene. The primers used for *Ly6C* were: forward, 5′-GAAGCCATCACCATCTT-3′; GADPH sense, 5′-ACAATCAGCGCAGCGGTC-3′; MPYS sense, 5′-CAGGAAACCGGTCTAGGAA-3′; and MPYS antisense 5′-GCCAACATCCACTGAGGT-3′. Statistical analysis

All data are expressed as means ± SEM. Statistical significance was evaluated using Prism 4.0 software (GraphPad) to perform a Student t test (unpaired, two-tailed) for comparisons between mean values.

**Results**

Increased bacterial burden in the liver of *L. monocytogenes*–infected *Tmem173<sup>−/−</sup>* (MPYS<sup>−/−</sup>) mice

MPYS mediates *L. monocytogenes* induction of IFN-β in cultured BMDM and BM-derived dendritic cells (BMDC) (Supplemental Fig. 1A) (12) via activation of transcriptional factor IRF3 (Supplemental Fig. 1B, 1C) (18). Previous studies have demonstrated that IFN-I is detrimental to the host defense against *L. monocytogenes* (20–22). We thus hypothesized, initially, that MPYS<sup>−/−</sup> mice will be resistant to *L. monocytogenes* infection, similar to the IFNAR<sup>−/−</sup> mice. To test this hypothesis, we infected the MPYS<sup>−/−</sup> mice (*Tmem173<sup>−/−</sup>*<sup>−/−</sup>) (18) with a sublethal dose of *L. monocytogenes* (i.v.). To our surprise, we found that MPYS is only partially required for *L. monocytogenes* induction of IFN-β production in vivo (Fig 1A). Furthermore, unlike the IFNAR<sup>−/−</sup> mice, which have ~1200-fold less bacterial load in the spleen at 72 hpi (22), spleens from *L. monocytogenes*–infected MPYS<sup>−/−</sup> mice have a similar bacterial burden to wild-type (WT) mice (Fig 1B).

The spleen and liver are the major sites of infection during systemic *L. monocytogenes* infection in vivo. We found that MPYS<sup>−/−</sup> mice have increased bacterial burden in the liver by 72
and 96 hpi compared with WT mice (Fig. 1C). This observation is in contrast to IFNAR−/− mice, which showed ∼1000-fold reduced bacterial burden in the liver compared with WT mice by 72 hpi (22). We also examined cytokine production during L. monocytogenes infection. We found that MPYS−/− mice have normal serum IFN-γ, IL-1β, and TNF levels for 3 d following infection (Fig. 1D–F, Supplemental Fig. 1D). Interestingly, L. monocytogenes–infected MPYS−/− mice have decreased IL-12 production (Fig. 1G), which is also different from the increased IL-12 production found in L. monocytogenes–infected IFNAR−/− mice (22). We concluded that MPYS mediates host defense against L. monocytogenes through a mechanism distinct from its role in activating IFN-I signaling.

MPYS is highly expressed in CD45+ liver cells but not in CD45− hepatocytes

L. monocytogenes–infected MPYS−/− mice have increased bacterial load in the liver but not in the spleen relative to WT mice (Fig. 1). To understand the seemingly different roles of MPYS in host defense against L. monocytogenes in the spleen and liver, we examined MPYS expression in spleen and liver cells by intracellular staining with a polyclonal Ab that recognizes the cytoplasmic tail of MPYS (18). Using the cells from MPYS−/− mice as negative controls, we found that MPYS is highly expressed in CD4+ T cells, CD8+ T cells, and IgM+ B cells in the spleen (Fig. 2A). The deficiency of MPYS does not affect B cell and T cell development (12) (data not shown). Interestingly, MPYS is barely detectable in the thymus, where most cells are CD4+CD8+ T cells (Fig. 2A). Surprisingly, we found that, in the liver, MPYS is only expressed in CD45+ cells, whereas the majority of liver cells (CD45− hepatocytes) do not express MPYS (Fig. 2B). RT-PCR analysis of MPYS level in CD45− hepatocytes confirms the lack of expression (Fig. 2C). Thus, the increased liver bacterial burden in MPYS−/− mice is likely due to the deficiency of MPYS in CD45+ liver cells.

MPYS deficiency does not affect the ability of CD45+ liver cells to clear L. monocytogenes

To examine the ability of residential CD45+ liver cells to clear L. monocytogenes, we infected ex vivo CD45+ liver cells with Listeria-GFP strain and tracked GFP-positive cells over time. We found that, unlike BMDM and BM neutrophils (Fig. 3), CD45+ liver cells do not efficiently take up L. monocytogenes (Supplemental Fig. 2A). Furthermore, there is no difference in the number of L. monocytogenes–positive CD45+ WT and MPYS−/− liver cells after overnight culture (Supplemental Fig. 2A). The MPYS−/− mice have similar populations of CD3+ T cells, B220+ B cells, and NK1.1+ NK cells in the liver to WT mice (Supplemental Fig. 2B).

MPYS is expressed in BM Ly6C+ monocytes but absent in Ly6G+ neutrophils

During systemic L. monocytogenes infection, there is an influx of monocytes from BM into the liver. These cells can represent...
∼50% of total CD45+ cells in the liver (23). Recent studies revealed that it is these Ly6Chi monocytes, but not Ly6G+ neutrophils, that mediate bacterial clearance in the liver (5). We found that MPYS is expressed in Ly6Chi monocytes, but not Ly6G+ neutrophils (Fig. 2D). The expression of MPYS in Ly6C<sup>hi</sup> Ly6G<sup>−</sup> BM cells is also low (Fig. 2D). RT-PCR of MPYS in Ly6C<sup>hi</sup> and Ly6G<sup>+</sup> cells confirmed these results (Fig. 2E).

**MPYS deficiency does not affect bacterial clearance by BM cells**

To examine their ability to clear *L. monocytogenes*, we infected ex vivo BM cells with a *Listeria*-GFP strain and tracked GFP-positive *L. monocytogenes*–infected cells over time. We found that *L. monocytogenes* was taken up mainly by Ly6G<sup>+</sup> neutrophils (Fig. 3A). Furthermore, there was no difference in bacterial clearance by Ly6G<sup>+</sup> neutrophils cells from MPYS<sup>−/−</sup> and WT mice (Fig. 3A). This is consistent with our finding that Ly6G<sup>+</sup> neutrophils do not express MPYS (Fig. 2).

**MPYS deficiency does not affect bacterial clearance by BMDM**

As shown in Fig. 3A, Ly6C<sup>hi</sup> monocytes isolated from BM do not phagocytose bacteria well. However, Ly6C<sup>hi</sup> monocytes can differentiate into phagocytic macrophages in peripheral tissues. We examined bacterial clearance in BMDM. We did not find significant differences in phagocytosis of *L. monocytogenes* by BMDM from WT and MPYS<sup>−/−</sup> mice (Fig. 3B, 3C). Macrophages can be further primed by IFN-γ and LPS to generate activated macrophages that efficiently clear bacteria. Using the *Listeria*-GFP strain, we found that activated BMDM from MPYS<sup>−/−</sup> mice have a similar ability to clear bacteria to WT mice (Fig. 3D). We concluded that MPYS deficiency did not affect bacterial clearance by resting or activated BMDM.

**Decreased *L. monocytogenes* infection–induced recruitment of Ly6C<sup>hi</sup> monocytes to liver in MPYS<sup>−/−</sup> mice**

MPYS deficiency does not affect macrophage clearance of *L. monocytogenes*, which is consistent with the observation that there is no defect in bacterial clearance in spleens of infected MPYS<sup>−/−</sup> mice (Fig. 1). However, we did see increased bacterial burden in livers of infected MPYS<sup>−/−</sup> mice (Fig. 1). Unlike spleen, we found that MPYS is only expressed in CD45<sup>+</sup> liver cells (Fig. 2). Furthermore, during *L. monocytogenes* infection, ∼50% of these CD45<sup>+</sup> liver cells are Ly6C<sup>hi</sup> monocytes newly migrated from BM. We hypothesize that the increased bacterial load in the liver is due to the impaired recruitment of Ly6C<sup>hi</sup> monocytes. Indeed,
we found that both the percentage and total numbers of Ly6C^{hi} monocytes in the liver are decreased in L. monocytogenes–infected MPYS^{−/−} mice compared with WT mice over a 3-d period, postinfection (Fig. 4A, 4B).

NK cells play an important role in the innate immunity phases to L. monocytogenes infection, producing IFN-γ required for the maturation of Ly6C^{hi} monocytes and generation of activated macrophages that ensure effective clearance of bacteria (23). We found that the number of CD11b^+NK1.1^+ activated NK cells in the liver, from infected MPYS^{−/−} mice, is not significantly different from WT mice (Supplemental Fig. 3). This is consistent with the observation of normal IFN-γ production in MPYS^{−/−} mice (Fig. 1D, Supplemental Fig. 1D).

There is no difference in Ly6C^{hi} monocyte infiltration in the spleens of infected WT and MPYS^{−/−} mice (Fig. 4C, 4D). This is consistent with the observation that MPYS^{−/−} mice have comparable bacterial burden in spleen to WT mice (Fig. 1).

**Diminished MCP-1 and MCP-3 production in MPYS^{−/−} mice**

The recruitment of Ly6C^{hi} monocytes to foci of infection is critical for the eradication of the bacteria (6). Previous studies have established that the chemokine receptor CCR2 is essential for monocyte emigration out of BM (9). However, we found that MPYS^{−/−} Ly6C^{hi} monocytes have normal CCR2 signaling (Fig. 5A).

CCR2 is a receptor for the chemokines MCP-1 and MCP-3. Recent studies suggest that MPYS is required for MCP-1 production in mouse embryonic fibroblast cells (24). In view of this, we determined whether MPYS deficiency affects in vivo MCP-1 and MCP-3 production during L. monocytogenes infection. We found that the production of both MCP-1 and MCP-3 are diminished in L. monocytogenes–infected MPYS^{−/−} mice (Fig. 5B, 5C). In agreement with this finding, we found that MPYS^{−/−} mice have decreased Ly6C^{hi} monocytes in the bloodstream during systemic L. monocytogenes infections (Fig. 5D, 5E).

**Impaired Ly6C^{hi} monocytes migration from bloodstream to livers in MPYS^{−/−} mice**

We then addressed why Ly6C^{hi} monocyte infiltration into livers, but not spleens, is impaired in MPYS^{−/−} mice. Migration of Ly6C^{hi} monocytes from the bloodstream to the liver is CCR2 independent, but requires CD11b expression on Ly6C^{hi} monocytes (10). However, migration of Ly6C^{hi} monocytes from the bloodstream to the spleen depends on CX3CR1 expression on monocytes and CX3CCL1 expression in the marginal zone on T cells (25). To begin to address this selectivity of infiltration, we performed the competitive adoptive-transfer experiments. Sorted Ly6C^{hi} monocytes from WT and MPYS^{−/−} mice were differentially labeled with CFSE, then mixed at a 1:1 ratio, and adoptively transferred into MPYS^{−/−} mice (i.v.) (Fig. 6A). After 17 h, labeled positive cells in the liver, spleen, and BM of the recipient mice were quantified. We found that there were fewer MPYS^{−/−} Ly6C^{hi} monocytes than WT Ly6C^{hi} monocytes in the liver (Fig. 6B, left panel). Interestingly, there were more MPYS^{−/−} Ly6C^{hi} monocytes in the spleen than WT Ly6C^{hi} monocytes (Fig. 6B, middle panel). The more efficient migration of MPYS^{−/−} Ly6C^{hi} monocytes from the bloodstream to the spleen may explain why the numbers of Ly6C^{hi} monocytes in MPYS^{−/−} and WT spleens are similar. There was no significant difference in the recovery of WT and MPYS^{−/−} Ly6C^{hi} monocytes in BM (Fig. 6B, right panel). The CD11b expression on WT and MPYS^{−/−} monocytes Ly6C^{hi} was also similar (Fig. 6C).

**FIGURE 4.** MPYS^{−/−} mice exhibit diminished Ly6C^{hi} monocyte recruitment to liver but not spleen during systemic L. monocytogenes infection. MPYS knockout (KO) and their WT littermate controls were infected (i.v.) with L. monocytogenes (∼7000 CFU). Representative FACS plots of CD45^+ leukocytes in livers (A) and spleen (C) stained for Ly6C and CD11b expression are shown. The number of liver (B) or spleen (D) Ly6C^{hi} monocytes from infected mice during the time period is shown. The data are presented as mean ± SEM. A representative of three independent experiments is shown.
The impaired MPYS$^{-/-}$ Ly6C$^{hi}$ monocytes migration from bloodstream to liver is cell intrinsic

To determine whether it is the lack of MPYS expression in Ly6C$^{hi}$ monocytes or other cell types that leads to the impaired MPYS$^{-/-}$ Ly6C$^{hi}$ monocyte migration, we adoptively transferred CFSE-labeled WT and MPYS$^{-/-}$ Ly6C$^{hi}$ monocytes to WT or MPYS$^{-/-}$ mice and examined the ratio of CFSE-labeled cells. We found that, in both cases, there were fewer MPYS$^{-/-}$ Ly6C$^{hi}$ monocytes than WT Ly6C$^{hi}$ cells in the liver (Fig. 6D). We concluded that the homing defect is monocyte intrinsic (i.e., due to the lack of MPYS expression in Ly6C$^{hi}$ monocytes).

IFNAR$^{-/-}$ Ly6C$^{hi}$ monocytes migrate normally to liver

MPYS is an IFN-I stimulator. To determine if IFN-I signaling is required for Ly6C$^{hi}$ monocytes migration to liver, we performed a competitive adoptive transfer experiment in which WT and IFNAR$^{-/-}$ Ly6C$^{hi}$ monocytes were transferred to recipients and localization in liver compared. We found that, in both cases, there were fewer MPYS$^{-/-}$ Ly6C$^{hi}$ monocytes than WT Ly6C$^{hi}$ cells in the liver (Fig. 6D). We concluded that the homing defect is monocyte intrinsic (i.e., due to the lack of MPYS expression in Ly6C$^{hi}$ monocytes).

Adoptive transfer of WT Ly6C$^{hi}$ monocytes to MPYS$^{-/-}$ mice decreases liver bacterial burden

It was shown that the number of Ly6C$^{hi}$ monocytes recruited directly correlates with bacterial clearance efficiency in the liver (10). To test our hypothesis that the increased liver bacterial burden in MPYS$^{-/-}$ mice is due to the decreased recruitment of Ly6C$^{hi}$ monocytes, we adoptively transferred WT Ly6C$^{hi}$ monocyte into L. monocytogenes-infected MPYS$^{-/-}$ mice and assessed bacterial burden. We found that injecting (i.v.) WT Ly6C$^{hi}$ monocyte into L. monocytogenes-infected MPYS$^{-/-}$ mice indeed increased the numbers of liver Ly6C$^{hi}$ monocyte (Fig. 8A). We next examined the liver bacterial burden in adoptively transferred MPYS$^{-/-}$ mice at 96 hpi. Indeed, liver bacterial burden in these MPYS$^{-/-}$ mice is significantly decreased by the addition of WT Ly6C$^{hi}$ cells (Fig. 8B). There is no significant change in the spleen (Fig. 8C). We concluded that the increased bacterial burden in livers from MPYS$^{-/-}$ mice is due to the decreased liver localization of Ly6C$^{hi}$ cells.

Discussion

MPYS is critical for host defense against DNA virus infection due largely to its ability to mediate IFN-I production induced by the
sensing of cytosolic viral DNA (12). In this report, we report a new in vivo mechanism by which MPYS mediates host defense against *L. monocytogenes* infection by controlling Ly6Chi monocytes recruitment in the liver. MPYS controls Ly6Chi monocytes migration by the regulation of chemokines production and Ly6C<sup>hi</sup> monocytes intrinsic homing to liver.

This novel function of MPYS described in this study is distinct from its ability to activate IFN-I signaling. This conclusion is supported by following observations: 1) MPYS<sup>−/−</sup> mice have largely normal *L. monocytogenes*-induced IFN-I production; 2) whereas IFNAR<sup>−/−</sup> mice have >1000-fold reduced bacterial load in liver during *L. monocytogenes* infection (22), MPYS<sup>−/−</sup> mice have increased bacterial loads in the liver relative to WT; 3) whereas IFNAR<sup>−/−</sup> mice, which have elevated *L. monocytogenes*-induced IL-12 production (22), MPYS<sup>−/−</sup> mice have decreased IL-12 production in vivo; and 4) unlike monocytes from IFNAR<sup>−/−</sup> mice, MPYS<sup>−/−</sup> monocytes have intrinsic defect in homing to the liver. Thus, our results reveal a novel biologic function of MPYS by regulating Ly6Chi monocytes migration in vivo.

MPYS is essential for IFN-I production by *L. monocytogenes* in BMDM and BMDC. It is rather surprising that MPYS<sup>−/−</sup> mice have normal IFN-I production at 24 hpi during *L. monocytogenes* infection in vivo, though IFN-I production at 6 hpi was reduced. Several reports have shown that in vivo production of IFN-β is restricted to monocyte/macrophages during *L. monocytogenes* infection (26–28). Interestingly, naïve and activated macrophages adopt different mechanisms to produce IFN-β in response to *L. monocytogenes* infection (29). In naïve macrophages, *L. monocytogenes* activates host IFN-β production after escaping from phagosomes and entering the cytosol (30). Cytosolic *L. monocytogenes* DNA or cyclic-di-AMP, secreted by *L. monocytogenes* (31, 32), then activates MPYS/STING-mediated IFN-β production (31, 32).

The pore-forming protein listeriolysin O–deficient *L. monocytogenes* mutant, which fails to escape from phagosomes, fails to activate IFN-I production by BMDM and BMDC. It is rather surprising that MPYS<sup>−/−</sup> mice have normal IFN-I production at 24 hpi during *L. monocytogenes* infection in vivo, though IFN-I production at 6 hpi was reduced. Several reports have shown that in vivo production of IFN-β is restricted to monocyte/macrophages during *L. monocytogenes* infection (26–28). Interestingly, naïve and activated macrophages adopt different mechanisms to produce IFN-β in response to *L. monocytogenes* infection (29). In naïve macrophages, *L. monocytogenes* activates host IFN-β production after escaping from phagosomes and entering the cytosol (30). Cytosolic *L. monocytogenes* DNA or cyclic-di-AMP, secreted by *L. monocytogenes*, then activates MPYS/STING-mediated IFN-β production (31, 32).

FIGURE 6. MPYS<sup>−/−</sup> monocytes have impaired ability migrating from bloodstream to liver. (A) Ly6C<sup>hi</sup> BM cells from WT and MPYS<sup>−/−</sup> mice were sorted, labeled with low dose of CFSE (0.5 μM, WT BM cells) or high dose of CFSE (2.5 μM, MPYS<sup>−/−</sup> cells), and then mixed at a 1:1 ratio and injected (i.v.) into MPYS<sup>−/−</sup> mice. CFSE<sup>+</sup> cells were analyzed after 17 h. (B) Representative FACS plots of CFSE<sup>+</sup> monocytes in the liver, spleen, and BM of the recipient mice. (C) Representative FACS plots of CD11b expression in Ly6C<sup>hi</sup> monocytes from WT and MPYS<sup>−/−</sup> livers. (D) Ly6C<sup>hi</sup> BM cells from WT and MPYS<sup>−/−</sup> mice were sorted, labeled with low dose of CFSE (0.5 μM, MPYS<sup>−/−</sup> cells) or high dose of CFSE (2.5 μM, WT cells), mixed at a 1:1 ratio, and injected (i.v.) into MPYS<sup>−/−</sup> or WT mice. CFSE<sup>+</sup> cells were analyzed after 17 h. Shown are representative FACS plots of CFSE<sup>+</sup> monocytes in the liver of recipient mice. A representative of three independent experiments is shown. KO, Knockout.

FIGURE 7. IFNAR<sup>−/−</sup> monocytes migrates normally to liver. Ly6C<sup>hi</sup> BM cells from WT and IFNAR<sup>−/−</sup> mice were sorted, labeled with low dose of CFSE (0.5 μM, IFNAR<sup>−/−</sup> Ly6C<sup>hi</sup> cells) or high dose of CFSE (2.5 μM, WT Ly6C<sup>hi</sup> cells), mixed at a 1:1 ratio, and injected (i.v.) back into WT mice. CFSE<sup>+</sup> cells were analyzed after 17 h. Shown are representative FACS plots of total cells and CFSE<sup>+</sup> liver, spleen, and BM of the recipient mice. Experiments were repeated twice.
IFN-β in naive macrophages (30). However, a listeriolysin O–deficient L. monocytogenes mutant induces similar IFN-β production to WT L. monocytogenes in IFN-γ-treated BMDM (activated macrophage) (29). This response is partially dependent on nucleotide-binding oligomerization domain-containing protein 2–mediated recognition of L. monocytogenes peptidoglycan in phagosome (29). Our result, which shows an early-phase (6 hpi), but not late-phase (24 hpi), deficiency of IFN-β production during L. monocytogenes infection is consistent with the presence of these two distinct IFN-β activation signaling pathway in vivo and suggests that MPYS may be only required for IFN-β production in response to L. monocytogenes in naive macrophages but not activated macrophages.

A previous study by Sauer et al. (33) found a major defect in IFN-1 activity in the N-ethyl-N-nitrosoare–induced goldenticket STING mutant mouse (STING<sup>Gatk</sup>) during L. monocytogenes infection. The STING<sup>Gatk</sup> mouse contains a T596A mutation in their MPYS/STING gene that results in an isoleucine to asparagine substitution (I199N) in the protein (33). It is noteworthy that, although BMDM from a STING<sup>Gatk</sup> mouse has no detectable STING protein, the I199N STING mutant transfection in 293T cells leads to detectable STING protein expression (33). It is possible that additional mutations exist in the N-ethyl-N-nitrosoare–induced STING<sup>Gatk</sup> mouse and confer the difference in our MPYS<sup>−/−</sup> mouse and distinct from the IFNAR<sup>−/−</sup> mice, the STING<sup>Gatk</sup> mice have the same L. monocytogenes burden in the spleen as the WT mice (33).

Ly6C<sup>hi</sup> monocyte recruitment is critical for host defense against L. monocytogenes infection (6). CCR2<sup>−/−</sup> as well as MCP-1<sup>−/−</sup> and MCP-3<sup>−/−</sup> mice, which are defective in Ly6C<sup>hi</sup> monocytes emigration from BM, are susceptible to L. monocytogenes infection (6). Accumulating evidence has demonstrated that both CCR2-dependent and -independent mechanisms control Ly6C<sup>hi</sup> monocyte migration (6). We show in this study that following L. monocytogenes infection, MPYS<sup>−/−</sup> mice have diminished MCP-1 and MCP-3 production and decreased levels of Ly6C<sup>hi</sup> monocytes in bloodstream. Thus, MPYS participates in CCR2-dependent regulation of monocyte migration.

Many cell populations, including macrophages, fibroblasts, and endothelial cells are capable of producing MCP-1 in vitro (34). We found that MPYS<sup>/−</sup> BMDC and BMDM have decreased MCP-1 and MCP-3 production in response to L. monocytogenes infection (Supplemental Fig. 4). Using WT and Ccl2<sup>−/−</sup> (Ccl2 encodes MCP-1) BM chimeric mice, Shi et al. (35) demonstrated that, in vivo, the radiation-insensitive and presumably nonhematopoietic cells produce MCP-1 in response to L. monocytogenes infection. Specific ablution of the Cx32 gene in mesenchymal stem cells, CXCL12-abundant reticular cells, or endothelial cells decreases the numbers of Ly6C<sup>hi</sup> monocytes in circulation during L. monocytogenes infection (35). However, unlike BMDM or BMDC, L. monocytogenes infection of these CD45<sup>−</sup> nonhematopoietic BM cells, in vitro, does not induce MCP-1 production (Supplemental Fig. 4). This is likely due to the poor uptake of L. monocytogenes by these Ly6G<sup>−/−</sup> BM cells (Fig. 3A). The MPYS<sup>−/−</sup> mice (Tnem173<sup>−/−</sup>Cam<sup>b−</sup>) studied in this paper can be used to generate conditional MPYS<sup>−/−</sup> mice (18). Thus, future studies, by specifically ablating MPYS expression in mesenchymal stem cells, CXCL12-abundant reticular cells, or endothelial cells, can determine whether MPYS expression in these cells is required for MCP-1 production during L. monocytogenes infection in vivo.

We observed nearly equivalent bacterial burden in the spleens of WT and MPYS<sup>−/−</sup> mice. A previous study found that though CCR2<sup>−/−</sup> mice have increased bacterial burden in the liver 1 d postinfection, the bacterial burden in the CCR2<sup>−/−</sup> spleen does not differ from WT mice until day 3 postinfection (36). This is likely due to the fact that the spleen has more resident Ly6C<sup>hi</sup> monocytes than the liver. Thus, the decrease of Ly6C<sup>hi</sup> monocyte recruitment into the liver would have a greater impact on bacterial clearance. Also, we found that MPYS<sup>−/−</sup> Ly6C<sup>hi</sup> monocytes home more efficiently from the bloodstream to the spleen than WT cells, which could also account for the lack of effect of MPYS deficiency on the spleen.

MPYS also affects CCR2-independent monocyte migration. We further show that this migration defect is MPYS<sup>−/−</sup> monocytes intrinsic. It was demonstrated that reactive oxygen species (ROS) enhance the migration of monocytes across the blood–brain barrier (37). MPYS is an ROS sensor (38). We suggest that the impaired migration of MPYS<sup>−/−</sup> monocytes from the bloodstream to the liver may be a result of disrupted ROS signaling in these cells.

In summary, our findings reveal a novel in vivo mechanism by which MPYS regulates Ly6C<sup>hi</sup> monocyte migration and modulates host defense. Increasing evidence, mostly from in vitro studies, suggests that MPYS may play a role in host defense against many nonviral pathogens (13–17). Future studies will be focused on understanding the in vivo mechanism by which MPYS mediates host defense against these pathogens, whether by the production of IFN-1 or regulation of monocytes migration or both or some other as-yet-undefined mechanisms.