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Critical Roles of Myeloid Differentiation Factor 88-Dependent Proinflammatory Cytokine Release in Early Phase Clearance of Listeria monocytogenes in Mice

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Listeria monocytogenes (LM) is a facultative intracellular bacterium, often causes life-threatening infections in an immunocompromised host, including newborns and elderly people (1–3). In host liver, LM invades and replicates in hepatocytes and Kupffer cells, tissue macrophages (2). The activation of the host’s innate immune system is critical for LM eradication in the early phase of infection (2–4). LM-infected macrophages produce IL-12 and IL-18 that synergistically induce IFN-γ production, leading to normal LM clearance in the host. IFN-γ knockout (KO) mice were highly susceptible to LM infection. IL-12/IL-18 double knockout mice were also highly susceptible. Their susceptibility was less than that of IFN-γ KO mice, but more than that of single IL-12 or IL-18 KO mice. Mice deficient in myeloid differentiation factor 88 (MyD88), an essential adaptor molecule used by signal transduction pathways of all members of the Toll-like receptor (TLR) family, showed an inability to produce IL-12 and IFN-γ following LM infection and were most susceptible to LM. Furthermore, MyD88-deficient, but not IFN-γ-deficient, Kupffer cells could not produce TNF-α in response to LM in vitro, indicating the importance of MyD88-dependent TNF-α production for host defense. As TLR2 KO, but not TLR4 KO, mice showed partial impairment in their capacity to produce IL-12, IFN-γ, and TNF-α, TLR2 activation partly contributed to the induction of IL-12-mediated IFN-γ production. These results indicated a critical role for TLRs/MyD88-dependent IL-12/TNF-α production and for IL-12- and IL-18-mediated IFN-γ production in early phase clearance of LM. The Journal of Immunology, 2002, 169: 3863–3868.

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Listeria monocytogenes (LM) is a Gram-positive facultative intracellular bacterium, often causes lethal infection of the host. In this study we investigated the molecular mechanism underlying LM eradication in the early phase of infection. Upon infection with LM, both IL-12 and IL-18 were produced, and then they synergistically induced IFN-γ production. These results indicated a critical role for MyD88-dependent TNF-α production in host defense. MyD88-deficient, but not IFN-γ-deficient, Kupffer cells could not produce TNF-α in response to LM in vitro, indicating the importance of MyD88-dependent TNF-α production for host defense. As TLR2 KO, but not TLR4 KO, mice showed partial impairment in their capacity to produce IL-12, IFN-γ, and TNF-α, TLR2 activation partly contributed to the induction of IL-12-mediated IFN-γ production. These results indicated a critical role for TLRs/MyD88-dependent IL-12/TNF-α production and for IL-12- and IL-18-mediated IFN-γ production in early phase clearance of LM.


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isteria monocytogenes (LM) is a Gram-positive facultative intracellular bacterium, often causes life-threatening infections in an immunocompromised host, including newborns and elderly people (1–3). In host liver, LM invades and replicates in hepatocytes and Kupffer cells, tissue macrophages (2). The activation of the host’s innate immune system is critical for LM eradication in the early phase of infection (2–4). LM-infected macrophages produce IL-12 and IL-18 that synergistically induce IFN-γ (4–10), which subsequently activates macrophages and neutrophils to kill LM via production of listericidal molecules, such as NO (2, 11). Therefore, IL-12 and IL-18 seem to play an essential role in early phase clearance of LM by induction of IFN-γ.

Toll-like receptors (TLRs) have been identified as signaling receptors of the innate immune system and recognize corresponding pathogen-associated molecular patterns (12). TLR2 recognizes Gram-positive bacterial components, including peptide glycan and lipoproteins (12, 13). Studies using TLR2-transfected Chinese hamster ovary cells revealed TLR2 as a signaling receptor for LM products (14). TLR4 is reported to act as a signaling receptor of LPS, Gram-negative bacterial component (13, 15–17).

Myeloid differentiation factor 88 (MyD88) is an adaptor molecule essential for signaling through TLR (12, 18, 19). Pathogen-associated molecular patterns induce recruitment of MyD88 to the cytoplasmic domain of TLR, resulting in a relay of signal and activation of NF-κB that causes transcription of IL-12, TNF-α, and other inflammatory cytokines. Furthermore, MyD88 plays a critical role in IL-18R-mediated IL-18 signaling that induces IFN-γ production from IL-12-stimulated innate or acquired immune cells (20). These results suggest the importance of MyD88 in host defense against various pathogens. Indeed, MyD88 knockout (KO) mice are highly susceptible to Staphylococcus aureus infection and show the disability to produce proinflammatory cytokines (13). However, our recent study demonstrated the presence of MyD88-independent IL-18 release in LM-infected mice (9). Therefore, it is important to understand the protective role of TLR/MyD88 in LM infection.

In this study we have investigated molecular mechanisms of clearance of LM in mice. IL-12- or IL-18-deficient (KO) mice lacked the capacity to produce detectable levels of IFN-γ in their sera. These mice showed marked and moderate susceptibility to LM, respectively. IFN-γ KO mice entirely lacking the ability to produce IFN-γ; IL-12/IL-18 double KO (DKO) mice, lacking upstream cytokines for IFN-γ induction, showed much higher susceptibility to LM. However, we found that MyD88 KO mice that cannot produce IFN-γ, IL-12, and TNF-α are the most susceptible to LM. These results suggested that both IFN-γ and TNF-α are essential for host defense against LM.
We conclude that MyD88 is a critical adaptor molecule essentially required for full activation of innate immune system.

Materials and Methods

Mice

Specific pathogen-free female C57BL/6 mice (6–8 wk old) were purchased from Clea Japan (Osaka, Japan). IL-12 KO mice were provided by Dr. J. Magram (Roche Institute of Molecular Biology, Nutley, NJ) (21) and were backcrossed with C57BL/6 mice, and female F1 mice (6–9 wk old) were used for this study. IL-18 KO mice (22) and IFN-γ KO mice (23) were backcrossed with C57BL/6 mice, and female F1 mice (6–9 wk old) were used. To obtain C57BL/6-background IL-12/IL-18 DKO mice (6–8 wk old), F1 mice from C57BL/6-backcrossed IL-18 KO mice were crossed with F2 mice of C57BL/6-backcrossed IL-12 KO mice. IL-18Rα KO mice (24) were backcrossed with C57BL/6 mice, and female F10 mice (6–9 wk old) were used. TLR2 KO mice (13), TLR4 KO mice (15), and MyD88 KO mice (20) were backcrossed with C57BL/6 mice, and F2 female mice (6–9 wk old) were used. To obtain TLR2/TLR4 DKO mice (6–9 wk old), F2 mice from C57BL/6-backcrossed TLR2 KO mice were crossed with F2 mice from C57BL/6-backcrossed TLR4 KO mice.

Assay for cytokines

Concentrations of IFN-γ, IL-12p40, and TNF-α were determined by ELISA kits (Genzyme, Boston, MA).

Infection with LM

LM 43251 (American Type Culture Collection, Manassas, VA) was cultured in Trypsinase Soy Broth (BD Biosciences, Cockeysville, MD). Mice with various genotypes were i.v. inoculated with 5 × 10^7 LM. At the indicated time points serum was sampled for measurements of various cytokines. On day 3 livers from mice with various genotypes were homogenized and the supernatants were collected. Their liver specimens were sampled and homogenized. Culture medium was added to the culture medium at additional 24 h (5), and cytokine levels in each supernatant were determined according to the method described previously (4). The survival of various genotype mice inoculated with 5 × 10^7, 5 × 10^6, or 5 × 10^5 LM was judged every 12 h until day 14 after infection.

Kupffer cell preparation

Kupffer cells were prepared as previously shown (9). Culture medium was RPMI 1640 supplemented with 10% FCS, 50 μM 2-ME, and 2 mM l-glutamine. The cells (1 × 10^6/ml) were cultured with live LM (1 × 10^8 or 1 × 10^7/ml). After 1 h in culture, penicillin and streptomycin were added to the culture medium at final concentrations of 100 U/ml and 100 μg/ml, respectively, to limit the growth of LM, the cells were incubated for an additional 24 h (5), and cytokine levels in each supernatant were determined by ELISA.

Histology

Mice were inoculated with 5 × 10^7 LM and were killed 72 h after infection. Their liver specimens were sampled and fixed in 10% buffered formalin, and the slides were stained with H&E.

Statistics

All data are shown as the mean ± SD of triplicate determinations. Significance between control and experimental groups was examined with unpaired Student’s t test. A value of p < 0.05 was regarded as significant.

Results

Both IL-12 and IL-18 are required for induction of IFN-γ production at the early infectious phase of LM

It is well known that IFN-γ is critical for early phase clearance of LM (1–4, 25). We wanted to clarify whether IL-12 and/or IL-18 play an essential role in early induction of IFN-γ production following LM infection. We measured IFN-γ levels in the sera of mice with various genotypes after LM infection (Fig. 1). In wild-type (WT) mice the serum IFN-γ level increased and peaked at 24 h postinfection, while IFN-γ was undetectable in the sera of IL-12/IL-18 DKO mice. IL-12 or IL-18 single-KO mice also showed undetectable levels of IFN-γ in their sera. These results strongly indicated the requirement for both IL-12 and IL-18 for IFN-γ production in LM-infected mice. LM-infected MyD88 KO mice also displayed an undetectable level of IFN-γ in their sera, further substantiating our previous reports that MyD88 is required for IL-12-mediated IFN-γ induction (9, 20).

Critical role of MyD88 in eradication of LM in the liver

As both IL-12 and IL-18 contribute to IFN-γ accumulation (Fig. 1), we investigated whether both IL-12 and IL-18 are also responsible for early phase LM clearance in the liver. IL-18 KO mice and IL-12 KO mice showed partial and severe impairment in LM eradication, respectively, compared with WT mice (Fig. 2A). As expected, IL-12/IL-18 DKO and IFN-γ KO mice showed striking impairment in LM eradication. IFN-γ KO mice had the largest CFU counts in their livers (Fig. 2A), indicating that IFN-γ plays an essential role in LM eradication from the liver.

IL-18 seemed to weakly contribute to early LM eradication in the liver (Fig. 2A). Furthermore, the absence of expression of IL-18 or IL-18Rα did not affect IL-12 production in LM-infected mice (Fig. 2C and data not shown). In contrast, MyD88 KO mice did not produce IL-12 (Fig. 2C), although they could produce IL-18 (9), suggesting a contribution of MyD88 to IL-12 production.

Because MyD88 is an essential adaptor molecule for IL-18R-mediated signaling (20), we compared CFU counts in the livers of IL-18Rα KO mice with those in MyD88 KO mice. Like IL-18 KO mice (Fig. 2A), IL-18Rα KO mice showed much better LM clearance than MyD88 KO mice, indicating only moderate contribution of IL-18 signaling to LM clearance (Fig. 2B).

MyD88-dependent TNF-α production is required for LM eradication

We next investigated whether defects in LM clearance in MyD88 KO mice are solely due to their disability to produce IFN-γ. For this purpose we compared listerial burden in the liver between IFN-γ KO and MyD88 KO mice. As shown in Fig. 2B, bacterial burden in the liver of MyD88 KO mice was significantly heavier than that in IFN-γ KO mice (p < 0.05).

Listerial burden in the liver of MyD88 KO mice was larger than that in IFN-γ KO mice (Fig. 2B), indicating the importance of an additional factor(s) that was produced in a MyD88-dependent manner. As TNF-α is well known as another important factor for
MyD88 is indispensable for LM clearance via induction of both IFN-γ and TNF-α. A. On day 3 after inoculation, liver was sampled from WT, IL-12 KO, IL-18 KO, IL-12/IL-18 DKO (DKO), and IFN-γ KO (GKO) mice. Bacterial burden in the liver was calculated. Data are the mean ± SD of five mice in each experimental group. Similar results were obtained in three independent experiments. On day 3 after inoculation, liver was sampled from WT, IL-12Rα KO (IL-12RKO), IFN-γ KO (GKO), or MyD88 KO mice. Bacterial burden in the livers was calculated. Data are the mean ± SD of five mice in each experimental group. Similar results were obtained in three independent experiments. Bacterial burden in the liver was calculated. Data are the mean ± SD of five mice in each experimental group. Similar results were obtained in three independent experiments. A. *, p < 0.05; **, p < 0.01. B. On day 3 after inoculation, liver was sampled from WT, IL-12 KO, IL-18 KO, IL-12Rα KO (IL-12RKO), IFN-γ KO (GKO), or MyD88 KO mice. Bacterial burden in the livers was calculated. Data are the mean ± SD of five mice in each experimental group. Similar results were obtained in three independent experiments. A. *, p < 0.05; **, p < 0.01. C. Sera were obtained from WT, IL-12 KO, IL-12 KO, IL-18 KO, IL-12Rα KO (IL-12RKO), IFN-γ KO (GKO), or MyD88 KO mice at 24 h postinfection for the measurement of IL-12p40 levels by ELISA. Data are the mean ± SD of five mice in each experimental group. D. Kupffer cells from WT, IL-12 KO, IL-12 KO, IL-12/IL-18 DKO (DKO), IL-18 KO, IFN-γ KO (GKO), or MyD88 KO mice were incubated with live LM (1 × 10^7/ml C or 1 × 10^6/ml D) for 24 h, and TNF-α levels in each supernatant were determined by ELISA. Data are presented as the mean ± SD of triplicate cultures. Similar results were obtained in three independent experiments. ND, not detectable.

LM clearance (26–28), we simultaneously investigated the capacity of MyD88 KO mice to produce TNF-α upon LM infection. TNF-α was undetectable in the sera of LM-infected WT mice. Therefore, we examined the capacity of macrophages to produce TNF-α in response to LM in vitro. As shown in Fig. 2D, WT Kupffer cells produced TNF-α comparably when stimulated with LM. IL-12-, or IL-12-deficient or IL-12/IL-18 doubly deficient Kupffer cells produced TNF-α similarly to WT Kupffer cells (Fig. 2D), indicating that IL-12 and IL-18 are dispensable for TNF-α production. IFN-γ-deficient cells secreted slightly less TNF-α (Fig. 2D), indicating a minor role of IFN-γ in TNF-α production. In contrast, MyD88-deficient Kupffer cells did not produce TNF-α, suggesting the involvement of TNF-α in LM clearance.

TLR/MyD88 is required for IL-12 release upon LM infection

MyD88 is essential for IL-12 and TNF-α production upon LM infection (Fig. 2, C and D) (9). Next, we investigated what types of TLRs are involved in the induction of IL-12 and TNF-α. As shown in Fig. 3A, TLR2 KO mice, but not TLR4 KO mice, showed reduced IL-12p40 production following LM infection. Similarly, TLR2/TLR4 DKO mice showed a reduction in their capacity to produce IL-12, indicating that TLR2 partly contributes to IL-12 production independently of TLR4. Consistent with our previous report (9), MyD88 KO mice failed to produce IL-12 (Fig. 3A), although they secreted IL-18 (data not shown). TLR2 KO mice and TLR2/TLR4 DKO mice, but not TLR4 KO mice, displayed less IFN-γ in their sera than did WT mice post-LM infection (Fig. 3B). As expected from the results with IL-12 (Fig. 3A), MyD88 KO mice were incapable of producing IFN-γ in their sera (Fig. 3B). Serum levels of IFN-γ appeared to correlate well with those of IL-12 in various mutant mice, suggesting that IL-12 is principally responsible for determining the IFN-γ level. These results suggested the importance of TLR2 and possibly other TLRs, except for TLR4, in IL-12-dependent IFN-γ production after LM challenge.

We investigated whether TLR2 is also important for TNF-α production in response to LM. After being stimulated with a small number of the bacteria, TLR2-deficient Kupffer cells, unlike TLR4-deficient cells, produced much less TNF-α, as in MyD88-deficient cells (Fig. 3C). However, upon challenge with a large number of LM, TLR2-deficient cells produced more TNF-α than MyD88-deficient Kupffer cells (Fig. 3D). Collectively, LM infection induces both IFN-γ and TNF-α production, dependent in part on TLR2 and perhaps other TLRs.

MyD88 is critical for neutrophil recruitment into the liver

As both IFN-γ and TNF-α are involved in the recruitment of neutrophils, potent effector cells (29–32), we investigated inflammatory...
responses in the liver of the various mutant mice postinfection. LM-infected WT mice had large inflammatory foci consisting of numerous neutrophils in the liver (Fig. 4, A and B). In livers of IL-18 KO or IL-12KO mice we could not find significant differences in the levels of inflammatory responses compared with WT mice (data not shown). The inflammatory foci in livers of IFN-γ KO mice contained fewer neutrophils in their population (Fig. 4, C and D), indicating the importance of IFN-γ in the recruitment of neutrophils. Somewhat surprisingly, IL-12/IL-18 DKO mice showed a dense infiltration of neutrophils (data not shown), while IFN-γ KO mice did not, suggesting the failure of IL-12 and IL-18 to completely cover the function of IFN-γ. MyD88 KO mice had much smaller foci, composed of few neutrophils, than the other mutant mice, even including IFN-γ KO mice (Fig. 4, C–F), suggesting that MyD88 participates in neutrophil recruitment via induction of both IFN-γ and TNF-α. There were no remarkable differences in the pathological changes in livers between WT and TLR4 KO mice, but the foci in TLR2 KO mice contained fewer neutrophils than those in WT or TLR4 KO mice (Fig. 4, G–J). This may be explained by the partial impairment in the production of both IFN-γ and TNF-α in TLR2 KO mice (Fig. 3, B and C). Collectively, these results suggest the essential role of both IFN-γ and TNF-α in the early phase clearance of LM via activation and recruitment of effector cells in the liver.

MyD88 is essential for resistance to LM

Finally, we investigated the mortality of mice with various genotypes after inoculation with LM. As shown in Fig. 5, WT mice inoculated with the highest number of LM survived up to 14 days postinfection, at which time they were sacrificed. The survival rate of IL-18 KO mice was less than that of WT mice only when they were infected with the highest number of LM (5 × 10⁵ bacteria; Fig. 5A). All IL-12 KO and IL-12/IL-18 DKO mice died by day 5 postinfection with the largest number of LM (Fig. 5A). However, inoculation with a lesser number of LM (5 × 10⁴ bacteria) revealed that IL-12/IL-18 DKO mice were more susceptible than IL-12 KO mice (Fig. 5B). Moreover, all the IL-12/IL-18 DKO mice survived, but 80% of IFN-γ KO mice succumbed upon infection with the smallest number of LM (5 × 10³ bacteria; Fig. 5C). These results strongly indicated the essential involvement of IFN-γ in host defense against LM infection. Consistent with their disability to produce both IFN-γ and TNF-α (Figs. 1–3), MyD88 KO mice were most susceptible to LM (Fig. 5). The mortality well paralleled the LM titer in the liver (Fig. 2) and the intensity of neutrophil recruitment (Fig. 4). Collectively, LM-induced MyD88-mediated IL-12 and TNF-α production is essential for protection of mice from fatal LM infection.

Discussion

Initial infection with microbes activates host innate immunity through TLRs to promptly expel the pathogen (12). Insufficient early eradication of the pathogen is sequentially restored by activated adaptive immunity, resulting in successful clearance of the pathogens. In contrast to the involvement of innate immunity in the initial infection, adaptive immunity promptly participates in clearance of the pathogen upon reinfection. LM is an intracellular parasitic bacterium against which Th1 cells have been reported to be essentially required (3). However, the innate immune responses developing in initial LM infection often determine the outcome of the host, especially the immunocompromised host (2). The effector mechanism in the innate immune response to LM consists of phagocytosis and NK cell activation. As previously reported, innate immunity effectively exerts its protective action against pathogen when they are efficiently activated by endogenous and/or exogenous IL-12 and IL-18, which in combination have capacity to induce IFN-γ production by innate and adaptive immune cells, including T cells, B cells, NK cells, macrophages, and dendritic cells (10). Here, we illustrated the importance of endogenous IL-12 and IL-18 in early phase clearance of LM (Figs. 2 and 5). Although the relative roles of IL-12 and IL-18, which are essential for IFN-γ production in vivo (Fig. 1), are still unclear in innate immunity, our present study clearly showed that IL-12 is much more important in local LM elimination than IL-18 (Fig. 2). We also revealed that MyD88 is essential for induction of IL-12 plus IL-18-dependent IFN-γ production (Figs. 1 and 2), because it allows signaling through TLR and IL-18R, which are activated by LM and IL-18, respectively. In studies using IFN-γ KO and MyD88 KO mice we showed an additional role of MyD88 as an essential molecule to induce production of TNF-α (Fig. 2D). MyD88-dependent IFN-γ in combination with MyD88-dependent TNF-α accomplish host defense against LM with efficient recruitment of neutrophils in the liver (Figs. 4 and 5). Our present results revealed the importance of innate immune responses that are definitively triggered by TLR/MyD88-dependent pathways.

Very recently, Neighbors et al. (33) reported that treatment of BALB/c mice with mAb against IL-18Rβ, a signaling component of IL-18R, renders them highly susceptible to sublethal numbers of LM compared with neutralizing anti-IL-12p40 mAb. Our present
FIGURE 5. MyD88 determines host resistance to LM. The survival rates of MyD88 KO (△), IFN-γ KO (GKO; ○), IL-12/IL-18 DKO (DKO; •), IL-12 KO (▲), IL-18 KO (○), and WT (●) mice after i.v. injection of 5 × 10^5 (A), 5 × 10^4 (B), or 5 × 10^3 (C) CFU of LM/mouse are shown. Survival was assessed every 12 h for 14 days. Each experimental group consisted of eight (A) or five (B and C) mice. Similar results were obtained in two independent experiments.

results using IL-12 KO mice on the C57BL/6 background indicated that endogenous IL-12p40 is more dominantly involved in LM clearance than IL-18. There are several possibilities that account for this discrepancy. First, as noted above, they used LM-susceptible BALB/c mice, while they used LM-resistant C57BL/6 background mice. Second, they used anti-IL-18Rβ mAb-treated mice, while they used IL-18 KO and IL-18Rα KO mice. Both IL-18 KO and IL-18Rα KO mice had the same immune responses after LM infection (Fig. 2, A and B), demonstrating that IL-18Rα is necessary and sufficient for IL-18 signaling. Therefore, we suspect that their mAb might inhibit not only IL-18 signaling but also signaling of an unknown factor(s) that uses IL-18R β-chain. Third, the discrepancy might be due to the difference in the target tissues examined. The liver, which we manipulated, is composed of unique residential lymphocytes, including NK cells and NKT cells, which are meager in the spleen they used.

As previously reported, IL-18 has capacity to induce TNF-α production by macrophage upon LM infection (33). However, IL-18 deficiency does not reduce TNF-α production in response to LM (Fig. 2D), indicating that endogenous IL-18 does not participate in TNF-α production, at least in macrophage levels. Furthermore, we showed that the absence of IL-18 does not influence IL-12p40 production (Fig. 2C). Thus, apparently IL-18 does not play a major role in the production of TNF-α or IL-12 after LM infection.

IL-12 KO mice were more susceptible than IL-18 KO mice (Fig. 5), although both failed to show a detectable serum level of IFN-γ (Fig. 1). At present we have no definitive explanation for this difference. However, IL-12 KO mice may have no capacity to develop Th1 cells, while IL-18 KO mice have such potentiality to develop Th1 cells following infection in the target organs (10, 22).

We measured serum levels of IFN-γ. Measurement of local levels of IFN-γ and determination of cells that produce IFN-γ in the liver might be important for understanding the difference in the host resistance to LM infection of IL-18 KO and IL-12 KO mice.

Livers of IFN-γ KO mice contain more LM titer than those of IL-12/IL-18 DKO mice (Fig. 2). Other factors, in addition to IL-12 and IL-18, might contribute to IFN-γ-dependent LM clearance. IL-15 is a candidate because IL-15 synergizes with IL-18 and/or IL-12 for induction of IFN-γ (Ref. 34 and our unpublished observations) and is synthesized by macrophages upon stimulation with microbes and microbe products, including LM (34, 35).

Furthermore, we tested the involvement of TLR members in LM infection to induce proinflammatory cytokine production. Because most bacterial cell wall components, either from Gram-positive or -negative bacteria, are recognized by TLR2 or TLR4, respectively, we used TLR2 KO mice and TLR4 KO mice as host (13). We found that TLR2 KO mice showed a relatively poor response to LM infection in terms of IL-12, IFN-γ, and TNF-α production (Fig. 3). Therefore, TLR2 seems to be partly involved in LM signaling. In contrast, MyD88 KO mice completely lacked the capacity to produce IL-12 and TNF-α upon LM infection and to respond to IL-12 plus IL-18 by IFN-γ production (Fig. 3). Thus, TLR2 might form heterodimer by association with another TLR member(s) to exert its role as a signaling molecule. Further study is required for identification of other TLRs involved in the response to LM.

Recruitment of neutrophils in the liver requires both TNF-α and IFN-γ. WT mice had large inflammatory foci composed of many neutrophils in their livers, while IFN-γ KO and MyD88 KO mice showed intermediate-sized foci with a small number of neutrophils, respectively (Fig. 4). These results substantiate further that MyD88-dependent IFN-γ and TNF-α production plays an essential role in LM clearance in the early infectious phase via activation and recruitment of effector cells.

In summary, our study showed that both IL-12 and IL-18 contribute to IFN-γ production in LM infection. In addition, IL-12 is more profoundly involved than IL-18 in LM eradication in the liver. Furthermore, MyD88 plays a pivotal role in early phase clearance of LM through induction of IFN-γ and TNF-α via TLR signaling. Taken together, these findings show that MyD88 is essential for determining host resistance to various microorganisms, including LM, during innate phase immune response.
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