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IL-23 Is Required for Protection against Systemic Infection with *Listeria monocytogenes*¹

Karen D. Meeks,* Amy N. Sieve,* Jay K. Kolls,† Nico Ghilardi,‡ and Rance E. Berg²*

*Listeria monocytogenes* (LM) is a Gram-positive, intracellular bacterium that can induce spontaneous abortion, septicemia, and meningitis. Although it is known that neutrophils are required for elimination of the bacteria and for survival of the host, the mechanisms governing the recruitment of neutrophils to LM-infected tissues are not fully understood. We demonstrate here that IL-23 and the IL-17 receptor A (IL-17RA), which mediates both IL-17A and IL-17F signaling, are necessary for resistance against systemic LM infection. LM-infected IL-23p19 knockout (KO) mice have decreased production of IL-17A and IL-17F, while IFN-γ production is not altered by the lack of IL-23. LM induces the production of IL-17A from γδ T cells, but not CD4, CD8, or NK cells. Furthermore, a lack of efficient neutrophil recruitment to the liver is evident in both IL-23p19 KO and IL-17RA KO mice during LM infection. Immunocytochemical analysis of infected livers revealed that neutrophils were able to localize with LM in IL-23p19 KO and IL-17RA KO mice, indicating that IL-23 and IL-17RA do not regulate the precise localization of neutrophils with LM. The importance of IL-23-induced IL-17A was demonstrated by injecting IL-23p19 KO mice with recombinant IL-17A. These mice had reduced LM bacterial burdens compared with IL-23p19 KO mice that did not receive IL-17A. These results indicate that during LM infection, IL-23 regulates the production of IL-17A and IL-17F from γδ T cells, resulting in optimal liver neutrophil recruitment and enhanced bacterial clearance. *The Journal of Immunology*, 2009, 183: 8026–8034.

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*Listeria monocytogenes* (LM)¹ is a Gram-positive bacterium that can induce septicemia, meningitis, or spontaneous abortion in infected individuals. Infection with this facultative intracellular pathogen invokes a complex immune response characterized by the influx of neutrophils and a predominantly proinflammatory cytokine response (1). Mice deficient in neutrophils have increased bacterial burden in the spleen and liver and increased susceptibility to LM (2–5). Additionally, neutrophils are more critical for protection against LM in the liver than in the spleen or peritoneal cavity (4). Little is known regarding the signals necessary to influence the mobilization of neutrophils to sites of LM infection. One possible mechanism involves the homologous members of the IL-17 cytokine family, IL-17A and IL-17F, which act to influence the production of chemokines (CXCL1, CXCL2, CXCL5, CXCL8) and cytokines (IL-6, GM-CSF, and G-CSF) that ultimately induce the recruitment, differentiation, and activation of neutrophils in other models of infection (6, 7).

A critical role for IL-17A secreted by γδ T cells in the liver has recently been established during infection with LM (8). Likewise, another study has demonstrated that, during LM infection, systemic levels of IL-17A are increased in the absence of LFA-1, a leukocyte adhesion molecule. This results in LFA-1-independent liver neutrophilia which enhances listerial clearance (9). In addition, our published data have demonstrated that during a primary chronic infection with *Mycoplasma pulmonis*, IL-17A provides a novel form of cross-protection against a secondary infection with LM. This protection is mediated by increased recruitment of neutrophils to sites of infection (10). Further evidence suggests a role for cytokines and chemokines dependent upon the actions of IL-17A and IL-17F during LM infection. The cytokine IL-6 is critical for optimal mobilization of neutrophils and clearance of LM (11, 12). Additionally, G-CSF and GM-CSF are induced by IL-17A and IL-17F and have been shown to be produced during LM infection (13). In mice lacking G-CSF or GM-CSF, neutrophil recruitment and mobilization are decreased and mice demonstrate increased susceptibility to LM infection (14, 15). Furthermore, Abs against CXCL1 or the mouse IL-8 receptor homolog (CXCR2) blocked the recruitment of neutrophils to LM-infected livers (16). Taken together, it is likely that IL-17A and IL-17F are involved in an optimal immune response against LM by inducing a multitude of cytokines and chemokines, ultimately influencing the recruitment of neutrophils to sites of infection.

The IL-12 family member IL-23 is responsible for the maintenance of cells that secrete both IL-17A and IL-17F (17). As a heterodimeric cytokine, IL-23 shares the p40 subunit with IL-12 and expresses a unique p19 subunit (18). Although originally thought to act in a similar fashion to IL-12 (18), IL-23 has been shown to possess independent effector functions from IL-12. The pathogenic roles for IL-23 are primarily studied in models of allergic and autoimmune disease (19), while its protective roles are primarily studied during models of infection. Evidence that the IL-23/IL-17 axis is protective during infection has been demonstrated with extracellular and vacuole-bound bacteria: *Klebsiella pneumoniae* (20), *Citrobacter rodentium* (21, 22), *Salmonella enterica* (23, 24), and *Mycobacterium tuberculosis* (17, 25). Additionally, the IL-23/IL-17 cytokine axis has been demonstrated to be protective during infection with *Toxoplasma gondii* (26, 27) and
Candida albicans (28–30). The role of IL-23, IL-17A, and IL-17F has not been researched during systemic infection or with a cytoplasmic pathogen such as LM. Important preliminary evidence demonstrates that IL-23 can be secreted by LM-infected dendritic cells (31). Given that IL-23 maintains IL-17-secreting cells in other infectious models and the importance of neutrophils for resistance to LM infection, it is of interest to delineate the role of the IL-23 and IL-17A/F axis during LM infection and the impact these cytokines may have on neutrophil recruitment. To investigate the role of the IL-23 and IL-17A/F axis, mice rendered deficient in either IL-23 (IL-23p19 knockout (KO)) or the IL-17 receptor A (IL-17RA KO), thus devoid of both IL-17A and IL-17F signaling, were systemically infected with the cytoplasmic pathogen LM. We demonstrate here that IL-23 is required for an optimal immune response against LM through a mechanism dependent on IL-17A and IL-17F production and the recruitment of neutrophils to the liver.

Materials and Methods

Mice
C57BL/6 (B6) mice were purchased from the National Cancer Institute. IL-23p19 KO and IL-17RA KO backcrossed on a B6 genetic background have previously been described (32, 33). All studies used male mice between 6 and 12 wk of age, which were housed with food and water ad libitum in sterile microisolator cages with sterile bedding at the University of North Texas Health Science Center American Association for the Accreditation of Laboratory Animal Care accredited animal facility. All animal studies were performed under the approval of the Institutional Animal Care and Use Committee at the University of North Texas Health Science Center.

LM infections and in vivo procedures

LM 10403s and LM-expressing full-length OVA (Lm/OVA) were grown on brain-heart infusion (BHI) agar plates (BD Bacto) and virulent stocks were maintained by repeated passage through B6 mice. For infection of mice, log-phase cultures of LM or LM/OVA were grown in BHI broth, washed twice, and diluted in PBS to the desired concentration. To determine survival kinetics, a dose of $3.5 \times 10^4$ LM was injected via the lateral tail vein. To determine bacterial burden, cytokine secretion, and neutrophil numbers and localization, a sublethal dose of $1 \times 10^6$ LM was used. To determine LM CFUs, spleens and livers from infected mice were homogenized in sterile double distilled H2O. Serial dilutions (1/10) of the homogenate of infected spleens and livers were plated on BHI agar plates. After overnight incubation at 37°C, colonies were counted and the LM CFUs recovered from each tissue were calculated. For the administration of exogenous IL-17A (R&D Systems) and IFN-γ (R&D Systems), mice were fixed and permeabilized at 4°C for 20 min using an intracellular cytokine staining kit from BD Pharmingen. After washing in permeabilization buffer, the cells were fixed and permeabilized at 4°C for 20 min using an intracellular cytokine staining kit from BD Pharmingen. After washing in permeabilization wash buffer, the cells were incubated in saturating amounts of anti-IFN-γ or anti-IL-17A at 4°C for 20 min. Data were acquired and analyzed using a Beckman Coulter Cytomics FC500.

Quantitative measurements of IL-17A, IL-17F, and IFN-γ

ELISAs were performed on serum or filtered, cell-free splenocyte and liver culture supernatants. Quantification of IL-17A and IFN-γ were performed using ELISAs purchased from BD Biosciences and Quantification of IL-17F was performed using an ELISA duo set from R&D Systems. Cytokine levels were determined by comparison with standard curves generated from rIL-17A (R&D Systems), rIL-17F (R&D Systems), or rIFN-γ (PeproTech) and were analyzed using a Biotek EL808 spectrophotometer.

Immunocytochemistry and microscopy

Immunocytochemistry of spleens and livers was performed by making 7-μm sections of frozen organs from LM-infected B6, IL-23p19 KO, and IL-17RA KO mice using a Leica CM 1850 cryostat. Spleen and liver sections were acetone fixed before staining. The Ab combination used to stain spleen and liver sections was purifi-antibody to Ly6G (1A8; BD Pharmingen) and Difco Listeria O polysaccharide (Fisher Scientific). The anti-Ly6G Ab was developed with anti-rat Alexa Fluor 594 (Molecular Probes). The anti-O polysaccharide was developed with anti-rabbit Alexa Fluor 488 (BD Pharmingen). Stained sections were then visualized on an Olympus AX70 fluorescence microscope and images were captured with an Olympus DP70 digital camera.

Statistical analyses

ANOVARs were conducted on the data. Bonferroni t tests and Tukey-Kramer analyses were used for post hoc analyses. LM CFU data was log transformed before analysis and is represented as such in the figures. Kaplan-Meier plots and log-rank tests were used to compare the survival curves between groups. A p value of 0.05 or less was considered significant in all cases.

Results

IL-23 is required for protection against LM infection

Although IL-23 has been shown to be secreted by LM-infected dendritic cells (31), its role during systemic LM infection is unknown. Therefore, B6 and IL-23p19 KO mice were infected i.v. with $3.5 \times 10^4$ LM to perform a survival study. By day 10 p.i., the LM infection was fatal for 100% of the IL-23p19 KO mice (Fig. 1A). In contrast, only 9% of the B6 mice succumbed to infection. Given that the majority of the IL-23p19 KO mice succumbed to infection by day 5, a sublethal dose of $1 \times 10^3$ LM was used to determine the kinetics and distribution of bacterial burden in the spleens and livers of mice infected for 1, 3, 5, or 7 days. At days 1 or 3 p.i., IL-23p19 KO mice did not show significant increases in bacterial burden in both the spleen and liver compared with B6 mice (data not shown). However, at days 5 and 7 p.i. with LM, significant increases in bacterial burden in both the spleens and the livers of the IL-23p19 KO mice were evident (Fig. 1, B and C). These data demonstrate that IL-23 plays a vital role in protecting against systemic LM infection.

The secretion of IL-17A and IL-17F, but not IFN-γ, is regulated by IL-23 during LM infection

To understand the increased susceptibility of the IL-23p19 KO mice to LM, cytokines were investigated in mice that were infected with LM.
response to infection and affected due to a lack of IL-23 production. IL-23 has been shown to be necessary for driving the expansion and maintenance of IL-17-secreting cells which produce both IL-17A and IL-17F (35–37). Therefore, the production of IL-17A and IL-17F was measured from uninfected B6, LM-infected B6, and LM-infected IL-23p19 KO mice at day 5 p.i. Serum levels of IL-17A from LM-infected B6 and IL-23p19 KO mice were undetectable (data not shown). Spleen and liver cell cultures were left unstimulated or were provided HKLM or exogenous IL-23 for 2 days. Infection of B6 mice with LM induces the production of IL-17A and IL-17F from the spleen during LM infection. Furthermore, when the phenotype of the IL-17A-secreting cells was analyzed, γδ T cells were found to be the primary, if not exclusive, source of IL-17A at both day 1 (Fig. 3B) and day 5 (Fig. 3D) after LM infection. Percentages of IL-17A-producing CD4, CD8, and NK cells are <0.5% at days 1 and 5 p.i. after stimulation with IL-23 and do not differ between uninfected and LM-infected B6 mice (data not shown). γδ T cells were also the primary source of IL-17A production from leukocytes isolated from the liver (data not shown). Therefore, during LM infection, γδ T cells respond rapidly by producing IL-17A and IL-23 regulates the production of IL-17A from γδ T cells.

To more closely analyze Ag-specific CD8 T cell responses to LM, B6 and IL-23p19 KO mice were infected with LM/OVA. At day 7 p.i., splenocytes from the infected mice were cultured with the SIINFEKL peptide to stimulate cytokine production from OVA-specific CD8 T cells. In accordance with previous reports (41, 42), we were unable to detect appreciable amounts of Ag-specific IL-17A or IL-17F production from splenocytes stimulated with the SIINFEKL peptide (Fig. 3, E and F). Both IL-17A and IL-17F were produced from B6 splenocytes in response to the nonspecific stimulus HKLM/OVA and this response was reduced in the IL-23p19 KO mice. However, no differences in IFN-γ production from LM-infected B6 and IL-23p19 KO mice were evident when measured with ELISA (Fig. 3G). Additionally, when intracellular cytokine staining was utilized, there were no differences in the percentages of OVA-specific CD8 T cells secreting IFN-γ from LM/OVA-infected B6 (Fig. 3H) and IL-23p19 KO (Fig. 3I) mice when stimulated with SIINFEKL. These data indicate that
Ag-specific CD8 T cells do not contribute to the production of IL-17A or IL-17F and that IL-23 does not regulate Ag-specific production of IFN-γ during LM infection. 

Signaling through the IL-17RA is required for protection against LM infection

Considering that IL-23p19 KO mice infected with LM failed to induce optimal secretion of IL-17A or IL-17F in spleen and liver cultures, we next wanted to determine whether IL-17A and IL-17F were required for protection against LM infection. Therefore, B6 and IL-17RA KO mice, which are unable to signal through IL-17A or IL-17F, were infected with \(10^4\) LM to perform a survival study. As seen in Fig. 4A, by day 9 p.i., the LM infection had proved fatal for 90% of the IL-17RA KO mice. In contrast, only 20% of the B6 mice succumbed to the infection. Given that the majority of the IL-17RA KO mice succumbed to infection by day 6, a sublethal dose of \(10^4\) LM was used to determine the kinetics and distribution of bacterial burden in the spleens and livers of mice infected for 1, 3, 5, or 7 days. At days 1 or 3 p.i., IL-17RA KO mice did not show increased bacterial burden compared with B6 mice (data not shown). However, at both day 5 (Fig. 4B) and day 7 (Fig. 4C) p.i., IL-17RA KO animals had increased bacterial burden in the spleen and liver similar to the bacterial kinetics of the IL-23p19 KO mice shown in Fig. 1. IL-17A and IL-17F are known to play a role in the mobilization and recruitment of neutrophils (6, 7) and neutrophils are necessary for appropriate clearance of LM (2–5). Given this, we next wanted to test whether neutrophil numbers would be reduced in mice lacking IL-23 or IL-17RA and whether this might provide the mechanism for the increased bacterial burden and increased susceptibility to LM in the absence of these cytokines.

IL-23 and IL-17RA are required for optimal recruitment of neutrophils to the liver during LM infection

To determine whether neutrophil recruitment to the liver and spleen, or the maintenance of neutrophils in the peripheral blood, is affected by the absence of IL-23 or IL-17A/F signaling during LM infection, flow cytometry was used to analyze the coexpression of Ly6G and CD11b on activated neutrophils (43, 44). As seen in Fig. 5, there are no differences in the percentages or total numbers of neutrophils in the livers of uninfected B6, IL-23p19 KO, and IL-17RA KO mice and no differences at 3 h p.i. Interestingly, at 24 h (1 day) and 72 h (3 days) p.i., the livers of the IL-23p19 KO and IL-17RA KO mice contained reduced percentages (Fig. 5A) and total numbers (Fig. 5B) of neutrophils compared with the B6 mice. By 120 h (5 days) p.i., B6, IL-23p19 KO, and IL-17RA KO mice had similar percentages and numbers of neutrophils in the liver (Fig. 5). These data suggest that the optimal recruitment of neutrophils to the liver at 1 and 3 days p.i. requires IL-23 and IL-17A/F signaling.

In contrast to the liver, IL-23p19 KO and IL-17RA KO mice did not have reduced percentages of neutrophils in the spleen or peripheral blood at 0, 3, 24, 72, or 120 h after infection (data not shown). Therefore, we propose that an IL-23- or IL-17A/F-independent mechanism must exist to regulate neutrophil recruitment to the spleen and maintain neutrophil numbers in peripheral blood.

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**FIGURE 2.** IL-23 is required for optimal production of IL-17A and IL-17F during LM infection. B6 and IL-23p19 KO mice were infected with \(10^4\) LM for 5 days. On the day of sacrifice, splenocytes and liver leukocytes from LM-infected and uninfected B6 mice were left unstimulated (US) or were cultured with HKLM or 10 ng/ml IL-23. Two days after culture, spleen (A, C, and E) and liver (B, D, and F) supernatants were obtained and the concentrations of IL-17A (A and B), IL-17F (C and D), and IFN-γ (E and F) were measured. Two-way ANOVAs detected significant effects of mouse strain. An asterisk (**) indicates a significant difference from LM-infected B6 mice (\(p < 0.05\)). Double asterisks (**) indicate a significant difference from both LM-infected B6 and IL-23p19 KO mice (\(p < 0.05\)). These data are representative of two independent experiments. All data are expressed as the mean ± SEM (\(n = 5\)/group). ND, Not detectable.
As has been previously demonstrated, the recruitment of neutrophils to the liver is more important than the recruitment of neutrophils to the spleen for the clearance of LM (4). Therefore, our data suggest that the reduced recruitment of neutrophils to the liver at days 1 and 3 p.i. contributes to the increased susceptibility of IL-23p19 KO and IL-17RA KO mice to infection with LM.

To further analyze the recruitment of neutrophils to the livers and spleens of mice lacking IL-23 and IL-17RA, immunocytochemistry was performed on organ sections at days 1 and 3 after LM infection. In the spleen, neutrophils were evident and in equivalent numbers at days 1 and 3 after LM infection in B6, IL-23p19 KO, and IL-17RA KO mice (data not shown). In accordance with
the flow cytometry data, mice lacking IL-23 or IL-17RA had reduced total numbers of neutrophils in the liver at days 1 and 3 after LM infection compared with B6 mice (data not shown). At the dose used, the presence of LM in the liver is difficult to visualize at day 1 after LM infection. However, at day 3 p.i., LM can be visualized in the liver (45). There did not appear to be differences in the lesions in the three strains of mice, likely due to the fact that the number of LM in the livers is not higher at day 3 p.i. in the IL-23p19 KO and IL-17RA KO mice. Furthermore, the localization of neutrophils around the LM lesions was not different in the livers of B6 (Fig. 6, A and B), IL-23p19 KO (Fig. 6, C and D), and IL-17RA KO mice (Fig. 6, E and F). Collectively, these data suggest that IL-23 and the IL-17RA regulate the optimal recruitment of neutrophils to the liver during LM infection, but that these cytokines do not regulate precise neutrophil localization in LM-infected livers.

**Discussion**

In the present study, we provide evidence that IL-23 and IL-17A/F signaling are necessary for resistance against systemic infection with LM. A deficiency in either IL-23 or the IL-17RA leads to increased mortality and decreased bacterial clearance from the spleen and liver. IL-17A and IL-17F levels are decreased in liver and splenocyte culture supernatants from infected IL-23p19 KO mice compared with B6 mice, and IL-17A is produced by γδ T cells in the spleen and liver of LM-infected B6 mice. In the absence of IL-23p19 or IL-17RA, neutrophil percentages and total numbers in the liver are reduced at 1 day and 3 days p.i.; however, the ability of neutrophils to localize to sites of LM infection is not altered in these mice. Additionally, the administration of exogenous IL-17A can reduce LM CFUs in IL-23p19 KO mice.

*rlIL-17A can reduce LM CFUs in IL-23p19 KO mice*

To determine whether the lack of IL-17A production in infected IL-23p19 KO mice results in increased CFUs, exogenous IL-17A was administered to IL-23p19 KO animals during infection with LM. IL-23p19 KO mice were infected with LM and were given daily doses of 1 μg of IL-17A or PBS. IL-23p19 KO mice with or without IL-17A were sacrificed on day 5 p.i. and bacterial burden was assayed in both the spleen and liver compared with wild-type B6 controls (±PBS). As seen in Fig. 7, upon administration of IL-17A during infection with LM, protection was evident in the spleen and liver of the IL-23p19 KO animals. The resistance of the IL-23p19 KO mice against LM infection in the spleen and liver provided by the IL-17A administration was similar to the level of resistance against LM evident in the B6 animals. These data suggest that exogenous IL-17A is able to provide protection during LM infection in IL-23p19 KO mice.
IL-23p19 KO mice to levels equivalent to those of B6 mice. We propose here that IL-23 regulates the production of IL-17A and IL-17F from γδ T cells, thereby inducing the early (days 1 and 3 p.i.) presence of neutrophils in the liver during LM infection. For the first time, we outline a critical role for IL-23 during systemic infection with the cytoplasmic bacterium LM.

The regulation of IL-17A and IL-17F by IL-23 during systemic infection with LM is critical in both the spleen and the liver. Reduced levels of IL-17A and IL-17F in IL-23p19 KO mice are evident in both the spleen and the liver (Figs. 2 and 3). Furthermore, when exogenous IL-17A is administered to IL-23p19 KO animals during LM infection, both spleen and liver CFUs are reduced (Fig. 7). Interestingly, our data also indicate that the IL-23/IL-17 axis may serve a different role in the spleen than in the liver of LM-infected mice. A decrease in the percentage of neutrophils recruited to the liver is evident in IL-23p19 KO and IL-17RA KO mice compared with their wild-type counterparts. No such difference is demonstrated when comparing the percentages of neutrophils recruited to the spleens of IL-23p19 KO, IL-17RA KO, and B6 mice infected with LM. This indicates that IL-23 and IL-17A/F play a critical role in the spleen during infection with LM that is independent of mediating neutrophil recruitment and mobilization.

IL-17A and IL-17F are known to induce antimicrobial peptides in addition to their ability to influence the recruitment and mobilization of neutrophils to sites of infection (7, 36, 46). It is possible that a lack of IL-17A and IL-17F in the spleen during systemic infection with LM may reduce antimicrobial peptide production, thus affecting bacterial killing. This potential mechanism warrants further examination during infection with LM.

Previously, it has been demonstrated that the recruitment of neutrophils to the liver is more important for clearance of LM than the recruitment of neutrophils to the spleen (4). Viable LM can be recovered from the liver as early as 10 min after i.v. injection, and the presence of LM is associated with an increase in neutrophil recruitment (47). We demonstrate here that the percentage and total numbers of neutrophils in the liver at 0 and 3 h.p.i. in mice lacking either IL-23 or IL-17RA compared with wild-type B6 mice was not significantly decreased (Fig. 5). Presumably, the early presence of neutrophils in the livers of the IL-23p19 KO and IL-17RA KO mice contributes to the control of listerial growth resulting in no increases in bacterial burden at days 1 and 3 p.i. (our unpublished data). However, mice lacking either IL-23 or the IL-17RA are unable to maintain continued recruitment of neutrophils to the liver at days 1 and 3 p.i. with LM. Previous publications and our unpublished data have demonstrated that percentages of neutrophils in the blood and spleen peak at 1 day p.i. with LM (48, 49). Likewise, in the liver, peak recruitment of neutrophils occurs at day 1 p.i. in the B6 mice (Fig. 5). In contrast, the peak LM burden does not occur until days 3 and 4 p.i. (1, 50, 51). Given that neutrophil recruitment does not appear to directly overlap with the kinetics of bacterial burden, the reduced recruitment of neutrophils to the livers of IL-23p19 KO and IL-17RA KO mice at days 1 and 3 p.i. likely contributes to the increased bacterial burden not evident until day 5 p.i. Therefore, our data suggest that the IL-23- and IL-17RA-dependent continual recruitment of neutrophils to the liver is critical for resistance against LM infection.

A protective role for IL-17A and neutrophil recruitment during infection with LM has recently been established. When LFA-1− is absent during LM infection, increased systemic IL-17A and increased neutrophil recruitment to the liver enhances bacterial clearance (9). In addition, our recent study of coinfection with M. pulmonis and LM implicated IL-17A in the facilitated clearance of LM (10). These two reports provide evidence that IL-17-dependent neutrophilia can enhance listerial clearance. Additional evidence exists to support a protective role for IL-17A during LM infection. Recently, it has been demonstrated that IL-17A-deficient mice infected i.p. with the EGD strain of LM have increased bacterial burden in the liver at day 5 p.i. and decreased liver neutrophil localization during LM infection in the liver. B6 (A and B), IL-23p19 KO (C and D), and IL-17RA KO (E and F) mice were infected with −1 × 10^6 LM for 3 days. Mice were sacrificed and 7-μm liver sections were stained for Ly6G+ neutrophils (red) and LM (green, yellow when merged). Original magnification, ×200. B, D, and F depict a higher magnification of the boxed areas from A, C, and E, respectively. Data are representative of four mice per group.

**FIGURE 6.** The IL-23 and IL-17A/F axis does not regulate the precise localization of neutrophils during LM infection in the liver. B6 (A and B), IL-23p19 KO (C and D), and IL-17RA KO (E and F) mice were infected with −1 × 10^6 LM for 3 days. Mice were sacrificed and 7-μm liver sections were stained for Ly6G+ neutrophils (red) and LM (green, yellow when merged). Original magnification, ×200. B, D, and F depict a higher magnification of the boxed areas from A, C, and E, respectively. Data are representative of four mice per group.

**FIGURE 7.** Exogenous IL-17A provides protection to LM-infected IL-23p19 KO mice. B6 and IL-23p19 KO mice were infected with −1 × 10^6 LM. On 0, 1, 2, 3, and 4 days p.i., IL-23p19 KO mice received either PBS or 1 μg of IL-17A. B6 mice received PBS in all cases. On day 5 p.i., animals were sacrificed and CFUs were obtained from the spleen and liver. Two-way ANOVAs detected significant effects of mouse strain (p < 0.05). An asterisk (*) indicates a significant difference from IL-23p19 KO + PBS (p < 0.05). These data are representative of three independent experiments. All data are expressed as the mean ± SEM (n = 5/group).
numbers in response to HKLM (8). Collectively, these data suggest that IL-17A is required for optimal neutrophil recruitment and protection from LM.

A recent study did not find differences in survival between IL-17RA KO mice and B6 mice infected i.v. with the LM strain 43251 (American Type Culture Collection). Furthermore, there were no differences in CFUs between the IL-17RA KO animals at day 4 p.i. and the wild-type B6 animals in this model (52). It is important to note that ATCC LM strain 43251 is 10-fold less virulent than LM strain 10403s, which is the strain used in our study (53). In this study, we demonstrated an increased susceptibility of IL-17RA KO mice to infection with the highly virulent LM strain 10304s. Furthermore, we do not detect an increase in bacterial burden between the IL-17RA KO and B6 mice until day 5 p.i. Although our data clearly show that the IL-17RA is required for protection against virulent LM infection, less virulent strains of LM may be controlled in a fashion independent of IL-17RA.

The role of IL-23 and its downstream cytokines has been investigated in a variety of infectious models. However, the focus has been on mucosal infections. Furthermore, research has been limited to extracellular C. rodentium (21) and K. pneumoniae (20) and vacuole-bound bacteria M. tuberculosis (17) and S. enterica (23, 24). To our knowledge, this is the first report detailing a role for both IL-23 and IL-17A/F signaling during systemic infection with a cytoblastic bacteria. Although IL-23 can be protective in multiple infectious models, not all protective roles of IL-23 are found to be dependent upon IL-17A. Further evidence suggests that additional cytokines are dependent on IL-23. Mice rendered deficient in IL-23 have an increased susceptibility to C. rodentium that is not attributed to a lack of IL-17A but to the IL-10 family cytokine IL-22 (22). IL-22 has been found to induce the production of antimicrobial peptides in several infectious models (36, 54). To this point, a role for IL-22 during LM infection has not been defined, and no difference in bacterial clearance exists at 3 days p.i. in IL-22 KO mice compared with B6 mice (55). The investigation of IL-22 during LM infection at alternate time points could potentially yield other results because we have discovered that LM-infected IL-23p19 KO mice produce less IL-22 than their wild-type counterparts (our unpublished results). Taken altogether, the role of IL-22, antimicrobial peptides, and other cytokines that function downstream from the actions of IL-23 requires further examination during infection with LM and other Gram-positive, cytoblastic bacteria.

Our data support a critical role for IL-23 and IL-23-dependent IL-17A/F production during systemic infection with a cytoblastic bacteria. The previously unrecognized role for IL-23 and IL-17A/F likely complements the actions of IL-12 during infection with LM. Both the unique IL-12p35 subunit and the shared IL-12/IL-23, IL-23p40 subunit are required for optimal clearance during early LM infection (56, 57). However, there has not been a direct comparison of the susceptibility of IL-12p40-deficient, IL-12p35-deficient, and IL-23p19-deficient mice during LM infection. IL-12 is critical during early LM infection because it is necessary, along with IL-18, for the induction of innate IFN-γ secretion (34, 58). IFN-γ is required for resistance against LM (34, 59) and activates macrophages to be bactericidal (60, 61). Upon infection with LM, we propose that two separate immune pathways are activated: 1) IL-12 is secreted to induce the production of IFN-γ, thus activating macrophages to be bactericidal and 2) IL-23 is secreted to induce the production of IL-17, influencing the mobilization and recruitment of neutrophils to sites of infection. Both macrophages and neutrophils are required for resistance against LM (4, 62–64). Divergent roles for IL-12 and IL-23 have been demonstrated during infection with M. tuberculosis, K. pneumoniae, Francisella tularensis, and S. enterica (20, 25, 65, 66). IL-12 is necessary for the expression of IFN-γ while IL-23 is critical for the production of IL-17 during infection with K. pneumoniae and M. tuberculosis (20, 25). We suggest that the IL-23/IL-17 axis is necessary for the early recruitment of neutrophils to the liver and that this axis exists as a separate, yet complementary, branch of the immune system to the IL-12/IFN-γ axis during LM infection. Failure in either the IL-12 or IL-23 pathway can result in increased susceptibility to LM infection. Further evidence is needed to delineate the divergent roles of these cytokines during LM infection, which will ultimately aid in the rational design of effective therapies against intracellular bacterial infections.

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Disclosures

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