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The Interaction between IL-18 and IL-18 Receptor Limits the Magnitude of Protective Immunity and Enhances Pathogenic Responses following Infection with Intracellular Bacteria

Purnima Ghose,* Asim Q. Ali,* Rong Fang,† Digna Forbes,* Billy Ballard,* and Nahed Ismail*‡

The binding of IL-18 to IL-18Rα induces both proinflammatory and protective functions during infection, depending on the context in which it occurs. IL-18 is highly expressed in the liver of wild-type (WT) C57BL/6 mice following lethal infection with highly virulent Ixodes ovatus ehrlichia (IOE), an obligate intracellular bacterium that causes acute fatal toxic shock-like syndrome. In this study, we found that IOE infection of IL-18Rα−/− mice resulted in significantly less host cell apoptosis, decreased hepatic leukocyte recruitment, enhanced bacterial clearance, and prolonged survival compared with infected WT mice, suggesting a pathogenic role for IL-18/IL-18Rα in Ehrlichia-induced toxic shock. Although lack of IL-18R decreased the magnitude of IFN-γ producing type-1 immune response, enhanced resistance of IL-18Rα−/− mice against Ehrlichia correlated with increased proinflammatory cytokines at sites of infection, decreased systemic IL-10 production, increased frequency of protective NKT cells producing TNF-α and IFN-γ, and decreased frequency of pathogenic TNF-α-producing CD8+ T cells. Adoptive transfer of immune WT CD8+ T cells increased bacterial burden in IL-18Rα−/− mice following IOE infection. Furthermore, rIL-18 treatment of WT mice infected with mildly virulent Ehrlichia muris impaired bacterial clearance and enhanced liver injury. Finally, lack of IL-18R signal reduced dendritic cell maturation and their TNF-α production, suggesting that IL-18 might promote the adaptive pathogenic immune responses against Ehrlichia by influencing T cell priming functions of dendritic cells. Together, these results suggested that the presence or absence of IL-18R signals governs the pathogenic versus protective immunity in a model of Ehrlichia-induced immunopathology. *The Journal of Immunology, 2011, 187: 000–000.

Human monocytotropic ehrlichiosis (HME) is an emerging tick-borne disease caused by Ehrlichia chaffeensis (1, 2), a Gram-negative obligate intracellular bacterium that lacks LPS (3, 4). HME can manifest as an acute mild disease with nonspecific flu-like symptoms or as an acute severe multisystem disease that progresses to multiorgan failure and fatal toxic shock-like syndrome (1, 2, 5). Doxycycline treatment is frequently ineffective in preventing disease progression when administered late in the course of illness (6). Animal models of HME include wild-type (WT) C57BL/6 mice infected with Ehrlichia muris, which causes mild, self-limited disease (7, 8), or WT C57BL/6 mice infected with Ixodes ovatus ehrlichia (IOE), which causes acute fatal toxic shock-like syndrome or mild disease, depending on the dose and route of inoculation (7, 9–11). The pathologic changes in patients with fatal HME, as well as in murine models of fatal ehrlichiosis, suggested that the severity of HME is closely associated with immune-mediated pathology, as indicated by the severe tissue injury and multiorgan failure that occurs in the absence of large quantities of Ehrlichiae in the blood or tissues (1, 2, 5, 7, 12). Protective immunity against Ehrlichia is mediated by Ehrlichia-specific IFN-γ–producing CD4+ Th1 cells, and IFN-γ–producing NKT cells are critical for effective bacterial elimination (7, 11, 13–18). However, these cells undergo apoptosis at the end stages of fatal disease (7, 16, 19). In contrast, Ehrlichia–induced shock in mice is associated with systemic overproduction of pro- and anti-inflammatory cytokines and chemokines (e.g., TNF-α, IL-10, MCP-1, and MIP-1) (7, 13, 19). Furthermore, cytotoxic NK and TNF-α–producing CD8+ T cells play pathogenic roles during fatal murine ehrlichiosis because they directly mediate tissue injury and suppress anti-Ehrlichia protective immunity and, thus, impede effective bacterial clearance (7, 16, 19).

IL-18, formerly termed IFN-γ–inducing factor, is a member of the IL-1 superfamily and is initially synthesized as an inactive 24-kDa precursor protein (pro–IL-18) (20). Stimulation and secretion of IL-18 are mediated by a number of inflammatory mediators and cytolsolic proteins that regulate the cysteine protease caspase-1 within a multiprotein complex known as the inflammasome (21–23). Activation of caspase-1 (also called IL-1–converting enzyme) leads to the cleavage of pro–IL-18 into its mature and biologically active 18-kDa form. A wide range of cells (mainly activated blood and tissue monocytes/macrophages, Kupffer cells, B cells, dendritic cells [DCs], epithelial cells, and T cells) are capable of producing IL-18 upon stimulation (21–23). IL-18 binds to IL-18Rα, originally described as an IL-1R–related protein because of...
its homology to the IL-1/Toll receptor family. IL-18Rβ subunit, which is also a member of the IL-1R family, is responsible for signal transduction mediated by the IL-18R complex (23). The binding of IL-18 to the heterodimeric IL-18Rα/β complexes expressed on T lymphocytes, NK cells, macrophages, neutrophils, and endothelial cells induces downstream signals leading to the activation of NF-κB (20–23). IL-18 has pleiotropic functions, depending on the context of stimulation, cytokine milieu, and genetic predisposition. Some studies suggested that IL-18 is a Th1-promoting and proinflammatory cytokine that promotes the production of IFN-γ from T and NK cells, particularly in the presence of IL-12p70 (23–26) and, thus, plays a role in the protection against several infectious diseases caused by intracellular bacteria (24–27). Other studies demonstrated that IL-18 promotes Th2 and increases allergic sensitization (28, 29). IL-18 increases FAS (CD95) expression on host cells in a murine hepatitis model (30) and promotes the secretion of TNF-α, IL-1β, IL-8, and GM-CSF and, as a consequence, enhances expansion, migration, and activation of neutrophils during infections (31, 32). In addition, IL-18 was defined as an important cofactor for enhanced cytotoxic activity and proliferation of CD8+ T and NK cells (33–35).

Several studies showed that elevated serum levels of IL-18 are associated with poor clinical outcome in severe inflammatory and septic conditions (36–38). Neutralization of IL-18 via caspase-1 intervention or through the administration of IL-18–binding protein was postulated to be a promising therapeutic approach (38, 39). However, the factors that influence the functional outcomes of IL-18 expression remain poorly defined; thus, additional studies are required to evaluate its full potential in acute inflammatory and infectious diseases.

Our recent studies demonstrated a significant association between elevated IL-18 levels and development of fatal ehrlichiosis caused by i.p. infection with high doses of virulent IOE (7). In the current study, we examined the contribution of IL-18 to anti-Ehrlichia protective immunity and the pathogenesis of Ehrlichia-induced tissue injury and toxic shock. In this article, we show that IL-18/IL-18Ro interaction negatively regulates protective immunity and promotes immunopathology during lethal ehrlichial infection. Furthermore, this study established a critical role for IL-18/IL-18R in the maturation of DCs, cytokine production by NKT and NK cells, and induction of pathogenic TNF-α- and IL-18-producing CD8+ T cells following infection with obligate intracellular bacteria that cause toxic shock.

Materials and Methods

Mice and Ehrlichia infection

Female WT C57BL/6 mice and IL-18Rα−/− mice (strain B6.129P2-Il18r1tm1Aki), 8–12 wk of age, were obtained from The Jackson Laboratories (Bar Harbor, ME). All animals were housed under specific pathogen-free conditions at the Animal Research Center at Meharry Medical College in accordance with the institutional guidelines for animal welfare. Two species of monocytotropic Ehrlichia were used in this study: the highly virulent IOE and the mildly virulent E. muris. Both strains were provided by Dr. Y. Rikihisa (Ohio State University, Columbus, OH). IOE and E. muris stock were propagated by passage through WT C57BL/6 mice.

Single-cell suspensions from the spleens of day 7 infected mice were stored in liquid nitrogen and used as stocks. Mice were infected i.p. with 1 × 10^7 bacteria/mouse E. muris inoculum (nonlethal infection) or 5 × 10^7 bacteria/mouse IOE (lethal infection). Mice were monitored daily for signs of illness and survival. On the indicated days postinfection (p.i.), three to six mice/group were sacrificed, and selected organs were harvested for further analysis.

Preparation of host cell-free Ehrlichia

Host cell-free IOE Ags were prepared from IOE-infected spleens and livers harvested on day 7 p.i., as previously described (7, 11, 40). Spleen and liver of naive mice were prepared and used as a negative control in all experiments using cell-free IOE Ags (mock Ag). The E. muris Ag was prepared from an E. muris-infected DH82 canine macrophage cell line. Ehrlichiae were harvested when ~90–100% of the cells were infected, and cell-free ehrlichiae were prepared as previously described (3, 4, 7, 11, 13). Cell lysates from uninfected DH82 cells were similarly prepared and used as a negative control (mock Ag) for all experiments using E. muris Ag. The total protein concentration of the resulting bacterial preparations was determined using a bicinchoninic acid protein assay kit (Pierce, Rockford, IL) and was used as the Ag in flow cytometry and the in vitro culture of splenocytes or peritoneal exudate cells (PECs).

Isolation of PECs and preparation of culture supernatant from PECs following in vitro Ag stimulation

The peritoneal cavities of infected mice were washed with 10 ml sterile PBS. The peritoneal wash/avage was centrifuged at 275 × g for 5 min to separate the cell fraction (PECs). As negative controls, PECs were prepared from uninfected mice injected with 1.5 ml 3% thioglycolate medium (Difco Laboratories), and resident PECs were harvested 4 h after the injection. PECs from IOE-infected WT and IL-18Rα−/− mice and uninfected controls were directly processed for flow cytometry and intracellular cytokine staining, as described below. In addition, PECs from infected WT and IL-18Rα−/− mice and uninfected cultures were cultured at a concentration of 5 × 10^5 cells/well with host cell-free IOE (5 × 10^7 bacteria/ cell) in a 24-well plate. The culture supernatants were harvested at 24 h and assayed through a membrane filter (pore size: 0.22 μm), and the levels of several cytokines in the peritoneal culture supernatant were measured by ELISA.

In vitro stimulation of splenocytes

Spleens were excised on the indicated days p.i. from groups of three or four Ehrlichia (E. muris or IOE)-inoculated C57BL/6 and IL-18Rα−/− mice. Splenocytes were isolated as previously described (7, 11). Briefly, spleens were homogenized, and RBCs were lysed with 0.84% ammonium chloride treatment. A total of 5 × 10^7 cells was resuspended in 2 ml DMEM supplemented with 10% FBS and 100 μg/ml penicillin and streptomycin (HyClone) and plated into 12-well tissue-culture plates. Splenocytes were cultured with or without cell-free E. muris or IOE Ags. After 48 h, the supernatants were collected, and cytokine levels were analyzed by ELISA, as described below.

Cytokine ELISA

The concentrations of IFN-γ, IL-4, TNF-α, IL-6, IL-10, and IL-12 in the sera or PEC culture supernatant were measured using Quantikine ELISAs (R&D Systems, Minneapolis, MN). The minimum detection limit of the mouse cytokines was as follows: IFN-γ (2 pg/ml), IL-4 (2 pg/ml), TNF-α (5.1 pg/ml), IL-6 (1.6 pg/ml), IL-10 (4.0 pg/ml), IL-12 p40 (4 pg/ml), and IL-18 (25.0 pg/ml).

Measurements of bacterial burden by real-time PCR

Ehrlichial stock and bacterial burden in different organs were measured using an iCyter IQ Multicolor real-time detection system (Bio-Rad, Hercules, CA), as previously described (7, 11). Ehrlichial burdens were determined using two primer sets (Table I). The first set included primers targeting both E. muris and IOE dsb (a thio-disulfide oxidoreductase) gene and eukaryotic housekeeping gene GAPDH, and real-time PCR was performed using specific probes, as described previously (11). The second set included primers targeting a different sequence of the dsb gene of Ehrlichia and the same housekeeping GAPDH gene, and real-time PCR was performed using the SYBR Green IQ Supermix (Bio-Rad), as described previously (41, 42). The results were normalized to the levels of expression of the GAPDH gene in the same sample and expressed as copy number per 10^9 GAPDH (7, 11). PCR analyses were considered negative for ehrlichial DNA if the critical threshold values exceeded 40 cycles.

Neutralization of IL-18Ra

C57BL/6 WT mice were injected i.p. with 100 μg/mouse anti–IL-18Ra mAb (clone 112624; R&D Systems) on days 0, 1, 3, and 5 following IOE infection. Control mice were injected with 100 μg/mouse rat IgG2a isotype control mAb at the indicated time points. Anti–IL-18Rα–treated and isotype control mice were infected with a high lethal dose of IOE (5 × 10^7 bacilli/mouse). Of note, JOE-infected mice treated with the isotype control Ab had higher bacterial burdens than did IOE-infected untreated WT mice in some experiments, especially when measured by the first dsb primer set.
Flow cytometry and intracellular cytokine staining

Splenocytes were harvested, counted, and resuspended in fluorescence-activated cell sorter-staining buffer (Dulbecco’s PBS without Mg2+ or Ca2+ containing 1% heat-inactivated FCS and 0.09% sodium azide (pH 7.4–7.6)) at a concentration of 10^6 cells/well. FeRs were blocked with a mAb (clone 2.4G2) against mouse cell surface Ags CD16 and CD32 for 15 min. The following FITC-, PE-, PerCP-Cy5.5-, Alexa Fluor-, and allophycocyanin-conjugated Abs were purchased from BD Biosciences: anti-CD4 (clone 69), anti-CD3 (clone 145-2C11), anti-CD11c (clone HL3), anti-CD4 (clone H-450), anti-CD8a (clone 53-6.7), anti-CD11b (clone M1/70), anti-CD4 (clone RM4–4), anti-CD8 (clone YTS169), anti-CD19 (clone 1D3), anti–TNF-α (clone MP6-XT2), anti–IFN-γ (clone XMG102), anti–CD95 (clone Jo2), anti–Ly6G (clone 1A8), anti–IFN-γ (M5/114.15.2), anti–H2Dd (KH95), anti–CD40 (3/23), anti–CD80 (16–10A1), and anti–CD86 (GL1). Isotype control mAbs, including FITC-, PE-, or allophycocyanin-conjugated hamster IgG1 (A9-13), rat IgG1 (R3-34), rat IgG2a (R35-95), mouse IgG2a (X39), mouse IgG2b (MPC-11), mouse IgG1 (X40), and rat IgG2b (A95–1) were purchased from BioLegend (San Diego, CA). For intracellular cytokine staining, splenocytes were incubated at 37˚C for 4 h in complete medium with the addition of BD Golgi Plug (BD Biosciences, CA), according to the manufacturer’s recommendations. Lymphocyte and granulocyte populations were gated based on forward and side-scatter parameters; 50,000–200,000 events were collected using BD-LSR or BD FACScalibur (BD Immunocytometry Systems, San Jose, CA) flow cytometer, and data were analyzed using FlowJo software (TreeStar, Ashland, OR).

Generation of bone marrow-derived DCs and in vitro Ehrlichia stimulation

Immature CD11b^+CD11c^+ cells were isolated from the bone marrow of naive WT C57BL/6 or IL-18R^−/− mice. Bone marrow-derived DCs (BMDCs) were generated as originally described (43–45), with slight modifications. Briefly, a single-cell suspension from bone marrow was prepared from mouse femurs and adjusted to 2 × 10^6 cells/10 ml complete IMDM containing 10% FBS, 1 mM sodium pyruvate, 50 μm L-arginine, 100 μg/ml streptomycin sulfate, and 100 U/ml penicillin. DC culture medium was supplemented with 20 ng/ml rGM-CSF (BD Biosciences, CA). On day 3, 6 ml fresh GM-CSF–containing medium was added. On day 6, 10 ml the culture medium was replaced with fresh GM-CSF–containing medium. On day 8, the cultures were examined by FACS analysis and were used for experiments if they contained 70–75% CD11c^+ cells. Cell-free IOE organisms were added to each well at a multiplicity of infection of 5.1. The cells were harvested 24 h following infection, and the expression of various mature markers (MHC-I, MHC-II, and costimulatory molecules, including CD80, CD86, and CD40) were examined by flow cytometry. In addition, the levels of TNF-α and IL-6 in BMDC culture supernatant were determined by ELISA, as described above.

Histopathology and TUNEL assays

Liver segments were fixed in 10% neutral buffered formalin, dehydrated in graded alcohols, and embedded in paraffin wax. Sections (3 μm thick) were collected on coated slides and stained with H&E. Liver lesions were assessed semiquantitatively using two main parameters that were shown to be associated with Ehrlichia-induced immunopathology and toxic shock: the number of apoptotic and/or necrotic cells and the number of inflammatory foci. TUNEL staining was performed on liver sections to quantify the number of apoptotic cells, as described previously (7, 11, 16).

Adoptive cell transfer

For adoptive transfer of cells, splenocytes were collected from IOE-infected WT mice on day 7 p.i. (5 × 10^5 IOE organisms/mouse) or from naive mice. Splenocytes were enriched for the CD8^+ population using mouse CD8 microbeads (MACS; Miltenyi Biotec, Auburn, CA) before injection, resulting in a cell population of 75–85% CD8^+, as determined by cell surface staining and flow cytometry. A total of 10^6 purified CD8^+ cells in 200 μl was injected i.v. into groups of six C57BL/6 or IL-18R^−/− mice (in two separate experiments) through retro-orbital route 2 h before inoculation with a high dose of IOE (5 × 10^5 organisms/mouse).

rIL-18 therapy

C57BL/6 mice were infected i.p. with a high dose of E. muris (i.e., nonlethal infection) and were treated with rIL-18 (MBI International, Woburn, MA). To mimic the in vivo increase in IL-18 production at late stages of lethal IOE infection, multiple IL-18 injections (1 μg/mouse) were administered to E. muris-infected mice on days 3–6 p.i. These time points correspond to time points at which a significant elevation in IL-18 in the sera and livers of lethally/IOE-infected WT mice was observed. The sham controls consisted of E. muris-infected mice injected with PBS only or uninfected mice injected with rIL-18 only at the same time points.

Statistical analyses

The two-tailed t test was used for comparison of mean values for two experimental groups, and p values were calculated using GraphPad Prism (GraphPad Software, San Diego, CA). One-way ANOVA was used for comparisons of multiple experimental groups. Post hoc group pairwise comparisons were conducted using the Bonferroni procedure, and an overall α level of significance of 0.05. The p values < 0.001 were considered highly significant (**), p values < 0.01 were considered moderately significant (**), and p values < 0.05 were considered significant (*).
infected mice on day 5 p.i. were lower than those detected in the spleens of nonlethally infected mice. However, IL-12 increased in the spleens of lethally infected mice on day 7 p.i. (Fig. 1F). Interestingly, regardless of the levels of IL-12 and IL-18 in the sera or spleens, IFN-γ levels in the spleens of lethally infected mice were significantly lower on days 5 and 7 p.i. compared with nonlethally infected mice (Fig. 1G).

We next examined whether altered systemic and local production of Th1 cytokines during lethal *Ehrlichia* infection is due to changes in kinetics of IL-10 production. Both lethal and nonlethal infections increased serum levels of IL-10 on day 1 p.i. compared with uninfected controls, with a significantly higher level in nonlethally infected mice. IL-10 dramatically declined in the sera of nonlethally infected mice on days 2, 5, and 7 p.i. (Fig. 1D). In contrast, the transient decline in serum levels of IL-10 in lethally infected mice on days 2 and 5 p.i. was followed by a substantial increase on day 7 p.i. (Fig. 1D). IL-10 was also significantly higher in the spleens of lethally infected mice than in nonlethally infected mice on days 5 and 7 p.i. (Fig. 1H). Decreased IFN-γ in the spleens and increased systemic and local IL-10 production in lethally/IOE-infected mice was associated with higher bacterial burdens in all organs at late stages of infection compared with nonlethally *E. muris*-infected mice (Fig. 2). Collectively, these data suggested that IOE infection induced a late burst in the levels of circulating proinflammatory/Th1 and anti-inflammatory cytokines that coincided with lethality and defective bacterial clearance.

**Absence of IL-18R enhances bacterial elimination and prolongs survival following infection with a lethal dose of IOE**

To examine the role of IL-18 in the control of ehrlichial infection, C57BL/6 WT and IL-18R−/− mice were infected i.p. with a lethal dose of IOE, and the bacterial burden in different organs at early and late stages of infection was determined by real-time PCR using two sets of primers (Table I). Unexpectedly, we found that IL-18R−/− mice had significantly lower bacterial burdens in the liver, lung, and kidney compared with WT mice on days 3 and 7 p.i. (Fig. 3A, 3C). Furthermore, although IOE-infected WT mice succumbed to lethal infection on days 8–10 p.i., IOE-infected IL-18R−/− mice survived longer; 100% of mice succumbed to infection on day 16 p.i. (Fig. 3E). To ensure that enhanced resistance to IOE infection in IL-18R−/− mice was not due to changes in the immune system or compensatory mechanisms of these knockout (KO) animals, WT infected mice were treated with anti–IL-18R mAb. Similar to IL-18R−/− mice, WT mice treated with anti–IL-18R mAb contained lower bacterial burdens in their lungs, livers, and kidneys on days 3 and 7 p.i. (Fig. 3B, 3D) compared with isotype-sham controls. Using the two primer sets, we consistently detected a significant difference in the bacterial burden between IL-18R−/− and anti–IL-18R−/−–treated mice compared with infected WT and isotype control mice. However, the ehrlichial copy number in organs in all groups of mice was higher when measured by the first primer set than when measured by the second set of primers (data not shown). Taken together, these results demonstrated that IL-18/IL-18R interaction contributes to ineffective bacterial elimination and mortality following acute lethal ehrlichial infection.
**FIGURE 3.** Enhanced resistance to IOE infection in IL-18Rα−/− and anti-IL-18Rα mAb-treated mice compared with infected WT or sham control mice. C57BL/6 WT and IL-18Rα−/− mice (A, C) or anti-IL-18Rα Ab-treated mice and isotype controls (B, D) were infected i.p. with a high dose of IOE (i.e., lethal infection). On days 3 (A, B) and 7 (C, D) p.i., tissues from lung, liver, and kidney were collected, and bacterial burden was determined by real-time PCR using the second primer set. The copy number of IOE was normalized to the housekeeping gene GAPDH. Bacterial burden in all organs were lower in IOE-infected IL-18Rα−/− and anti-IL-18Rα mAb-treated mice compared with WT and isotype controls on days 3 and 7 p.i. Data represent the mean ± SEM of three mice/group and are representative of three independent experiments with similar results. E, Survival of WT and IL-18Rα−/− mice after i.p. infection with a high dose of IOE. Data shown represent one of three independent experiments with a total of 12 mice/group. **p < 0.01, ***p < 0.001.

**IL-18Rα is required for induction of Ehrlichia-induced liver inflammation and tissue injury**

One of the main pathologic features of HME in humans and mice is the development of severe tissue injury, followed by multigorgan failure and toxic shock (7, 11–13). Hence, we examined the impact of the IL-18/IL-18Rα interaction on acute liver damage and inflammation following lethal ehrlichial infection on days 3 and 7 p.i. Our data showed that the differences in hepatic pathology between IOE-infected C57BL/6 WT mice and IL-18Rα−/− mice were minimal on day 3 p.i. (data not shown). However, on day 7 p.i., at the peak of the disease, the livers of IOE-infected WT mice showed an increased influx of inflammatory cells, including lymphocytes and macrophages, which was associated with marked apoptosis of hepatic cells (Fig. 4C, 4D) compared with uninfected mice (Fig. 4A, 4B). In contrast, IOE-infected IL-18Rα−/− mice developed an attenuated pathology, with significantly less cellular infiltration and fewer apoptotic cells (Fig. 4E, 4F) in the livers compared with infected WT controls. Quantitative data on the number of cellular foci determined by H&E staining and the number of apoptotic cells among different groups of mice determined by TUNEL assay are presented in Fig. 4G and 4H, respectively. These results suggested that the absence of IL-18Rα signals confers protection against Ehrlichia-induced immune-mediated pathology. Consistent with the results from IL-18Rα−/− mice, IOE-infected WT mice treated with anti-IL-18Rα mAbs exhibited decreased cellular recruitment to the liver and fewer disruptions in liver architecture on day 7 p.i. compared with IOE-infected sham control mice (data not shown). Collectively, these data indicated that IL-18/IL-18Rα interaction contributes to the pathogenesis of lethal Ehrlichia-induced tissue injury and inflammation during fatal disease.

**Lack of IL-18Rα influences innate immune responses against Ehrlichia**

During the initial phase, the antiehrlichial immune response is mediated by elements of the innate immune system and involves a complex interaction between cytokines, such as TNF-α, and IFN-γ, and innate immune cells (7, 13, 16, 46, 47). We hypothesized that enhanced bacterial elimination in IL-18Rα−/− mice is due to enhanced protective immunity at the sites of infection. To test this hypothesis, we examined effects of IL-18 signals on the expansion and function of phagocytic cells at the primary site of infection, the peritoneum. Our data indicated that the absence of IL-18R signals in IOE-infected KO mice on day 3 p.i. resulted in a decrease in the percentage (Fig. 5B) and absolute number (Fig. 5E) of TNF-α–producing CD11c+ DCS, whereas it did not influence the percentage or absolute number of TNF-α–producing CD11c− CD11b+ cells (consistent with the phenotype of macrophages) (Fig.
Furthermore, the absence of IL-18R signals decreased the percentage and absolute number of Ly6G+ cells expressing CD11b activation marker, which is consistent with the phenotype of activated neutrophils (48, 49), compared with infected WT mice (Fig. 5D, E). Interestingly, resistance of WT mice to nonlethal infection with *E. muris* was associated with reduced expansion of activated neutrophils (Ly6G+CD11b+F4/80−) in the spleen on day 3 p.i. compared with lethal IOE infection (Fig. 5F). These data suggested that the lack of IL-18/IL-18R interactions can shift the immune response against *Ehrlichia* from a pathogenic to a protective phenotype.

**FIGURE 5.** The lack of IL-18/IL-18R interaction influences the frequency and function of innate immune cells in the peritoneum. Peritoneal exudates were collected from naive and IOE-infected IL-18Rα−/− and WT mice on day 3 p.i. A. Cells were gated based on forward and side scatter, and the frequencies of macrophages, DCs, and granulocytes were analyzed by flow cytometry. Flow cytometric analysis of peritoneal cells from uninfected, infected WT, and infected IL-18Rα−/− mice stained for CD11C and TNF-α (B), CD11b+ CD11c− and TNF-α (C), and Ly6G+ CD11b+ (D). CD11b expression was measured to quantify the degree of neutrophil activation in the peritoneal fluid in response to *Ehrlichia*, as previously described. E. Absolute number of different cell subsets in the peritoneal fluid of naive, IOE-infected WT, and IOE-infected IL-18Rα−/− mice on day 3 p.i. F. Spleen cells from naive and lethally (IOE) or nonlethally (*E. muris*) infected mice were harvested on day 3 p.i. and gated on granulocytes based on side and forward scatter. Dot plot showing gated granulocytes stained for F4/80 and Ly6G as markers of macrophages and neutrophils, respectively. Graphs (bottom row) show the percentage of activated neutrophils, as marked by the expression of CD11b, an activation marker, on gated Ly6G+ F4/80− neutrophils. Data shown are from a representative mouse from each group (n = 4), and the numbers indicate the percentage of cells within each quadrant. Data shown are from one experiment that is representative of three different experiments. **p < 0.01.
and that activated neutrophils may play a role in the pathogenesis of *Ehrlichia*-induced fatal shock.

We subsequently analyzed the systemic and local cytokine responses on day 7 p.i. in serum and culture supernatant from PECs and spleens of infected mice following in vitro Ag stimulation. Our data showed that a lack of IL-18Rα resulted in a significant increase in levels of IL-6, TNF-α, and IFN-γ in the culture supernatant of PECs from IOE-infected IL-18Rα−/− mice compared with WT mice (Fig. 6A–C). Although the serum levels of IL-6 and TNF-α were not significantly different between infected WT and IL-18Rα−/− mice (Fig. 6E, 6F), the serum level of IFN-γ was lower in IL-18Rα−/− mice compared with WT mice (Fig. 6G). The data showed that a lack of IL-18Rα resulted in a decrease in the absolute numbers of total CD3+ T cells, CD4+ T cells, CD8+ T cells, and CD8+CD4+ T cells compared with infected WT mice (7.85 ± 2.6% compared with 13.45 ± 2.2%, Fig. 7B), indicating that IL-18R signal is critical for activation of CD4+ T cells. However, the expression of apoptotic markers (CD95/FAS) on CD4+ T cells was also lower in IOE-infected IL-18Rα−/− mice compared with infected WT mice (Fig. 7B), suggesting that the decline in the number of effector CD4+ T cells is unlikely to be caused by apoptotic cell death. Surprisingly, attenuated pathology and enhanced resistance in IOE-infected IL-18Rα−/− mice correlated with a levels of IL-10 in the peritoneum of IL-18Rα−/− mice (Fig. 6D); however, it significantly decreased its serum levels compared with infected WT mice (Fig. 6H). Taken together, our data suggested that IL-18/IL-18R interaction is critical for systemic production of IL-10, as well as suppression of proinflammatory and Th1 cytokines at the peripheral site of IOE infection.

**Enhanced resistance to infection in IL-18Rα−/− mice is not linked to elevated type-1 immune responses in the spleen**

We next examined whether attenuated pathology and enhanced bacterial elimination in IL-18Rα−/− mice on day 7 p.i. was due to an enhanced protective Th1 response in the spleen. At the single-cell level, the lack of IL-18Rα in IOE-infected IL-18Rα−/− mice resulted in decreased absolute numbers of CD3+ , CD8+ , and CD4+ T cells on day 7 p.i. (Fig. 7A). In particular, IOE-infected IL-18Rα−/− mice have a lower percentage of CD4+CD25+ effector T cells compared with IOE-infected WT mice (7.85 ± 2.6% compared with 13.45 ± 2.2%, Fig. 7B), indicating that IL-18R signal is critical for activation of CD4+ T cells. However, the expression of apoptotic markers (CD95/FAS) on CD4+ T cells was also lower in IOE-infected IL-18Rα−/− mice compared with infected WT mice (Fig. 7B), suggesting that the decline in the number of effector CD4+ T cells is unlikely to be caused by apoptotic cell death. Surprisingly, attenuated pathology and enhanced resistance in IOE-infected IL-18Rα−/− mice correlated with a

**FIGURE 6.** Altered pro- and anti-inflammatory cytokines in the sera and peritoneum of IL-18Rα−/− mice compared with infected WT or sham control mice. PECs harvested from day-7 IOE-infected WT and IL-18Rα−/− mice were cultured in vitro in the presence of IOE Ags for 48 h. Peritoneal cell culture supernatant (A–D) and sera (E–H) were collected on day 7 p.i. from all infected mice and tested for IL-6, TNF-α, IFN-γ, and IL-10 using ELISA. Data are mean ± SEM for three mice in each group. Data shown are from one experiment and are representative of three different experiments. **p < 0.05, ***p < 0.001.

**FIGURE 7.** IL-18/IL-18Rα interaction is essential for the expansion and activation of type-1 cells during *Ehrlichia* infection. Splenocytes harvested from naive and IOE-infected IL-18Rα−/− and WT mice on day 7 p.i. were stimulated with IOE Ags. Lymphocyte population was gated based on forward and side scatter and analyzed by flow cytometry. A, IOE infection in IL-18Rα−/− mice resulted in a decrease in the absolute numbers of total CD3+ T cells, CD4+CD3+ cells (CD4+ T cells), and CD8+CD3+ cells (CD8+ T cells) compared with infected WT mice. B, Dot plots showing surface expression of activation marker CD25 and apoptotic marker CD95/FAS on CD4+ lymphocytes, as well as intracellular IFN-γ production by CD3+ T cells (type-1 T cells). C, Serum cytokine levels of IL-18 and IL-12 in naive and IOE-infected WT and IL-18Rα−/− mice on days 3 and 7 p.i. Data shown are from a representative mouse from each group (n = 4), and the numbers in B indicate the percentage of cells within each quadrant. Data shown are representative of three different experiments. **p < 0.05, ***p < 0.001.
decreased percentage (Fig. 7B) and absolute number (data not shown) of Ehrlichia-specific IFN-γ–producing CD3+ type-1 cells in the spleen. Decreased type-1 responses in IOE-infected IL-18Ra−/− mice did not shift the responses toward a Th2 response, because the percentage of IL-4–producing Trop-2 cells was minimal and not significantly different from that in naive or infected WT mice (data not shown).

Because IL-12 and IL-18 are produced by the same APCs and can act cooperatively in the induction and expansion of Th1 responses (20–24), we examined whether the decreased type-1 immune response in IL-18Ra−/− mice was due to reduced production of Th1-promoting cytokines, mainly IL-12. Our data showed that on day 3 p.i., there was no significant difference in the levels of IL-12 and IL-18 between the two groups of mice (Fig. 7C). In contrast, on day 7 p.i., we detected an increase in the serum level of IL-18 in IOE-infected IL18Ra−/− mice compared with WT mice, whereas IL-12 levels were comparable between the two groups of infected mice (Fig. 7C). Thus, decreased frequency of type-1 cells in IL-18Ra−/− mice is less due to decreased production of Th1-promoting cytokines.

Enhanced resistance to infection in IL-18Ra−/− mice is linked to altered NK and NKT cell responses

Our previous study indicated that IFN-γ– and TNF-α–producing NKT cells promote effective bacterial elimination, whereas NK cells producing IFN-γ inhibit protective anti-Ehrlichia immunity and mediate pathogenic responses and tissue injury during fatal disease (16–18). To further address the effect of IL-18R on NK and NKT responses, we measured the frequency of and cytokine production by these cells in the spleens of WT and IL-18Ra−/− mice during lethal IOE infection. On day 3 p.i., lethal IOE infection in IL-18Ra−/− mice resulted in a significant increase in the percentage of NKT cells compared with IOE-infected WT mice (Fig. 8A). A similar increase in the percentage (Fig. 8B) and absolute number (Fig. 8F) of NKT cells in IL-18Ra−/− mice was observed on day 7 p.i. compared with WT mice. Within the NKT cell population, we detected a significantly greater percentage and absolute number of NKT cells producing TNF-α only and NKT cells producing TNF-α and IFN-γ (Fig. 8C, 8F), as well as the absolute number of NKT cells producing IFN-γ only (Fig. 5F), in infected IL-18Ra−/− mice compared with infected WT mice. In contrast, the lack of IL-18R signals did not significantly influence the percentage and absolute number of NK cells in the spleen of IL-18Ra−/− mice on day 3 (Fig. 8A) or day 7 p.i. (Fig. 8B, 8E). Within the NK population, IL-18Ra−/− mice have a lower percentage and absolute number of IFN-γ–producing, but not TNF-α–producing, NK cells than do IOE-infected WT mice (Fig. 8D, 8E). These data suggested that IL-18/IL-18R interaction contributes to a decreasing frequency of protective NKT cells producing both TNF-α/IFN-γ, while enhancing the frequency of IFN-γ–producing pathogenic NK cells during fatal ehrlichial infection.

IL-18Ra is required for induction and expansion of pathogenic TNF-α–producing CD8+ T cells during fatal ehrlichial infection

Our previous studies showed that Ehrlichia-induced toxic shock and tissue injury are directly mediated by cytotoxic pathogenic CD8+ T cells producing TNF-α (7, 13, 19). We assessed whether IL-18Ra acts as a limiting factor for the activation and expansion of pathogenic Ehrlichia-specific CD8+ T cells during the peak stage of the disease. Compared with infected WT mice, the absence of IL-18Ra in KO mice resulted in the decreased percentage (Fig. 9A) and absolute numbers (Fig. 7A) of CD8+ T cells in the
spleen, with a significant decrease in the percentage and absolute number of TNF-α–producing CD8+ T cells on day 7 p.i. (Fig. 9B, 9C).

Adoptive transfer of CD8+ T cells from immune WT mice increases IOE loads in IL-18Rα−/− mice

Based on the above data, we hypothesized that enhanced resistance against IOE in 18Rα−/− mice is due to reduction in the number of pathogenic CD8+ T cells and that adoptive transfer of immune CD8+ cells from WT mice may reverse the observed resistance and protective immunity against IOE in IL-18Rα−/− infected mice. To test this hypothesis, we purified CD8+ T cells from spleens of IOE-infected WT mice on day 7 p.i., transferred these cells or naive WT CD8+ cells to WT or 18Rα−/− mice, and challenged these mice i.p. with IOE. Bacterial burdens were determined in different organs 7 days later. IL-18Rα−/− mice treated with naive WT CD8+ splenocytes harbored significantly less IOE in their organs compared with similarly treated IOE-infected WT mice (compare Fig. 10B and 10A). WT mice treated with naive or immune CD8+ cells had a similar burden of IOE in their organs, suggesting that additional immune CD8+ cells are not sufficient to further inhibit protective immunity and lead to increased bacterial burden (Fig. 10A). In contrast, bacterial burdens in different organs of 18Rα−/− mice that received immune WT CD8+ splenocytes were significantly increased compared with 18Rα−/− mice given naive CD8+ T splenocytes (Fig. 10B). Furthermore, bacterial burden in immune CD8+ cell-treated IL-18Rα−/− mice

![Figure 9](http://www.jimmunol.org/)

**FIGURE 9.** IL-18/IL18Rα interaction is essential for the induction of pathogenic TNF-α–producing CD8+ T cells during severe *Ehrlichia* infection. Spleen cells were harvested from IOE-infected IL-18Rα−/− and WT mice on day 7 p.i. and stimulated in vitro with IOE Ags, and the frequency of IOE-specific CD8+ T cells, as well as TNF-α–producing CD8+ T cells, was analyzed by flow cytometry. A. Contour plot shows the percentage of CD3+CD8+ T cells in naive and IOE-infected WT and IL-18Rα−/− mice. B, CD8+ T cells were gated and analyzed for intracellular TNF-α production, naive mice have <1% of TNF-α–producing CD8+ T cells, and infected WT mice have a higher percentage of TNF-α–producing CD8+ T cells than do IOE-infected IL-18Rα−/− mice. C, The absolute number of TNF-α–producing CD8+ T cells in the three groups of mice. Data shown are from a representative mouse from each group (n = 3). Data shown are from one experiment that is representative of three different experiments. ***p < 0.001.

was not statistically significantly different from that in WT mice receiving naive or immune CD8+ cells (compare Fig. 10B and 10A), suggesting that the protective responses of IL-18Rα−/− mice in the control of IOE infection can be inhibited or abrogated by pathogenic immune WT CD8+ T cells.

![Figure 10](http://www.jimmunol.org/)

**FIGURE 10.** Adoptive transfer of splenocytes from immune WT mice increases IOE numbers in IL-18Rα−/− mice. A total of 10⁶ naive splenocytes and immune splenocytes was harvested from WT mice on day 7 post-high-dose IOE infection, enriched for CD8+ population, and injected i.v. into groups of three to seven WT mice (A) or IL-18Rα−/− mice (B) 2 h prior to IOE challenge. Bacterial loads in lungs, livers, and spleens on day 7 p.i. are expressed as copy number/10⁶ GAPDH. Data shown are from one experiment that is representative of two different experiments with similar results. **p < 0.01, ***p < 0.001.

**rIL-18 inhibits bacterial elimination and exacerbates pathology**

Based on the above data, we hypothesized that the late burst of IL-18 following lethal ehrlichial infection promotes immunopathology and negatively regulates protective immunity. To further test this hypothesis, we examined the effects of administering a high concentration of IL-18 on the host defense following nonlethal *E. muris* infection. WT mice were infected with a high dose of *E. muris* and subsequently treated with rIL-18. Compared with IL-18–treated, but uninfected, control mice (Fig. 11A) or untreated *E. muris*-infected mice (Fig. 11B), rIL-18 therapy of *E. muris*–infected mice (Fig. 11C) resulted in an enhanced inflammatory cellular infiltration in the liver, increased numbers of apoptotic cells, and the development of marked fatty changes in the liver (Fig. 11D). In addition, rIL-18 therapy of *E. muris*-infected mice resulted in a significantly increased ehrlichial burden in different organs on days 7 p.i. (Fig. 11E) compared with untreated *E. muris*-infected mice. These results supported the notion that *Ehrlichia*-induced inflammation and tissue injury are maintained in the liver under conditions of IL-18 overproduction, which negatively influences protective antiehrlichial immunity.

**Decreased frequency of CD4+ and CD8+ T cells in IL-18Rα−/− mice is associated with decreased DC maturation**

To determine whether the decreased numbers of TNF-α–producing CD8+ T cells in KO mice are due to activation-induced cell death (AICD), we examined the expression of apoptotic receptor (FAS) on CD8+ T cells. Similar to expression levels of FAS/CD95 on CD4+ T cells, lack of IL-18R signals in IOE-infected KO mice...
increased tissue injury, and inhibit protective anti-ehrlichial immunity following nonlethal ehrlichial infection. C57BL/6 mice were infected with mildly virulent E. muris, and rIL-18 was administered at different time points p.i. On day 7 p.i., mice were sacrificed, and liver sections from rIL-18–treated mice were stained with H&E. Enhanced inflammatory infiltration (arrow) and apoptotic cell death (arrowheads) in rIL-18–treated E. muris–infected mice were associated with fatty changes (arrowheads) compared with naive or untreated, but E. muris–infected, mice. E. Bacterial burden in the lungs, liver, kidney, and spleen, as determined by real-time PCR, was higher in treated E. muris–infected mice compared with untreated controls on day 7 p.i. Data are shown as the means ± SD of three mice/group. Data are representative of three independent experiments with similar results. **p < 0.01.

decreased the expression of FAS on CD8+ T cells compared with infected WT mice (data not shown), suggesting that FAS-mediated AICD is unlikely to be responsible for decreased CD8+ T cells in KO mice.

Next, we examined whether the decreased absolute number of both CD4+ and CD8+ T cells is due to defective priming by APCs, mainly DCs. To this end, we analyzed the maturation and proinflammatory cytokine production of DCs from WT and IL-18R−/− mice following in vivo and in vitro stimulation with IOE. Compared with IOE-infected WT mice, IOE infection in IL-18R−/− mice resulted in lower expression levels of MHC-II and CD40 on splenic CD11c+ DCs in vivo (Fig. 12A). Consistent with the in vivo findings, IL-18R−/−–deficient BMDCs infected in vitro with cell-free Ehrlichia had lower surface expression of MHC-II, CD86, and CD40 at 24 h p.i. (Fig. 12B), but not MHC-I and CD80, compared with IOE-stimulated IL-18R+–expressing BMDCs from WT mice. As a positive control, LPS-stimulated BMDCs from IL-18R+–/− mice had comparable levels of maturation markers compared with LPS-stimulated BMDCs from WT mice (data not shown), suggesting that defective maturation of BMDCs from IL-18R−/− mice is not due to intrinsic defects in TLR4/MyD88-signaling pathways. Interestingly, decreased maturation of IOE-stimulated IL-18R+–deficient BMDCs was associated with a significant decrease in their production of TNF-α, but not IL-6, compared with IOE-stimulated BMDCs from WT mice (Fig. 12C). Thus, our data suggested that IL-18R+ signals promote DC maturation and TNF-α production by these cells, and both of these factors might contribute to the induction of pathogenic CD8+ T cells and subsequent immunopathology during fatal Ehrlichia-induced shock.

Discussion

Acquired immune responses against several pathogens are known to be regulated by innate immunity involving cytokines. Protection against lethal systemic infection with LPS-lacking Ehrlichia requires a balance between several protective (mediated by CD4+ Th1 cells and IFN-γ–producing NKT cells) and pathogenic (mediated by cytotoxic, IFN-γ– and TNF-α–producing NK and CD8+ T cells) components of innate and adaptive immune systems. We identified an unexpected pathogenic role of IL-18Rα in promoting Ehrlichia–induced toxic shock. Lack of IL-18/IL-18R interaction in KO or Ab-treated mice resulted in enhanced protective immunity against Ehrlichia, as revealed by decreased bacterial burden and prolonged survival. Enhanced resistance in IL-18Rα−/− mice was associated with elevated levels of proinflammatory and Th1 cytokines (TNF-α, IL-6, IFN-γ) only at the sites of infection (peritoneum), decreased systemic IL-10 production, and increased numbers of protective NKT cells producing both TNF-α and IFN-γ. More intriguing, IL-18Rα deficiency resulted in abrogation of multiple pathogenic components of Ehrlichia–induced toxic shock, including decreased leukocyte infiltration into liver and host cell apoptosis and necrosis, and decreased numbers of pathogenic TNF-α–producing CD8+ T cells. Consistent with the pathogenic role of IL-18 in our model, systemic administration of rIL-18 to WT mice infected with mildly virulent E. muris resulted in increased host cell apoptosis and ineffective bacterial elimination in different organs compared with untreated controls. Thus, it is clear that IL-18 signaling plays negative and detrimental regulatory roles in Ehrlichia–induced toxic shock.

Several studies suggested a role for IL-18 in promoting IFN-γ production, which enhances the elimination of intracellular pathogens (22–26), whereas others suggested a role for IL-18 in mediating autoimmune and inflammatory disorders (36–39). Our data demonstrated a significant correlation between enhanced clearances of Ehrlichiae, decreased IFN-γ production by type-1 T cells (with no bias toward Th2 response), and decreased IL-10 production in IL-18Rα−/− mice compared with WT controls. These results were unexpected because IFN-γ is a hallmark of protective immunity against Ehrlichia. However, the enhanced bacterial elimination in IL-18Rα−/− mice could be attributed, in part, to a lack of immunosuppressive effect of IL-10 on microbicidal functions of macrophages. Studies showed that IL-6 contributes to host defense against various pathogens, including Streptococcus pneumoniae, Escherichia coli, Listeria monocytogenes, and Borrelia burgdorferi (50–53). Thus, it is also possible that the local levels of IFN-γ, in addition to the levels of other inflammatory cytokines, such as TNF-α and IL-6, are optimal for effective elimination of bacteria at local sites of infection. Alternatively, other anti-Ehrlichia effector molecules, such as NKT cells producing both IFN-γ and TNF-α, may be compartmentalized to the sites of infection in peripheral organs and, thus, compensate for the lack of IFN-γ production by type-1 T cells. This conclusion is supported by our data in Fig. 8, showing expansion of NKT cells producing both IFN-γ and TNF-α in IOE-infected IL-18Rα−/− mice compared with infected WT mice. NKT cells are critical for host defense against various pathogens, including Ehrlichia, by killing infected cells and producing proinflammatory cytokines that enhance bacterial clearance (16–18). This study showed evidence suggesting that IL-18/IL-18R interaction influences the frequency and functions of NKT cells following systemic and severe Ehrlichia infection and that the absence of IL-18R
signals leads to restoration of splenic NKT cells. Interestingly, we demonstrated previously that doxycycline treatment resulted in expansion of NKT cells following lethal IOE infection of WT mice (16). Thus, the lack of IL-18R signals could indirectly influence the response of NKT cells by enhancing protective immunity and bacterial clearance. These data are consistent with other studies showing that IL-18 influences the frequency and function of the invariant NKT cell population (20–24).

The present study suggested that neutrophils are unlikely to mediate ehrlichial elimination, but they may contribute to tissue injury following systemic ehrlichial infection, as evidenced by a decreased number of neutrophils in IL-18Rα−/− mice compared with WT mice following lethal infection with IOE and decreased expansion of neutrophils in nonlethally infected mice compared with lethally infected mice. In support of this conclusion, studies showed that IL-18 delays apoptosis of neutrophils and enhances neutrophil recruitment to peripheral organs during stressful and inflammatory conditions, such as a burn wounds and liver inflammation, and this can lead to prolonged release of harmful enzymes and multiple organ dysfunction (57, 58).

Systemic IL-10 production was decreased in IOE-infected IL-18Rα−/− mice (Fig. 6). We previously demonstrated that IL-10 overproduction is associated with increased bacterial burden, immunopathology, and mortality following lethal ehrlichial infection (7, 13, 16). However, the direct correlation between decreased levels of both IFN-γ and IL-10 in IL-18Rα−/− mice is paradoxical in the context of known regulatory function of IL-10 on Th1 responses during infections with other intracellular pathogens. It is possible that during the course of systemic and severe Ehrlichia infection, IL-18 secreted by APCs induces immune cells to produce IL-10, which, in turn, inhibits further production of Th1-promoting cytokines, such as IL-12 and IL-18, by APCs or IFN-γ production by T cells. Although the source of IL-10 is not well defined, we argue that NK cells could be a major source. This conclusion is consistent with a recent study suggesting that during acute infection with diverse rapidly disseminating pathogens, IL-12 production by DCs stimulates IL-10 production by NK cells as an innate, negative feedback loop in which IL-12 limits its own production (59). Further support to this conclusion are the following findings. Fig. 1 shows positive and

![Figure 12](http://www.jimmunol.org/)

**FIGURE 12.** The lack of IL-18Rα signals causes downregulation of T cell costimulatory functions of DCs during severe ehrlichial infection. A, Splenocytes were harvested from IOE-infected IL-18Rα−/− and WT mice on day 3 p.i., and the expression levels of CD40 and MHC-II costimulatory molecules on CD11c+ DCs were analyzed by flow cytometry. B, BMDCs were isolated from WT and IL-18Rα−/− mice and were propagated in vitro. Cells were either left unstimulated or infected with IOE at a multiplicity of infection of 5:1 for 24 h. Graphs show different maturation markers; MHC-I, MHC-II, CD80, CD86, and CD40 on CD11c+ BMDCs from naive (filled line), WT (solid line), and IL-18Rα−/− (dashed line) mice. C, TNF-α and IL-6 production in culture supernatant of IOE-infected BMDCs from WT and IL-18Rα−/− mice. Uninfected BMDCs from WT mice were used as negative controls. In vivo experiments showing results from four mice/group; similar results were obtained in three independent experiments. In vitro data are representative of one of three different experiments with similar results. **p < 0.01.
negative feedback loops between increased systemic production of IL-12 and IL-18 in IOE-infected WT mice and increased IL-10 production at late stages of the disease, which coincide with decreased level of IFN-γ in the spleens. Lack of IL-18R signals in infected IL-18R−/− mice resulted in decreased systemic IL-10 production and enhanced IFN-γ and TNF-α production by NK cells (Fig. 8). Interestingly, although infected IL-18R−/− mice had a lower frequency of splenic type-1 T cells and a lower serum level of IFN-γ than did WT mice, they produced higher or similar concentrations of IFN-γ in the peritoneum and spleens (Fig. 6), respectively, compared with WT mice. These data suggested that during the course of systemic ehrlichial infection, this IL-10/Th1 negative feedback loop may be compartmentalized and effective at the sites of infection in response to inflammation and tissue damage. Recently, we showed that depletion of NK cells following IOE infection resulted in abrogation of tissue injury and enhanced bacterial elimination, which correlated with decreased systemic IL-10 production (7, 13, 16), suggesting that IL-10 could be derived from NK cells.

Studies showed that IL-18 contributes to enhanced cytotoxicity of NK and CD8+ T cells, particularly when combined with IL-10 (60–62), which, in turn, drives apoptosis of hepatocytes in vivo via a FAS-dependent mechanism (29, 33, 61, 63). We did not directly measure the cytotoxic functions of NK and CD8+ T cells against Ehrlichia in this study. However, decreased IFN-γ and TNF-α production by NK and CD8+ T cells, respectively (Figs. 8, 9), abrogation of tissue injury, and decreased FAS expression on CD4+ T cells (Fig. 7B) and CD8+ T cells (data not shown) in IL-18R−/− mice compared with infected WT mice suggested that IL-18/IL-18R interaction may play a synergistic role with IL-10 in promoting pathogenic cytotoxic functions of NK and CD8+ T cells following severe Ehrlichia infection.

We previously showed that TNF-α–producing CD8+ T cells play a pathogenic role in fatal ehrlichiosis. Data in Fig. 9 suggested that the enhanced bacterial elimination in IOE-infected IL-18R−/− mice could be due to defective induction and/or expansion of pathogenic CD8+ T cells or other cellular responses that influence the outcome of infection. Adoptive transfer of splenocytes enriched for CD8+ T cells from immune WT mice increased IOE numbers in different organs of IL-18R−/− mice to numbers similar to those in infected WT mice. These data suggested that pathogenic CD8+ T cells are sufficient to inhibit the protective anti-Ehrlichia immune responses of IL-18R−/− mice. The mechanisms by which IL-18/IL-18R interaction mediates the induction and/or expansion of pathogenic TNF-α–producing CD8+ T cells are not known. However, decreased numbers of CD8+ T cells in IL-18R−/− mice (Fig. 7A) could be due to AICD, which might occur via FAS-mediated mechanisms, as suggested previously (16, 17, 19); migration of cells to peripheral sites of infection; or defective induction and expansion of naive and effector T cells, respectively. Our data supported the third possibility for the following reasons. First, the expression of FAS on CD8+ T cells was decreased in IL-18R−/− mice compared with infected WT mice. Second, the absence of IL-18R signals resulted in marked attenuation of leukocyte accumulation in the liver, which could be due to the effect of IL-18 on the production of several chemokines, as suggested by other studies (22–25). Thus, migration of T cells to liver is unlikely to account for the observed decrease in T cells. Third, IOE infection of IL-18R−/− mice resulted in decreased splenic DCs, decreased maturation of BMDCs, as well as in vivo and in vitro production of TNF-α by DCs. In the context of known functions of DCs (64–70), these results suggested that absence of IL-18R signals in IL-18R−/− mice influenced the T cell-priming functions of DCs, which, in turn, influenced the induction of Ehrlichia-specific CD8+ T cells. DCs are known to effectively prime CD8+ T cells against intracellular bacteria that reside within endosomes and cannot access the endogenous MHC-I pathway via cross-presentation pathways (64–70). Ehrlichia (IOE) reside within phagosomes and do not escape into the cytosol, and it is not known whether they secrete proteins into the cytoplasm that access the endogenous pathway of Ag presentation (71, 72). Furthermore, lethal ehrlichial infection induced marked host cell apoptosis and necrosis, which are pathologic events that support cross-presentation of exogenous pathogen-derived Ag to CD8+ T cells (64–68). Furthermore, IOE-stimulated BMDCs from IL-18R−/− mice had lower expression of MHC-II, but not MHC-I, compared with BMDCs from WT mice (Fig. 12). Taken together, we envisaged that IL-18/IL-18R interaction is critical for maturation of infected DCs, which, in turn, prime pathogenic CD8+ T cells, most likely via a cross-presentation pathway.

The data presented in this study are novel because they unravel a previously unidentified pathogenic role for IL-18 in the immunopathogenesis of infection-induced inflammatory diseases and toxic shock-like syndrome. As we demonstrated, targeting IL-18/IL-18R interaction would enhance protective immunity, as well as abrogate the immune-mediated pathology that causes multiorgan failure following infections with intracellular pathogens. Although further studies are needed to dissect the IL-18–dependent molecular and cellular interactions, our data suggest the fascinating proposition that targeting IL-18/IL-18R interaction could represent an effective treatment for LPS-lacking Gram-negative, intracellular bacterial infection-induced toxic shock, primarily in patients who do not respond to antibiotic treatment.

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Disclosures

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References


