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Type I IFN Modulates Host Defense and Late Hyperinflammation in Septic Peritonitis

Heike Weighardt,† Simone Kaiser-Moore,* Sylvia Schlautkötter,* Tanja Rossmann-Bloeck,* Ulrike Schleicher,† Christian Bogdan,† and Bernhard Holzmann*

TLRs are considered important for the control of immune responses during endotoxic shock or polymicrobial sepsis. Signaling by TLRs may proceed through the adapter proteins MyD88 or TIR domain-containing adaptor inducing IFN-β. Both pathways can lead to the production of type I IFNs (IFN-αβ). In the present study, the role of the type I IFN pathway for host defense and immune pathology in sepsis was investigated using a model of mixed bacterial peritonitis. Systemic levels of IFN-αβ protein were markedly elevated during septic peritonitis. More detailed analyses revealed production of IFN-β, but not IFN-α subtypes, and identified CD11b+CD11c− macrophage-like cells as major producers of IFN-β. The results further demonstrate that in IFN-αβ receptor I chain (IFNAR1)-deficient mice, the early recruitment of neutrophils to the infected peritoneal cavity was augmented, most likely due to an increased local production of MCP-1 and leukotriene B4. In the absence of IFNAR1, peritoneal neutrophils also exhibited enhanced production of reactive oxygen intermediates and elevated expression of Mac-1. Conversely, administration of recombinant IFN-β resulted in reduced leukotriene B4 levels and decreased peritoneal neutrophil recruitment and activation. Analysis of the cytokine response to septic peritonitis revealed that IFNAR1 deficiency strongly attenuated late, but not early, hyperinflammation. In accordance with these findings, bacterial clearance and overall survival of IFNAR1−/− mice were improved. Therefore, the present study reveals critical functions of the type I IFN pathway during severe mixed bacterial infections leading to sepsis. The results suggest that type I IFN exerts predominantly adverse effects under these conditions. The Journal of Immunology, 2006, 177: 5623–5630.
LTB₄ levels and neutrophil recruitment. Importantly, IFNARI deficiency caused an attenuation of the late, but not early, sepsis-induced hyperinflammatory reaction. Systemic protein levels of type I IFN were elevated during septic peritonitis and further analysis of type I IFN subtypes revealed induction of IFN-β, but not IFN-α. CD11b⁺ CD11c⁺ macrophage-like cells could be identified as main IFN-β producers in spleen. We therefore conclude that the type I IFN pathway stimulates mostly adverse activities during septic peritonitis.

Materials and Methods

Mouse strains and colon ascendens stent peritonitis (CASP) model of polymicrobial septic peritonitis

Mice with a deletion of the IFN-αβ receptor I chain (IFNAR1−/−) (26) and 129Sv control mice were obtained from B&K Universal Group. Mice at 8 to 12 wk of age were used for all experiments. The CASP used for induction of septic peritonitis and the characteristics of this model were described in detail elsewhere (25, 27). Briefly, the colon ascendants was exteriorized and a 7/0 Ethil thread (Ethicon) was stitched through the antimesenteric portion of the colon ascendants ~10 mm distal of the ileocecal valve. A 16-gauge venous catheter was punctured antimesenterically through the colonic wall into the intestinal lumen, directly proximal of the prepared knot, and fixed. To ensure proper intraluminal position of the stent, stool was milked from the cecum into the colon ascendants until a small drop appeared. Fluid resuscitation of the animals was performed by flushing 0.5 ml of sterile saline into the peritoneal cavity before closure of the abdominal wall. For some experiments, mice were treated with 10² or 10⁴ CFUs of L. monocytogenes into the peritoneal cavity before closure of the cavity. In addition, peritoneal lavage cells were counted and differentiated by staining by flow cytometry (FACSCalibur; CellQuest software; BD Biosciences) with Abs against Mac-1 (clone M1/70) and Ly-6C/G (Gr-1, clone RB6-8C5) using appropriate isotype-matched controls (all obtained from BD Pharmingen).

Bacterial counts and peritoneal neutrophil accumulation

Mice were sacrificed before (0 h) and 3, 6, and 12 h after CASP, and peritoneal lavage fluid was collected. Serial dilutions of lavage fluids 12 h after CASP were plated on blood agar plates. CFUs were counted after incubation at 37°C for 24 h and calculated as CFU per whole peritoneal cavity. In addition, peritoneal lavage cells were counted and differentiated by staining by flow cytometry (FACS Calibur; CellQuest software; BD Biosciences) with Abs against Mac-1 (clone M1/70) and CD11c (clone HL30). CD11b⁺ cells were isolated using a MoFlo cell sorter (DakoCytomation). RNA was prepared immediately after sorting using the RNeasy kit (Qiagen) and real-time PCR analysis was performed as described above.

Detection of apoptotic cells

Peritoneal exudate cells were collected 12 h after CASP. Cells were stained for detection of granulocytes with anti-Ly-6C/G (Gr-1, clone RB6-8C5). After a washing, cells were stained with annexinV-FITC (BD Pharmingen) and 5 μg/ml propidium iodide (Sigma-Aldrich) in 10 mM HEPES (pH 7.4), 140 mM NaCl, 2.5 mM CaCl₂, for 15 min. Flow cytometry analysis was performed immediately thereafter.

Analysis of cytokine and chemokine production

Peripheral blood, spleen, liver, and peritoneal lavage were collected before (0 h) and 3, 6, and 12 h after CASP. Solid organs were snap-frozen in liquid nitrogen and homogenized after thawing in 1 ml of PBS containing complete protease inhibitors (Roche Diagnostics). Organ extracts or sera were preincubated with L929 fibroblasts for 24 h at 37°C before the addition of VSV for a subsequent culture period of 48 h. Afterwards, the viability of L929 cells was measured by a MTT assay as described (29). For quantification of type I IFN purified mouse IFN-αβ (provided by Ion Gresser, Institute Curie, Paris, France) was used as a standard in this assay. IFN-γ-mediated protection of L929 cells from virus-induced cell lysis was excluded by demonstrating that cell culture supernatants and sera were negative for IFN-γ using a commercial capture ELISA (R&D Systems; data not shown).

Real-time PCR analysis

Spleens and livers were collected at different time points after sepsis induction and stored in RNA later buffer (Qiagen). RNA extractions were conducted using the RNeasy miNikit (Qiagen) according to the manufacturer’s instructions. First-strand cDNA was synthesized from 1 μg of total RNA using a mixture of oligo(dT)₁₂₋₁₈ and random hexamer primers and SuperScript reverse transcriptase (Invitrogen Life Technologies). The reaction was incubated for 60 min at 42°C and terminated by heating to 95°C for 5 min. SYBR Green master mix was used to detect accumulation of PCR products during cycling on an SDS7700 cycler (Applied Biosystems). RNA expression levels of samples of septic animals were normalized to β-actin and were displayed as fold change relative to samples of control mice used as the calibrator (set to 1). Primers for β-actin and IFN-β were designed using PrimerExpress software (Applied Biosystems). Primers for amplification of specific cDNA fragments were as follows: β-actin: sense, 5'-ACCCACTAGTGTGCACTTAC-3'; antisense, 5'-AGGCAAATGCCAGGCAGGG-3'. IFN-β: sense, 5'-TCCAAGAAAGGACGACATTGC-3'; antisense, 5'-TGGGACATCTGCGCCTCAAA-3'. IFN-α subtypes (including IFN-α1, -α2, -α7, -α11, and -α12 subtypes) were detected with published primer sets (30).

Isolation of spleen cell subsets

Spleens were collected before and 6 h after CASP induction. T and B cells were removed by magnetic cell sorting using anti-Thy1- and anti-CD19-conjugated beads (Miltenyi Biotec) according to the manufacturer’s protocols. Cell suspensions depleted for T and B cells were stained with Abs against CD11b (clone M1/70 and CD11c (clone HL30), CD11b⁺CD11c⁻ cells and CD11b⁻CD11c⁺ cells were isolated using a MoFlo cell sorter (DakoCytomation). RNA was prepared immediately after sorting using the RNeasy minikit (Qiagen) and real-time PCR analysis was performed as described above.

Quantification of type I IFN protein

The IFN-αβ content of cell culture supernatants and sera of CASP-treated and control mice was determined with a bioassay based on the protection of L929 fibroblasts against the cytotoxic effect of vesicular stomatitis virus (VSV) (19). Briefly, duplicates of serially 2-fold diluted cell culture supernatants or sera were preincubated with L929 fibroblasts for 24 h at 37°C before the addition of VSV for a subsequent culture period of 48 h. Afterwards, the viability of L929 cells was measured by an MTT assay as described (29). For quantification of type I IFN purified mouse IFN-αβ (provided by Ion Gresser, Institute Curie, Paris, France) was used as a standard in this assay. IFN-γ-mediated protection of L929 cells from virus-induced cell lysis was excluded by demonstrating that cell culture supernatants and sera were negative for IFN-γ using a commercial capture ELISA (R&D Systems; data not shown).
Enhanced innate immune defense against polymicrobial peritonitis in IFNARI−/− mice

To analyze the mechanisms that underlie improved survival of IFNARI-deficient mice in septic peritonitis, the innate cellular immune response was analyzed. Antibacterial defense in septic peritonitis critically depends on the influx of effector granulocytes at the site of infection (28, 33–35). Comparing the number of neutrophils infiltrating the peritoneal cavity of IFNARI-deficient and control mice revealed a markedly enhanced accumulation of IFNARI−/− granulocytes at 6 and 12 h after sepsis onset (Fig. 2a).

To further examine the antimicrobial activity of newly recruited peritoneal neutrophils, the production of reactive oxygen intermediates was measured directly ex vivo without additional in vitro stimulation as described previously (34). The results depicted in Fig. 2b show that neutrophil production of reactive oxygen intermediates was significantly increased in IFNARI−/− mice as compared with wild-type controls. Moreover, cell surface density of the integrin CD11b/CD18 (Mac-1), which has been implicated in neutrophil recruitment during peritonitis (36), was significantly greater on IFNARI-deficient than wild-type neutrophils (Fig. 2c).

Together, these results indicate that improved survival of IFNAR-deficient mice from septic peritonitis is associated with an increased accumulation and activation of neutrophils in the peritoneal cavity.

Previous studies have shown that the chemokine MCP-1 and its receptor CCR2 as well as LTB4 are involved in mediating immigration of neutrophils during septic peritonitis (37, 38). To investigate whether these pathways may also contribute to enhanced neutrophil recruitment in IFNAR−/− mice, peritoneal levels of these mediators were measured. As shown in Fig. 2d, production of both MCP-1 and LTB4 was significantly augmented in peritoneal cavities of IFNAR-deficient mice early (3 h) after sepsis induction, suggesting that accelerated production of chemotactic factors may facilitate immigration of neutrophils. In contrast, peritoneal levels of KC were not altered at any time point, indicating that type I IFN signaling does not influence KC expression.

In addition to an accelerated production of chemotactic factors, enhanced accumulation of neutrophils in the peritoneal cavity could be due to diminished apoptosis (34). However, peritoneal neutrophils isolated from septic IFNARI−/− and wild-type mice exhibited similar ex vivo staining profiles for annexin V and propidium iodide, suggesting that IFNAR deficiency does not influence apoptosis or necrosis of these cells during septic peritonitis (Fig. 2e).

Increased peritoneal neutrophil numbers and elevated production of reactive oxygen metabolites by these cells may improve host defense during septic peritonitis. To address this question directly the bacterial load of peritoneal cavities of IFNAR-deficient and wild-type mice subjected to CASP was analyzed. The results

**FIGURE 1.** Improved survival of IFNAR−/− mice in polymicrobial septic peritonitis. IFNAR-deficient and wild-type (WT) mice were subjected to CASP surgery, and survival was monitored. Data were pooled from three independent experiments yielding comparable results.

**FIGURE 2.** Increased peritoneal neutrophil recruitment and activation in IFNAR-deficient mice. a. Peritoneal cells from IFNAR−/− (△) and wild-type mice (□) were quantified before (0 h) as well as 3, 6, and 12 h after CASP. Neutrophils were identified by high expression of Gr-1 and Mac-1. b. Production of reactive oxygen intermediates (ROI) of Gr-1+ cells was determined ex vivo without additional stimulation and was expressed as mean fluorescence intensity (MFI). c. Surface expression levels of Mac-1 on Gr-1+ cells were quantified as mean fluorescence intensity. d. Peritoneal lavage fluid was obtained before (0 h), as well as 3, 6, and 12 h after CASP from IFNAR−/− (△) or control mice (□), and mediators were determined by ELISA. e. Peritoneal cells were harvested 12 h after CASP, and apoptotic and necrotic cells were determined by staining with annexin V and propidium iodide. All data are derived from four to six independent mice per group and time point. *, p < 0.05; #, p < 0.01; §, p < 0.001 (IFNAR−/− vs wild type; WT).
in Fig. 3 show that bacterial numbers were significantly lower in IFNARI−/− mice than in wild-type counterparts.

**IFNARI deficiency attenuates late systemic inflammation in septic peritonitis**

Sepsis leads to the induction of numerous cytokines which are beneficial for stimulating antibacterial host defense, but overwhelming inflammation during sepsis may lead to organ failure and subsequent death (39–43). Therefore, the systemic levels of inflammatory mediators were measured over the course of sepsis in IFNARI−/− and wild-type mice. The results in Fig. 4 reveal marked, but comparable, induction of TNF, KC, MCP-1, MCP-5, and IP10 at 3 and 6 h after sepsis induction in serum of both strains of mice. However, 12 h after sepsis onset, serum levels of TNF, MCP-5, and IP10 were significantly lower in IFNARI-deficient mice than in wild-type controls. In contrast, levels of MCP-1 and KC were not significantly different between both groups (Fig. 4). These data show that the hyperinflammatory reaction was attenuated in IFNARI-deficient mice at late, but not early, time points after sepsis onset.

To analyze the influence of IFNAR1 deficiency on cytokine regulation during sepsis in more detail, expression levels of KC, MCP-1, MCP-5, and IP10 were analyzed in protein extracts of spleen and liver. Analysis of local immune mediator expression yielded results that were mostly consistent with those obtained with serum samples. The induction of MCP-1 and MCP-5 in spleen and liver was IFNAR1 independent at early time points (3 and 6 h) after sepsis induction. However, 12 h after CASP, the amounts of MCP-1 and MCP-5 proteins were reduced in spleen and liver of IFNAR1-deficient mice compared with wild-type controls, indicating type 1 IFN-dependent expression during the late phase of sepsis (Fig. 5). Expression of IP10 in liver of septic IFNAR1-deficient mice was also significantly attenuated only at the late time point (Fig. 5a). In spleen, however, IP10 production was reduced not only 12 h after sepsis induction but also at the 3-h time point (Fig. 5b), indicating organ-specific regulation of IP10 expression. Local induction of KC in both liver and spleen was not influenced by IFNAR1 deficiency, demonstrating type 1 IFN-independent regulation of KC during the entire observation period.

Collectively, these data indicate that sepsis-induced cytokine and chemokine production is largely independent of type I IFN signaling at early time points, whereas marked dependency on type I IFN is observed during the late course of sepsis. Thus, attenuation of the late hyperinflammatory response may contribute to the improved survival of IFNAR1-deficient mice from polymicrobial septic peritonitis.

**Production and cellular source of type I IFN during septic peritonitis**

To analyze the expression of type I IFN during polymicrobial sepsis, mRNA levels of IFN-β and IFN-α subtypes (IFN-α1, -α2, -α7, -α11, and -α12) in septic spleen and liver were quantified by real-time PCR (Fig. 6, a and b). Strong up-regulation of IFN-β mRNA could be detected in both organs during the septic course. In the spleen, maximal levels of IFN-β expression were reached as early as 3 h after sepsis induction, whereas in liver peak levels were observed after 6 h. In contrast, expression of IFN-α1 could not be detected in either liver or spleen. To confirm the results on type I IFN expression in polymicrobial sepsis on the protein level, serum samples were analyzed 9 h after induction of peritonitis. This time point was chosen, because peak mRNA levels were observed after 6 h (Fig. 6, a and b). The results in Fig. 6c clearly show that high systemic levels of type I IFN activity were observed in septic mice at this time point, whereas in nonseptic mice type I IFN protein was not detectable. Thus, mixed bacterial sepsis leads to a marked production of type I IFN. The results also suggest that IFN-β rather than IFN-α subtypes are produced and may be responsible for the late cytokine burst during septic peritonitis.

To further identify the cellular source of IFN-β during septic peritonitis, CD11b+CD11c− and CD11b+CD11c+ cells were purified by cell sorting from spleen cell suspensions 6 h after CASP induction. This time point was chosen because high levels of IFN-β expression were found in septic organs 6 h after CASP (Fig. 6, a and b). Quantification of mRNA levels showed that both CD11b+CD11c− and CD11b+CD11c+ cells contribute to the sepsis-induced production of IFN-β (Fig. 6d). However, IFN-β mRNA levels were ~2-fold higher in splenic CD11b+CD11c− than in with CD11b+CD11c− cells (Fig. 6d). Thus, IFN-β is the main type I IFN produced during polymicrobial septic peritonitis and CD11b+CD11c− macrophage-like cells appear to be a more prominent source of IFN-β than CD11b+CD11c+ myeloid dendritic cells.
Administration of IFN-B attenuates accumulation and activation of neutrophil at the septic focus

Having established that IFNARI-deficient mice show enhanced host defense during mixed bacterial sepsis, we next addressed the question of whether administration of exogenous type I IFN may lead to opposite effects. Different doses of recombinant murine IFN-β/H9252 were injected i.v. into wild-type mice, and the inflammatory response in the infected peritoneal cavity was analyzed 3 h later. This time point was chosen because peritoneal mediator concentrations as well as neutrophil activation were significantly elevated in IFNARI-deficient mice at this time point, whereas neutrophil numbers were already weakly increased (Fig. 2). The results in Fig. 7, a and b, show that sepsis-induced accumulation of neutrophils at the site of infection as well as the production of reactive oxygen intermediates were significantly reduced after administration of IFN-β in a dose dependent manner. Similarly, peritoneal levels of LTB4 were decreased in IFN-β-treated compared with control PBS-treated mice (Fig. 7c). Consistent with these results, levels of the IFN-regulated chemokine IP10 were elevated after injection of high (10^4 U), but not low (10^2 U), doses of recombinant murine IFN-β (Fig. 7d). These observations confirm the data obtained with IFNARI-deficient mice, suggesting that IFN-β negatively contributes to host defense mechanisms during mixed bacterial infection.

Discussion

Although induction of cell-autonomous antiviral immunity is considered a predominant activity of type I IFN (16–18), they also have been reported to exhibit diverse functions during infection with nonviral pathogens. Thus, type I IFNs were shown to be crucial for pathogen clearance and NO production during infection with the protozoan parasite L. major (19). Moreover, type I IFNs are produced during innate immune responses to most bacterial infections (13, 31). In bacterial infections, however, both beneficial and adverse effects of type I IFN have been documented. Whereas type I IFN promote resistance of mice against S. pneumoniae (23), they were found to impair host defense against the intracellular bacterium L. monocytogenes during the first days of infection, which in one study was attributed to an increased susceptibility of T lymphocytes to apoptosis (20–22). Using a model of septic peritonitis, the present study reveals critical functions of type I IFN during severe mixed bacterial infections. Polymicrobial peritonitis was shown to result in marked production of type I IFN protein with mRNA for IFN-β/H9252, but not IFN-α/H9251.
Conversely, administration of exogenous IFN-α h after CASP and LTB₄, and IP₁₀ levels were determined by ELISA.

macrophage-like cells and CD₁₁b⁺CD₁₁c⁺ dendritic cells were found to express IFN-β. We further show that in the absence of IFNARI the early activation and recruitment of neutrophils to the infected peritoneal cavity was augmented, whereas the late hyper-inflammatory response was attenuated. Consequently, bacterial clearance and overall survival of IFNARI⁻/⁻ mice were improved. Conversely, administration of exogenous IFN-β was found to reduce peritoneal chemoattractant production as well as neutrophil recruitment and activation. Thus, our findings suggest that type I IFN exert predominantly adverse functions during severe mixed bacterial infections.

Immediate recruitment of neutrophils to the site of infection is considered an essential mechanism to eliminate certain classes of pathogens, and neutrophils were shown to be of critical importance for the immune defense against intra-abdominal bacterial infections in mice (28, 33–35). In the present study, increased accumulation of neutrophils in the peritoneal cavity has been associated with an improved bacterial clearance, suggesting that this mechanism contributes to the enhanced survival of IFNARI⁻/⁻ mice in septic peritonitis. Accumulation of neutrophils at the site of infection may be influenced by various processes including apoptosis as well as cell recruitment mediated by chemoattractant stimuli and adhesion receptors. Because type I IFNs have been implicated in sensitizing T cells for apoptosis after infection of mice with the Gram-positive bacterium L. monocytogenes (21, 22) and because our previous work indicated that peritoneal neutrophil accumulation during mixed bacterial septic peritonitis may be influenced by apoptosis (34), we asked whether IFNARI deficiency could augment peritoneal neutrophil accumulation by lowering the sensitivity of these cells to apoptosis. However, we could not observe any differences in the induction of apoptosis when comparing neutrophils of septic IFNARI-deficient and wild-type mice. In marked contrast, IFNARI⁻/⁻ mice had elevated peritoneal levels of MCP-1 and LTB₄ during the early phase of septic peritonitis, suggesting that increased production of chemoattractants contribute to augmented peritoneal neutrophil accumulation in these mice. Consistent with this notion, injection of IFN-β decreased both peritoneal LTB₄ levels and neutrophil numbers. The involvement of MCP-1 would be consistent with our previous work showing that blockade of CCR2, which functions as a receptor for MCP-1, strongly attenuates peritoneal neutrophil recruitment in the CASP model (37). The induction of MCP-1 during septic peritonitis induced by cecal ligation and puncture was found to amplify the macrophage production of the neutrophil chemoattractant LTB₄ (38), suggesting that elevated peritoneal levels of LTB₄ in IFNARI⁻/⁻ mice may result from increased MCP-1 production. In addition to its chemotactic activity, LTB₄ was found to activate β₂ integrins such as CD₁₁b/CD₁₈ (Mac-1) on neutrophils (44). It is therefore conceivable that enhanced production of LTB₄ may also contribute to up-regulation of Mac-1 on IFNARI-deficient neutrophils, thereby further supporting neutrophil migration to the infected peritoneal cavity. Consistent with this notion, Mac-1 was shown to be involved in the immigration of immune cells into the peritoneal cavity during septic peritonitis (36).

In addition to enhanced cell recruitment, IFNARI deficiency was also associated with increased neutrophil activation in the infected peritoneal cavity. The influence of the type I IFN pathway on neutrophil activation during septic peritonitis was shown by elevated ex vivo production of reactive oxygen intermediates and up-regulation of cell surface CD₁₁b/CD₁₈ (Mac-1) on IFNARI⁻/⁻ neutrophils and, in addition, by the reduced neutrophil oxidant production upon IFN-β treatment. Reactive oxygen intermediates were previously shown to play an important role for the defense of various bacterial infections (45, 46). It is therefore tempting to speculate that increased neutrophil production of reactive oxygen intermediates contributes to improved bacterial clearance in IFNARI⁻/⁻ mice.

Induction of septic peritonitis leads to the rapid induction of various cytokines and chemokines that are considered important for the resolution of the infection. However, overwhelming production of inflammatory mediators may cause hyperinflammation, organ failure, and lethal septic shock (39–43). The present study shows that absence of IFNARI-induced signaling leads to a substantial reduction of the hyperinflammatory reaction during the late phase of polymicrobial septic peritonitis. Reduced systemic and local production was observed for TNF, IP₁₀, and MCP-5, but not for KC, which is consistent with previous findings on the role of the type I IFN pathway for the TLR-stimulated production of these mediators (5, 9, 47). During the early phase of septic peritonitis, however, systemic and local production of TNF, IP₁₀, and MCP-5 was mostly unaffected in IFNARI⁻/⁻ mice. These findings are in accordance with the results obtained in a murine model of endotoxic shock, in which deficiency of Tyk2 kinase or IFN-β protected from death without affecting the release of various proinflammatory cytokines (24). However, the results of the present report also extend these earlier reports by establishing a critical role of type I IFN for septic hyperinflammation induced by mixed bacterial infection and by identifying type I IFN as mediators of the late, but not early, phase of hyperinflammation. The data suggest that different phases of cytokine production in sepsis may be distinguished based on the involvement of type I IFN. Whereas the early cytokine release is almost entirely type I IFN independent, the late cytokine burst is strongly promoted by type I IFN.

Sepsis induction led to marked systemic production of type I IFN protein. Production of IFN-β was stimulated in liver and
spleen of septic mice, whereas IFN-α subtypes were not detected. It therefore appears that IFN-β produced during septic peritonitis does not stimulate the amplification loop of the type I IFN response seen in other infectious disease models, which would result from the production of IFN-α (13–15). The highest expression levels of IFN-β in either liver and spleen could be observed 3–6 h after sepsis induction, which would be consistent with the hypothesis that IFN-β contributes to the late hyperinflammatory reaction in sepsis. To further determine the cellular source of sepsis-induced IFN-β, CD11b+CD11c− and CD11b+CD11c+ cells were isolated from spleen cell suspensions 6 h after sepsis induction and monitored for their capacity to produce IFN-β. These subsets were chosen for analysis, because both macrophages and myeloid dendritic cells were previously shown to be critically involved in the immune response during septic peritonitis (48–50). Our results revealed that both cell types contribute to IFN-β production but that CD11b+CD11c− macrophage-like cells represent the more prominent source of IFN-β during septic peritonitis.

The phenotype of IFNAR1−/− mice in polymicrobial septic peritonitis resembles that observed for MyD88−/− mice (32). It was previously shown that MyD88-deficient mice also exhibit a survival advantage in the CAPS model and that the hyperinflammatory reaction was reduced in these mice, whereas the bacterial clearance was intact due to MyD88-independent host responses. Together, these studies would be consistent with the view that different thresholds of TLR/type I IFN-mediated innate immune activation may have to be reached to trigger either protective or detrimental responses. Whereas full activation of TLR/type I IFN signaling appears to be necessary for generating deleterious hyperinflammatory reactions, partial activation of TLR/type I IFN responses represented by individual signaling pathways may be sufficient to combat bacterial infection and to yield intact host defense. The present and previous investigations would also suggest that there is a considerable degree of redundancy between MyD88 and type I IFN-dependent signaling pathways in mediating these responses.

**Disclosures**

The authors have no financial conflict of interest.

**References**


