Pharmacological Inhibition of Type I Interferon Signaling Protects Mice Against Lethal Sepsis

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Current research on new therapeutic strategies for sepsis uses different animal models, such as the lipopolysaccharide-induced endotoxemia model and the cecal ligation and puncture (CLP) peritonitis model. By using genetic and pharmacologic inhibition of the type I interferon (IFN) receptor (IFNAR1), we show that type I IFN signaling plays a detrimental role in these sepsis models. Mortality after CLP was reduced even when type I IFN responses were blocked after the onset of sepsis. Our findings reveal that type I IFNs play an important detrimental role during sepsis by negatively regulating neutrophil recruitment. Reduced neutrophil influx likely occurs via the induction of the CXC motif chemokine 1. Moreover, human white blood cells exposed to heat-killed Pseudomonas aeruginosa secrete IFN-β and stimulate type I IFN signaling. We provide data that support pharmacologic inhibition of type I IFN signaling as a novel therapeutic treatment in severe sepsis.

Keywords: sepsis; type I interferons; cecal ligation and puncture; endotoxemia; neutrophils.

Sepsis is a systemic inflammatory reaction to microbial infections. Despite advances in supportive care, the rate of mortality from sepsis remains high [1]. After decades of intensive research, only few new therapies have proven relatively beneficial [2]. To gain more insight into the complex pathophysiology of sepsis, animal models of sepsis, such as the lipopolysaccharide (LPS)–induced endotoxemia model and the cecal ligation and puncture (CLP) model, are essential [3].

Type I interferons (IFNs) are a family of cytokines consisting of multiple IFN-α proteins, IFN-β, and several others. All type I IFNs bind to a heterodimeric cell surface receptor complex consisting of 2 subunits, type I IFN receptor 1 (IFNAR1) and 2 (IFNAR2) [4]. Whereas type I IFNs are well known for their antiviral actions [5, 6], recent research has revealed their critical role in immune regulation against nonviral pathogens [7–9].

The central role of type I IFNs in endotoxemia was demonstrated by showing that IFN-β and IFNAR knockout mice resist the lethal effects of LPS [10–13]. Although these data highlight the central role of type I IFNs in endotoxemia, the role of type I IFNs during bacterial sepsis remains controversial [14]. IFNAR1 knockout mice are resistant to colon ascendens stent peritonitis (CASP) but sensitive to nonsevere CLP, both of which are superior sepsis models compared to endotoxemia [8, 15].

Here, we further investigated the role of type I IFN responses in the immunopathology of sepsis by using two mouse models of sepsis, the LPS model and a severe CLP model. We used IFNAR1 knockout mice, as well as a neutralizing antibody (Ab) against IFNAR1. We report that type I IFNs are critical mediators of sepsis. Human white blood cells incubated with Pseudomonas aeruginosa produced IFN-β and showed expression of type I IFN-dependent genes. We propose a mechanism by which IFN reduces neutrophil influx and propose IFNAR1 as a potential drug target for sepsis.

MATERIAL AND METHODS

Mice and Human Studies

C57BL/6 mice were purchased from Janvier (Le Genest-St. Isle, France). IFNAR1 knockout mice were provided by D. Bonaparte (Gulbenkian Institute of Science, Oeiras,
Portugal). The mice were housed in individually ventilated cages under a constant dark-light cycle. All animal experiments were approved by the institutional ethics committee for animal welfare of the Faculty of Sciences, Ghent University (Ghent, Belgium; ethics committee number 2010-040), in accordance with European guidelines (directive 2010/63/EU; Belgian Royal Decree of 6 April 2010). Fresh human blood was collected from 3 healthy human volunteers in compliance with the guidelines of the MedImmune institutional review board. All donors provided written, informed consent before study-related procedures were performed.

Reagents
LPS from Salmonella abortus equi was purchased from Sigma-Aldrich (Saint Louis, MO). For the IFNAR blocking experiments, mice were injected intraperitoneally with 1 mg of IFNAR1 monoclonal Ab (MAR1-5A3) [16] or an irrelevant isotype-matched monoclonal Ab.

Endotoxemia Model
Mice were injected intraperitoneally with LPS in 0.2 mL of pyrogen-free phosphate-buffered saline (PBS). Blood was withdrawn with a glass capillary from the retro-orbital plexus and allowed to clot overnight at 4°C.

CLP sepsis Model
Mice were subjected to CLP to induce polymicrobial septic shock. Mice were anesthetized by isoflurane inhalation, and a 1-cm midline incision was made on the abdomen, after which the cecum was exposed and ligated. This was followed by making 2 punctures in the cecum with a 21-gauge needle. The abdominal musculature and skin were closed with simple running sutures and metallic clips, respectively. Ten and 24 hours after CLP, mice were injected intraperitoneally with both ceftriaxone (25 mg/kg; Sigma) and metronidazole (12.5 mg/kg; Sigma). For CXCR2 inhibition studies, mice received an intraperitoneal injection of 10 mg/kg SB225002 (Cayman Chemical, Ann Arbor, MI), a CXCR2 antagonist.

Cytokine Measurement
The serum interleukin 6 (IL-6) level was determined with a 7TLD1 bioassay. Samples were assayed for cytokines and chemokines, such as KC and Mip2, using Luminex technology (Bio-Rad, Nazareth, Belgium) in accordance with the manufacturer’s protocol. Levels of IFN-β in serum were determined by an enzyme-linked immunosorbent assay (PBL Biomedical Laboratories, Piscataway Township, New Jersey).

Quantitative Real-Time Polymerase Chain Reaction (qPCR) Analysis
Tissues samples were collected in RNA Later (Qiagen, Limburg, The Netherlands), and RNA was isolated with the RNeasy Mini Kit (Qiagen). RNA concentration was measured with the Nanodrop 1000 (Thermo Scientific, Zellik, Belgium), and complementary DNA was prepared with Superscript II (Invitrogen). qPCR was performed using the Roche LightCycler 480 system (Applied Biosystems, Foster city, California).

Differential Cell Counts in Peritoneum and Circulation
Blood was collected in ethylenediaminetetraacetic acid–coated tubes (Sarstedt, Essen, Belgium). Cells were counted with a hematology analyzer. Peritoneal lavage was performed with 4 mL of PBS containing 1 mM ethylenediaminetetraacetic acid. Neutrophil migration into the peritoneal cavity was assessed by fluorescence-activated cell sorter analysis.

Determination of Bacterial Load
Serial dilutions of peritoneal lavage were prepared in sterile PBS for plating on brain-heart-infusion agar plates. Plates were incubated at 37°C overnight. Viable counts of bacteria were expressed as log10 colony-forming units per peritoneal cavity.

P. aeruginosa Infection of Human Blood
Anti-IFNAR Ab or isotype control was added to each well containing 2.5 mL of blood, for a final concentration 25 µg/mL. After incubation for 30 minutes, heat-killed P. aeruginosa was added at final concentration 3 × 106 colony-forming units/mL. The plates were incubated at 37°C. After 4 and 20 hours of incubation, plasma samples were collected, and the cell pellet from each well was resuspended in 2.5 mL of PBS and transferred to PAXGene tubes (Qiagen). RNA was extracted according to the manufacturer’s recommendations, using the PAXGene Blood RNA kit (Qiagen), and purified RNA was used for expression analysis for IFN-stimulated genes. For detection of intracellular keratinocyte chemoattractant (KC; CXCL1), human whole blood was treated with P. aeruginosa overnight in the presence of transport inhibitor cocktail (eBioscience, Vienna, Austria) and stained with anti-CD14 or anti-CD16. After red blood cells were lysed, cells were fixed and permeabilized and stained with anti-human CXCL1 Ab (eBioscience). Samples were acquired on an LSRII flow cytometer (Becton Dickinson, Erembodegem, Belgium) and analyzed using FlowJo software.

Statistical Analysis
Survival curves (Kaplan-Meyer plots) were compared by a log-rank test, and final outcomes were compared by a χ2 test. Data were expressed as means ± standard error. The statistical significance of differences between groups was evaluated with the Student t test (with 95% confidence intervals) and with 1-way or 2-way analysis of variance.

RESULTS
Genetic Deficiency and Ab-Mediated Neutralization of IFNAR1 Protect Mice Against LPS
We confirmed the detrimental role of type I IFNs in the acute inflammatory response to LPS [12]. IFNAR1 knockout and
control mice were injected with different doses of LPS (100 μg [Figure 1A] and 250 μg [Figure 1B]), and mortality was monitored. IFNAR1 knockout mice were resistant to high doses of LPS and exhibited lower levels of circulating IL-6 than control mice (Figure 1E). Blocking IFNAR1 with the MAR1-5A3 monoclonal Ab 2 hours before LPS administration resulted in similar resistance (100 μg [Figure 1C] and 250 μg [Figure 1D]). The resistance of Ab-treated mice to LPS was also reflected in lower serum IL-6 levels, compared with control mice (Figure 1E). The specificity of the anti-IFNAR1 Ab was confirmed by showing that the Ab has no protective effect against LPS in IFNAR1 knockout mice (Figure 1F).

Blocking IFNAR1 significantly reduced the induction of proinflammatory genes, such as Il12, in the liver (Figure 1G). In addition, the production of various IFN-stimulated response element (ISRE)–dependent genes, such as Ifit2, was absent in the anti-IFNAR1 treated mice after injection of LPS (Figure 1H). Collectively, these data suggest that type I IFNs exert toxic effects during endotoxemia, leading to increased cytokine production and eventually death.

**Genetic Deficiency and Ab-Mediated Neutralization of IFNAR1 Protects Mice Against CLP-Induced Sepsis**

Next, we examined the effects of type I IFN signaling on host immunity in a model of polymicrobial sepsis, namely the CLP sepsis model [3]. IFNAR1 knockout mice treated with broad-spectrum antibiotics were significantly protected from lethal CLP in comparison to similarly treated control mice (Figure 2A). IFN-β was detected in the circulation of C57BL/6 mice at different time points following CLP (Figure 2B).

In addition, the IFNAR1-neutralizing Ab can protect mice from death following CLP. Male C57BL/6 mice were injected with anti-IFNAR1 Ab, control Ab, or PBS, and 2 hours later all mice were subjected to CLP. At different times (8 and 24 hours) after surgery, the mice were again injected with Ab or PBS. Survival was significantly higher in mice treated with the anti-IFNAR1 Ab than in control mice (Figure 2C). In addition, IL-6 levels, especially at the later time points, were lower in the anti-IFNAR1–treated group (Figure 2D). These data suggest that the Ab that neutralizes type I IFN signaling might be considered as therapy for sepsis.

The anti-IFNAR1 Ab was also effective when given after the initiation of sepsis. Mice were subjected to severe CLP and treated with antibiotics, and 6 and 24 hours after the onset of sepsis they were injected with either anti-IFNAR1 Ab or control IgG Ab (Figure 2E). In addition, we found that the antibiotic regimen of 2 doses, as used in all other experiments, improved the survival rate significantly in the Ab-treated group, in contrast to an early treatment with 1 dose of antibiotics (Figure 2F).

In conclusion, our data illustrate that type I IFN signaling decreases host resistance against CLP-induced sepsis, in combination with an adequate regimen of broad-spectrum antibiotics.

**Neutralization of IFNAR1 Results in Increased Bacterial Clearance Due to Enhanced Neutrophil Recruitment**

First, we quantified the bacterial content of peritoneal lavage 6, 12, and 48 hours after CLP. Six and 48 hours after CLP, mice treated with anti-IFNAR1 had significantly less bacteria than those treated with control Ab (Figure 3A). This indicates that type I IFNs decrease the host’s ability to clear the bacteria.

Early after CLP, the numbers of circulating white blood cells, more specifically, neutrophils, dropped, particularly in mice treated with anti-IFNAR1 Ab. Twelve and 48 hours after CLP, this drop was similar in mice treated with anti-IFNAR1 or control immunoglobulin G Ab (Figure 3B). Upon CLP-induced sepsis, there was a steady accumulation of leukocytes in the peritoneum, mainly due to the influx of neutrophils. The number of peritoneal neutrophils early after CLP (6 hours) was higher in mice with neutralized IFNAR1 signaling than in control mice (Figure 3C).

We found that the circulating levels of the chemokines CXCL1 (KC) and CXCL2 (macrophage inflammatory protein 2 [MIP-2]) increased rapidly after CLP. Interestingly, the KC levels were found to be higher in mice treated with anti-IFNAR1 Ab than in control mice, whereas the MIP-2 levels were similar in both groups (Figure 3D). Next, by blocking neutrophil migration by using a selective nonpeptide inhibitor of CXCR2, which is the chemokine receptor for KC [17], we showed the involvement of neutrophils in the increased resistance of IFNAR1 knockout mice (Figure 3E). In addition, the presence of the CXCR2 antagonist resulted in reduced recruitment of neutrophils into the peritoneal cavity in anti-IFNAR Ab–treated mice (Figure 3F).

**P. aeruginosa Infection of Human Blood Samples Leads to IFN-β Production and IFN Responses**

Four and 20 hours after exposure of heat-killed *Pseudomonas aeruginosa* to human whole blood, expression of IFN-dependent genes such as Ifit2 (Figure 4B) and Rsad2 (Figure 4C) was upregulated in cells. Additionally, increased production of IFN-β (Figure 4A) and CXCL10 (Figure 4D) was observed in the plasma 20 hours after treatment. Consequently, IFNAR1 blockade significantly blocked the expression of Ifit2 and Rsad2 transcripts (Figure 4B and 4C) and CXCL10 protein (Figure 4D). The higher levels of IFN-β after neutralization of type I IFN signaling (Figure 4A) are consistent with previous findings by Rathinam et al [18]. No IFN-α was detectable in the plasma after blood was exposed to *P. aeruginosa* (data not shown), likely because IFN-α production was below the detection threshold or because the IFN-β priming required for significant IFN-α production was absent [19, 20]. Furthermore, in human whole blood samples we showed that KC is produced by monocytes and to a lesser extent by neutrophils after treatment with *P. aeruginosa* (Figure 4E). Note that the production of KC by other peripheral blood cellular subsets cannot be excluded by this analysis.
Figure 1. Genetic and pharmacologic inhibition of type I interferon (IFN) receptor 1 (IFNAR1) protects mice against lipopolysaccharide (LPS)–induced shock. A and B, Genetic inhibition of IFNAR1 improves survival from endotoxemia. Female IFNAR knockout (KO) mice (○, n = 9) and wild-type (WT) mice (▪, n = 8) were injected intraperitoneally with 100 μg (A) or 250 μg (B) of LPS. C and D, Pharmacologic inhibition of IFNAR1 improves survival against cecal ligation and puncture (CLP)–induced sepsis. Female C57BL/6 mice were treated with either 1 mg of anti-IFNAR1 antibody (Ab; ○, n = 7), 1 mg of control immunoglobulin G (IgG) Ab (▲, n = 7), or phosphate-buffered saline (PBS; ■, n = 6). Two hours later, they were injected intraperitoneally with 100 μg (C) or
DISCUSSION

Sepsis is a serious condition that is highly prevalent in intensive care units and is associated with high mortality rates. Current management of sepsis relies on supportive treatment, so research has focused on finding new therapeutic targets, which requires a better understanding of the pathophysiological and immunological features of sepsis. IFNAR1 knockout mice are resistant to multiple inflammatory models, such as endotoxemia [12], TNF-α-induced shock [21], infectious models (CASP-induced sepsis [8]), and sterile sepsis models (liver ischemia/reperfusion model [22]). These findings clearly illustrate that type I IFN signaling is central in the innate immune responses.

In addition to the use of mice deficient for IFNAR1, we used a specific monoclonal Ab to block IFNAR1 (MAR1-5A3) [16], which is therapeutically more relevant. We demonstrate that this Ab can protect mice against LPS, which validates our data obtained with IFNAR1 knockout mice, as well as the use of this Ab in acute inflammation in mice. Our data also demonstrate that type I IFNs act as mediators of LPS-induced shock, likely by amplifying the production of proinflammatory molecules, eventually resulting in organ failure and death.

As the LPS model does not completely reflect the complexity of human sepsis, we also investigated the role of type I IFN signaling in sepsis by using a severe CLP model of polymicrobial sepsis [3, 23, 24]. Similar to therapeutic treatment of patients with sepsis, we treated the mice with broad-spectrum antibiotics twice during the process of sepsis to prevent bacteremia [25]. IFNAR1 knockout mice were significantly protected against CLP-induced sepsis, compared with control mice. This result is at odds with a report by Kelly-Scumpia et al, which showed that type I IFNs are necessary for host defenses against CLP-induced sepsis [15]. The discrepancy between our data and the dataset of Kelly-Scumpia et al is likely due to the use of different CLP procedures, such as the severity of CLP, determined by the percentage of cecum that is ligated and the number and size of punctures in the cecum [3]. We used a severe CLP model, whereas Kelly-Scumpia et al used a mild CLP procedure [15]. Recently, Qiu et al noted that patients with sepsis who are more likely to die will benefit from a therapeutic strategy [26]. Accordingly, blocking type I IFN responses will probably be more effective in our severe CLP model than in a nonsevere model [15]. Another variable that influences the mortality rate is the genetic background, which was different in the 2 studies: we used C57BL/6, whereas Kelly-Scumpia et al used 129S6/SvEv [15]. The genetic background is an important element in the response to sepsis, because allelic variability between inbred strains may modulate the response to injury [27]. Additionally, the optional use of supportive treatments, such as antibiotics, has a great influence on the outcome of CLP-induced sepsis [3]. In the study by Kelly-Scumpia et al no antibiotic treatment was used [15], whereas in our study antibiotics were given, which correlates better with the real-world clinical situation [28]. It has been shown that antibiotics can greatly change the penetration of a certain gene knockout in mice, compared with their littermate controls, in part because it causes the release of endotoxin from gram-negative bacteria [29, 30]. Accordingly, we found that the role of type I IFNs is indeed different depending on the antibiotic regimen used. In addition, the use of antibiotics prevents bacteremia and causes the release of endotoxins from gram-negative bacteria. Because we showed that blocking type I IFN signaling protects mice against endotoxic shock, we believe that the mouse sepsis model that uses antibiotics is closest to the real-world clinical situation and that the mechanism of protection is at the level of inhibition of the biological effects of endotoxins.

Neutralization of IFNAR1 function resulted in substantial survival benefit in the CLP sepsis model. Accordingly, blocking type I IFN signaling also clears persistent viral infections [31, 32]. The results of the studies described above allow us to conclude that type I IFN signaling may be detrimental in the systemic response to some infections, which is contrary to the initial thought that innate immune responses, including the induction of type I IFNs, are required to inhibit pathogen spread. These findings suggest that type I IFNs might be an interesting therapeutic target for the treatment of bacterial sepsis. However, the efficacy of new therapeutic agents should be tested when they are given after the initiation of sepsis. Previously, some therapies, such as Ab directed against macrophage migration inhibitory factor [33], high mobility group box 1 [34], and interleukin 17A [35], were beneficial in the CLP model even when treatment was delayed for several hours after the infection started. In our study, the anti-IFNAR1 Ab significantly prevented death even when it was administered up to 6 hours after CLP. Thus,

Figure 1 continued. 250 μg (D) of LPS. E, Serum interleukin 6 (IL-6) levels in IFNAR WT mice (left black bars, n = 8) and KO mice (left white bars, n = 9), as well as in mice treated with PBS (right black bars, n = 6), control IgG Ab (grey bars, n = 7), or anti-IFNAR1 Ab (right white bars, n = 7). All mice were injected intraperitoneally with 100 μg of LPS, and blood was sampled 6 hours later. F, Female IFNAR KO mice were treated with 1 mg of anti-IFNAR1 Ab (○, n = 6), 1 mg of control IgG Ab (△, n = 5), or PBS (■, n = 5), and 2 hours later all mice were injected intraperitoneally with 500 μg of LPS. Mortality in all experiments was monitored for 92 hours, after which no further deaths occurred. All experiments were performed at least twice. G and H, LPS-induced gene expression in liver is reduced in mice treated with 1 mg of anti-IFNAR1 Ab (white bars, n = 5), compared with mice treated with PBS (black bars, n = 5) or 1 mg of control IgG Ab (grey bars, n = 5). Two hours after administration of PBS or Ab, mice were challenged with 100 μg of LPS. Next, livers were obtained 0, 6, and 12 hours after LPS injection. Quantitative real-time polymerase chain reaction analysis was used to measure liver messenger RNA levels of Il12p70 (G) and Ifih1 (H). Significance levels were calculated for differences between anti-IFNAR-treated mice and each control group, as indicated in the figures. Error bars represent the mean ± standard error. *P < .05, **P < .01, and ***P < .001.
an anti-IFNAR1 Ab could also be effective for the management of ongoing acute severe sepsis.

The survival benefit granted by the Ab against IFNAR1 was associated with a reduction of the systemic inflammatory response, as shown by a decrease in serum IL-6 concentrations. However, there was much variation in IL-6 levels within the groups. IL-6 levels can be used to predict outcome in CLP sepsis [24, 36], with high levels strongly correlating with mortality [28]. Consistently, we observed high IL-6 levels in mice that eventually died from CLP, and the number of dead mice was lower in the group treated with the anti-IFNAR Ab.

Neutralization of IFNAR1 activity in the CLP model was also associated with reduced bacterial counts in the peritoneum. It has been reported that phagocytes, especially neutrophils, are the
Figure 3. Neutralization of type I interferon (IFN) receptor 1 (IFNAR1) reduces the peritoneal bacterial load due to increased neutrophil recruitment, which is in turn caused by increased levels of keratinocyte chemoattractant (KC).

A. Improved bacterial clearance in mice injected with antibody (Ab) against IFNAR1. Peritoneal lavage fluid was obtained 6, 12, and 48 hours after cecal ligation and puncture (CLP) from male C57BL/6 mice treated with 1 mg of anti-IFNAR1 Ab (○) or 1 mg of control immunoglobulin G (IgG) Ab (▪), and total bacterial counts were determined. Data are expressed as colony-forming units (CFU) per peritoneal cavity.

B. The numbers of white blood cells (left panel) and neutrophils (right panel) in circulation drop after the induction of septic peritonitis by CLP. The decrease was greater among mice treated with 1 mg of anti-IFNAR1 Ab (white bars) than among those treated with 1 mg of control IgG Ab (black bars; n = 5–14 per time point). The differences between the 0-hour time point and each additional time point in both treatment groups were significant (not depicted in graph).

C. Neutralization of IFNAR1 leads to a greater influx of neutrophils into the peritoneum. Peritoneal cells from male C57BL/6 mice treated with 1 mg anti-IFNAR1 Ab (white bars) or 1 mg control IgG Ab (black bars; n = 6–12 per time-point) were quantified 6, 12, and 48 hours after CLP.

D. Kinetics of KC (CXCL1) levels (full lines) and macrophage inflammatory protein 2 (MIP-2 [CXCL2]) levels (dotted lines) in peritoneal washes of male C57BL/6 mice subjected to CLP and treated with 1 mg of anti-IFNAR1 Ab (○) or 1 mg of control IgG Ab (▪; n = 5–20 per time point).

E. Resistance of IFNAR1-deficient mice to CLP is dependent on neutrophil attraction. IFNAR1 knockout (KO) mice (○; n = 14–15) were injected with solvent (dimethyl sulfoxide [DMSO]; full lines) or CXCR2 antagonist (dotted lines) 1 hour before CLP. Significance levels were calculated for differences between the anti-IFNAR Ab–treated group and the control group at the different time points. All experiments were performed at least twice, and the results from the independent experiments were pooled.

F. The greater influx of neutrophils into the peritoneum after neutralization of IFNAR1 is dependent on CXCR2 activity. Neutrophils from male C57BL/6 mice treated with 1 mg of anti-IFNAR1 Ab or 1 mg of control IgG Ab in the presence of DMSO or CXCR2 antagonist (n = 6 per group) were quantified 6 hours after CLP. NS, no significant difference. Error bars represent the mean ± standard error. *P<.05, **P<.01, and ***P<.001.
primary mediators of bacterial clearance during septic peritonitis [17]. A reduction in early neutrophil migration to infection sites is associated with more peritoneal bacteria and a poor outcome in patients with sepsis [37]. Also, severe sepsis after CLP is associated with the failure of neutrophil migration [38].

We consistently observed improved neutrophil migration into the peritoneal cavity after blocking IFNAR1. Increased neutrophil recruitment into the peritoneum has been linked to better control of bacterial growth and consequently improved survival of the host [17], so we speculate that the inhibition of neutrophil

**Figure 4.** Heat-killed *Pseudomonas aeruginosa* induced interferon β (IFN-β) production from human whole blood, and anti-type I IFN receptor 1 (IFNAR1) treatment blocked IFN-mediated inflammation. Fresh human blood samples (n = 3) were incubated with phosphate-buffered saline (PBS), control antibody (Ab), or anti-IFNAR Ab for 30 minutes, and heat-killed *P. aeruginosa* was added. After 20 hours, levels of IFN-β (A) and CXCL10 (D) were measured in plasma, and expression of IFN-dependent genes, such as *Ift21* (B) and *Rsad2* (C), in whole blood cells was measured 4 and 20 hours later. Significance of the difference was calculated between the anti-IFNAR–treated group and the control group, as indicated in the graphs. E, Expression of keratinocyte chemo-attractant (KC; CXCL1) in monocytes and neutrophils. Monocytes and neutrophils were identified from human blood treated with PBS or *P. aeruginosa* with anti-CD14 or anti-CD16 staining, respectively. Next, these cells were fixed, permeabilized, and stained intracellularly with an anti-CXCL1 antibody. This experiment was repeated using specimens from 2 different donors. Error bars represent the mean ± standard error. *P < .05, **P < .01, and ***P < .001.
influx by type I IFNs contributes to their toxic effects in severe sepsis. It has been reported that type I IFNs play an important inhibitory role in regulating neutrophil recruitment and turnover during CASP-induced sepsis [8], Leishmania infections [39], Listeria infections [40], and Yersinia pestis infections [41].

Furthermore, neutrophils obtained from severely septic patients displayed a marked reduction in the neutrophil chemotactic response [37]. Normally, immune system activation by bacteria results in increased levels of circulating chemokines, of which KC and MIP-2 contribute greatly to neutrophil migration and, consequently, to clearance of bacteria and improved survival after CLP [17, 42]. Accordingly, reduced neutrophil migration during severe sepsis has been associated with down-regulation of CXCR2, the receptor for KC and MIP-2 [37, 43]. We also observed higher levels of KC when type I IFN signaling was blocked with the anti-IFNAR1 Ab. This finding is in agreement with previous reports that claim IFNAR1 knockout mice are resistant to secondary Streptococcus pneumoniae and Listeria monocytogenes infections because of higher production of KC and MIP-2 and subsequent adequate neutrophil responses during the early phase of host defense [40, 44]. These findings suggest an inhibitory effect of type I IFNs on the production of CXCR2-binding chemokines and subsequent neutrophil accumulation. A repressive effect of IFN-β on the expression of several chemokines, such as KC, CXCL3, and CXCL8, has already been reported in peripheral blood mononuclear cells [45]. However, the mechanism by which type I IFNs inhibit KC production remains to be elucidated. A recent report showed that type I IFNs inhibit FOXO3 [46]. Because in silico analysis of the Cxcl1 promoter shows the presence of FOXO3 binding sites [47], the inhibition of KC induction by type I IFNs via FOXO3 is a possible mechanism to further investigate. In addition, because KC is functionally related to CXCL8 (ie, by recruiting neutrophils to areas of inflammation), they might share a common mechanism of regulation. It was shown that IFN-β increases binding of corepressors to the promoter region of CXCL8, leading to reduced histone acetylation and subsequent lower transcription of CXCL8 [48]. A causative role of increased neutrophil migration after IFNAR1 blocking in the protection against sepsis was supported by our results obtained with the CXCR2 receptor antagonist [17]. Figure 5 depicts a model that shows how type I IFNs play a detrimental role in the outcome of CLP-induced sepsis.

In support of a role of type I IFNs in human sepsis, we showed that human blood cells produced IFN-β upon infection with the gram-negative bacterium P. aeruginosa and that these...
cells express typical ISRE genes in a strictly IFNAR1-dependent way. Because gram-negative bacteria are among the most common isolates in patients with sepsis [49], we believe that this suggests a role for type I IFN responses in the pathology of human sepsis. Therefore, blocking type I IFNs or their receptors in combination with antibiotics might offer a new strategy for the management of patients with severe sepsis.

Notes

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