Recombinant Human Interleukin-11 Has Anti-inflammatory Actions Yet Does Not Exacerbate Systemic Listeria Infection

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To determine whether recombinant human (rh) interleukin (IL)–11 disrupts the clearance of microbial pathogens, mice were challenged with Listeria monocytogenes after receiving high-dose rhIL-11, anti–tumor necrosis factor (TNF) monoclonal antibody (MAb), anti–IL-11 MAb, or saline control. The LD₅₀ was not affected by rhIL-11 but was 10-fold lower in the anti–TNF MAb group (P < .001). Plasma IL-6, IL-1β, and TNF-α levels were not different between rhIL-11-treated animals and the control group; however, interferon-γ levels were significantly reduced by IL-11 treatment (2477 vs. 0 pg/mL, P < .01). Compared with the control group, the quantitative level of L. monocytogenes in hepatic and splenic tissue was unchanged by rhIL-11 but was significantly increased by TNF or IL-11 inhibition. The results indicate that IL-11 down-regulates cytokine production but does not exacerbate systemic infection in the murine Listeria infection model.

Interleukin (IL)–11 is a multifunctional cytokine with a number of favorable attributes that may prove therapeutically useful in clinical medicine. In addition to its recognized platelet restorative properties following cytoreductive chemotherapy [1], IL-11 acts as a general hematopoietic growth factor and works in concert with other growth factors to support erythocyte and platelet precursors. IL-11 is a member of the Gp130 receptor ligand family and possesses numerous physiologic activities that affect a wide variety of tissue responses. It promotes epithelial cell growth [2], cellular recovery after exposure to cytotoxic radiation and chemotherapeutic agents [3], activation of hepatic acute phase protein synthesis [1, 3], and has immunomodulatory activities [4].

IL-11 up-regulates the synthesis of IκB-α and IκB-β in mononuclear cell lines resulting in diminished nuclear translocation of NFκB and reduction in proinflammatory cytokines [5]. It also has immunologic activities similar to Th2 type cytokines and attenuates interferon (IFN)–γ synthesis by CD4 T lymphocytes [4, 6]. A number of these advantageous properties of recombinant human (rh) IL-11 may be therapeutically useful in a variety of immunologically mediated proinflammatory disorders [3–6].

In the process of evaluating anti-inflammatory modulators, it is important to assess the potential of these agents to disrupt host defense mechanisms and exacerbate systemic infection. Inhibition of tumor necrosis factor (TNF) [7], IL-1 activity [8], combinations of inhibitors [9], and administration of other anti-inflammatory cytokines [10] can potentiate systemic infections. The following experiments were undertaken to determine the potential risk of rhIL-11 to facilitate systemic bacterial infection in the murine Listeria infection model. Listeria monocytogenes is an intracellular bacterial pathogen that requires a coordinated cell-mediated host immune response with contributions from TNF, IL-1, and IFN-γ for effective clearance [7, 8, 11].

Methods

Animal model. Specific pathogen-free female C57/BL6 mice (Charles River Breeding Laboratories, Wilmington, MA) weighing 15–20 g were intravenously challenged with L. monocytogenes (ATCC strain 19115) via tail vein injection at doses of 10⁷–10⁸ cfu/mL. Before experiments were initiated, animals were given 1 week to acclimatize to the laboratory environment. They were kept in a pathogen-free environment in 12 h day/night light cycles in biosafety hoods. The animals were allowed to eat and drink sterile water ad libitum.

The L. monocytogenes cultures were maintained on McBride Listeria agar (Becton Dickinson Microbiology Systems, Cockeysville, MD). The bacteria were grown to mid-log growth in trypticase soy broth (Becton Dickinson Microbiology Systems), centrifuged at 800

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The experimental protocol was approved by the university animal care committee and was in compliance with Institution Animal Care and Use Committee guidelines for use of animals in biomedical research.

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g for 15 min, and resuspended in PBS prior to injection. Bacterial concentration in PBS was measured photospectrometrically and confirmed by standard dilution and plating methods.

One hour before intravenous challenge with *Listeria monocytogenes*, the animals were pretreated intravenously with 1 of the following agents: 150 mg/kg rhIL-11 (Genetics Institute, Cambridge, MA) every 24 h for 7 days; 20 mg/kg anti-TNF monoclonal antibody (MAb) (anti-TNF, gift of Celltech, Slough, UK); 10 mg/kg anti–IL-11 monoclonal antibody (MAb) (anti–IL-11), and anti–tumor necrosis factor MAb (anti-TNF).

Forty-eight hours after intravenous *Listeria* challenge, animals (*n* = 10 in each group) were sacrificed for cytokine analysis and quantitative colony counts of liver and spleen tissue. Each animal underwent necropsy. Quantitative colony counts for *L. monocytogenes* from liver and spleen tissue were made by weighing the organs, suspending homogenized tissue in PBS, and serially diluting the samples on *Listeria* agar. For lethality assays, the animals were followed for 7 days before sacrifice. The LD₅₀ was determined after logarithmic transformation of the data by the method of Reed and Muench [12].

Cytokine determinations were measured from plasma at 48 h. EIA's were used to measure murine IL-1β, IL-6, and IFN-γ (Bio-Science International, Camarillo, CA). The limit of detection of each assay was 2 pg/mL. TNF-α determinations were measured by bioassay using the L929 cytotoxicity assay (limit of detection, 1 pg/mL).

**Statistical methods.** Since the results of the cytokine analyses and quantitative colony counts were not normally distributed, the data are shown as median values with 25%-75% interquartile ranges. Data were analyzed by nonparametric 1-way analysis of variance for multiple groups. *P < .05* was considered significant.

**Results**

**LD₅₀ and microbiology findings.** The LD₅₀ of *L. monocytogenes* in mice treated with rhIL-11 (150 mg/kg daily for 7 days) did not differ significantly from the control group (log₁₀ 4.67 [rhIL-11] vs. 4.74 [control group]; *P* = not significant [NS]) for each group of 40 animals. The LD₅₀ in the anti–TNF MAb–treated group (*n* = 25) was 10-fold lower than in the IL-11 treatment group or the control group (2.97; *P < .001*). The LD₅₀ in the mice treated with anti–IL-11 MAb (*n* = 25) was unchanged from the control group at 4.77 vs. 4.74 (*P* = NS).

The quantitative colony counts of hepatic and splenic tissue from each treatment group are shown in figure 1. Organ tissue colony counts were markedly increased with anti-TNF treatment, whereas the IL-11–treated animals showed no differences compared with the control group. There was a modest but significant elevation of colony counts in liver and splenic tissue in animals treated with anti–IL-11 antibody (figure 1).

The IL-6 and IFN-γ levels in the circulation 48 h after the *Listeria* challenge were significantly increased in the anti–TNF MAb–treated group compared with the control group (*P < .01*) (shown in figure 2 as the median with 75th percentile interquartile range). IL-6 levels were highly variable and did not differ in animals treated with rhIL-11 or with anti–IL-11 MAb compared with the control group. IFN-γ levels were significantly reduced by rhIL-11 treatment (*P < .01*), significantly increased by anti–TNF MAb (*P < .01*) and unchanged by anti–IL-11 MAb treatment (*P* = NS). The median values (pg/mL) in each group were 2447 (control), 0 (rhIL-11), 4170 (anti–TNF MAb), and 947 pg/mL (anti–IL-11 MAb). Median values of TNF by bioassay were below the limits of detection (1 pg/mL) in each treatment group and did not differ between groups. Similarly, the circulating levels of IL-1β were <50 pg/mL 48 h after bacterial challenge in each treatment group. The only difference observed was slightly higher blood levels of IL-1β in the anti–TNF MAb–treated group (median, 45 [anti–TNF MAb–treated group]) compared with the control (median, 9 [control group]; *P < .01*).

**Figure 1.** Quantitative colony counts of *Listeria monocytogenes* strain in hepatic and splenic tissues after 48 h in mice treated with saline (PBS control), interleukin (IL)-11 (recombinant human IL-11), anti–IL-11 monoclonal antibody (MAb) (anti–IL-11), and anti–tumor necrosis factor MAb (anti-TNF).

**Figure 2.** Plasma interleukin (IL)-6 and interferon (IFN)–γ in circulation of mice 48 h after challenge with *Listeria monocytogenes*. Treatment groups: control (PBS), IL-11 (recombinant human IL-11), anti–tumor necrosis factor monoclonal antibody (MAb) (anti–TNF), and anti–IL-11 MAb (anti–IL-11). *P < .01*, IFN-γ–control vs. IL-11.
TNF] vs. 0 pg/mL [control]; \( P < .05 \). TNF-\( \alpha \) and IL-1\( \beta \) levels were low in all treatment groups.

Discussion

The results indicate that despite the capacity of rhIL-11 to attenuate the production of proinflammatory cytokines, the cytokine does not exacerbate infection or lead to excess lethality in the presence of a systemic challenge with \textit{L. monocytogenes} in mice. In these experiments, rhIL-11 treatment significantly reduced the systemic release of IFN-\( \gamma \) compared with the control group. This was previously observed in other in vitro and animal experiments and appears to be a direct effect of IL-11 on the synthesis of IFN-\( \gamma \) by activated lymphocytes [5, 6]. Administration of the anti–IL-11 MAb had minimal effects on cytokine responses and microbial clearance in these animals. This indicates that systemic levels of endogenous IL-11 are not essential in the primary innate immune response during systemic \textit{L. monocytogenes} infection in mice.

In contrast to the findings with rhIL-11 and anti–IL-11 MAb, the anti–TNF MAb markedly disrupted the host response to \textit{L. monocytogenes} infection with exacerbation of the infection and increased lethality. Despite the difficulty in measuring systemic levels of TNF in the animals, the removal of TNF activity greatly exacerbated \textit{L. monocytogenes} infection. These observations were previously observed with inhibitors of TNF [7] and with other inhibitors of the proinflammatory immune response [8–10]. The increase in colony counts in the anti–IL-11 MAb group remains unexplained and is the focus of further investigation.

The lack of worsening of systemic infection by rhIL-11 administration may have several potential explanations. First, rhIL-11 promotes an acute phase protein response and serves as a hematopoietic growth factor that supports granulocyte production and lymphocyte activity [13]. These attributes may assist in the host defense against invasive microbial pathogens. Second, IL-11 attenuates the host proinflammatory cytokine response but does not ablate the proinflammatory response to inflammatory stimuli [14]. This immune modulation may allow an appropriate host response to infection to occur while limiting the potentially deleterious effects of systemic release of proinflammatory cytokines. Third, IL-11 has no effect on the surface expression of major histocompatibility complex class II molecules, accessory molecules, such as B7.2, or a membrane-bound CD14 [13]. In this respect, IL-11 may not be as effective as a monocyte/macrophage deactivator as the potent anti-inflammatory cytokine IL-10 [10]. There is no increased synthesis of IL-10 after administration of IL-11 in experimental systems [6, 13]. Finally, rhIL-11 will diminish IL-2 and IFN-\( \gamma \) production in mixed lymphocyte cultures yet does not limit the generation of cytotoxic T lymphocytes [6].

As for the administration of any new immunobiologic agent, its potential to disrupt the host immune response to systemic infection must be carefully analyzed in the preclinical development of new treatments for human medicine [10]. The absence of exacerbation of \textit{Listeria} infection at doses up to 50-fold greater than those recommended in human clinical trials is reassuring [15]. Nonetheless, the potential for adverse host responses to infection must be carefully monitored as this and other potent immunomodulatory agents undergo extensive clinical testing in immunocompromised patients.

References

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