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Interleukin-10 suppresses natural killer cell but not natural killer T cell activation during bacterial infection

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Abstract

Interleukin (IL)-10 is an anti-inflammatory cytokine known to modulate the outcome of sepsis by decreasing pro-inflammatory cytokine production, including IL-12, a main activator of natural killer (NK) cells. We hypothesized that neutralization of IL-10 would increase NK and natural killer T (NKT) cell activation through increased IL-12 in a mouse model of bacterial peritonitis. NK and NKT cell activations were measured by CD69 expression on NK1.1+/CD3− and NK1.1+/CD3+ cells after cecal ligation and puncture (CLP). NK cells were significantly more activated in mice treated with anti-IL-10 antibodies, whereas no such effect was observed in NKT cells. Similarly, intracellular interferon gamma (IFN-γ) levels were increased in NK cells of anti-IL-10-treated mice, but not in NKT cells. IL-12 and IL-18 levels were increased in both CLP groups, but in anti-IL-10-treated mice, early IL-12 and late IL-18 levels were significantly higher than in controls. Survival at 18 h after CLP was lower in anti-IL-10 mice, which was associated with increased liver neutrophil accumulation. In summary, these data show an activating effect of IL-10 on NK, but not on NKT cells after CLP, which corresponded with decreased survival, higher IFN-γ production, and increased remote organ neutrophil accumulation. These effects were not mediated by IL-12 and IL-18 alone, and reinforce a role for NK cells in remote organ dysfunction following peritonitis.

Keywords: Sepsis; Peritonitis; CD69; Interferon gamma; Lymphocyte

1. Introduction

Despite dramatic advances in contemporary intensive care treatment, mortality from bacterial sepsis remains high, and was recently estimated to be over 50% in patients at 12 months following hospital discharge [1]. Multiple organ dysfunction syndrome (MODS) as a consequence of sepsis represents the main reason for this high mortality [2]. The pathogenesis of MODS has not been completely elucidated, but is thought to result in part from an imbalance of pro- and anti-inflammatory cytokines [3]. Interleukin (IL)-10 is a cytokine produced by T-helper (Th)-2 cells, natural killer (NK) cells, B-cells and macrophages, which promotes anti-inflammatory and immunosuppressive responses. IL-10 suppresses the production of pro-inflammatory cytokines such as IL-12 and IL-18 from macrophages, and thereby antagonizes pro-inflammatory stimuli to prevent potential harmful consequences of an over-exuberant inflammatory response [4].

The level of IL-10 has been shown to be a valid indicator of the severity of sepsis in human studies, and increased levels were associated with a higher onset of MODS [5] and septic

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shock [6]. This is thought to be secondary to an inability of IL-10 to control the pro-inflammatory response to overwhelming sepsis. When it is able to fully compensate for the inflammatory response, IL-10 reduces morbidity and mortality from multiple organ failure [7,8]. The therapeutic benefit of IL-10 in the treatment of bacterial sepsis, however, remains controversial. Pretreatment with IL-10 was shown to improve survival following cecal ligation and puncture (CLP) in mice, a widely used and clinically relevant rodent model of polymicrobial peritonitis [9]. In line with these findings, treatment of mice with anti-IL-10 decreased survival after CLP [10]. Others have not found such survival benefits by treatment with exogenous IL-10 [11], and together with the wide range of potential side-effects of IL-10, have dampened the enthusiasm for use of this cytokine as a treatment for septic patients.

NK and natural killer T (NKT) cells are major producers of cytokines during bacterial infection, in particular the pro-inflammatory cytokine interferon gamma (IFN-γ) during CLP [12]. NK and NKT cells have also been shown to regulate the immune response in different autoimmune and inflammatory disorders by altering the cytokine responses [13,14]. The potential of NK and NKT cells to produce both pro- and anti-inflammatory cytokines also provides them with potential for promoting or inhibiting multiple organ failure following bacterial sepsis. However, the control of the cytokine production by NK and NKT cells during bacterial sepsis has not been widely investigated.

IL-12 is considered a main activator of NK cells, and was shown to increase their cytotoxic capacity in vitro and in vivo [14,15]. IL-12 is also known to be important in survival following CLP [16], and levels of IL-12 can be suppressed by IL-10. IL-18 is another important pro-inflammatory cytokine produced by macrophages, and has also been shown to be suppressed in part by IL-10 [17]. IL-18 acts alone, or synergistically with IL-12, to activate NK cells [18,19].

In this study we investigated the effects of blocking IL-10 in mice with a neutralizing antibody on the activation and cytokine production of NK and NKT cells following CLP. Our data indicate that IL-10 regulates the activation of NK cells but not NKT cells, and that this activation of NK cells is associated with remote organ damage following CLP. This may provide further evidence for the role of NK and NKT cells in organ dysfunction, as well as a novel mechanism for IL-10 in preventing such dysfunction following bacterial infection.

2. Materials and methods

2.1. Animals

C57BL/6 mice (aged 6–8 weeks) were obtained from Jackson Laboratories (Bar Harbor, ME). All animals were housed in a facility approved by the American Association for Accreditation of Laboratory Animal Care and provided with food and water ad libitum. Studies were carried out according to the National Institutes of Health guidelines under supervision of a certified veterinarian.

2.2. Cecal ligation and puncture

Mice were anesthetized with inhaled isofluorane (Abbott Laboratories, North Chicago, IL). CLP was performed as previously described [20]. Briefly, the cecum was exposed through a midline laparotomy incision and ligated just below the ileocecal junction with 4-0 silk suture and two 18-gauge through-and-through needle punctures were made in the cecum. The cecum was then returned to the peritoneal cavity, and the abdominal incision was closed in layers. This provides a relatively severe infectious stimulus in which most animals die within 48 h. For immunoneutralization experiments, 60 μg of either rat anti-mouse IL-10 monoclonal antibody or control immunoglobulin (IgG; Biosource, Camarillo, CA), was injected into the tail vein immediately prior to CLP.

2.3. Timed harvests

Mice were euthanized at 1.5, 3, 6 or 18 h after CLP under ketamine (80 mg/kg) and xylazine (16 mg/kg) anesthesia by intramuscular injection. Peritoneal exudate cells (PEC) were recovered by peritoneal lavage with 4 mL of ice-cold, heparinized, RPMI 1640 medium (GIBCO/BRL, Bethesda, MD) and were counted manually using a hemocytometer. Cells were collected by centrifugation and analyzed by flow cytometry as described below. PEC supernatants were collected and stored at −20 °C for later cytokine analysis. Liver and lung tissues were collected for determination of myeloperoxidase (MPO) levels, and bacterial counts were determined in liver tissue. Blood was collected in a heparinized syringe by cardiac puncture for evaluation of bacterial and leukocyte counts. Bacterial levels in the lavage fluid were determined as described below. Serum was collected by centrifugation and stored at −20 °C for later cytokine analysis. Spleen cells were isolated from whole spleen through 0.7 μm sterile filters, and counted manually using a hemocytometer. Cells were analyzed by flow cytometry, NK and NKT cells were isolated, and splenic production of IFN-γ was determined by cell culture with 5 μg/mL of the mitogen concanavalin A for 48 h at 37 °C/5% CO_2. Spleen cells were plated on 24-well plates at 1×10^6 cells/mL following hypotonic lysis of red blood cells and two washes in 10 mL phosphate buffered saline (PBS; Sigma, St. Louis, MO).

2.4. Myeloperoxidase assay

Myeloperoxidase (MPO) was used as an indirect measure of tissue neutrophil accumulation. Liver and lung tissues (40–60 mg) were homogenized in 20 mM phosphate buffer (pH 7.4), and then centrifuged at 10,000 rpm for 15 min. Tissue cell pellets were resuspended in 50 mM phosphate buffer (pH 6.0) with 10 mM EDTA and 0.5% hexadecyltrimethylammonium bromide. The solubilized pellets were frozen, thawed, and heated for 2 h at 60 °C, sonicated for 2 s, and then refrozen. The myeloperoxidase levels were determined spectrophotometrically as previously described, using tetramethylbenzidine as the color reagent [21].