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Interleukin 17A plays a role in lipopolysaccharide/D-galactosamine–induced fulminant hepatic injury in mice

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ABSTRACT

Background: Lipopolysaccharide/D-galactosamine (LPS/GalN)–induced hepatic injury is an experimental model of fulminant hepatic failure in which tumor necrosis factor alpha (TNF-α) plays a pivotal role. Moreover, it was reported from our laboratory that interleukin (IL) 17A enhanced production of TNF-α by the Kupffer cell.

Objective: The purpose of this study was to determine the role of IL-17A in LPS/GalN-induced hepatic injury in mice.

Methods: LPS/GalN was injected into three mouse models: wild-type (WT) mice, IL-17A knockout (KO) mice, or IL-17A KO mice treated with recombinant mouse (rm) IL-17A homodimer (KO + rmIL-17A). Survival was assessed for 24 h after LPS/GalN injection, and histopathologic findings were evaluated at various time points after LPS/GalN injection for neutrophil and apoptosis markers. After LPS/GalN injection, expression of the inflammatory mediators TNF-α, IL-6, monocyte chemotactic protein 1, IL-17A, high-mobility group box 1, and soluble intercellular adhesion molecule 1 was assessed in serum by enzyme-linked immunosorbent assay.

Results: Survival was higher in KO mice compared with WT mice after LPS/GalN injection. However, in KO + rmIL-17A mice, mortality was not significantly different compared to the other groups. Neutrophil infiltration and apoptosis were significantly greater in WT mice than KO mice. Furthermore, serum alanine aminotransferase, serum TNF-α, monocyte chemotactic protein 1, IL-17A, high-mobility group box 1, and soluble intercellular adhesion molecule 1 levels were also significantly greater in WT mice than KO mice. In KO + rmIL-17A mice, these levels were similar to those in WT mice.

Conclusions: IL-17A is a key regulator in hepatic injury caused by neutrophil-induced inflammatory responses after LPS/GalN injection.

1. Introduction

Fulminant hepatic injury is often a fatal condition resulting in hepatocellular apoptosis and hemorrhagic necrosis, leading to multiple organ failures [1,2]. Fulminant hepatic injury has a variety of causes, such as hepatitis virus infection, toxic insult, autoimmunity, liver transplantation, drugs, or ischemia and/or reperfusion [3–6]. Hepatic injury, such as

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dysfunctions of hepatic microcirculation, metabolic changes, direct cytotoxicity, and macrophage-mediated hepatic injury, occurs in lipopolysaccharide (LPS)-treated mice [7]. Furthermore, D-galactosamine (GalN; as a specific hepatotoxic agent) has been shown to increase sensitivity to the lethal effects of endotoxin [8]. Hepatic injury induced by LPS in combination with GalN is similar to acute hepatic failure in the clinical setting [9]. Therefore, LPS/GalN-induced hepatic injury is used as an animal model of fulminant hepatic injury in humans [10–12].

The hepatic macrophage, the Kupffer cell, is known to produce a variety of growth and immunomodulating mediators that have stimulatory and inhibitory effects on hepatic injury [13]. It has been reported that hepatic macrophages are the major source of tumor necrosis factor alpha (TNF-α) and interleukin (IL) 6, and depletion of hepatic macrophages impairs hepatic injury [14–16]. IL-17A was originally described and cloned by Rouvier *et al.* [17] and named cytotoxic T-lymphocyte-associated serine esterase 8. IL-17A, a cytokine produced by various cells including Th17, T cells, and CD8-positive T cells, is elevated in inflammatory conditions, such as inflammatory myopathies, rheumatoid arthritis, asthma, ulcerative colitis, and multiple sclerosis [18–22]. It was reported from our laboratory that IL-17A increases TNF-α production by isolated Kupffer cells *in vitro* [23]. These results support the hypothesis that IL-17A may be involved in fulminant hepatic failure; however, its role has not been fully elucidated. Therefore, the specific purpose of the present study is to investigate the role of IL-17A in fulminant hepatic failure.

2. Materials and methods

2.1. Animals

Male wild-type (WT) mice (C57BL/6, 20–25 g, 8–9 weeks of age, obtained from Jackson Laboratories, Bar Harbor, ME) and male IL-17A knockout (KO) mice (backcrossed onto C57BL/6) were housed in a clean, temperature-controlled environment with a 12-h light-dark cycle and were given free access to regular laboratory chow diet and water for several days. All animals received humane care, and the study protocols were approved by the Committee of Laboratory Animals at University of Yamanashi according to institutional guidelines. Mortality study was performed with eight animals of each groups, and time point study was performed with six to eight animals at each time points (0, 1, 2, 4, and 8 h after LPS/GalN injection).

2.2. Treatment with recombinant mouse IL-17A homodimer or phosphate-buffered saline in IL-17A KO mice before LPS/GalN injection

Recombinant mouse (rm) IL-17A homodimer (R&D Systems, Minneapolis, MN) was diluted in sterile phosphate-buffered saline (PBS) and administered intraperitoneally (i.p.) at a dose of 0.5 μg per mouse in a total volume of 100 μL at 6 h before LPS/GalN i.p. ($n = 8$). The concentration of rmIL-17A homodimer to be administered was determined in preliminary dose-response experiments, which showed that 0.5-μg IL-17A consistently produced a significant influx of neutrophils [23].

2.3. Treatment of mice with LPS/GalN injection

LPS and D-GalN were purchased from Sigma Aldrich (St. Louis, MO). The mice were divided into the following groups: WT mice treated with LPS/GalN and PBS (WT mice group), IL-17A KO mice treated with LPS/GalN and PBS (KO mice group) and IL-17A KO mice treated with LPS/GalN and rmIL-17A (KO [rmIL-17A] mice group). LPS/GalN was dissolved in 200-μL saline and injected i.p. at 2.5-μg/kg LPS and 300-mg/kg GalN.

2.4. Collection of samples

Blood samples were collected via the inferior vena cava at 0, 1, 2, 4, and 8 h after LPS/GalN injection, centrifuged, and stored at −80°C until assay. Tissue samples were also collected and weighed at designated times after i.p. and were stored at −80°C for further analysis. Samples were fixed in formalin or acetone, embedded in paraffin, and serially sectioned.

2.5. Immunohistochemical staining for neutrophils or Tdt-mediated dUTP-biotin nick labeling assay

For immunohistochemical staining of IL-17A in the liver, formalin-fixed, paraffin-embedded tissue specimens were cut into 4-μm sections. Each section was mounted on a silane-coated glass slide, deparaffinized, and soaked for 15 min at room temperature in 0.3% H_2O_2/methanol to block endogenous peroxidase. The rat antimouse neutrophil Ab (1/500, Caltag Laboratories, South San Francisco, CA) was applied overnight at 4°C. Peroxidase-linked secondary antibodies and diaminobenzidine (VECTASTAIN Elite ABC Kit; Vector Laboratories, Burlingame, CA) were used to detect specific binding. Sections were counterstained with hematoxylin. Morphometric analysis of immunoreactive neutrophils in liver sections was performed by quantifying the total numbers of immunoreactive cells in five random at 400 × magnification fields (four independent animals were analyzed for each experimental group).

Apoptosis was evaluated by the Tdt-mediated dUTP-biotin nick labeling (TUNEL) method using ApopTag Plus Peroxidase in Situ Apoptosis Detection Kit (Millipore, Billerica, MA) according to the manufacturer's instructions. Methyl green (0.5% wt:vol) was used for counterstaining. TUNEL-positive cells were observed under microscopy. At least five fields were examined for calculation of apoptosis index.

2.6. Measurement of plasma alanine aminotransferase

Plasma alanine aminotransferase (ALT) levels were measured to assess hepatic parenchymal damage using FUJI DRI-CHEM analyzers (Fujifilm Co, Tokyo, Japan).

2.7. Measurement of serum cytokines

Determination of serum TNF-α (R&D Systems, Minneapolis, MN), IL -6 (R&D Systems, Minneapolis, MN), sICAM-1(CD54) (R&D Systems, Minneapolis, MN), CCL2/JE/MCP-1 (R&D