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Inflammasome activation involved in early inflammation reaction after liver transplantation

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ABSTRACT

Liver transplantation has emerged as a vital therapy for end-stage liver diseases. Acute −phase inflammation play an important role in liver graft injury.Recent studies have revealed that inflammasome are responsible for initiating inflammation in early stage of acute organ rejection in liver transplantation, however the underlying mechanism remains unclear. Here we explored to block inflammasome activation to see whether it can alleviate early inflammation reaction during rejection of allgrafts in a rat model and gain further insights into the mechanism of inhibiting inflammation in allografts. By using Ac-YVAD-CMK, a highly selective caspase-1 inhibitor, to inhibit inflammation reaction involved in allograft rejection in a rat model. Our results showed that the rejection activity index (RAI) of Ac-YVAD-CMK-treated allografts is significantly diminished in similar magnitude to that of isografts. Compared with isografts, the expression of apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) and IL-1β in allograft group increased significantly with the development of rejection, exhibiting apparent correlation. Expression of IFN-γ mRNA in untreated allografts was maximal on day 3 while in Ac-YVAD-CMK-treated allografts and isografts, IFN-γ mRNA levels remained low over the duration of the time course. ELISA results revealed serum elevation of IL-1β by day 7 after othotopic liver transplantation (OLT) in comparison with isografts. There were no statistically significant differences between isografts and Ac-YVAD-CMK-treated allografts. For the first time, our data reveal that inhibition of the inflammasome activation pathway attenuates inflammation reaction of hepatic transplant rejection.

1. Introduction

Liver transplantation has become one significant treatment modality for patients with end-stage hepatic diseases, including liver cancer and acute liver failure, among others [1]. The survival rate after liver transplantation is today markedly elevated with improvement surgical techniques and development of new immunosuppressant agents since the first liver transplantation in 1963 [2]. However, rejection in liver transplant recipients still presents difficulties for clinicians and patients. Rejection may require re-transplantation, which further diminishes the pool of available organs [3–6].

Rejection after liver transplantation is usually characterized histologically by inflammatory cell infiltration, along with injury to the bile duct and parenchyma cells, including cell degeneration and tissue necrosis [7]. Hence, inflammation plays an important role in hepatic allograft transplantation. Cytokine secretion is one of the main factors involved in the inflammation process [8]. It has been reported that interleukin-1β (IL-1β) can mediate a wide range of immune and inflammatory responses [9]. Also, IL-1β expression is upregulated during allograft rejection as are certain other cytokines such as IFN-γ and IL-6 [10]. IL-1β is known to be involved in inflammation in graft rejection and ischemia/reperfusion injury (IRI) [11].

Secretion of mature IL-1β is a central event in the initiation of inflammation. It requires both intracellular up-regulation of pro-IL-1β and secretion of the bioactive molecule. The release of pro-IL-1β is a complex process involving proteolytic cleavage by caspase-1 [12,13] and the exact machnism of secretion is still poorly understood. Multiple accumulating reports have identified the important role of a cellular multisubunit complex, the inflammasome [14–16] that activates caspase-1, which goes on to further process the secreted cytokines IL-1β and IL-18. In the activation pathway involving IL-1β processing by the inflammasome, the "adaptor apoptosis-associated speck-like protein

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containing a caspase recruitment domain" (ASC) is critical in the assembly of the inflammasome and regulation of cytokine secretion [17].

The roles of the inflammasome in organ transplantation have been identified in certain reports. Inflammasome genetic variants identified in donors and recipients are closely related to clinical outcome after allogenic stem cell transplantation and can be a prognostic factor in donor selection [18]. In cardiac allograft rejection, ASC is upregulated [19], and inflammasomes are generated [20]. Moreover, gene silencing of one of the inflammasome structural components, nucleotide binding and oligomerization domain-like receptor (NLRP; NACHT, leucine-rich repeat, and pyrin domain-containing protein-3), can protect against IRI in mice [21].

Ac-YVAD-CMK is a selective irreversible inhibitor of caspase-1 (interleukin-1β converting enzyme, ICE) with some activity also against caspase-4, it has effects of anti-apoptosis, anti-inflammatory and neuroprotective resulting from its preventing caspase-1 activation of the proinflammatory cytokine interleukin-1β. In a rat liver transplantation model, we have demonstrated that the inflammatory reaction in allograft cohorts treated with Ac-YVAD-CMK was lessened compared to the control chort. The RAI of Ac-YVAD-CMK-treated allografts was reduced similar to the level of isografts. Compared with isografts, the expression of ASC and IL-1β in allografts, when investigated immunohistochemically, was significantly increased with the onset of organ rejection, exhibiting apparent linear correlation. ASC was mainly localized to the cytoplasm of inflammatory cells, and IL-1β was distributed in the interspace of hepatocytes around blood vessels and ducts. In Ac-YVAD-CMK-treated allografts, the expression pattern was similar to that seen in isografts. Expression of IFN-γ mRNA in untreated allografts was maximal on day 3, while in Ac-YVAD-CMK-treated allografts and isografts levels remained low during the time course examined. ELISA results demonstrated significant elevation of IL-1β in serum by day 7 after OLT, compared with isografts. There was no significant difference between isografts and Ac-YVAD-CMK-treated allografts. These results indicated that inhibition of inflammasomes activation may attenuate inflammation reaction of liver transplantation rejection.

2. Material and methods

2.1. Animals

Male (DA) and Lewis rats, aged between 10 and 12 weeks and weighing 220–250 g, were obtained from Vital River Laboratory Animal Technology Co. Ltd. in Beijing and were maintained in a pathogen-free animal facility. The rats were allowed free access to tap water and food. All procedures involving animals were approved by the Institutional Animal Care Committee according to the Animal Protection Act of China.

2.2. Orthotopic liver transplantation

Rats were randomly divided into three groups, one for each time point. DA livers were transplanted into Lewis recipients as allografts (DA Lewis), and Lewis livers were transplanted into the same strain as isografts (Lewis–Lewis). To detect the inflammasome activation pathway, we treated recipients of allogenetic OLT with Ac-YVAD-CMK (10 mg/kg/day, Bachem Co. Ltd.) through intraperitoneal injection each day after OLT (DA Lewis treated with Ac-YVAD-CMK served as another group). The time points for sample collection were days 1, 3, and 7 post-OLT. $n = 5$ for each time point and treatment. Naive hearts were used as controls ($n = 5$).

A rat OLT model followed the method described previously by Kamada and Calne [22,23] with minor modifications and without anastomosis of the hepatic artery. The donor and recipient animals were anesthetized through intraperitoneal injection of pentobarbital sodium (40 mg/kg, Shanghai No. 1 Biochemical & Pharmaceutical, China). The donor livers were infused with precooling saline with heparin (25 U/ml) via the inferior caval vein after the donor rat was dissected. The obtained livers were reserved in saline and surrounded with ice until transfer to the abdomens of recipient animals. After the suprahepatic vena cava and portal vein was inosculated and hemostatic clamping relieved, the livers were re-perfused with blood. Then the bile ducts were anastomosed with a stent made using a venous indwelling needle. All the recipients were injected with 2-ml sterile saline through the penile vein after the operation. To help the recipients recover more quickly, they were warmed using infrared lamp illumination.

2.3. Histopathologic and immunohistochemical analysis

Sections of donor livers were fixed in 10% neutral formalin PBS solution, dehydrated, embedded in paraffin, and sectioned into 3-mm slices. The sections were de-paraffinized with xylene and ethanol and then stained with hematoxylin and eosin using standard practice. Immunochemistry analysis was performed as previously described. Briefly, after de-paraffinization, the slices were irradiated in citrate buffer (pH 6.0) in a microwave oven for approximately 20 min and then incubated with 0.3% hydrogen peroxide to minimize nonspecific interactions for 15 min. Nonspecific binding sites were blocked with 5% fetal bovine serum for 50 min at 37 °C. The sections were incubated with anti-IL-1β (R & D, 1:100) and ASC (Abcam, 1:800) primary antibody dilution overnight in a humidified chamber at 4 °C. The sections were then incubated with secondary antibody for 1 h. The slices were stained with dimethylbenzene, and hematoxylin was used to counterstain the nuclei and finally dehydrated. Normal rabbit IgG and rat IgG were used as negative controls.

2.4. Rejection scores

The Banff schema incorporates a semiquantitative scoring system for grading of acute cellular rejection (ACR) of the liver allograft [7]. The Banff rejection activity index (RAI) comprises 3 components scored from 0 to 3: venous endothelial inflammation (E); bile duct damage (B); and portal inflammation (P); the scores are combined to an overall score (the RAI). RAI of the transplanted livers harvested at days 1, 3, and 7 after OLT was determined by Banff RAI.

2.5. Real-time RT-PCR assay for ASC, IL-1β, and IFN-γ mRNA quantification

Total RNA was isolated from hepatic grafts using Trizol reagent (Takara), and reverse transcription was undertaken using Kit (Takara) following the manufacturer's instructions. Real-time RT-PCR was performed using SYBR Kit (Takara) using the ABI 7500 System (Applied Biosystems, Inc.) according to the manufacturer's instructions at 95 °C 30 s; 95 °C 10 s, 56 °C 15 s, and 72 °C 45 s for 40 cycles. The copy numbers of target cDNA were normalized to β-actin expression. Results are expressed as relative gene expression using the ΔΔCt method. Primers for ASC, IL-1β, IFN-γ, and β-actin are listed as follows:

ASC F: 5′-GCAATGTGCTGACTGAAGGA-3′
ASC R: 5′-TGTTCCAGGTCTGTCACCAA-3′
IL-1β F: 5′-CTGTGACTCGTGGGATGATG-3′
IL-1β R: 5′-GGGATTTTGTCGTTGCTTGT-3′
IFN-γ F: 5′-GCCCTCTCTGGCTGTTACTG-3′
IFN-γ R: 5′-CTGATGGCCTGGTTGTCTTT-3′
β-actin F: 5′-AGCCATGTACGTAGCCATCC-3′
β-actin R: 5′-CTCTCAGCTGTGGTGGTGAA-3′

2.6. ELISA

Blood samples were taken from the inferior caval vein. Blood samples were allowed to clot at room temperature for 30 min and then centrifuged at 2000*g* for 15 min at 4 °C to obtain serum. Serum samples