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Involvement of LSECtin in the hepatic natural killer cell response

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ABSTRACT

Accumulating evidence has indicated that natural killer cells (NK cells) play an important role in immune responses generated in the liver. However, the underlying molecular basis for local immune regulation is poorly understood. Mice were intraperitoneally injected with polyinosinic-polycytidylic acid (PolyI:C) at a dose of 20 mg/kg body wt. The percentage and absolute number of NK cells in the liver were analysed with flow cytometry. LSECtin knockout mice and LSECtin cDNA plasmids were used for analyze the role of LSECtin in hepatic NK cell regulation in vivo. Here, we show that the C-type lectin LSECtin, a member of the DC-SIGN family, is a novel liver regulator for NK cells. LSECtin could bind to NK cells in a carbohydrate-dependent manner and could regulate the number of hepatic NK cells. In the NK cell-mediated acute liver injury model induced with PolyI:C, the exogenous expression of LSECtin accelerated NK cell-induced liver injury, whereas the absence of LSECtin ameliorated this condition. Our results reveal that LSECtin is a novel, liver-specific NK cell regulator that may be a target for the treatment of inflammatory diseases in the liver.

1. Introduction

Natural killer cells (NK cells) are large granular lymphocytes that display cytotoxic activity against most pathogens [1]. NK cells are present in the blood and the spleen, and they are enriched in the population of resident liver lymphocytes [2,3]. Mouse liver lymphocytes contain approximately 10% NK cells, whereas rat and human liver lymphocytes contain approximately 30%–50% NK cells [4]. The signals transmitted by stimulatory and inhibitory receptors control the activity of NK cells [5,6]. However, the mechanisms of NK cell trafficking to the liver and recruitment within the liver during inflammation are still poorly understood.

Recently, we reported a novel c-type lectin, LSECtin (liver node sinusoidal endothelial cell lectin), that is specifically expressed on LSECs and Kupffer cells. LSECtin is a member of a family comprising CD23, DC-SIGN and DC-SIGNR, possesses a typical carbohydrate recognition domain (CRD) and binds to mannose, *N*-acetylglucosamine (GlcNAc) and fucose in a Ca^{2+}-dependent manner [7]. We found that LSECtin recognises activated T cells and negatively regulates T-cell receptor-mediated signalling *in vitro* and the hepatic T cell immune response in vivo [8,9]. Then, we sought to determine whether LSECtin can regulate other immune cells and if it contributes to hepatic immune regulation.

Here, we show that LSECtin can recognize to NK cells in a carbohydrate-dependent manner. We observed decreased accumulation of NK cells in the liver in the absence of LSECtin. Upon treatment with the double-stranded RNA polyinosinic-polycytidylic acid (polyI:C), LSECtin expression accelerates inflammatory liver injury in vivo, with slight elevation of serum alanine transaminase (ALT)/aspartate aminotransferase (AST) levels. In contrast, the absence of LSECtin ameliorated this condition. Thus, our results reveal that LSECtin plays an important role in the local recruitment of hepatic NK cells.

Abbreviations: LSECtin, liver node sinusoidal endothelial cell lectin; NK cells, natural killer cells; PolyI:C, polyinosinic-polycytidylic acid; LSECs, liver node sinusoidal endothelial cells; DC-SIGN, dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin; ALT, alanine transaminase; AST, aspartate aminotransferase; CRD, carbohydrate recognition domain.

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2. Materials and methods

2.1. mAbs, reagents and plasmids

The mAb against human CD3 (clone 515), CD56 (clone B159) were from BD Pharmingen; the mAb against mouse CD3 (145-2C11), pan-DX5 were (clone DX5) from e-bioscience; Recombine LSECtin protein were from R&D systems (LSECtin extracellular region Ser54-Cys293). The mAb against LSECtin were prepared as described [8]. Polyinosinic–polycytidylic acid (polyI:C) sodium were from Sigma. The cDNA coding full-length mLSECtin were subcloned into the expression vector pcDNA3.1a (Invitrogen).

2.2. Mice treatment

Male C57 mice, 6–8 week-old, were purchased from Vitonglihua Co. (Beijing, China). LSECtin knock-out mice was backcrossed to C57BL/6 strains as describe [8]. All mice were maintained under controlled conditions (22 °C, 55% humidity, and 12 h day/night rhythm) in compliance with the regulations of animal care of China. PolyI:C was dissolved in the pyrogen-free saline at the concentration of 1 mg/ml. For in vivo stimulation of NK cells, mice were intraperitoneally injected with polyI:C at dose of 20 mg/kg body. Serum ALT/AST levels were estimated using a detection kit. The mLSECtin cDNA were delivered in vivo using a modified 'hydrodynamic transfection method [9]. The mice were injected with full-length mLSECtin cDNA plasmid or control plasmid (1 mg/kg body weight i. v. in 2 ml of saline).

2.3. Cell preparations

Murine livers were removed and passed through a 200-gauge

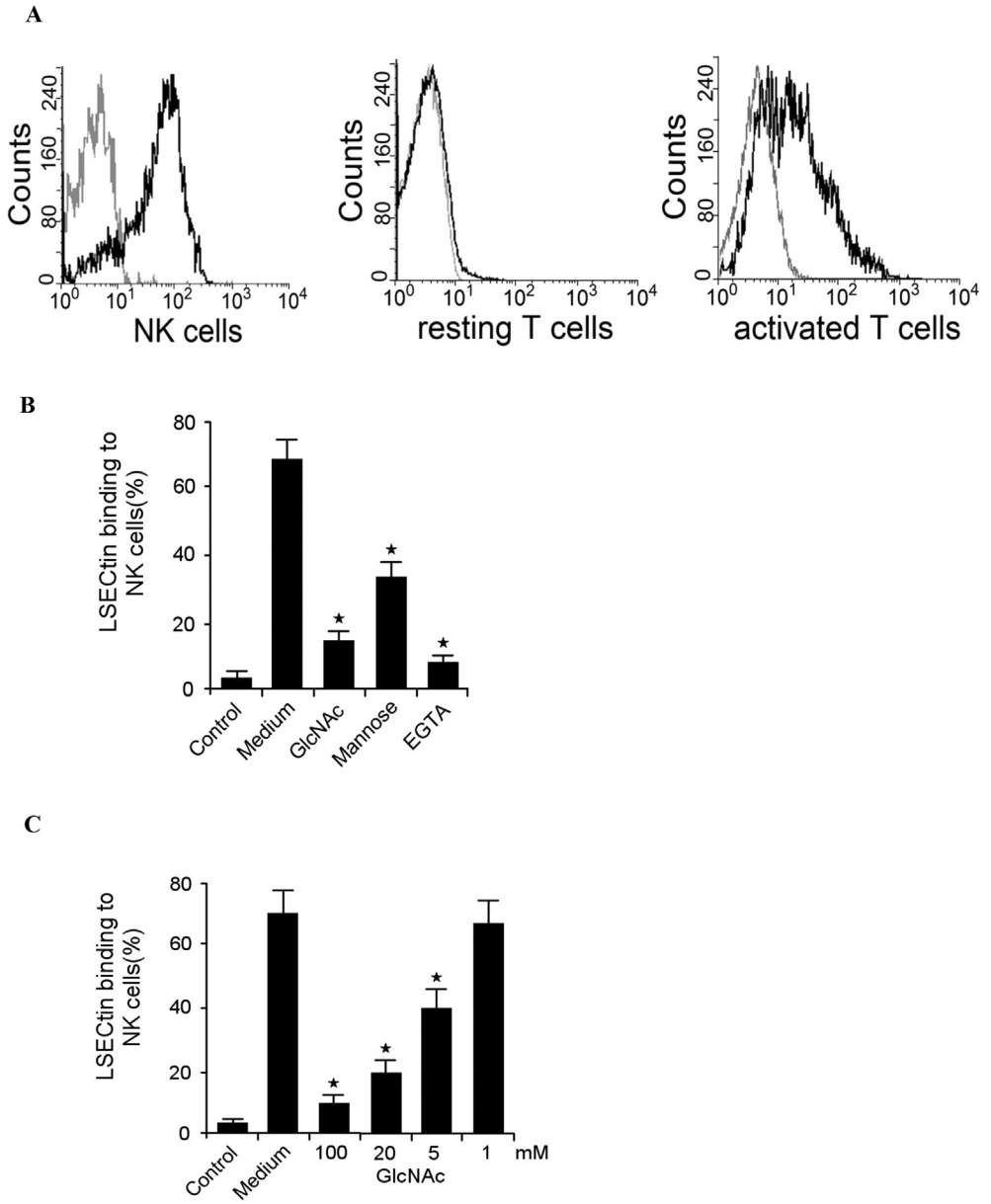

Fig. 1. LSECtin binds to NK cells (A) Adhesion of LSECtin to NK cells. Human PBMCs were isolated and subsequently analysed for CD3 and CD56 expression using specific mAbs. LSECtin binding to activated T cells as positive control. **(B)** Inhibition of LSECtin binding to NK cells by D-mannose, GlcNAc and EGTA. **(C)** Inhibition of LSECtin binding to NK cells by GlcNAc at different concentrations. Results are the mean ± S.D. from three independent experiments. ★, $p < 0.05$.