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Possible role of LECT2 as an intrinsic regulatory factor in SEA-induced toxicity in D-galactosamine-sensitized mice

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Abstract To elucidate whether leukocyte cell-derived chemotaxin 2 (LECT2) controls the progression of staphylococcal enterotoxin A (SEA)-induced toxicity, we examined the role of LECT2 in a mouse model. Almost all the C57BL/6 J (B6) mice survived for 72 h after the injection of 0.1 μg of SEA and 20 mg of D-galactosamine (D-GalN). However, the same treatment protocol in LECT2$^{-/-}$ mice produced a high lethality (~90%), severe hepatic apoptosis, and massive hepatic and pulmonary hemorrhage, similar to the situation observed in B6 mice treated with 1.0 μg SEA/D-GalN. The plasma LECT2 levels in B6 mice treated with 1.0 μg SEA/D-GalN were inversely correlated with the plasma cytokine levels and were associated with prognosis. LECT2 administration increased the survival of B6 mice and down-regulated TNF-α and IL-6. These results suggest the involvement of LECT2 in the regulation of fatal SEA-induced toxicity in D-GalN-sensitized mice.

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1. Introduction

Toxic shock syndrome (TSS) is an acute systemic disease caused by *Staphylococcus aureus* infection [1–3]. A variety of clinical symptoms and laboratory data [1,2], as well as histopathological findings in deceased cases [4,5], have indicated the involvement of disseminated intravascular coagulation (DIC) and multiple organ failure (MOF) in TSS. Since TSS toxin-1 (TSST-1), one of the superantigens (SAgs) [6,7] that stimulate T cells in a manner restricted by the TCR β chain variable region (Vβ) [8], is produced by causative cocci, the overactivation of T cells by TSST-1 has been implicated in the pathogenesis of TSS [3,9,10]. In support of this assumption, the massive proliferation of TSST-1-reactive Vβ2$^+$ T cells has been observed in patients with TSS during the acute clinical phase [11,12], which may persist for 4–5 weeks [12]. The cytokines that are produced in excess amounts are assumed to abrogate physiological homeostasis, leading to the severe clinical symptoms seen in patients [13,14]. The abnormal reactions in TSS have been reproduced in animal model experiments, indicating not only T-cell-dependency but also disease susceptibility defined by the non-T-cell compartment [15]. The administration of SAgs such as TSST-1, staphylococcal enterotoxins A (SEA) or SEB together with D-galactosamine hydrochloride (D-GalN) induces T-cell-dependent lethal reactions in mice [16–18]. The overproduction of inflammatory cytokines is induced by SAgs and is strongly associated with disease severity in this model. When antibodies to the inflammatory cytokines TNF-α or IFN-γ are injected into mice, the mortality of mice treated with bacterial toxins and D-GalN is suppressed [18,19]. Since hepatotoxic agents such as D-GalN sensitize animals to bacterial toxins, hepatic failure is the most likely cause of mortality in this model, as observed in LPS and D-GalN-treated mice [20]. As for the protracted expansion of SAg-reactive T cells in patients with TSS, we have established a mouse model using an osmotic pump containing SEA (SEA-pump) [21]. The SEA-pumps implanted in the mice supply SEA continuously and induce the marked expansion of SEA-reactive Vβ3$^+$ CD4$^+$ T cells for 30 days.

Leukocyte cell-derived chemotaxin 2 (LECT2) was originally identified as a neutrophil chemotactic factor produced *in vitro* from PHA-activated human T-cell leukemia SKW-3 cells [22] and was subsequently found to be identical to chondromodulin II, a growth factor for chondrocytes [23]. Recently, Con A-induced hepatitis was reported to be exacerbated in mice depleted of the LECT2 gene (LECT2$^{-/-}$ mice) [24]. Con A is a potent polyclonal stimulator of T cells, and a severe type of hepatitis has also been seen in mice injected with SAgs together with D-GalN. Therefore, LECT2 may also control the progression of SAg and D-GalN-induced abnormal reactions.

In the present study, we evaluated the role of LECT2 in the above-mentioned mouse model, and identified a suppressive effect of LECT2 on lethality and the production of inflammatory cytokines, but not on protracted T cell expansion. Based on these results, we discuss the possible regulatory mechanism of LECT2 and its therapeutic potential.

2. Materials and methods

2.1. Mice

C57BL/6J (B6) mice were purchased from CLEA (Tokyo, Japan). LECT2$^{-/-}$ mice were described previously [24]. All animals were bred in the animal facility at the Department of Microbiology and Immunology, Tokyo Women's Medical University, and 8- to 12-week-old female mice were used in the studies. All animal care and experimentation was performed in accordance with the guidelines of the ethics review committee for animal experiments of Tokyo Women's Medical University.

2.2. Monoclonal antibodies (mAbs) and reagents

Biotin-conjugated anti-TCR Vβ3 (KJ-25), Vβ6 (RR4-7), Vβ8 (F23.1), Vβ11 (RR3-15) and Vβ12 (MR-11), PE-conjugated anti-CD8 (53-6.7), FITC-conjugated anti-CD4 (L3T4) (RM4-5) and Cy5-conjugated streptavidin were purchased from BD Biosciences (San Jose, CA). SEA was purchased from Toxin Technology (Sarasota, FL). D-GalN was obtained from Wako (Tokyo, Japan).

2.3. Induction of SEA-induced lethal reactions

LECT2$^{-/-}$ or B6 mice were injected intraperitoneally (i.p.) with SEA (indicated amounts/mouse) and D-GalN (20 mg/mouse). The severity of disease and the fates of these mice were followed for 72 h.

2.4. Histology and TUNEL staining

Formalin-fixed and paraffin-embedded sections of tissue samples (liver and lungs) were stained with H&E. Apoptotic cells were detected with TUNEL staining using the In Situ Cell Death Detection Kit, AP (Roche, Mannheim, Germany) according to the manufacturer's instructions.

2.5. Preparation of recombinant LECT2 (rLECT2) and treatment protocol

A fragment encompassing the lect2 gene was amplified using the sense primer 5′-AGCAGAACCTAGATGATTCCC-3′ and the 6xHis encoded-antisense primer 5′-TTAGTGATGGTGATGGTGATGCAGGTATGCTGT-3′ from mouse lect2 cDNA [25]. The PCR products were cloned into a pCR8/GW/TOPO vector (pCR8/GW/TOPO TA Cloning Kit; Invitrogen, Merelbeke, Belgium). Proteins were produced using a Baculoviral Expression System (BAC-to-BAC, Invitrogen) according to the manufacturer's instructions. His-tagged rLECT2 protein in the culture supernatants of infected Sf9 cells was purified using Ni^{2+} affinity resin, followed by size-exclusion chromatography. B6 mice were injected with rLECT2-His protein (5 μg/mouse) i.p. at 0.5 h and 6 h after SEA/D-GalN treatment. The control mice underwent the same procedure, receiving an equivalent volume of vehicle (PBS).

2.6. Measurement of LECT2

Plasma LECT2 was quantified using a sandwich ELISA method with anti-mouse LECT2 mAb (8G2) as a capture antibody and another biotinylated-anti-mouse LECT2 mAb (4C3) for detection (these mAbs were kindly provided by Medical and Biological Laboratories Co., Ltd. Nagoya, Japan). After binding with streptavidin-conjugated peroxidase (Sigma, St