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Activated NKT cells facilitated functional switch of myeloid-derived suppressor cells at inflammation sites in fulminant hepatitis mice

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ABSTRACT

Myeloid-derived suppressor cells (MDSCs) confer immunosuppressive properties, but their roles in fulminant hepatitis have not been well defined. In this study, we systematically examined the distribution of MDSCs in bone marrow (BM), liver and spleen, and their functional and differentiation status in an acute fulminant hepatitis mouse model induced by lipopolysaccharide and D-galactosamine (LPS-GalN). Moreover, the interaction between NKT cells and MDSCs was determined. Our study revealed that BM contained the largest pool of MDSCs during pathogenesis of fulminant hepatitis compared with liver and spleen. MDSCs in liver/spleen expressed higher levels of chemokine receptors such as CCR2, CX3CR1 and CXCR2. At inflamed tissues such as liver or spleen, activated NKT cells induced differentiation of MDSCs through cell–cell interaction, which markedly dampened the immunosuppressive effects and promoted MDSCs to produce pro-inflammatory cytokines and activate inflammatory cells. Our findings thus demonstrated an unexpected pro-inflammatory state for MDSCs, which was mediated by the activated NKT cells that precipitated the differentiation and functional evolution of these MDSCs at sites of inflammation.

1. Introduction

Fulminant hepatitis, also called acute liver failure, is characterized by peripheral vasodilatation, development of encephalopathy and coagulopathy (Bernal and Wendon, 2013). Accumulating evidence demonstrates that systemic inflammatory response syndrome is a predictor of progressive encephalopathy, renal dysfunction and poor prognosis in fulminant hepatitis patients (Leithead et al., 2009; Rolando et al., 2000), suggesting that excessive immune response may play a pivotal role in the pathogenesis and prognosis of fulminant hepatitis.

Recently, expansion of myeloid-derived suppressor cells (MDSCs), a heterogeneous population of myeloid progenitor cells and immature myeloid cells (Solito et al., 2014), have been frequently reported in patients with cancer or those with autoimmunity diseases (Greten et al., 2011). For example, MDSCs showed immunosuppressive effects in cancer patients through interacting with T cells and natural killer (NK) cells (Arina and Bronte, 2015; Hoechst et al., 2009; Mao et al., 2014). Meanwhile, the accumulation of MDSCs in liver could inhibit the progression of liver injury and dampen T-cell mediated-hepatitis (Conrad et al., 2014; Sarra et al., 2013). However, little is known about the functional and differentiation status of MDSCs in the pathogenesis of fulminant hepatitis.

As an important immuno-modulator of myeloid cells (Lindau et al., 2013a,b), NKT cell is a key regulator of hepatic immune response and constitutes about 15–20% of total lymphocytes in liver (Mattner, 2013). It is known that the regulation of NKT on MDSC function depends on its functional state. Type II NKT cells

Abbreviations: BM, bone marrow; DC, dendritic cell; MDSC, myeloid-derived suppressor cell; Treg, regulatory T cell; MLN, mesenteric lymph node; MNC, mononuclear cell; VEGF, vascular endothelial growth factor; MFI, mean fluorescence intensity.

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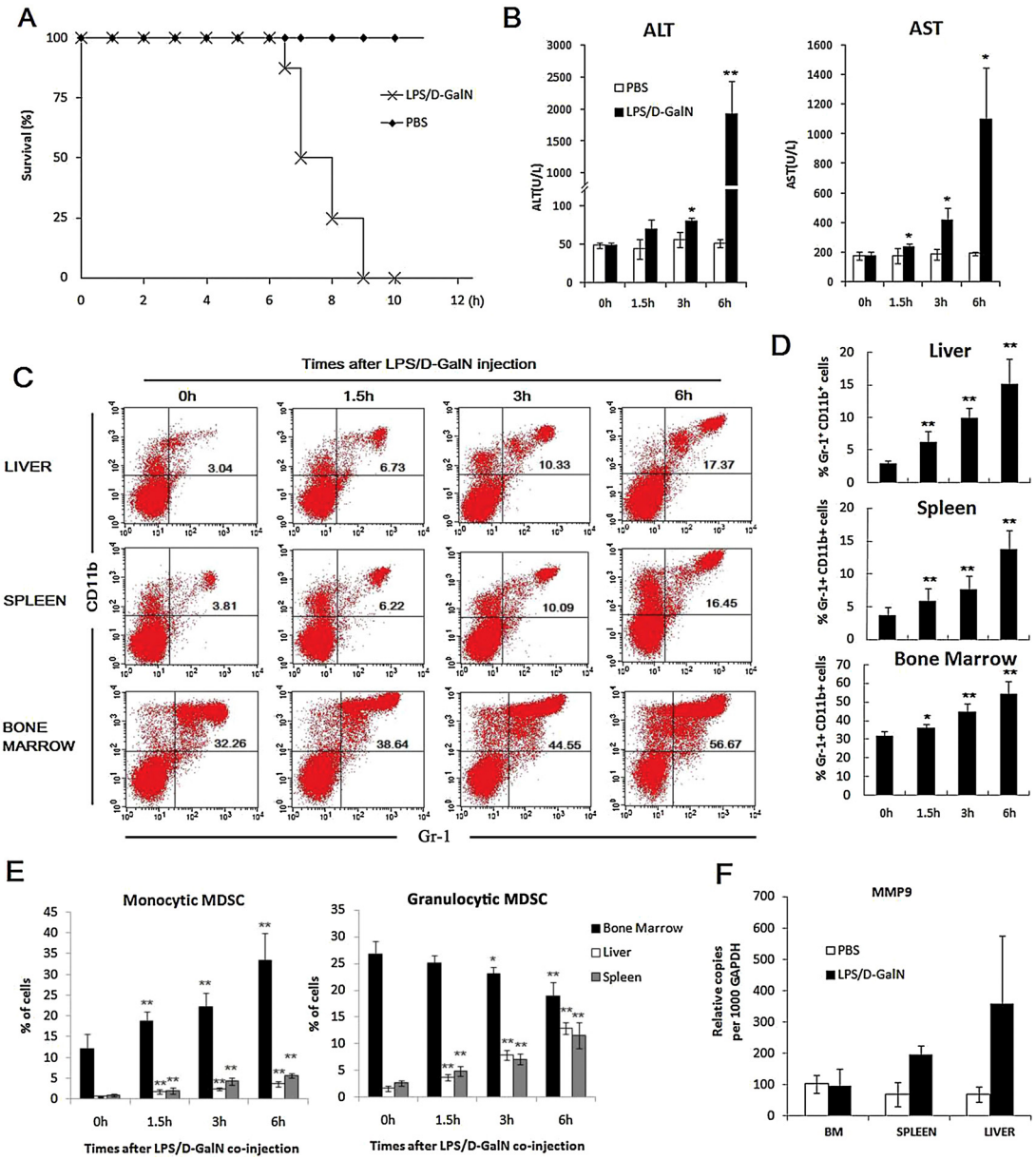

Fig. 1. Dynamic expansion of MDSCs and their subsets in bone marrow (BM), spleen and liver in mice with fulminant hepatitis (n = 6 for each group). (A) Survival of fulminant hepatitis mice. (B) Serum ALT and AST at 0 h, 1.5 h, 3 h and 6 h after LPS/D-GalN injection. Each experiment in (A) and (B) was repeated at least in triplicate. (C-D) Flow cytometry was performed to examine the percentage of MDSCs in mononuclear cells (MNCs). (E) The frequency of monocytic MDSCs and granulocytic MDSCs measured by flow cytometry. (F) MMP9 mRNA expression in MDSCs at 0 h and 6 h after onset of fulminant hepatitis quantified by real-time PCR (F). Each experiment in (C) to (F) was repeated at least 6 times.*, $P < 0.05$ versus baseline level; **, $P < 0.01$ versus baseline level.

can enhance the suppressive activity of MDSCs (Terabe et al., 2005). Meanwhile, activated NKT cells contribute to the reduction of immunosuppressive activity of MDSCs (De Santo et al., 2008) and facilitate the conversion of immunosuppressive MDSCs into immunogenic antigen presenting cells (APCs) (Ko et al., 2009). Therefore, in this study we aimed to investigate the potential roles of NKT cells in regulating the differentiation and function of MDSCs in the pathogenesis of mouse fulminant hepatitis.

2. Materials and methods

2.1. Animals

Male C57BL/6 mice (6–8 weeks) were purchased from Shanghai Laboratory Animal Center, Chinese Academy of Science (Shanghai, China). The study protocols were approved by the Ethic Committee of Zhejiang University School of Medicine.

2.2. Induction of fulminant hepatitis

Fulminant hepatitis was induced by co-injection of 5 μg/kg lipopolysaccharide (LPS) (Escherichia coli, 0111:B4, Sigma, US) and 400 mg/kg D-Galactosamine (GalN) (Sigma, US) via intraperitoneal injection. Anesthesia was performed using pentobarbital sodium (45 mg/kg). After sacrifice, blood was collected from eyeballs. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured by an automatic biochemical analyzer (Olympus AU400, Japan).