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IL-33 protects murine viral fulminant hepatitis by targeting coagulation hallmark protein FGL2/fibroleukin expression

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ABSTRACT

Fulminant hepatitis (FH) is characterized by rapid liver failure and high mortality. The pathogenesis of viral FH includes virus-induced immune activation, inflammation, and subsequent hepatic apoptosis and necrosis. However, the mechanisms that underlie FH progression are unclear. IL-33 is a member of the IL-1-related cytokines, considered to be an "alarmin" that participates in various diseases, but its precise role in the coagulation of FH is not very clear. In our study, we found that IL-33 is significantly elevated in mice infected with murine hepatitis virus strain 3 (MHV-3). This is accompanied by an increase in pro-coagulant fibrinogen-like protein 2 (FGL2) in the liver. Previous studies have suggested that an increase in FGL2 is diagnostic of FH and liver necrosis, and animals with no FGL2 had better survivorship during FH. Our studies showed that IL-33 administration in a MHV-3 infection promoted survival during FH, with a significant reduction in FGL2 expression and liver inflammation. In vitro IL-33 treatment abrogated MHV-3 and IFN-γ induced FGL2 expression in RAW264.7 and THP-1 cells, respectively. In conclusion, our research suggests that IL-33 protects against viral fulminant hepatitis in mice by antagonizing expression of the pro-coagulant protein FGL2.

1. Introduction

Owing to limited treatment options, liver transplantation has been suggested as the primary therapeutic approach to treat viral fulminant hepatitis (FH). However, global FH mortality is still notably high (Lee, 1993). Pathogenesis includes immune cell activation and infiltration, as well as increased secretion of inflammatory cytokines, such as TNF-α and IFN-γ. This appears to play an important role in the initiation and maintenance of the immune response during FH (Liu et al., 2001). Additionally, TNF-α null mice demonstrate increased resistance to MHV-3 infection and survival rates greater than 80% (Liu et al., 2015). IFN-γ is primarily produced by Th1 CD4+ cells, NK cells, and macrophages. Several lines of evidence suggest that IFN-γ is involved in the development of acute hepatitis in both patients and animal models (Kimura et al., 2008; Sun and Ran, 2004; Wang and Liu, 2003; Zheng et al., 2017). These include the observation that IFN-γ deletion relieved acute LPS-induced liver injury (Tsuji et al., 1999).

Fibrinogen-like protein 2 (FGL2) is expressed by several immune cells, including macrophages, CD4+ T cells, and endothelial cells. It has been verified to play an essential role in the progression of many diseases, including FH (Shalev et al., 2009). In a mouse model of MHV-3 viral infection, severe hepatitis is observed that mirrors many aspects of FH, including hepatocellular necrosis, sinus thrombosis, and a significant increase in FGL2 expression (Bernal et al., 2010; Ding et al., 1997). Another histological feature of FH is fibrinogen deposition, consistent with the pro-coagulant activity (PCA) of FGL2 (Zhu et al., 2006). Furthermore, targeted depletion of FGL2 increases mouse survival following MHV-3 infection and the subsequent development of FH, suggesting that the protein is potentially a molecular target for FH therapy (Yang and Hooper, 2013).

IL-33 is a member of the IL-1 super-family of cytokines that includes IL-1ß and IL-18, sharing homologous sequence and structure (Garlanda et al., 2013; Sims and Smith, 2010). IL-33 binds to a heterodimeric receptor complex composed of IL-1R and IL-1 receptor-like 1 (IL1RL1 or

Abbreviations: FH, fulminant hepatitis; MHV-3, murine hepatitis virus strain 3; FGL2, Fibrinogen-like protein 2; ST2, IL-1 receptor-like 1; ALF, acute liver failure; PFU, plaque-forming units; PBS, phosphate-buffered saline; ALT, alanine aminotransferase; AST, aspartate aminotransferase; PCA, pro-coagulant activity; HRP, horseradish peroxidase; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling

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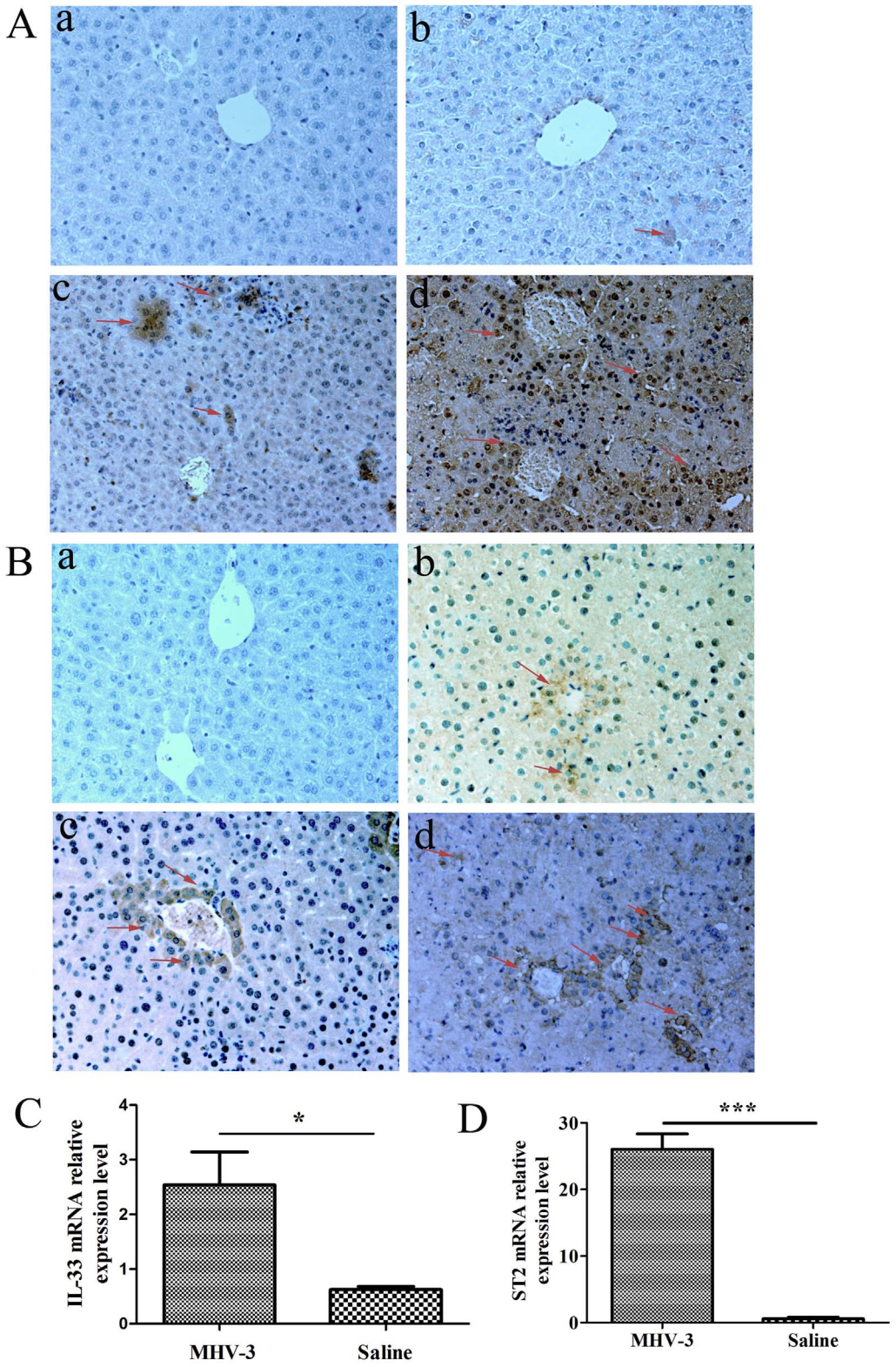

Fig. 1. IL-33 and IL1RL1 (ST2) are increased in MHV-3 infected BALB/c mice.Mice were euthanized 0, 24, 48, and 72 h after infection with MHV-3. IL-33 and IL1RL1 (ST2) levels in the liver tissues were assessed using immunohistochemistry (A: IL-33, B: IL1RL1 (ST2), 400× magnification)(n = 6 per group) and Real-time PCR (C for IL-33, D for IL1RL1 (ST2), n = 6 per group). Panels a–d demonstrate representative liver tissue harvested at 0, 24, 48, and 72 h post virus infection, respectively. Arrows indicate cells with high expression of IL-33 and IL1RL1 (ST2) (primarily endothelial cells and hepatocytes). Experiments were performed in triplicate. Values shown are means ± SD. A two-tailed Student's *t*-test was used for statistical analysis. *P < 0.05 and ***P < 0.001 for MHV-3 injected mice versus saline-injected mice.

ST2) proteins (Tominaga, 1989). Binding of IL-33 to this complex activates several intracellular signaling pathways, including NF-κB and MAP kinase. This induces a Th2 immune response and cytokine profile that leads to increased IL-4, IL-5, and IL-13 secretion, resulting in several downstream effects, such as eosinophilia and elevated mucus production (Schmitz et al., 2005; Moussion et al., 2008). IL-33 is expressed in a variety of cells, including epithelial cells, fibroblasts, and macrophages (Mirchandani et al., 2012). It has recently been reported