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New Aspect of Liver IL-17$^+$ γδ T Cells[☆]

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ABSTRACT

Liver is a critical organ where comprehensive immune regulation or defense occurs. γδ T cells in liver represent indispensable population and are found to be regulating a variety of diseases including autoimmunity, cancer, fibrosis, and infections. IL-17$^+$ γδ T cells in liver is a functional subset which has been found to have pro-inflammatory effect or anti-infection ability. However, how their function is activated or maintained, isn't well understood. Microbiota in the intestine provide continual source of metabolites to the liver, through at least the portal vein. The interaction between intestinal microbiota and liver γδ T cells has not been well studied. Li et al found that lipid antigen derived from intestinal microbiota was presented by hepatocyte CD1d, and activated liver-resident γδ T cells and maintained the IL-17 production. Moreover, microbiota promoted NAFLD through IL-17$^+$ γδ T cells. Thus, new aspect of IL-17$^+$ γδ T cells in the liver was characterized.

γδ T cells represent important subset of immune cells, which regulate a variety of immune responses including inflammation, autoimmunity, and cancer (Born et al., 2010). Recent study found that thymic education influences the γδ T cell effector fate choice before going into periphery (Jensen et al., 2008). With TCR-ligation or CD70 engagement, γδ T cells develop to IFN-γ producing cells, while antigen-naïve cells are directed to IL-17 fate (Jensen et al., 2008; Ribot et al., 2009). However, mechanisms of peripheral γδ T cells maintaining the fate or being activated isn't well known.

Liver is an important organ for metabolism and immunity. Immune cells are enriched in liver, regulating liver functions. Immune dysfunctions in liver lead to several kinds of diseases. Several studies showed that γδ T cells in liver played a role in the specific condition, which has been reviewed previously (Hammerich and Tacke, 2014). Concanavalin A (Con A)-induced acute liver damage in mice is a good model to mimic human liver injury including autoimmune liver disease (Tiegs et al., 1992). Using this model, in our group's previous work, we found that γδ T cells, especially Vγ4 γδ T cell subset, produced IL-17 in the ConA-challenged liver (Zhao et al., 2011). The Vγ4 γδ T cell-derived IL-17 played a negative role in regulating pro-inflammatory NKT cell IFN-γ production. Thus, this study defined protective role of IL-17, which is derived from Vγ4 γδ T cells, in Con A-induced liver inflammation in mice. Very similarly, our group found that human Vδ2 γδ T cells played protective role in HBV infection induced liver inflammation (Wu et al., 2013). With progression of HBV infection from immune tolerance to immune activated, peripheral Vδ2 γδ T cell number decreased. To further analyze the functional change of Vδ2 γδ T cells, we found that with progression of disease, Vδ2 γδ T cells acquired CD45RA$^+$CCR7$^-$ terminally differentiated effector memory phenotype. Moreover, Vδ2 γδ T cells suppressed IL-17 secretion in CD4 T cells via IFN-γ production. However, the stimulus of IL-17 or IFN-γ production by γδ T cells in liver, is not known. Whether the functional portion of γδ T cells is from tissue-resident or recruited from circulating cells, is also not characterized.

Microbiota in the intestine has extensive interactions with host immune system (Hooper et al., 2012). Specifically, intestinal

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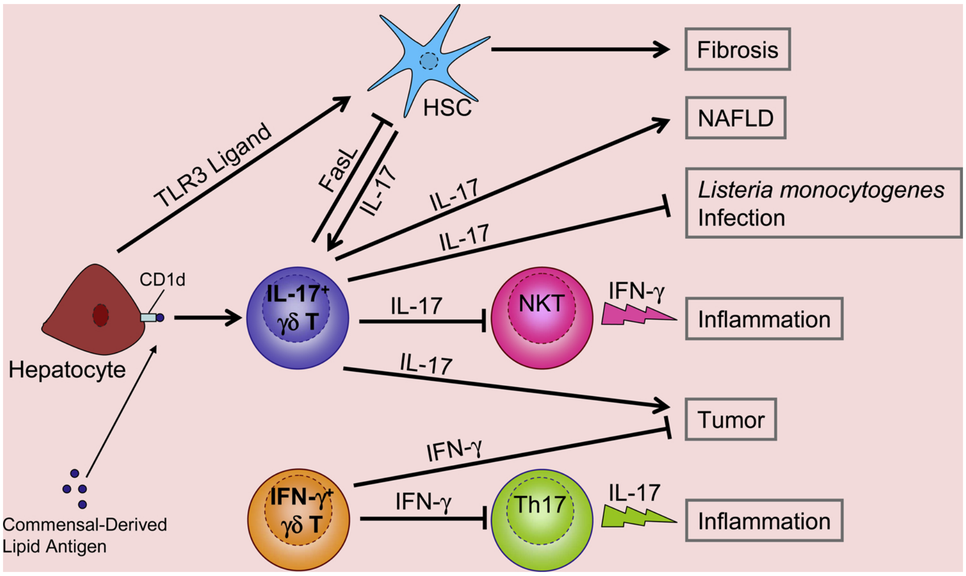

Fig. 1. Representative functions of γδ T cell subsets in liver diseases.
Liver resident IL-17$^+$ γδ T cells are maintained by hepatocyte CD1d-presented lipid antigen, which is derived from intestinal microbiota. Under stress condition, γδ T cell IL-17 production played a pathogenic role in NAFLD (Li et al., 2017). IL-17$^+$ γδ T cells play a protective role in *Listeria monocytogenes* infection, through recruiting neutrophils (Hamada et al., 2008). Differently, liver γδ T cell-derived IL-17 negatively regulates NKT cell function in Con A-induced fulminant hepatitis (Zhao et al., 2011). IL-17$^+$ γδ T cells produce FasL, induce HSC (hepatic stellate cell) apoptosis, and ameliorate liver fibrosis (Hammerich et al., 2014). HSCs which are activated by hepatocytes produce IL-17 and amplify γδ T cell IL-17 production (Seo et al., 2016). IFN-γ$^+$ subset of γδ T cells suppress Th17-mediated inflammation in HBV infection (Wu et al., 2013). IFN-γ$^+$ γδ T cells are tumor killers and potentially protect liver from cancer (Hoh et al., 2013), while IL-17$^+$ γδ T cells help tumor progression via facilitating immune suppression (Ma et al., 2014).

commensals regulate T cell properties through several ways, including regulating several subsets of T cells via divergent mechanisms (Hooper et al., 2012). Liver is a direct organ receiving microbial metabolites and other molecules from the intestine, via the portal vein. However, how liver γδ T cells are regulated by the microbiota is not known.

Li et al published their research in Nature Communications, describing a new mechanism of γδ T cell IL-17 production in the liver, which is regulated by commensal microbiota (Li et al., 2017). Firstly, they found that liver γδ T cells are powerful IL-17 makers. Using parabiotic paired mice, authors found the IL-17$^+$ γδ T cells are mostly tissue-resident in the liver, rather than circulating cells. To ask whether the IL-17$^+$ γδ T cells are regulated by intestinal microbiota, authors depleted commensals via antibiotics treatment. Result showed that with antibiotics treatment, IL-17$^+$ γδ T cells reduced significantly, and restoring gut bacteria into germ-free mice recovered IL-17$^+$ γδ T cells. Consistently, the mice which have higher amount of bacteria correlated with IL-17$^+$ percent in the γδ T cells in the liver. These suggested microbiota in the intestine plays a positive role in the presence of IL-17$^+$ γδ T cells in the liver. Through analyzing the IL-17$^+$ γδ T cells and bacteria under treatment of the combinations of antibiotics, authors found *E. Coli* was possible positive regulator of IL-17 in γδ T cells. To test the hypothesis, authors reconstituted antibiotics-treated mice with *E. Coli*, and found IL-17$^+$ γδ T cells in the liver are rescued by the *E. Coli*. These suggested *E. Coli* is sufficient to induce liver IL-17$^+$ γδ T cells. To uncover the IL-17-inducing molecules *in vivo*, authors screened several antigens from bacteria and also series of receptors from the host, using KO mice. These results indicated *E. Coli*-derived lipid antigen is very likely the key stimulator of IL-17$^+$ γδ T cells, by the presentation of CD1d molecule, with the phenotype of CD1d$^{-/-}$ or Jα18$^{-/-}$ mice have reduction of IL-17 production in liver γδ T cells. Using fetal liver chimeric mice, authors excluded the function of CD1d in hematopoietic cells, suggesting liver hepatocytes-expressed CD1d is important for presenting lipid antigens to liver γδ T cells. To test the hypothesis, authors loaded hepatocytes with lipids and co-cultured with γδ T cells, and found the essential role of hepatocyte CD1d-lipid for stimulating IL-17$^+$ γδ T cells. To elucidate the function of liver-resident IL-17-producing γδ T cells, authors use an NAFLD (nonalcoholic fatty liver disease) model. They found that IL-17 from γδ T cells played important pathogenic role in the NAFLD. Collectively, authors found a new aspect of liver resident γδ T cell IL-17 production which is stimulated by hepatocyte CD1d-presented lipid antigen from intestinal microbiota. IL-17$^+$ γδ T cells contributed to consequent pro-inflammatory function in NAFLD when mice were given high-fat diet. Controlling these processes may provide new treatment way for liver inflammation. A figure of representative functions of γδ T cells in liver diseases is shown (Fig. 1).

For future research on liver γδ T cells, there are still a lot of questions to be answered. Butyrophilins (BTNs) are a family of co-stimulatory molecules that are found to be important regulators for T cells (Abeler-Dorner et al., 2012; Arnett and Viney, 2014; Rhodes et al., 2016). Importantly, specific BTN family members were found to exert thymic selection function for the respective subset of γδ T cells. Skint1 selects epidermal Vγ5 γδ T cells (Boyden et al., 2008), and also shape peripheral γδ T cell cytokine production (Turchinovich and Hayday, 2011). BTNL1 was found to select mouse intestinal Vγ7 γδ T cells (Di Marco Barros et al., 2016), and further Vγ7 γδ T cell expansion and maturation is also dependent on BTNL1. In human, BTNL3 and BTNL8 jointly activate Vγ4 γδ T cells. Moreover, human BTN3A1, with phosphorylated antigens, is a potent activating protein for Vγ9Vδ2 T cells (Vavassori et al., 2013). These indicated that the BTNs have probable corresponding γδ TCRs for selection and shaping each subset. Among liver γδ T cells, there has been at least found Vγ1 and Vγ4 γδ T cells in the mouse (Zhao et al., 2011). It will be good hypothesis that specific BTNs regulate certain subset of γδ T cells in the liver. And investigating the expression of functional BTNs will be good direction for the mechanisms of γδ T cells regulation. Addressing these questions may open a new field in liver γδ T cell study.

Another important question remained for the field is the mechanisms of γδ T cell cytokine production. Although γδ T cells are found to be pre-determined before going to peripheral organs (Jensen et al., 2008; Ribot et al., 2009), peripheral γδ T cell effector differentiation are found (Sutton et al., 2009). Evidence was found that effector γδ T cells in the periphery are selectively expanded, due to the expression of innate receptors (Martin et al., 2009). These indicated organ-specific γδ T cells have unique feature to elicit immunity while responding to certain environmental challenge. From our early studies, γδ T cells predominantly produced IFN-γ (Yin et al., 2002; Yin et al., 2000), and Vγ4 subset played protective role in tumor immunity (Gao et al., 2003; He et al., 2010). Conversely, Vγ1 γδ T cells produced IL-4 and negatively regulated Vγ4 γδ T cell-mediated tumor surveillance (Hao et al., 2011). γδ T cells in liver provided necessary source of IL-17 to protect liver from acute inflammation induced damage (Zhao et al., 2011). However, how the cytokines are induced isn't well known. *In vitro* differentiation experiment indicated γδ T cells produced IFN-γ or IL-17 through signaling pathways which were different from those in αβ T cells (Ribot et al., 2009; Yin et al., 2002; Yin et al., 2000). It is worth investigating the cell differentiation difference between αβ and γδ T cells. While people utilize the cytokine production analysis for liver γδ T cells, arguments are made against the methodology of intracellular cytokine staining, which requires researchers to stimulate cells before staining by cytokine specific antibodies. Can this method faithfully reflect the cytokine production in the organ? Answers cannot be made perfectly. Intracellular cytokine staining so far only reflects the ability of cytokine production. However, the real stimuli of γδ T cells may be different ones, *in vivo*. Realizing this limitation, it is better to provide more evidences of the γδ T cell cytokine production by adding other assays, or using real ligands (if defined) to stimulate γδ T cells before analysis.

The functional molecules for CD4 and CD8 αβ T cells are well characterized and utilized clinically, with checkpoint therapy for cancer as an example, which was awarded The Nobel Prize in Physiology or Medicine 2018 (Ledford et al., 2018). New molecules often emerged as effectors for αβ T cells in a variety of models and diseases. In γδ T cells, however, investigation on searching new functional molecules isn't as active as that in αβ T cell field. People hardly believe γδ T cells exert effector function through only IFN-γ or IL-17 in most diseases. Researchers have much opportunity to find new players in the regulation of diseases by γδ T cells. It is important to find not only the functional molecules expressed by γδ T cells, but also consequential mechanisms of how the effector molecules act downstream. Paying attention to these questions will solve the weakness in γδ T cell research field.

Authors declare no financial conflict of interests

None.

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