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NKT cells in liver fibrosis: Controversies or complexities

To the Editor:
We read with great interest a recent article in the *Journal of Hepatology* by Ishikawa *et al.* investigating the role of CD1d-restricted natural killer T (NKT) cells in thioacetamide (TAA)-induced liver fibrosis, by using *CD1d* knockout (KO) mice that are associated with NKT cell deficiency [1]. First, the authors observed that *CD1d* KO mice were resistant to TAA-induced liver inflammation, damage, and hepatocyte apoptosis. Second, the authors observed *CD1d* KO mice were resistant to TAA-induced liver fibrosis, indicating that NKT cells play an important role in promoting liver fibrogenesis in mice after chronic TAA treatment. The pro-fibrotic effects of NKT cells were also recently suggested by the data from a murine model of HBV transgenic mice [2], primary biliary cirrhosis [3], nonalcoholic steatohepatitis (NASH), and patients with NASH [4].

Recently, we identified the double sword face of invariant NKT (iNKT, type I NKT) cells in liver fibrogenesis in a model of CCl4-induced liver injury [5]. On the one hand, iNKT-deficient mice had increased liver injury and fibrosis, especially in the early stage of CCl_4-induced liver injury, suggesting that natural activation of iNKT cells by endogenous lipid antigens plays a protective role in preventing CCl_4-induced liver injury and fibrosis. On the other hand, strong activation of iNKT cells by α-galactosylceramide (α-GalCer) accelerated CCl_4-induced hepatocellular injury and subsequently enhanced fibrosis. Our findings clearly suggest a complex role of iNKT cells in liver fibrosis: inhibiting liver fibrosis via the suppression of HSC activation or indirectly promoting liver fibrosis via enhancing liver injury. We believe that the final effect of iNKT cells on liver fibrosis is determined by the balance between the inhibitory and stimulatory effects as we discussed above, but may also dependent on the real context of developing stage human liver diseases or animal models used. In addition to iNKT (type I NKT) cells, other subtypes of NKT cells including type II and possible type III NKT cells also exist [6]. These different subtypes of NKT cells exert many similar functions but may also exert some opposing functions, which further complicates the role of NKT cells in liver fibrogenesis [6,7].

In the study by Ishikawa *et al.* [1], it appears that CD1d restricted NKT cells play an important role in inducing liver injury, which may contribute to the pro-fibrotic effects of NKT cells observed in the TAA-induced liver injury model. In the Supplementary material, Ishikawa *et al.* [1], reported that *CD1d* KO mice were also resistant to CCl_4-induced acute liver injury; however, we found that *CD1d* KO mice had similar liver injury and fibrosis after chronic treatment with CCl_4 (Park *et al.*, unpublished data). Further studies are required to clarify the complex role of NKT cells in liver injury and fibrosis.

Conflict of interest

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Reply to: "NKT cells in liver fibrosis: Controversies or complexities"

To the Editor:
Emerging attention has been paid to the role of natural killer T (NKT) cells in a variety of liver diseases including nonalcoholic steatohepatitis (NASH) [1]. The role of NKT cells in hepatic fibrogenesis, however, has been controversial [2–4]. In our latest manuscript entitled "CD1d-restricted natural killer T cells contribute

to hepatic inflammation and fibrogenesis in mice", we demonstrated that CD1d-restricted natural killer (NK) T cells play an exacerbating role in xenobiotics-induced liver injury and subsequent fibrogenesis using *CD1d*-knockout (KO) mice [5]. As Dr. Hua Wang *et al.* pointed out in their letter, however, the functions of NKT cells in liver pathophysiology appear to be more complex in nature.

It is an intriguing hypothesis that NKT cells act as a double-sword in liver pathophysiology. It is indeed quite reasonable, considering the fact that NKT cells produce a variety of mediators including both Th1 and Th2 cytokines. From these important characteristics, NKT cells are often called as "the regulator of Th1/Th2 balance"; however, the precise mechanisms by which NKT cells modulate the balance in quantity and timing to produce these cytokines following specific stimuli have not been well elucidated. Park *et al.* demonstrated the complicated roles of NKT cells in acute and chronic liver injury caused by CCl_4 [4]. Although their hypothesis is fascinating, the experimental design of their study needs attention. In their study, α-galactosylceramide (GalCer), a specific ligand to invariant T cell receptor (TCR) in NKT cells, was used to stimulate these cells. Indeed, it is well established that GalCer causes a variety of responses in NKT cells, from the early activation phase with cytokine production to the subsequent tolerant state. Typically, a single injection of GalCer induces inactivation and/or apoptotic death of NKT cells systemically, the effect lasting a few days. Moreover, GalCer itself induces considerable degree of liver injury in mice [6]. Therefore, the timing/interval of pretreatment is obviously critical for the alterations in the innate immune microenvironment in the liver caused by GalCer.

The discrepancies between our observations and theirs can be explained in part by the fact that NKT cells consist of heterogeneous subsets of lymphocytes which express both NK and T cell surface markers. Classical, type I NKT cells express invariant T cell receptor (TCR), which is a complex of Vα14-Jα18 chain and β2 macroglobulin heterodimer. In contrast, non-classical, type II NKT cells contain a different type of TCR, which also recognizes glycolipid antigens in a CD1d-restricted manner [7]. *CD1d*-KO, therefore, lack both type I and type II NKT cells, whereas *Vα14-Jα18*-KO mice are defective in type I NKT cells alone and maintain type II NKT cells intact. Therefore, it is quite important to note which type of KO mice are used in the study. The functional differences between type I and type II NKT cells in liver pathophysiology still remain unclear. Recently, we investigated the differences in acute thioacetamide (TAA) intoxication between *CD1d*-KO and *Vα14*-KO mice (unpublished observations). As described in our manuscript, *CD1d*-KO mice are protected against TAA-induced mortality; however, the mortality rates following a single injection of TAA in *Vα14*-KO mice were almost similar as in wild-type (WT) animals. The necro-inflammatory responses, as well as the elevation in serum aminotransferases levels, were significantly improved in *Vα14*-KO mice in comparison with WT mice, but the degree of liver injury was much severer as compared to *CD1d*-KO mice. The difference in TAA-intoxication between *CD1d*- and *Vα14*-KO mice suggests the differential role in type I and type II NKT cells. These findings indicate that type I NKT cells seem to play a major role in systemic inflammatory responses, which lead to mortality in both WT and *Vα14*-KO mice. On the other hand, type II NKT cells alone can promote TAA-induced liver injury in part, even in the absence of type I NKT cells. It is intriguing to investigate the differences in induction of cytokines in each subset, especially from the aspect of the Th1/Th2 balance. Collectively, it is suggested that type I and type II NKT cells synergistically participate in the development of acute TAA-induced liver injury.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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