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# Chemokines: potent mediators of hepatic inflammation and fibrosis in chronic liver diseases

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**Article comment:**

Wehr A, Baeck C, Heymann F, Niemietz PM, Hammerich L, Martin C, Zimmermann HW, et al. Chemokine receptor CXCR6-dependent hepatic NK T cell accumulation promotes inflammation and liver fibrosis. *J Immunol* 2013; 190(10): 5226-36.

**Comment:**

Chronic liver injury is characterized by inflammation and fibrosis. Unresolved progression of fibrosis can lead to hepatocellular carcinoma and end-stage liver disease.[1] Chronic inflammation is often associated with apoptosis and is thought to accelerate steatosis and fibrogenesis.[1,2] Several immune cell types in the liver such as infiltrating monocytes and resident Kupffer cells have been shown to mediate the propagation of inflammatory insults through production of potent cytokines (such as TNF-α and IL-1β),[3] while CD8 T lymphocytes have been shown to promote fibrosis via activation of hepatic stellate cells (HSC);[4] by contrast, natural killer (NK) cells have been shown to be inhibitory to fibrosis in some liver injury models.[5] To add to the repertoire of immune cells regulating hepatic injury and fibrogenesis, a recent publication in the *Journal of Immunology,* has delineated a previously undiscovered role for a chemokine receptor, CXCR6, and its ligand CXCL16, in the hepatic recruitment of NKT cells and promoting liver inflammation and fibrosis.[6]

NKT cells are a diverse population of T cells that bear markers of T cell receptors (TCRs) and NK cells.[7] NKT cells are abundant in the liver, and different subsets with diverse properties exist in humans and mice. They have been shown to be involved in varied functions such as maintenance of liver homeostasis, immune patrolling and production of potent cytokines such as IFN-γ and IL-4 upon activation.[7-11]

Chemokines are peptide mediators, which stimulate chemotaxis of target cells that bear their cognate receptors. A number of recent studies have highlighted an important role for chemokines and their corresponding chemokine receptors in regulating immune cell infiltration and inflammation in response to liver injury.[12,13]

Previous studies have demonstrated that mice lacking NKT cells showed a decrease in liver inflammation and fibrosis.[7,9,11] In this study, the authors wanted to investigate the molecular mechanisms underlying NKT cell recruitment and action during liver fibrogenesis. The authors hypothesized that the chemokine receptor, CXCR6 and its cognate ligand, CXCL16 played a vital role in regulating hepatic NKT cells during liver injury. In humans, high levels of CXCR6+ T cells have been detected in patients with chronic liver disorders, and its known ligand CXCL16 has been detected in hepatocytes of patients with liver disease.[14]

In this study, Wehr, *et al*. first showed that the expression levels of CXCR6 and CXCL16 are enhanced in livers of patients with different chronic liver diseases (CLD), suggesting a role for CXCR6-CXCL16 interaction in CLD. They also demonstrated that the expression levels of the two proteins were upregulated in mice subjected to either toxic liver injury by carbon tetrachoride ($CCL_4$, fibrosis model) or chronic diet-induced liver injury by methionine choline-deficient diet (MCD, nonalcoholic steatohepatitis model). The specific hepatic cell populations with elevated levels of CXCL16 were endothelial cells, recruited macrophages and resident Kupffer cells, whereas NKT cells were among the T lymphocytes that expressed the highest levels of CXCR6 in the mice.

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To determine the functional importance of CXCR6 expression in the recruitment of inflammatory cells to the injured liver, the authors used a state-of-the-art technique, namely intravital multiphoton microscopy, which allowed *in vivo* real-time imaging following $CCL_4$ mediated liver injury. For this experiment, Wehr, *et al*. utilized $CXCR6^{+/gfp}$ and $CXCR6^{gfp/gfp}$ mice in which they knocked-in green fluorescent protein (GFP) cDNA into the CXCR6 coding regions of one or both alleles of the CXCR6 gene. They showed that GFP expression was predominantly detected on NKT cells among the liver immune cell types. There were significantly higher levels of CXCR6/GFP+ NKT cells visualized in heterozygous $CXCR6^{+/gfp}$ mice showing high motility even in healthy liver; and they migrated rapidly in response to CXCL16 ligand in a chemotaxis assay in the mice treated with $CCL_4$. In contrast, the CXCR6 $^{gfp/gfp}$ mice (CXCR6 null mice) had significantly fewer intrahepatic GFP+ cells. Furthermore, the GFP+ cells from the CXCR6 null mice displayed a migratory defect in response to CXCL16, suggesting that CXCR6 was critically responsible for the hepatic NKT cell accumulation and migration under baseline conditions and upon liver injury by $CCL_4$.

The authors next investigated if CXCR6 regulates hepatic inflammation and fibrosis during liver injury, by assessing hepatic immune cell accumulation, steatohepatitis and fibrogenesis. They subjected WT and CXCR6 null mice to the two experimental models of liver injury ($CCL_4$ and MCD diet). As expected, WT mice developed liver fibrosis and inflammation with elevated accumulation of leukocytes, NKT cells and macrophages, in both models, whereas livers of CXCR6 null mice had greatly diminished numbers of total leukocytes, NKT cells and macrophages, and were protected from development of steatohepatitis. CXCR6 null mice also showed significantly reduced liver fibrosis (as assessed by Sirius red staining and hydroxyproline levels) demonstrating that CXCR6 regulates accumulation of hepatic inflammatory immune cell mediators such as NKT cells and macrophages; and development of steatohepatitis and fibrosis.

The authors next utilized FACS sorting (using both the CD4+NK1.1+ and the αGalCer-loaded CD1d tetramer-based analysis) to isolate and purify the hepatic NKT cells from WT and CXCR6 null mice from control and MCD diet fed mice and assessed the purified NKT cell population for cytokine production. They found that NKT cells from CXCR6 null mice from MCD model had greatly attenuated production of cytokines such as IFN-γ, TNF-α and MCP-1, relative to WT NKT cells, demonstrating that CXCR6 regulates not only NKT cell accumulation in the liver, but also their ability to produce inflammatory cytokines and thereby propagate inflammation during liver injury.

Finally, the authors wanted to confirm that NKT cells promote fibrogenesis in a CXCR6-dependent manner. To explore this, they conducted adoptive transfer studies, wherein they injected purified WT NKT cells or CD4 cells into MCD diet fed WT and CXCR6 null mice. Only the livers of CXCR6 null mice that received WT NKT cells developed significant fibrosis, while the transferred WT CD4 T cells showed no effect on fibrosis in CXCR6 null mice. Thus, the authors elegantly demonstrated that CXCR6-dependent NKT cells are required for fibrogenesis.

Chronic liver diseases are complex fibroinflammatory disorders. While numerous inflammatory molecules that contribute to and exacerbate steatohepatitis and fibrosis have been identified, the relative contribution of the various components is still unclear. Identifying and ascribing a hierarchy to the various inflammatory mediators has potential therapeutic implications.

Limitations to extrapolating these interesting data to complex human CLD should be recognized; even though in their model, adoptive transfer of CD4 T cells, for example, did not contribute to fibrosis, the possibility that under the complex *in vivo* milieu of CLD, the conditions necessary to render other cell types fibro-inflammatory may be met, and cannot be excluded.

While the authors have shown a crucial role of CXCR6 in the recruitment of NKT cells in the liver, and their involvement in promoting fibrosis, the precise molecular mechanism by which NKT cells activate stellate cells to accelerate fibrogenesis remains to be determined. Although targeting the CXCR6-CXCL16 interaction to control the hepatic NKT cell population has appeal as an area of therapeutic intervention to prevent fibrogenesis, it may be important to target the specific subset of NKT cells which are profibrogenic, without impacting beneficial subsets involved in normal immune functions.

In summary, these findings by Wehr, *et al*. uncover a novel role for CXCR6 and its ligand CXCL16, in liver inflammation and fibrosis, and highlight the pivotal role of NKT cells in the pathogenic mechanism regulating hepatofibrogenesis in CLD; providing a basis for future studies which could potentially investigate these chemokines as antifibrotics.

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