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REVIEW

# NKT cell subsets as key participants in liver physiology and pathology

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**Natural killer T (NKT) cells are innate-like lymphocytes that generally recognize lipid antigens and are enriched in microvascular compartments of the liver. NKT cells can be activated by self- or microbial-lipid antigens and by signaling through toll-like receptors. Following activation, NKT cells rapidly secrete pro-inflammatory or anti-inflammatory cytokines and chemokines, and thereby determine the milieu for subsequent immunity or tolerance. It is becoming clear that two different subsets of NKT cells—type I and type II—have different modes of antigen recognition and have opposing roles in inflammatory liver diseases. Here we focus mainly on the roles of both NKT cell subsets in the maintenance of immune tolerance and inflammatory diseases in liver. Furthermore, how the differential activation of type I and type II NKT cells influences other innate cells and adaptive immune cells to result in important consequences for tissue integrity is discussed. It is crucial that better reagents, including CD1d tetramers, be used in clinical studies to define the roles of NKT cells in liver diseases in patients.**
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## INTRODUCTION

The liver is a specialized tissue that, owing to its anatomical location, is the first recipient of gut-derived bacteria and their products, such as lipopolysaccharides. Therefore, the hepatic immune response to such products has to be carefully regulated to avoid liver injury. Liver inflammation is an integral part of the hepatic wound-healing response to injury due to, for example, excess fat, alcohol or viruses. Controlled inflammation may be beneficial in the short term in terms of the promotion of regeneration or an effective immune response against pathogens. However, chronic inflammation and the associated regenerative wound-healing response are strongly linked to the development of fibrosis, cirrhosis and cancer.[1] Compared with other peripheral organs, the liver is enriched in a number of innate immune cells, including resident macrophages, Kupffer cells (KCs), dendritic cells (DCs), natural killer (NK) cells and NK T (NKT) cells.[2,3] NKT cells are particularly enriched in the murine liver and form an important nexus that connects innate and adaptive immunities; these cells therefore have a crucial role in setting up the inflammatory response. Because the two NKT cell subsets can have opposing roles in immune responses that are mediated by the secretion of both pro-inflammatory and anti-inflammatory cytokines, it is necessary to distinguish their roles in acute and chronic inflammatory conditions of the liver, such as alcoholic hepatitis, autoimmune hepatitis (AIH) and steatohepatitis. In this review, we discuss the current knowledge about the roles of the two major subsets of NKT cells in inflammatory liver diseases and the maintenance of immune tolerance.

## TWO MAJOR SUBSETS OF NKT CELLS IN THE LIVER

NKT cells are innate-like T cells that express TCR-αβ chains in addition to the typical NK cell markers and have an important immunoregulatory role in inflammatory conditions, including autoimmune diseases, infectious diseases and cancer.[4–6] NKT cells act as a bridging system between innate and adaptive immunities.[7] These cells can recognize both exogenous and endogenous lipid antigens in the context of the major histocompatibility complex-like molecule CD1d.[8–10] Studies using knock-in $Cxcr6^{gfp/+}$ mice have demonstrated that type I NKT cells migrate to the liver sinusoids within minutes of α-galactosylceramide (αGalCer) injection.[11,12] The enrichment and constitutive activation of NKT cells in the liver sinusoids indicate that these cells participate in the mechanisms that

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control the induction and/or prevention of inflammation in the liver in various immunological responses.[4,13,14]

CD1d-restricted NKT cells are classified into two main subsets, that is, type I or invariant NKT cells and type II or diverse NKT cells. Type I NKT cells are more prevalent than type II NKT cells in mice and comprise ~50% of murine intrahepatic lymphocytes.[4,15–17] Type I NKT cells express a conserved semi-invariant αβTCR that is encoded predominantly by a germ line Vα gene (Vα24 in humans and Vα14 in mice) and Jα18 gene segments paired with a more diverse set of non-germ line Vβ genes (Vβ8.2, Vβ7, or Vβ2 in mice and Vβ11 in humans).[18] In humans, type I NKT cells comprise ~0.1–1% of the circulating T cells. CD4- and CD4+ type I NKT cells predominantly secrete Th1- and Th2-type cytokines, respectively, and Th1-like CD8α+ type I NKT cells have also been described.

Type II NKT cells are more abundant than type I cells in humans, and as in mice, they express relatively diverse TCR-α- and TCR-β chains. Recently, we demonstrated that one major subset of type II NKT cells that is reactive to the β-linked self-glycolipid sulfatide expresses an oligoclonal TCR repertoire that predominant uses the Vα3/Vα1-Jα7/Jα9 and Vβ8.1/Vβ3.1-Jβ2.7 gene segments.[19] Recent insights derived from the crystal structures of a type I NKT cell TCR-αGalCer/CD1d complex and a type II NKT cell TCR-sulfatide/CD1d complex suggest that distinct molecular motifs act in TCR recognition by type I and type II NKT cells.[21–23] The type I NKT TCR binds to CD1d in a parallel configuration that mainly involves the α-chain. The crucial residues within the CDR2β, CDR3α and CDR1α loops of the semi-invariant TCR of type I NKT cells have been demonstrated to be involved in the recognition of the αGalCer/CD1d complex.[24] In contrast, the sulfatide-reactive type II NKT TCR binds its ligands primarily with its β-chain by pinning them against the CD1d surface.

## ANTIGEN RECOGNITION AND ACTIVATION OF TYPE I NKT CELLS

Type I NKT cells were initially characterized as a major subset in mice that are reactive to a marine sponge-derived glycolipid αGalCer; this glycolipid stimulates these mice like a super-antigen in that it binds with high affinity to CD1d and type I NKT TCRs.[25] However, other glycolipids, such as isoglobotrihexosylceramide, are also able to activate type I NKT cells in a CD1d-dependent manner, but none of them are as effective as αGalCer.[27–30] In addition to lipid antigens, type I NKT cells are also activated following toll-like receptor (TLR)-mediated signaling and/or by cytokines (interleukin (IL)-12, IL-18 or type I interferon (IFN)) secreted by activated antigen-presenting cells (APC), such as KCs, hepatocytes and myeloid DCs.[31] Following activation, type I NKT cells can secrete Th1-, Th2- or Th17-like cytokines. Thus, depending upon the tissue milieu, antigen-presenting cell and lipid antigen, type I NKT cells can secrete different cytokine profiles. For example, type I NKT cells predominantly secrete IFN-γ following ischemia, toxin-induced injury or stimulation in the presence of IL-18 plus IL-12, but these cells secrete IFN-γ, IL-4 and IL-17 in response to αGalCer.[33]

Following activation, type I NKT cells can further stimulate DCs, NK cells, B cells, and conventional $CD4^+$ and $CD8^+$ T cells that can further mediate liver damage.[18,34] Furthermore, cytokines and chemokines secreted by activated type I NKT cells result in the recruitment of neutrophils, myeloid cells and monocytes to the liver.[35–40] Type I NKT cells can promote fibrogenesis involving the Hedgehog pathway,[41,42] and cytokines including osteopontin (OPN) lead to hepatic stellate cell (HSC) activation.[14,39] In addition, activated type I NKT cells can also kill hepatocytes directly via Fas/FasL interactions or indirectly by activating NK cells. KCs are located at the interface of the portal vein (within the sinusoidal vascular space) and the systemic circulation, and this critical location makes these cells key factors in the activation of the immune response by recognizing danger signals (pathogen-associated molecular patterns and danger-associated molecular patterns) through the expression of TLR and nucleotide-binding oligomerization domain-like receptors.[43] Activated KCs produce a variety of pro-inflammatory cytokines, such as IL-1β, IL-18, tumor necrosis factor (TNF)-α and IL-12, which have important roles in regulating the recruitment and activation of type I NKT cells.[44,45]

## ANTIGEN RECOGNITION AND ACTIVATION OF TYPE II NKT CELLS

Our laboratory has identified and characterized a major subset of type II NKT cells that is reactive to the self-glycolipid sulfatide.[4,19,20,36] Some type II NKT cells have also been demonstrated to recognize other β-linked glycolipids, including β-D-glucopyranosylceramide, βGluCer and βGalCer, as well as some pollen-derived lipids.[21,46,47] More recently, we demonstrated that other self-phospholipids, including lysophosphatidylcholine (LPC), lysosphingomyelin and lyso-platelet-activating factor, can effectively stimulate a subset of type II NKT cells in a CD1d-dependent manner both *in vitro* and *in vivo*.[48] IL-13-secreting LPC-reactive type II NKT cells have also been reported to be increased in myeloma patients relative to healthy donors.[49] A subset of type II NKT cells can also be stimulated by the lysophosphatidylethanolamine,[50] which is generated in hepatocytes following hepatitis B infection.[51] Phospholipids, such as phosphatidylglycerol, phosphatidylinositol and cardiolipin, can also activate murine type II NKT cell hybridomas.[52] Recently, murine and human type II NKT cells specific for glucosylsphingosine 1 have been found to be associated with disease severity in a murine model and also in peripheral blood mononuclear cells from Gaucher disease patients.[53]

In contrast to the predominantly pro-inflammatory role of type I NKT cells in liver damage, type II NKT cells are able to suppress the pro-inflammatory response induced by type I NKT cells and consequently protect against liver damage. Furthermore, the activation of type II NKT cells with sulfatide does not induce the activation of B, NK or T cells.[54] Therefore, the study of type II NKT cells following sulfatide activation has

uncovered a dominant immunoregulatory pathway that has been demonstrated to be involved in protection against hepatic ischemia reperfusion injury (IRI),[35] type 1 diabetes[55] and experimental autoimmune encephalomyelitis.[20]

## CROSS-REGULATION BETWEEN TYPE I AND TYPE II NKT CELLS

A major mechanism of cross-regulation between type I and type II NKT cells is revealed following the activation of type II NKT cells by a self-glycolipid or lysophospholipid. Following the administration of sulfatide, CD1d is upregulated on plasmacytoid DCs, but not conventional DCs (cDCs), which results in the activation of sulfatide-reactive type II NKT cells and the secretion of IL-12 and macrophage inflammatory protein-2, which in turn leads to the recruitment of type I NKT cells into the liver. Interestingly, these cellular interactions lead to the tolerization of cDCs and anergy induction in the recruited type I NKT cells.[36] Both anergic type I NKT cells and IL-10-secreting cDCs also inhibit adaptive immunity following sulfatide-mediated activation of type II NKT cells by suppressing the cytokine burst and neutrophil recruitment into the liver, which thereby attenuates concanavalin A-induced hepatitis, IRI and alcoholic liver disease (ALD).[36,38] A similar mechanism of immune regulation by type II NKT cells has been found to be involved following their activation by LPC.[48] Sulfatide-mediated activation of type II NKT cells can also modulate the activities of other immune cells, such as myeloid-derived suppressor cells (MDSCs), $CD11b^{+}Gr\text{-}1^{+}$ cells, B cells and neutrophils.[37,38,56] The opposing roles of type I and type II NKT cells have also been demonstrated in immune responses against parasites, antitumor immunity and autoimmunity (Figure 1).[4,57–59]

In contrast, there are experimental data that suggest that type I NKT cells could potentially cross-regulate type II NKT activity.[59] It has been demonstrated that the sulfatide stimulation of type II NKT cells completely inhibits the protective effect of αGalCer in the 15-12RM fibrosarcoma model but does not completely abrogate the protection afforded by αGalCer in CT26 colon cancer lung metastasis, and that type I NKT cells may exhibit moderate suppressive effects on the activity of type II NKT cells. A better understanding of the mechanisms involved in cross-regulation between type I and type II NKT cell subsets is crucial for the development of strategies to manipulate the outcome of the immune response in human inflammatory liver diseases.

More recently, type II NKT cell activation induced by IL-25 has been demonstrated to be involved in the regulation of inflammation in adipose tissue and the prevention of high fat diet-induced obesity in mice. The transfer of type II NKT cells into obese mice induces greater and more prolonged weight loss and improved glucose tolerance.[60]

Interestingly, similar to conventional T cells, type I NKT cells also become unresponsive or anergic after a secondary challenge following a primary TCR activation with αGalCer. Anergic type I NKT cells express greater levels of programmed death 1 (PD-1). Furthermore, blocking the PD-1/PD ligand 1 (PD-L1) pathway prevents αGalCer-induced but not bacterial lipid- or sulfatide-induced anergy in type I NKT cells[61,62] Recent studies have further indicated the involvement of the E3-ubiquitin ligase Cbl-b and mTOR signaling in anergy induction in type I NKT cells.[63]

## ROLES OF NKT CELL SUBSETS IN PATHOLOGICAL CONDITIONS IN THE LIVER

In the majority of experimental models of chronic liver diseases, including those of IRI,[64] con A-induced hepatitis,[36,54,65] primary biliary cirrhosis (PBC)[66] and nonalcoholic fatty liver disease (NAFLD),[67–69] type I NKT cells have been demonstrated to have a pathogenic role. However, in acute liver injury models, type I NKT cells can have a protective role. For example, in a mouse model of biliary obstruction and cholestasis, and in a model of acute $CCl_4$-induced fibrosis, type I NKT cell-dependent inhibition of macrophage inflammatory protein-2, KC and TNFα production leads to the inhibition of both neutrophil infiltration and liver injury.[70–72] Collectively, these data suggest that while in acute injury, type I NKT cell activation may be protective, in chronic conditions, type I NKT cells promote liver injury. Consistent with the dual role of type I NKT cells, Wang *et al.*[73] reported that αGalCer-mediated activation of these cells promotes neutrophil infiltration and hepatitis in a STAT-6 dependent manner, whereas this activation can also control liver injury by inducing neutrophil apoptosis via a STAT-1-dependent mechanism.

### Alcoholic liver disease

ALD is a common medical condition that results from chronic alcohol abuse and is among the most frequent causes of death in the general population. This disease can progress from hepatic steatosis (fatty liver) to alcoholic hepatitis (10–35%) and ultimately to alcoholic fibrosis or cirrhosis (8–20%). The cellular and molecular mechanisms underlying ALD involve complex interactions between innate immune cells (NK, NKT and γδ T cells), parenchymal cells (hepatocytes) and non-parenchymal cells (sinusoidal endothelial cells, KCs, HSCs and DCs).[74] Activation of KCs via LPS/TLR signaling-dependent mechanisms following alcohol consumption results in increased secretion of a variety of pro-inflammatory cytokines and chemokines in addition to eicosanoids and reactive oxygen species.[75,76] Indeed, TNFα and IL-1β levels are increased in the sera and livers of alcohol-fed mice, and the neutralization of IL-1β in KC attenuates type I NKT cell accumulation and steatosis. Moreover, Cui *et al.*[44] demonstrated that KC-derived NLRP3 inflammasome activation and IL-1β release are essential for hepatic NKT cell accumulation and activation in ALD. Consistently, gut-derived bacteria or their products, such as lipopolysaccharides, that are leaked into the circulation activate KCs via nucleotide-binding oligomerization domain-like receptors, specifically NLRP3, which could lead to NKT cell activation.[44]

Using a murine model of chronic plus binge ethanol feeding with a Lieber-DeCarli liquid diet, we recently demonstrated that an increased number of activated type I, but not type II

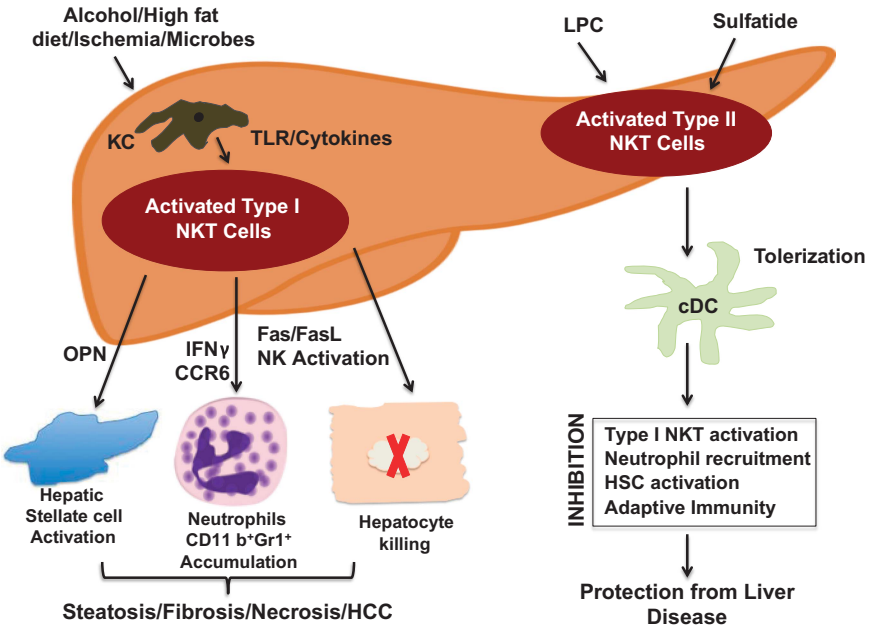


**Figure 1** A proposed model depicting the opposing roles of type I and type II NKT cells in inflammatory diseases in the liver. Type I NKT cells are rapidly activated following liver injuries induced by alcohol, high-fat diet, ischemia and/or gut-derived microbial products. Liver-resident antigen-presenting cells, such as KCs, and TLRs/cytokines mediate their activation, which results in the cytokine/chemokine-dependent recruitment of myeloid cells (CD11b+Gr-1+) and neutrophils, and the activation of HSC and NK cells. These cellular interactions lead to steatosis, fibrosis and hepatocyte necrosis. These events are also involved in the development of HCC. In contrast, type II NKT cells are activated following the presentation of self-lipids, such as sulfatide and LPC, which results in the induction of a cross-regulatory pathway that inhibits type I NKT cells, tolerizes cDCs and blocks the inflammatory cascade and liver disease. cDCs, conventional DCs; HCC, hepatocellular carcinoma; HSC, hepatic stellate cell; KC, Kupffer cell; LPC, lysophosphatidylcholine; NK, natural killer; NKT, natural killer T cells; OPN, osteopontin; TLR, toll-like receptor.

NKT, cells accumulate in the liver, which is consistent with another model of mice that are fed alcohol via an intragastric tube.[38,40,77,78] Moreover, J$\alpha$18$^{-/-}$ mice (which are deficient in type I NKT cells) and CD1d$^{-/-}$ mice (which are deficient in both NKT cell subsets) are protected against liver injury after alcohol intake.[38,40] The activation of type I NKT cells is required for the hepatic recruitment of inflammatory Gr-1$^{+}$CD11b$^{+}$ cells and neutrophils that results in liver tissue damage. Accordingly, several cytokines and chemokines that are associated with inflammation and neutrophil recruitment, including OPN, IL-1β, IL-6, TNFα, macrophage inflammatory protein-2, MIP-1β and CXCR1, are upregulated in the livers of alcohol-fed wild-type mice but not in type I NKT cell-deficient mice. Furthermore, the inhibition of type I NKT cells via a direct pathway involving all-*trans* retinoic acid or the indirect mechanisms of sulfatide-mediated activation of type II NKT cells or the administration of an anti-CD1d blocking antibody significantly suppresses this pro-inflammatory cascade and ameliorates ALD.[38,40] Importantly, the RARγ-signaling pathway is involved in the inhibition of type I NKT cells by ATRA owing to the greater expression of RARγ in these cells; accordingly, the clinically relevant RARγ agonist tazarotene blocks the development of ALD.[38]

In humans, the role of type I NKT cells in ALD has not been carefully investigated. However, consistent with the data from murine models, pro-inflammatory cytokines, including TNFα, IL-6, IL-8, OPN and IL-1, are increased in the sera and liver biopsies of humans with alcoholic hepatitis and may be correlated with disease severity/mortality.[13] Furthermore, E-selectin expression is highly upregulated in human alcoholic fatty livers but not in alcoholic cirrhosis, which suggests that E-selectin may have a role in hepatic neutrophil infiltration and injury in the early stages of disease.[77] Moreover, in patients with alcohol hepatitis, reduced NKG2D expression in CTLs, NK cells and type I NKT cells has been found to correlate with disease severity, which suggests that these cells are involved in promoting liver damage.[79] In contrast, increased frequencies of IL-22-producing cells and increased IL-17 plasma levels are associated with improved prognoses in patients with alcoholic hepatitis.[79–81]

### Nonalcoholic fatty liver disease

NAFLD is the most frequent chronic liver disease. NAFLD affects 10–20% of the population in developed countries, and its prevalence is increasing with the rise of diabetes and obesity. NAFLD is defined by the abnormal accumulation of fat within the liver, or steatosis, which can progress to severe inflammatory cell infiltration or nonalcoholic steatohepatitis (NASH) accompanied by fibrosis or necrosis or progress to liver cirrhosis and hepatocellular carcinoma (HCC).[82,83] Although a detailed CD1d tetramer-based analysis of the activation profiles of NKT cell subsets in NASH is lacking, reduced

numbers of type I NKT cells are found in mice fed methionine/choline-deficient or high-fat diets (CD-HFDs) and in ob/ob mice.[67,84] Activation of KC or the Tim-3/Gal-9 signaling pathway can lead to apoptosis in type I NKT cells in the liver, and could thus contribute to steatosis and insulin resistance.[45,67,85] Indeed, the depletion of KCs via treatment with gadolinium chloride reduces hepatic IL-12 expression and does not lead to type I NKT apoptosis, and thereby prevents diet-induced hepatic steatosis and insulin resistance. Consistently, the activation of the Hedgehog pathway and HSCs has been revealed to be associated with type I NKT cells in mice fed an MCD diet or a combination of a CD-HFD.[68,69,86] Similarly, the hepatic CD1d expression and increased numbers of $CD3^+$ $CD56^+$ cells in NASH patients suggest a potentially important role of NKT cells in this disease.[68,69,86,87]

**Autoimmune hepatitis**

AIH is a chronic autoimmune inflammation of the liver that is characterized by T-cell periportal and intralobular infiltration of the liver in the absence of other liver diseases and in association with increased serum transaminases, hypergammaglobulinemia and hepatocyte-specific autoantibodies. AIH is strongly linked to HLA-A1, -B8, -DR3 and -DRB1. There are at least two subtypes of AIH; type I is characterized by autoantibodies directed against smooth muscle antigens and antinuclear antibodies, and type II is characterized by autoantibodies directed against cytochrome p450 2D6 or formiminotransferase cyclodeaminase. Both types also share autoantibodies that recognize O-phosphoseryl-tRNA (Sec) selenium transferase/soluble liver antigen.[88–90] Earlier studies revealed that while type I NKT cells are pathogenic, type II NKT activation protects mice against ConA-induced hepatitis.[36,65] Recently, a strong correlation of high levels of IL-17 in the serum and liver with disease severity was found in patients with PBC and AIH, which suggests a role of IL-17-producing type I NKT cells.[91,92] Consistently, significant increases in the frequencies of IL-17+ expressing cells have been demonstrated in the portal areas of liver biopsies from PBC, chronic hepatitis C (HCV), NASH and AIH patients compared with control subjects.[88] In a murine model of αGalCer-induced liver injury, CD4+ type I NKT cells have been found to be the main source of IL-17, and accordingly, administration of anti-IL-17-neutralizing monoclonal antibodies before αGalCer injection significantly exacerbates hepatitis, the hepatic recruitment of neutrophils, and the production of IL-12 and TNFα by pro-inflammatory monocytes. In contrast, the administration of exogenous recombinant murine IL-17 before αGalCer injection attenuates hepatitis and prevents the recruitment of inflammatory monocytes to the liver. Thus, secretion of IL-17 by type I NKT cells could have a crucial role in AIH.[93]

**PBC and primary sclerosing cholangitis**

Biliary diseases such as primary sclerosing cholangitis (PSC) and PBC are characterized by an inflammatory immune response that leads to the destruction of the bile ducts. Cholangiocytes have been suggested to have an active role in immune responses.[94,95] Notably, murine and human cholangiocytes express CD1d and can present exogenous and endogenous lipid antigens to both type I and type II NKT cells.[66] Hepatic CD1d expression and type I NKT cell numbers have consistently been found to be elevated in patients with PBC 95. However, CD1d expression is downregulated in the biliary epithelia of patients with late PSC and PBC. Consistent with the pathogenic role of type I NKT cells in other liver diseases, an important role for type I NKT cells in the initiation of PBC was recently demonstrated in two murine models. In these models, NKT deficiency was found to attenuate the development of PBC induced by the overexpression of a dominant-negative TGF-βR in T cells or by infection with novosphingobium aromaticivorans. It is becoming clear that type I NKT cells cross talk with other autoreactive intrahepatic B cells and conventional CD4/CD8 T cells that are involved in these autoimmune diseases.[90,96,97]

**Chronic hepatitis B and HCV infection**

Chronic hepatitis B (HBV) and HCV infections account for 57% of the cases of liver cirrhosis and 78% of the cases of primary liver cancer worldwide, and cause one million deaths per year. Type I NKT cells may have a role in controlling HCV infections, particularly in the early stages of HCV infection in humans. Via IFN-γ secretion, type I NKT cells have been demonstrated to be capable of inhibiting HCV replication in hepatocytes,[98] and their activities are positively correlated with the outcome of acute HCV infection and the efficacy of IFN-α treatment in chronic HCV infection. Numerous studies have reported that type I NKT cells are significantly depleted during chronic HCV infection, which likely contributes to the failure of its resolution. Similarly high numbers of activated type I NKT cells have been found in the early stages of HBV infection in humans.[99–101] The inhibitory effect of type I NKT cells on HBV infection is likely mediated via the secretion of IFN-γ, which inhibits HBV replication and stimulates adaptive immune responses.[97] Although NKT cells can control HBV and HCV replication in the early stages of infection, NKT cells may also contribute to liver injury during chronic viral hepatitis infection via several mechanisms, including the lysis of hepatocytes, the production of pro-inflammatory cytokines, the induction of hepatocyte apoptosis, and the inhibition of hepatocyte proliferation.[102–104]

**Hepatocellular carcinoma**

HCC is frequently associated with chronic inflammatory liver diseases such as NASH and viral hepatitis.[105] Generally, NKT cells have a crucial dual role in cancer: they can promote an antitumor response via the activation of effector CD4/CD8+ T cells; and they can also promote tumor growth by recruiting suppressor or regulatory T cells to induce tolerance or by producing Th2 cytokines, which subsequently result in the inhibition of tumor antigen-specific CD8+ T-cell expansion.[106] Although NKT cells are abundant in the liver, relatively fewer studies have attempted to clarify their role in HCC.

It has been shown that long-term feeding with a CD-HFD induces the activation of intrahepatic CD8+ T cells and NKT cells, as well as the secretion of inflammatory cytokines in mice. NKT cells primarily cause steatosis via the secretion of LIGHT, whereas CD8+ and NKT cells cooperatively induce liver damage. Hepatocellular TLR4 and canonical nuclear factor-κB signaling facilitate the NASH-to-HCC transition, which suggests the involvement of distinct molecular mechanisms in NASH and HCC development. $CD4^+$ type I NKT cells can also mediate antitumor effects through the inhibition of the inflammatory response triggered by the activation of the oncogenic β-catenin pathway.[107] In addition, NKT cells suppress tumor growth in mice after adoptive transfer of HCC tumor lines.[50,108] In patients with HCC, there is an increase in the frequency of NKT in the tumor relative to the blood, and CD4+ Vα24/Vβ11 type I NKT cells secreting Th2 cytokines accumulate in human intrahepatic malignant tumors and inhibit tumor-specific CD8+ T-cell responses.[105]

**Liver injury induced by toxins and drugs**

The liver is specialized to metabolize circulating drugs or toxins, but the metabolism of these drugs frequently induces liver injury.[109] The pathological role of NKT cells in drug-induced liver injury has been investigated using the murine model of acetaminophen-induced liver injury. Type I NKT-deficient mice have been found to be more susceptible to acetaminophen-induced liver injury than wild-type mice.[110] Similarly, chronic $CCl_4$-, Con A- and paracetamol-induced liver injuries are blunted in type I NKT-deficient mice.[38,71] The drug or its metabolites trigger the activation of type I NKT cells that are responsible for the massive release of high levels of cytokines, including OPN, IFN-α and IL-4, and the increased expression of FasL by hepatocytes, which leads to massive hepatocellular necrosis.[4,14,39,109]

**Ischemia and reperfusion injury and liver transplantation**

IRI represents a complex inflammatory immune response that generally occurs in a sterile environment and results in tissue damage.[111] IRI is a major problem in liver resection and liver transplantation. IRI involves the activation of both innate and adaptive immunity that leads to the necrotic cell death of hepatocytes. The sterile immune response also involves signaling through pattern recognition receptors such as TCRs, for example, TLR4.[112] TLR4 likely mediates the early activation of type I NKT cells that secrete IFN-γ during liver IRI as demonstrated recently.[35] Accordingly, mice that are deficient in type I NKT cells or the cross-regulation of type I NKT cells following sulfatide-mediated activation of type II NKT cells leads to significantly reduced inflammatory infiltrate, including CD11b+Gr-1+ cells, and liver damage following reperfusion.[35] The tolerization of cDCs following the activation of type II NKT cells has a major role in the control of IRI because the depletion of cDCs leads to an increase in hepatic injury.[113] Consistent with the idea that NKT cells can control the accumulation of granulocytes and neutrophils in tissues, their excessive accumulation can promote uncontrolled inflammation and tissue damage during IRI.[114,115] It is also relevant to mention that because adaptive immune responses also become involved in IRI, the tolerization of DCs following the cross-regulation of NKT cells should be able to blunt the further detrimental effects of both conventional CD4+ and CD8+ T cells.

In liver transplantation, both the host-residual and donor-derived NKT cells exert protective functions that are particularly well described in graft-versus-host disease. Whereas the type I NKT subset is involved in protection mediated by host-residual T cells, type II NKT cells have critical roles in donor-derived protective effects.[116,117] Indeed, on the one hand, the adoptive transfer of type I NKT cells or the administration of αGalCer can attenuate graft-versus-host disease in recipient mice due to the vigorous secretion of IL-4 by type I NKT cells and the subsequent Th2 polarization of the immune response. On the other hand, donor type II NKT cells not only produce IL-4-like type I NKT cells but also produce IFN-γ, which induces apoptosis in donor $CD4^+$ and $CD8^+$ T cells in a Fas-dependent manner.[118] Moreover, human $CD161^+$ CD1d-reactive BM-derived type II NKT cells have been found to specifically suppress the mixed lymphocyte reaction and are able to induce tolerance to allografts.[119] Collectively, the appropriate targeting of the NKT cell subsets may have an important role in the development of strategies for reducing tissue damage due to IRI and liver transplantation.

**Liver regeneration**

The liver has a substantial ability to regenerate following tissue loss or injury. This regeneration process is controlled by various cytokines, growth factors and hormones.[120–122] The accumulation of type I NKT cells in the liver following partial hepatectomy suggests that type I NKT cells may have a role in liver regeneration. Accordingly, under inflammatory conditions (for example, partial hepatectomy in HBV transgenic mice), the depletion of NKT cells significantly enhances liver regeneration.[98,102] More recently, Yin *et al.*[123] also revealed that the activation of type I NKT cells with αGalCer strongly inhibits liver regeneration via a mechanism that depends on NKT-derived IFN-γ and IL-4 secretion. Similarly, the reduction of the commensal bacterial load after oral ampicillin treatment induces the expansion of IL-12-secreting KCs that overactivate hepatic type I NKT cells to produce higher IFN-γ levels that inhibit liver regeneration.[124] Collectively, the activation of type I NKT cells in inflammatory conditions appears to inhibit liver regeneration.

## FUTURE PERSPECTIVE

We have discussed many of the key aspects of NKT cell activation and their functions in inflammatory conditions. Most of the advances in the understanding of the roles of NKT cell subsets in the liver have come from studies with animal models. Experimental and clinical studies are complicated by the fact that there are at least two major subsets of CD1d-restricted NKT cells that have opposing functions and

can be differentially activated at different time points during the progression of the disease. Different degrees of NKT cell activation can also lead to the secretion of a wide array of cytokines, chemokines and other factors. Furthermore, because NKT cells also modulate the activity of other key immune cells, including KCs, macrophages, DCs and major histocompatibility complex-restricted CD4+/CD8+ cells, a molecular understanding of these cross-regulatory influences will be key for understanding how the liver is able to maintain a proper balance between immune tolerance and immunity. During chronic liver disease processes, the actions of the different NKT cell subsets are inextricably interconnected and change depending on the stage of the disease. It will be important to develop specific reliable reagents to identify and characterize both type I and type II NKT cell subsets in humans, and also to carefully use CD1d tetramers and other reagents that can differentiate their activation in peripheral blood mononuclear cells and liver tissues.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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