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Complexity and function of NKT cells with potential application to hepatic transplant survival

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Abstract

One of the major innate-like lymphocyte populations enriched in liver consists of Natural Killer T (NKT) cells, which recognize self and foreign lipid antigens presented by the non-polymorphic class I MHC-like molecule CD1d. NKT cells express NK cell markers as well as T cell receptors (TCR) and can be classified into 2 categories: type I NKT cells use a semi-invariant TCR whereas type II NKT cells express diverse but still limited TCRs. An emerging body of evidence points to their opposing roles in inflammation, including ischemic reperfusion injury. Improved understanding of their roles in experimental models as well as as in humans and the means by which their function can be manipulated may provide therapeutic benefit in liver diseases and in organ transplantation.

A major problem in transplant survival, on a par with host rejection, is organ deterioration due to oxidative stress, mitochondrial dysfunction and the burst in expression of inflammatory cytokines that follow prolonged ischemia [1] (ischemia reperfusion injury, IRI). This has led to examination of trial procedures to identify defensive treatments for mitochondrial preservation, reduction of cytokine expression or enhancing expression of stress protectors prior to organ harvesting, during transport, or directly in recipients. Fewer attempts to reduce IRI consider the function or manipulation of cytotoxic effector immune cells recruited into stressed tissue areas (e.g., NK, NKT). In this review we will summarize our findings on the reciprocal interactions between two NKT cell subsets which indicate that efforts aimed at controlling this effector arm of the innate immune system may prove beneficial in extending transplant survival.

NKT cells are part of the unconventional T cells enriched in the liver that are characterized by a higher frequency, faster responses, limited TCR diversity and ability to respond to molecular patterns or biochemical classes of antigenic ligands [2]. NKT cells recognize lipid antigens presented by a conserved, non-polymorphic class I MHC-like molecule CD1d and can be broadly categorized into two subsets: type I or invariant iNKT cells which express an invariant TCR α chain (TRAV11 and TRAJ18 in mice and TRAV10 and TRAJ18 in humans) and a limited number of TCR β chains; and type II NKT cells (also called diverse NKT or dNKT) which do not use the invariant TCR α chain but use diverse TCR α and β chains [3–

6]. In this brief review we will discuss the two TCR $\alpha\beta$ + NKT cell subsets and their emerging critical role in liver disease and in transplantation research.

NKT cells can recognize both self and foreign phospholipids and glycolipids. Type I and type II NKT cells are reactive to the α - or the β -anomeric linkage of a carbohydrate moiety of the lipid tail in glycolipids. Type I NKT cells recognize their prototypic ligand α GalCer. Type II NKT cells are not reactive to α GalCer, but a subset of type II NKT cells recognize sulfatide and its analogs in both mice and in humans. Accordingly, staining with α GalCer/CD1d- or sulfatide/CD1d-tetramers enable these two types to be distinguished by cytofluorography [7, 8]. Antigen-recognition mechanisms of type I and type II NKT cells also differ in terms of how their TCRs dock onto the CD1d molecule and predominance of the TCR α or TCR β chain in antigen binding [9]. Among phospholipids, lysophosphatidylcholine (LPC) has been shown to be recognized by both human and murine type II NKT cells but not by murine type I NKT cells [10, 11]. Since endogenous levels of lysophospholipids can be altered following phospholipid hydrolysis during inflammation [12], lysophospholipid-reactive type II NKT cells may play a role in the regulation of inflammation in liver. Therefore systematic analysis of changes in type II as well as type I NKT cells in the blood and liver tissue in different clinical conditions is needed to understand their potential roles in human immune-mediated pathologies.

There are several features of NKT cells that are important in relation to their role in immunity: (a) they are enriched in liver and are present at the basolateral sides of sinusoids where they can easily sense antigens coming into liver from the gut via portal veins; (b) unlike conventional MHC-restricted T cells, the transcriptional program in NKT cells is driven by promyelocytic leukemia zinc finger (PLZF), and an adaptor molecule called SAP (signaling lymphocyte activation-molecule associated protein) [13] enables them to respond very rapidly by producing cytokines and in some cases, cytotoxic activity within minutes to hours following activation. Notably while type I NKT cells can be activated either directly through TCR stimulation or indirectly without TCR signaling by cytokines (IL-12, IL-18, or type I IFN) through Toll-like receptor (TLR)- mediated signaling type II NKT cell activation appears to be primarily via TCR signaling following recognition of a lipid/CD1d complex [5–6]. Consistently in many experimental conditions in which type I NKT cells are activated by TLR signaling in APCs, type II NKT cells remain un-activated; (c) NKT cell subsets can secrete both Th1/Th17- or Th2-like cytokines; (d), activation and cytokine secretion of NKT cell subsets has a major impact on other immune and parenchymal cells in liver, including NK cells, dendritic cells (DC), macrophages, Kupffer cells, neutrophils, hepatic stellate cells and hepatocytes.

Activation of type II NKT cells following sulfatide or LPC administration induces a novel pathway of immune regulation which results in DC-mediated inactivation of type I NKT cells, as well as inhibition of the effector function of conventional T cells involved in autoimmunity, transplant rejection and anti-tumor immunity [3–6, 14]. In contrast, several studies have now suggested a pathogenic role for type I NKT cells as opposed to a protective role for type II NKT cells in inflammatory liver diseases (reviewed in [5,6, 14,15]). There are several mechanisms by which both NKT cell subsets modulate inflammation in liver. For example, following sulfatide or LPC-mediated activation of type II NKT cells in mice we

have found that there is a rapid IL-12 and MIP2-dependent accumulation of type I NKT cells into liver, but these cells are anergized, and consequently treated mice are protected from Concanavalin A-induced liver injury, IRI, alcoholic liver disease and CCL4-induced fibrosis [3–6,14,15]. Consequently type II NKT-mediated inactivation of type I NKT cells eventually results in a significant lack of pro-inflammatory macrophages and neutrophil accumulation and consequent inhibition of inflammation and IRI [15]. Therefore type I and type II cross-regulatory interactions are important and may be crucial for their successful functional manipulation of a variety of pathological conditions. An emerging body of experimental evidence (reviewed in [3–6]) suggests that inhibition of type I NKT cells directly, using retinoid signaling or use of anti-CD1d antibodies, or indirectly following sulfatide-, LPC- or their analogs-mediated activation of type II NKT cells should be useful for advancing clinical studies in humans.

It is also important to mention that type I NKT cells use different mechanisms to induce hepatic injury. For example, while type I NKT cells have enhanced IFN- γ secretion and accumulate in liver and lead to recruitment of neutrophils to cause hepatocyte injury following chronic plus binge alcohol feeding, they don't accumulate into liver but express higher levels of Fas/Fas-L resulting in direct killing of hepatocytes following chronic alcohol feeding. Similarly, depending upon different stimuli, for example acute vs. chronic CCl4 treatment or timing of analysis following feeding of mice with choline-deficient amino acid enriched diet, a model of NASH, hepatic type I NKT cells can secrete different cytokines (Maricic et al., unpublished data). Consistent with data in murine models, preliminary data from our ongoing studies related to analysis of type I NKT cells suggest their Th1-like cytokine secretion profiles in PBMCs derived from subjects with inflammatory liver diseases (Marrero et al., unpublished). Thus, It will be interesting to investigate NKT subset profile in the transplant as well as in the host.

Finally it is crucial to state a few points regarding analysis of NKT cell subsets in both mice and in humans. It is important to use CD1d/lipid tetramers in combination with cell surface markers, intracytoplasmic cytokine staining and semi-quantitative PCR for the invariant TCR chain to differentiate the two subsets. Staining with NK1.1/TCR (mouse) CD56/TCR(human) is not useful, as these markers vary and are expressed by other cells, including NK and activated T cells and can not differentiate between the two NKT subsets. In murine studies a combination of mice lacking type I NKT cells (Ja18 $^{-/-}$ mice) as well as adoptive transfer of purified NKT cells subsets from wild type mice are used to define the role of type I vs. type II NKT cells. However recently it has been shown that the defect in TCR repertoire in Ja18 $^{-/-}$ mice is broader than in just iNKT cells [16], which may impact interpretation of studies relying simply on comparing the phenotype in CD1d $^{-/-}$ mice (which lack both type I and II NKT cells) and Ja18 $^{-/-}$ mice. A newly developed mouse strain can be used to study loss of function in iNKT cells [17]. Similarly anti-CD1d antibodies that can also activate CD1d+ antigen-presenting cells and therefore can complicate interpretations and should be carefully used in combination with CD1d $^{-/-}$ mice. Additionally, caution should be exercised in interpretation of experiments using a super antigen-like agonist for type I NKT cells, α GalCer, as most known antigens bind with lower affinity and may behave differently.

In this brief review, we have summarized current evidence supporting the emerging role for both type I and type II NKT cells in liver inflammation. These cell's higher frequency than MHC-restricted T cells, rapid responses and ability to impact several other hepatic immune cell types is crucial to their function, and therefore there is an unmet need to better understand the biology of these cells and alterations of these cells in the setting of a variety of pathological conditions, particularly in human liver. Strategies to manipulate NKT cell subsets selectively in vivo using their agonists or antagonists should have important clinical implications for managing liver diseases as well as transplant survival.

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