Functionally distinct NKT cell subsets and subtypes

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Natural killer T (NKT) cells are a population of autoreactive cells that mediate both protective and regulatory immune functions. NKT cells comprise several subsets of cells, but it has been unclear whether these different NKT cell subsets possess distinct functions in vivo. New studies now demonstrate that subsets of NKT cells are indeed functionally distinct and that the specific functions of these cells may be dictated in part by organ-specific mechanisms.

During their development in the thymus, conventional αβ T cells acquire the expression of either CD4 or CD8, major histocompatibility complex (MHC) coreceptors that define functionally distinct MHC-restricted T cell subsets. In response to antigen stimulation, $CD4^+$ T cells differentiate into T helper (Th)-1 or Th2 cells. Th1 cells produce the signature cytokine interferon (IFN)-γ, whereas Th2 cells produce interleukin (IL)-4, IL-5, IL-13, and IL-10. Similarly, effector $CD8^+$ T cells can be classified as Tc1 or Tc2 cells, which produce type 1 or type 2 cytokines, respectively (1). From this perspective, the cytokine profiles of T lymphocytes are thought to reflect their functional activities.

Subsets and subtypes of natural killer T (NKT) cells

NKT cells express an invariant T cell receptor (TCR) α chain (Vα14-Jα281 in mice and Vα24-JαQ in humans) and recognize glycolipid antigens, such as the endogenous isoglobotrihexosylceramide (iGb3) (2) and the synthetic glycolipid, α-galactosylceramide (α-GalCer), in association with the MHC class I–like molecule CD1d. NKT cells are different from functionally differentiated conventional αβ T cells in that they are autoreactive and produce both Th1 and Th2 cytokines, including IL-4, IL-10, and IFN-γ, upon stimulation with their ligands (for review see reference 3). The invariant $V\alpha14^+$ NKT cells, also called type I NKT cells (4), include two defined populations: a $CD4^+$ and a $CD4^-CD8^-$ double negative (DN) population.

In addition to type I NKT cells, a population of CD1d-reactive NKT cells that express diverse non-Vα14 TCRs, referred to as type II NKT cells, has been described. These cells were identified based on the fact that NKT cell function was still detectable in mice lacking type I NKT cells but not in mice lacking CD1d (4). Although type II NKT cells are also restricted by CD1d, they do not recognize α-GalCer. It is important to note, however, that some type II NKT cells are also autoreactive, as they recognize the endogenous myelin-derived glycolipid sulfatide and help protect mice against the development of experimental autoimmune encephalitis (5). Thus, type I and type II NKT cells appear to have distinct functional capabilities, but these functional differences have not been well characterized.

Type I invariant $V\alpha14^+$ NKT cells (referred to hereafter as NKT cells) have been shown to mediate both protective and regulatory immune functions. These include tumor rejection, protection against infectious microbes, maintenance of transplant tolerance, and inhibition of autoimmune disease development (3). However, it remains unclear whether distinct subsets or subtypes of CD1d-reactive NKT cells mediate different functions. Two new studies, one in a recent issue (6) and one in this issue (7), suggest that a distinct subset of NKT cell mediates tumor rejection (6), and that type II NKT cells suppress antitumor immunosurveillance (7). These new studies might help explain the seemingly contradictory functions that have been ascribed to CD1d-reactive NKT cells. They also raise new questions about how subsets of NKT cells acquire distinct functional capabilities.

Tumor rejection by distinct NKT cell subsets

In a recent study, Crowe and colleagues compared antitumor activities of NKT cell subsets from different organs (liver, thymus, or spleen) against two different types of tumor cell (methylcholanthrene-1 sarcoma cells and B16-F10 melanoma cells) (6). Interestingly, they found that the DN subset of NKT cells from the liver were better able to reject tumor cells than were the $CD4^+$ liver-derived subset and both the $CD4^+$ and DN subsets from the thymus or the spleen, suggesting that organ-specific mechanisms might dictate the functional capabilities of resident NKT cells. Indeed, past studies have suggested that NKT cells in other organs may also have different functions. For example, thymic DN NKT cells have been shown to suppress type 1 diabetes development in NOD mice in an IL-4– and IL-10–dependent fashion (8). In addition, splenic $CD4^+$ NKT cells have been shown to induce $CD8^+$ regulatory T (T reg) cells that mediate systemic tolerance in the anterior chamber associated immune deviation (ACAID) model of tolerance (9). In the light of new data from Crowe et al. (6), it will be important to further clarify whether NKT cell subsets in different organs mediate different functions.

The finding that hepatic DN NKT cells are the primary mediators of antitumor responses is consistent with previous findings demonstrating that human DN NKT cells produce predominantly Th1 cytokines, whereas $CD4^+$ NKT cells produce predominantly Th2 cytokines (10, 11). In most

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models, tumor rejection depends on IFN-γ production by the NKT cells and the subsequent IFN-γ–induced activation of NK cells and $CD8^+$ T cells (12, 13). However, Crowe et al. found very little difference in cytokine production (IFN-γ and IL-4) among the subsets of NKT cells in the different tissues (6), suggesting that factors other than cytokine production might be involved in NKT cell–mediated tumor rejection. It is important to note, however, that although IL-4 production did not differ significantly between the various subsets of NKT cells, thymic NKT cells from IL-4–deficient mice acquired the ability to reject tumors (6), suggesting that the production of IL-4 might inhibit certain antitumor responses in vivo. However, molecules other than IL-4 must also contribute to the inhibition of NKT cell–mediated antitumor immunity, as hepatic DN NKT cells that mediated tumor rejection in this model were shown to produce significant levels of IL-4.

It is unclear whether the different subsets or types of NKT cells regulate each other's functions. However, it seems likely that the antitumor function of liver-derived DN NKT cells is controlled by other subsets or types of NKT cells, in a manner similar to the regulatory interplay between Th1 and Th2 cells.

Suppression of tumor rejection by type II NKT cells

A report by Terabe et al. in this issue shows that type II NKT cells are responsible for inhibiting tumor immunosurveillance in mice (7). This inhibition involved IL-13, myeloid cells, and transforming growth factor–β, but did not require naturally occurring $Foxp3^+$ T reg cells (14). Terabe et al. thus suggest that type II NKT cells may negatively regulate antitumor responses in mice, whereas type I NKT cells promote antitumor responses. Although mutual interactions between these cells have never been formally studied, it would be interesting based on these new results to evaluate whether type II NKT cells inhibit the protective function of type I NKT cells in the thymus or spleen, but not in the liver.

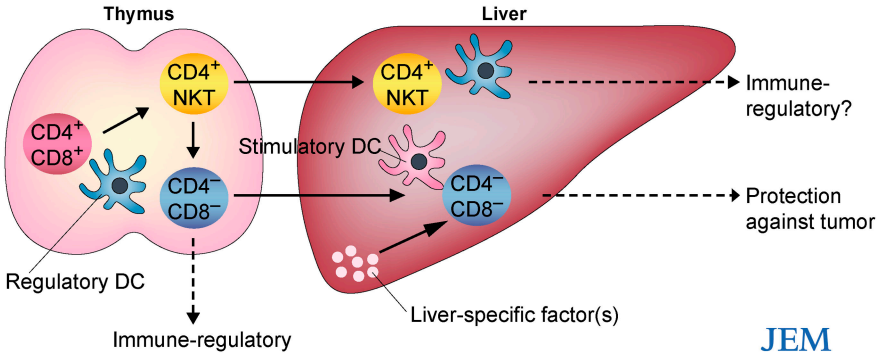

Figure 1. Potential mechanisms by which tissue-specific NKT cell subsets acquire distinct functions. NKT cells from different organs have been reported to have distinct functions. For example, double negative (DN) NKT cells in the liver mediate tumor rejection (reference 6), and DN NKT cells from the thymus regulate immune cell function in the NOD mouse model of type I diabetes (reference 8). These functionally distinct populations of NKT cells might arise from the same NKT cell precursors in the thymus. However, unidentified organ-specific factors might dictate the function of local NKT cell populations. Local dendritic cell populations (either stimulatory or regulatory) that interact with NKT cells might also influence local NKT cell function.

Organ-specific functional maturation of NKT cell subsets?

How do distinct NKT cell subsets, or NKT cells in different organs, acquire different functional capabilities? One possibility is that functionally distinct NKT cell subsets diverge after their development in the thymus. According to a recent report by Benlagha et al., both $CD4^+$ and DN NKT cells are derived from heat stable antigen $(HSA)^{high}$ double positive thymocytes (15). Interestingly, the $CD4^+$ subset is derived directly from HSA^{high} double positive thymocytes, whereas the DN subset appears to pass through a HSA^{low} $CD4^+$ stage. However, the two subsets of NKT cells do not seem to acquire different functional potentials in the thymus as, according to Crowe et al., neither $CD4^+$ nor DN NKT cells from this organ were able to reject tumors (6).

An alternative possibility is that a liver-specific mechanism may confer antitumor potential on resident DN NKT cells (Fig. 1). In mice, the liver contains more NKT cells than do other tissues and these NKT cells express high baseline levels of the adhesion molecule lymphocyte function-associated antigen (LFA)–1 and the chemokine receptor CXCR6. In LFA-1 or CXCR6 knockout mice, the numbers of NKT cells in the liver were significantly decreased (16–18), whereas the numbers of these cells in other organs were normal. These studies suggested that both LFA-1 and CXCR6 are selectively required for the development and/or survival of liver NKT cells. LFA-1 and CXCR6 also influence the fate and function of NKT cells by controlling their survival, cytokine production, and ability to induce tissue damage. In LFA-1–deficient mice, for example, Th2 cytokine production by NKT cells was significantly enhanced (19) and, in CXCR6 knockout mice, NKT cells underwent apoptosis and failed to induce hepatitis in response to concanavalin A stimulation (18). Therefore, the interaction between LFA-1–intercellular adhesion molecule–1 and/or CXCR6–CXCL16 may contribute not only to the accumulation of NKT cells in the liver but also to their acquisition of specialized functions (Fig. 1). In future studies, it will be important to examine the expression levels of LFA-1 and CXCR6 on distinct NKT cell subsets and to determine whether these molecules influence their function.

Expression levels of cytotoxic effector molecules (such as Fas ligand or TNF), NK cell receptors (such as CD94 or NKG2D), and chemokine receptors (such as CCR5 or CCR6) may also influence the effector function

of different NKT cell subsets (10, 11, 20). Consistent with this idea, our own microarray analyses have shown that mouse NKT cells from different organs show substantially different gene expression patterns, including cytokine- or chemokine-related and apoptosis-related genes (unpublished data).

It is also possible that organ-specific antigen-presenting cells (APCs) modulate NKT cell function (Fig. 1). Yang et al. suggested that different levels of costimulatory molecules expressed by organ-specific APCs cause different NKT cell responses (21). For example, thymic and splenic APCs induced similar level of IL-4 production by NKT cells, whereas only splenic APCs induced substantial IFN-γ production. APCs in different organs may also express different tissue-specific glycolipid ligands for NKT cells. A study of the TCRVβ repertoires of NKT cells from different organs showed that the DJ regions among TCRVβ8.2$^+$ NKT cells varied depending on the tissue of origin (bone marrow, spleen, or liver), suggesting the possibility that different endogenous ligands may exist in the different tissues (22). This idea is supported by the fact that several synthetic glycolipids have been shown to induce different cytokine profiles in NKT cells. OCH, which is a truncated version of the prototypic NKT cell ligand α-GalCer, induced the production of Th2 cytokines from NKT cells (23). In contrast, α-C-GalCer, a C-glycoside analogue of α-GalCer, mainly induced the production of Th1 cytokines (24). Thus, APCs in different tissues may express endogenous OCH- or α-C-GalCer–type ligands and thereby promote differential cytokine gene expression.

It also seems likely that the unique anatomical features of the liver could endow hepatic NKT cells with specialized functions. APCs in the liver might capture and present exogenous glycolipids from the alimentary tract, such as glycosphingolipids derived from Gram-negative bacteria including *Ehrlichia* and *Sphingomonas* (25, 26) These ligands might preferentially induce IFN-γ-dependent protective immune responses, including antitumor responses.

Other mechanism determining functions of NKT cell subsets

NKT cell functions or cytokine profiles can be altered by additional mechanisms, including the modulation of TCR signaling. Kojo et al. recently reported that the frequency of NKT cell stimulation through the TCR affects NKT cell function (27). A single stimulation with α-GalCer resulted in the production of high levels of IFN-γ by the NKT cell. This α-GalCer–induced IFN-γ production is the basis for the adjuvant effect of NKT cell activation on NK and CD8$^+$ T cells, and contributes to protective immune responses such as tumor rejection (28). In contrast, repeated stimulation with α-GalCer caused NKT cells to produce more IL-10 and less (if any) IFN-γ, thus favoring the development of regulatory NKT cells (27). These studies suggest that the intensity of TCR signals influences the function of NKT cells, in a manner similar to the effect of TCR avidity on positive and negative selection of developing T cells in the thymus (29).

Hayakawa et al. described similar tolerogenic changes in thymic NKT cells that resulted from chronic stimulation of the TCR (30). They showed that chronic exposure of mice to α-GalCer (weekly for 8 wk) resulted in the disappearance of existing NKT cells followed by the thymus-dependent repopulation of the mice with NKT cells that expressed increased levels of the inhibitory NK cell receptor Ly-49. These NKT cells displayed decreased cytokine production and a reduced ability to reject tumor cells. Therefore, the timing and duration of NKT cell stimulation also seems to be crucial in determining their functions during subsequent immune responses. It is not yet known whether the modulation of TCR stimulation has an equivalent effect on distinct subsets of NKT cells. It will be interesting to examine the possibility that chronic stimulation has different effects on different subsets of NKT cells, causing only certain subsets to become tolerogenic.

Concluding remarks

In considering any NKT cell–based immune therapy, it would be advantageous to be able to utilize only those NKT cells with the desired function. According to the findings by Crowe et al. (6) and Terabe et al. (7), it seems likely that different subsets or types of NKT cells and their tissue of origin will be important parameters in targeting particular NKT cell functions. For therapeutic purposes, the design of new glycolipid ligands for NKT cells that would preferentially activate a functionally specialized NKT cell subset, or that would activate NKT cells in a particular organ, would be warranted. Alternative approaches to activate NKT cells in a particular organ might include new drug delivery techniques, such as lipid particles or nanospheres. But before such therapeutic strategies are pursued, it will be important to determine the precise correlation between the phenotype, function, and anatomical distribution of mouse and especially human NKT cells.

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