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Jay A. Berzofsky and Masaki Terabe

NKT cells are true Ag-specific T cells that also have innate properties and form a bridge between the innate and adaptive immune systems. Distinct NKT cell subsets play positive and negative regulatory roles and define a new immunoregulatory axis with broad implications for tumor immunity and other immunological and disease settings. The Journal of Immunology, 2008, 180: 3627–3635.

The immune system appears to be built of a series of nested dichotomies, at least as viewed from the perspective of immunologists. Sometimes the cells from opposite categories support each other or even synergize, and in other cases they oppose each other. The largest dichotomy is between the innate and adaptive immune systems, which can both support and oppose each other. Within the innate immune system there are related pairs of cell types with opposite roles, such as M1 (protective) and M2 (suppressive) macrophages (1, 2) and stimulatory or tolerogenic dendritic cells (DC) (3, 4). Within the adaptive immune system many more nested dichotomies have been defined. The broadest is between T and B cells. Within the B cell compartment there are subsets that make different isotypes of Ig with different effector functions such as IgM, IgG, IgA, and IgE. Within the T cell category, CD4+ and CD8+ T cells play sometimes supportive and sometimes opposing roles. For example, CD4+ Th cells are critical for an optimal CD8+ T cell response and especially for long-lived memory. However, CD4+ T regulatory (Treg) cells can down-regulate a CD8+ T cell response. Within the CD4+ T cell family perhaps the best known polarity axis is between Th1 (IFN-γ-producing) and Th2 (IL-4-, IL-5-, and IL-13-producing) cells, which most often oppose and furthermore counter-regulate one another through the production of cross-regulatory cytokines (5). A more recently defined dichotomy is between CD4+CD25+Foxp3+ Treg cells (6–8) and Th17 cells (9, 10), which are both dependent on TGF-β but whose development is either suppressed or supported by IL-6. Within the CD8+ T cell compartment there have also been defined type 1 CD8+ T cell (Tc1) and type 2 CD8+ T cell (Tc2) subtypes (11), analogous in cytokine profile to Th1 and Th2 cells but both retaining lytic activity as well. In parallel with CD4+ regulatory T cells, CD8+ regulatory T cells (12) have also been defined that can inhibit other CD8+ as well as CD4+ T cells. Another dichotomy is between TCRαβ and TCRγδ T cells, which cuts across the adaptive and innate immune categories.

There are many interactions or bridges between the innate and adaptive immune systems. The innate immune system is the first responder, always armed and not requiring reactivation or expansion of memory cells, let alone the priming of naive cells as is required for adaptive immunity. Innate immune cells, such as DCs, are required for Ag presentation to activate naive T cells of the adaptive immune system to respond to Ag (3). Cytokines made by innate responders, such as macrophages, DCs, and NK cells, may profoundly influence the subsequent adaptive immune response. However, one cell that serves especially as a bridge between the innate and adaptive immune systems is the NKT cell (13–19). This cell not only plays a regulatory role in both innate and adaptive immune compartments, but it actually has a foot in each camp. It is a true T cell with a true TCR and CD3, but it also has NK receptors and other markers that allow it to respond in an innate fashion and to kill like an NK cell. NKT cells were first identified as T cells with a lower level of CD3 expression than most conventional T cells and also possessing NK cell markers such as NK1.1 (20–25). They were originally found to be CD1 restricted (26) and to be very rapid producers of cytokines, able to provide an early source of IL-4 to skew a conventional CD4+ T cell response toward Th2 cytokine production (27, 28). Subsequently, their use of a particular TCR α-chain Vα14 was discovered (29), defining a major subset of NKT cells now called type I or classical NKT cells. These NKT receptors cross-react with self so that even when it functions like a true T cell using its TCR, the NKT cell is preactivated by self-reactivity. Indeed, there is evidence that NKT cells already partially activated by recognition of self-antigens presented by CD1d can be induced to respond just by exposure to IL-12 produced by macrophages or DCs stimulated by LPS-containing bacteria (30). Accordingly, the NKT cell has...
preformed mRNA for such cytokines as IFN-γ and IL-4 so that it can respond more rapidly than can conventional T cells as a first responder on the scene during microbial infection (31, 32). Therefore, any cytokine bias (toward Th1 or Th2 cytokines) introduced by the initial NKT cell response may lead to a corresponding bias in the adaptive T cell response once it occurs (27). NKT cells also differ from conventional T cells in that rather than recognizing a classical class I or class II MHC molecule, they recognize a nonclassical class I molecule called CD1d (13–19). Also, rather than presenting a peptide Ag as do the classical MHC molecules, CD1d presents a glycolipid Ag. Therefore, NKT cells fill another important role of providing the cellular immune system with the capability to recognize glycolipid Ags from certain bacteria such as *Sphingomonas* organisms (33–36) whose lipid Ags would not be recognized by conventional T cells that focus on peptide Ags.

Within this innate-adaptive bridging population of NKT cells, several subsets also exist. Although NKT cells were originally defined as having both a TCR and NK cell markers, in order to distinguish them from activated conventional T cells that express NK1.1 as an activation marker, more recently it has been suggested that a better definition for NKT is a T cell that recognizes Ag presented by CD1d (17). Within this CD1d-restricted NKT cell population at least two subsets have been defined. The classical or type I NKT cell is characterized by a semi-invariant TCR using a unique TCR V\textalpha 14/J\textbeta 18 chain in the mouse and a V\textalpha 24/J\textbeta 18 chain in the human, along with a small number of possible TCR V\textbeta-chains. It is also characterized by its recognition of the glycolipid α-galactosylceramide (αGalCer) derived from a marine sponge or the microorganisms that are symbiotic with the sponge (37). This is not a mammalian gene product, and neither are most of the αGalCer analogues that have been studied. However, recently several endogenous mammalian self lipids have been defined as CD1d ligands recognized by type I NKT cells, including isoglobotrihexosylceramide (Gb3) and some phosphatidyl inositol compounds (38–41). Nested within the type I NKT subset is another dichotomy between CD4+ and CD4−CD8− double negative NKT cells. At least in humans the former have been described as making both Th1 and Th2 cytokines, whereas the latter are skewed more toward Th1 cytokines (42, 43). The other major category of NKT cell is the type II NKT cell, defined by what it is not, i.e., by having diverse TCRs rather than using the invariant V\textalpha 14/J\textbeta 18 combination, and by not responding to αGalCer, even though it still recognizes lipids presented by CD1d. These cells were first described more recently by Cardell and coworkers (44) and their physiological function has been less well studied and is still only poorly understood except in a few situations (see below). This review will focus on the dichotomy between type I and type II NKT cells in tumor immunity. In particular, the review will discuss the evidence that these two subsets of NKT cells not only have opposing functions but also counter-regulate each other and therefore define a new immunoregulatory axis of opposing forces analogous to that between Th1 and Th2 cells. Given the pivotal position of NKT cells as a bridge between the broad realms of innate and adaptive immunity, this NKT regulatory axis may play an important regulatory role in the immune system beyond its role in tumor immunity. Altering the balance along this axis may influence the outcome of infectious diseases, autoimmune diseases, and cancer.

**Type I NKT cells in tumor immunity and immune regulation**

**Protective role of type I NKT cells in tumor immunity.** The role of NKT cells in tumor immunity was not appreciated until it was discovered that αGalCer, which had been shown to have antitumor properties (45–47), was a strong agonist for NKT cells (37). Thereafter, a large number of studies confirmed the antitumor activity of NKT cells stimulated by αGalCer in vivo (48, 49) or by IL-12 (50). Furthermore, it was found that NKT cells play an important role in immunosurveillance against methylcholanthrene-induced tumors, even in the absence of αGalCer, based on the use of knockout (KO) mice deficient in either all NKT cells (CD1d KO mice) or NKT cells expressing the invariant V\textalpha 14/J\textbeta 18 TCR (J\textbeta 18 KO mice), as well as adoptive transfer of liver NKT cells (49, 51, 52). Stewart et al. also showed a requirement for J\textbeta 18+ NKT cells in early tumor immunity independent of stimulation by αGalCer (53). Crowe et al. found that the NKT cells’ ability to make IFN-γ was essential for their antitumor activity, whereas their expression of perforin was not (51). Further, it was shown that antitumor activity of the NKT cells required sequential production of IFN-γ by NKT cells and then NK cells (54, 55). This led to the understanding that the protective effect of NKT cells was not necessarily due to their own lytic activity and direct lysis of tumor cells, although they are capable of doing so (56), but by their production of IFN-γ and the recruitment thereby of NK cells and CD8+ T cells that mediated the protective effect.

In addition to the role of IFN-γ in recruiting NK cells, NKT cells were shown to activate DCs to make IL-12, and this activity also played an important role in antitumor activity (57). Indeed, DCs pulsed with αGalCer had potent antitumor activity even against 7-day established B16 melanoma tumors (48). This activity may be explained by the fact that αGalCer-pulsed DCs induced an NKT cell cytokine response skewed more toward IFN-γ and also more prolonged than that induced by the injection of free αGalCer, which appeared to energize the NKT cells after initially activating them (58) or skew them toward a Th2 cytokine profile (59). Moreover, the NKT cells fully matured and activated the DCs to induce a more effective adaptive immune response by conventional CD4+ and CD8+ T cells (60). Activated DCs also make IL-12 and IL-15, which can contribute along with IFN-γ to the induction of NK cells. In another approach, the administration of dying irradiated tumor cells along with αGalCer led to NKT cell-mediated induction of activated DCs that then presented Ags from the dying tumor cells to activate conventional CD4+ and CD8+ T cells (61). Likewise, B cells or tumor cells pulsed with tumor peptides and αGalCer were effective, with help from NKT cells, at inducing antitumor immunity (62, 63) that seems to involve cross-presentation by endogenous DCs (64). DCs pulsed with αGalCer also expanded and activated human NKT cells in vitro and skewed their cytokine profile more toward IFN-γ production (65).

In accord with the above findings in mice and in human cells in vitro, a number of clinical trials of αGalCer were tried in cancer patients. Some of these were conducted with soluble αGalCer (66, 67), but some were also performed with αGalCer-pulsed DCs (68–71). Also, NKT cells were found to be preserved in glioma patients and to be able to be expanded in vitro by αGalCer-pulsed DCs (72). The expanded NKT cells were able to lyse glioma cells, which retained expression of...
CD1d. Adoptive transfer of autologous NKT cells activated in vitro with αGalCer and IL-2 was also tested in a phase I clinical trial in cancer patients (73). However, none of these clinical trials to date has succeeded in achieving significant efficacy against human tumors in vivo, in contrast to the studies in mice. Whether this difference reflects a species-related difference in NKT cell activity, the known highly variable but generally lower frequency of NKT cells in humans (18), or a difference in experimental design associated with the treatment of advanced cancer patients compared with recently adoptively transplanted tumors in mice remains to be determined.

**Regulatory role of type I NKT cells in autoimmunity and the role of cytokine balance.** In contrast to their role in tumor immunity, which depends especially on their production of IFN-γ, the role of invariant (type I) NKT cells in asthma and autoimmune diseases seems to depend more on their ability to produce Th2 cytokines. NKT cells were implicated as contributing to the pathogenesis of asthma through their production of IL-13 and IL-4 in mice and were necessary despite the ability of mice deficient in NKT cells to still make a Th2 response (74). Indeed, the stimulation of invariant NKT cells with αGalCer or another lipid was sufficient to induce airway hypersensitivity in class II MHC-deficient mice, which lack conventional CD4+ T cells (75). Similarly, NKT cells appeared to be a major source of IL-13 and IL-4 in the lungs of human bronchial asthma patients because CD1d tetramer-binding T cells represented 60% of the CD3+CD4+ T cells in the lungs of patients with moderate to severe asthma, expressed the invariant TCR, and made Th2 cytokines (76), although this has not been observed in all asthmatics (77).

Likewise, in some autoimmune diseases NKT cells have been found to have a protective role based on their production of Th2 cytokines. Miyamoto et al. found that treatment of mice with OCH, a synthetic analog of αGalCer or another lipid was sufficient to induce airway hypersensitivity in class II MHC-deficient mice, which lack conventional CD4+ T cells (75). Similarly, NKT cells appeared to be a major source of IL-13 and IL-4 in the lungs of human bronchial asthma patients because CD1d tetramer-binding T cells represented 60% of the CD3+CD4+ T cells in the lungs of patients with moderate to severe asthma, expressed the invariant TCR, and made Th2 cytokines (76), although this has not been observed in all asthmatics (77).

Type I NKT cells have been implicated in protecting genetically diabetes-prone NOD mice from diabetes, although the role of Th2 cytokines is less clear. Activation of type I NKT cells with αGalCer protected mice from autoimmune encephalitis (EAE), a mouse model for multiple sclerosis (89). GalCer and IL-2 was also tested in a phase I clinical trial in cancer patients (73). However, none of these clinical trials to date has succeeded in achieving significant efficacy against human tumors in vivo, in contrast to the studies in mice. Whether this difference reflects a species-related difference in NKT cell activity, the known highly variable but generally lower frequency of NKT cells in humans (18), or a difference in experimental design associated with the treatment of advanced cancer patients compared with recently adoptively transplanted tumors in mice remains to be determined.

Paradoxical role of NKT cells in tumor immunity. With this background in which the predominance of the literature suggested that type I invariant NKT cells were protective against tumors based on a Th1 bias, it was surprising to find that NKT cells could also suppress tumor immunosurveillance based on their production of the Th2 cytokine IL-13 (93–97), even though they contributed to asthma or protected against some autoimmune diseases based on a Th2 bias. Recurrence of a murine regressor tumor was found not to occur if CD4+ regulatory cells were depleted, and these CD4+ cells were found to be CD1d-restricted NKT cells (93), not CD4+CD25+ Treg cells (98). Similar findings were made for protection against lung tumors derived from the CT26 colon carcinoma, in which spontaneous immunosurveillance was not apparent without blocking this regulatory pathway (97, 98). It was further found that the suppression of CD8+ T cell-mediated tumor immunosurveillance depended on NKT cell production of IL-13, but not IL-4 (93, 97). Because the CD8+ T cells mediating protection did not have IL-13 receptors, a search was undertaken for a downstream cell that responded to IL-13 and inhibited the CD8+ T cells. This cell turned out to be a CD11b+Gr-1+ myeloid cell that was induced to make TGF-β, and the TGF-β was sufficient to inhibit the CD8+ T cell response, suggesting that this was the effector molecule mediating the suppression (95). Besides this predominant suppressive mechanism involving IL-13 and TGF-β, NKT cells must be able to suppress by additional mechanisms as observed in the 4T1 mammary carcinoma in which CD1d KO mice and STAT6 KO mice were protected but IL-13 KO mice or mice treated with an IL-13 antagonist were not (99), and as observed in an orthotopic osteosarcoma model in which CD1d KO mice were protected but blockade of IL-13 or TGF-β had no effect (100). Studies in other tumor models supported a role for NKT cells in the suppression of tumor immunity, for example in the protection by IL-12 and IL-18 against the liver metastases of a mouse renal cell carcinoma (101). These findings that NKT cells could suppress tumor immunity led to a paradox in the role of NKT cells in tumor immunity (98, 102, 103). Were the same cells mediating opposite effects based on their cytokine balance, or were there two different types of NKT cells that were mediating these opposing effects? Attempts to resolve this paradox led to the discovery of the role of type II NKT cells in tumor immunity, as discussed in the next section.

**Type II NKT cells in tumor immunity and immune regulation**

Type II NKT cells and their role in autoimmune disease and infectious disease. A second type of NKT cell was first discovered by Cardell et al. (44) when examining the CD4+ T cells remaining in class II MHC-deficient mice. In the absence of conventional CD4+ T cells in these mice, many of the remaining cells recognized CD1d but had diverse TCR usage. Such noninvariant NKT cells were also observed again when
examining T cells elicited by immunizing mice with a tumor, RMA-S-CD1d1, that expresses neither conventional class I or II MHC molecules but was transfected to express CD1d (104). Such noninvariant NKT cells were the predominant cells elicited by this immunization. They still produced both IFN-γ and IL-4, as do most type I (invariant) NKT cells. This second subset of NKT cells, now called type II, were found to be CD1d restricted and function as early producers of both Th1 and Th2 cytokines (104, 105). However, they seemed to survey a set of self-lipids that differed from those recognized by type I NKT cells, in that type I required endosomal targeting of CD1d using a tyrosine-based motif in the intracytoplasmic tail, whereas presentation to type II NKT cells did not require such endosomal loading (105). The type II NKT cell is defined more by its CD1d specificity and lack of the invariant Vα14Jα18 receptor than by its lack of αGalCer reactivity, as some noninvariant NKT cells have also been found capable of response to αGalCer (106).

However, the physiologic functions of type II NKT cells are much less well studied than those of type I, except for a few specific cases in which they were found to play a role. Type II NKT cells found in human liver were mostly skewed toward Th1 cytokines and enriched in livers of patients with chronic hepatitis C infection (107). Similarly, in a mouse transgenic model of hepatitis B virus (HBV) infection, the induction of hepatitis in Rag-1 KO × HBV-env -transgenic mice required the transfer of cells that expressed both NK1.1 and a TCR, i.e., that were NKT cells but not NK cells and were not reactive with a CD1d tetramer loaded with αGalCer (108). Thus, type II NKT cells appeared to be required for the induction of the hepatitis in this model. Recent evidence suggests that their activation to produce hepatitis is dependent on NKG2D (109). Type II NKT cells were also found to play a proinflammatory role in the induction of human ulcerative colitis through their production of IL-13 (110).

Alternatively, type II NKT cells can also play an immunosuppressive role in some autoimmune diseases. In human bone marrow such noninvariant type II NKT cells were found to be skewed toward Th2 cytokines and to suppress an alloantigen-specific proliferative response (mixed lymphocyte response) (111). A TCR Vα3.2+Vβ9+ (type II) NKT cell was found able to suppress autoimmunity diabetes in NOD mice (112). Also, type II NKT cells reactive with sulfatide, a lipid derived from myelin sheaths, were found to protect against EAE, a mouse model of multiple sclerosis (113). Importantly, this study identified a lipid, sulfatide, that stimulates a substantial fraction of type II NKT cells and can be used to identify them using CD1d tetramers loaded with sulfatide instead of αGalCer. Moreover, the populations of αGalCer-CD1d tetramer-binding (type I) and sulfatide-CD1d tetramer-binding (type II) NKT cells were found to be nonoverlapping by flow cytometry (113). Unfortunately, this approach of identifying type II NKT cells has not yet met its full potential because of the low affinity of sulfatide for CD1d, resulting in instability of the tetramers and/or, because of the low affinity of the TCR for this tetramer, making these tetramers difficult to make and to use. However, the sulfatide-CD1d complex was stable enough to obtain crystals for determining an x-ray crystallographic structure at 1.9 Å that allows comparison with the crystal structure of CD1d loaded with αGalCer (114). This crystal structure may aid in the identification of other glycolipids that activate type II NKT cells. In any case, in using sulfatide reactivity as a marker it must be remembered that not all type II NKT cells are reactive with sulfatide (113).

Together with the fact that type II NKT cells are a mixture of cells with or without surface expression of NK1.1 (105, 115) and that not all type II NKT cells recognize sulfatide (113), it is possible that those type II NKT cells mediating proinflammatory responses, which may be self-destructive, or immunosuppressive responses, which might contribute to tolerance, are distinct subsets of type II NKT cells. It will be of great interest in the field to test this possibility.

In two parasitic diseases, type I and II NKT cells have been found to play different and possibly opposite roles based on the difference between CD1d KO mice that lack both types of NKT cells and Jα18 KO mice that lack only type I NKT cells. In murine Trypanosoma cruzi infection, CD1d KO mice were found to have a mild disease compared with Jα18 KO mice that had a much more severe inflammatory disease and died (116). This finding was interpreted to mean that type II NKT cells were proinflammatory in this disease whereas type I NKT cells might counteract the effect of type II when both were present. Opposite effects of these two types of NKT cells were also suggested in acute murine schistosomiasis after egg deposition, at which time CD1d KO mice lacking both subtypes had reduced Th2 cytokine production whereas Jα18KO mice lacking only type I NKT cells had reduced IFN-γ responses (117).

**Role of type II NKT cells in tumor immunity and the resolution of the paradox.** In tumor immunity, the evidence given above for the ability of NKT cells to protect against tumors as well as to suppress CD8+ T cell-mediated tumor immunosurveillance resulted in a paradox (16, 102). Was the same cell mediating different functions or were different types of NKT cells involved? Certainly, the protective cell was a type I NKT cell in most studies, especially in those using αGalCer, but type I NKT cells were also found to inhibit tumor immunity in a murine lymphoma model (118). Because NKT cells can make both Th1 and Th2 cytokines having potentially opposite effects and because IFN-γ was implicated in the protective mechanisms described above, whereas IL-13 was implicated in suppression of tumor immunosurveillance, it was possible that the same cell could be induced under different conditions to mediate opposite effects by altering the balance of cytokines secreted (16, 102). Furthermore, at least in humans, CD4+ and double negative type I NKT cells had different cytokine patterns (42, 43) in that double negative NKT cells expressed only Th1 cytokines whereas CD4+ NKT cells expressed both Th1 and Th2 cytokines. Also, in the mouse, liver-derived CD4−CD8− type I NKT cells were found to be more protective than splenic and thymic ones, suggesting the existence of different type I NKT cell subsets with different effects on tumor growth according to their tissue of origin (119) (Fig. 1). Recently it was also reported that NK1.1+ type I NKT cells are a mature population with different expression level of NK receptors compared with their NK1.1− counterparts (120).

Thus, one potential solution to the paradox was differential stimulation of type I NKT cells or subsets of type I NKT cells. Alternatively, Terabe et al. explored the possibility that the paradox could be resolved by invoking two different categories of NKT cells, type I and type II, both CD1d restricted (98). In four different mouse tumor models in which they found that...
CD4^+CD25^+ Treg cells did not seem to play a role they observed that tumor immunosurveillance was enhanced in CD1d KO mice that lack both types of NKT cells, but not in Jα18 KO mice that lack only type I NKT cells. In the Jα18 KO mice, the suppression of tumor immunosurveillance was as great as that in the wild-type mice. Thus, type II NKT cells were sufficient to suppress tumor immunosurveillance in the absence of type I NKT cells (98). This finding also provided a plausible explanation of an earlier observation that the antitumor immunity induced by CpG oligodeoxynucleotides was enhanced in CD1d KO mice but not in Jα18 KO mice, for which the initial interpretation was a novel regulatory role for CD1 independent of antigen recognition. Nevertheless, it remains possible that type I NKT cells can suppress as well under the right circumstances (118). However, when Ambrosino et al. stimulated type I NKT cells with OCH to skew their cytokine response toward more IL-4 and IL-13 and less IFN-γ production, the mice were still protected against tumor growth (122). Although this experiment could not completely exclude the possibility that a complete skewing to Th2 cytokine production would abrogate the protective effect of type I NKT cells, it does indicate that type I NKT cells can protect even when their cytokine profile is skewed more toward a Th2 balance.

Evidence for cross-regulation between type I and type II NKT cells forming a new immunoregulatory axis

Given that type I NKT cells can protect and type II NKT cells can suppress tumor immunosurveillance, Ambrosino et al. (122) asked whether these opposing subsets might cross-regulate each other and form a new immunoregulatory axis, like
Th1 and Th2 cells that not only have opposite functions but also cross-regulate each other. To address this question first in vitro, they stimulated simultaneously type I NKT cells with αGalCer and type II NKT cells with sulfatide and measured proliferation and cytokine production. Stimulation of type II NKT cells was found to suppress the proliferation and cytokine production by type I NKT cells stimulated with either αGalCer or OCH. This effect was not just on bystander cells in the spleen, because it could be detected by CFSE dilution in αGalCer-CD1d tetramer-binding type I NKT cells as well as by thymidine incorporation. Also, this effect was not just due to competition by sulfatide for binding to CD1d. First, sulfatide could be added to the cultures 30 min later than αGalCer without affecting the inhibition. Second, and most definitively, it was possible to separate pulse two sets of APCs with αGalCer or sulfatide and then wash and mix them to stimulate the two subsets of NKT cells simultaneously, but without the possibility of their competing for binding to CD1d on the same APC (122). Thus, in vitro, type II NKT cells could suppress the proliferation and cytokine production by type I NKT cells.

In vivo, it was noted that suppression was greater, especially early in tumor growth, in Jax18 KO mice than in wild-type mice, indicating that type I NKT cells in the wild-type mice might partially keep the suppressive effect of type II NKT cells in check. To ask whether type II suppression the protective effect of type I NKT cells, Ambrosio et al. treated mice with both glycolipids at the time of tumor challenge in two different tumor models (122). Strikingly, in the 15-12RM fibrosarcoma model the stimulation of type II NKT cells with sulfatide completely abrogated the protective effect of αGalCer. In the CT26 colon cancer lung metastasis model, sulfatide stimulation of type II NKT cells did not completely abrogate the protection afforded by αGalCer stimulation of type I NKT cells but did significantly reduce the protection, from complete to partial. In a patient this difference could make the difference between life and death. Thus, we conclude that not only do type I and type II NKT cells have opposing effects in tumor immunity, but also they do cross-regulate each other. Especially, type II can suppress type I NKT cells, but type I NKT cells seem to have a moderating effect on type II NKT cells. This ability to cross-regulate, therefore, defines a new immunoregulatory axis between type I and type II NKT cells (Fig. 1).

We do not know the exact mechanism by which type II NKT cells suppress type I NKT cells. Data with culture supernatants and anti-cytokine Abs suggest that it is not simply cytokine mediated (122). Halder et al. (123) also observed the ability of type II NKT cells to suppress type I NKT cells in a mouse hepatitis model. In this case, the suppression was mediated through plasmacytoid DCs. Thus, it is quite possible that the suppression is mediated through an intermediary cell and not through direct interaction between type I and type II NKT cells.

Conclusions and Implications

The recent studies described above help to resolve the paradox in the role of NKT cells in tumor immunity, define a new physiological function for the poorly understood type II NKT cell, and identify a new immunoregulatory axis between type I and type II NKT cells. This regulatory axis may have implications beyond tumor immunity, as it is known that early production of cytokines by NKT cells can skew subsequent adaptive immune responses by conventional CD4+ T cells, for example by providing a source of IL-4 for positive feedback in the induction of IL-4 production by class II MHC-restricted CD4+ T cells. Indeed, the type I-type II NKT regulatory axis could be a major determinant early in the immune response of the subsequent skewing of the Th1-Th2 immunoregulatory axis.

In the case of tumor immunity, clearly there are several dichotomies that can play a role in the outcome of the response (Fig. 1). Th1 cytokines are associated with protective responses whereas Th2 cytokines are associated with suppression of tumor immunity. This dichotomy may in part be associated with subsets of type I NKT cells in that CD4+ type I NKT cells may be more inclined to make Th2 cytokines than are double negative type I NKT cells. Although it is clear that type I NKT cells protect against tumors in a Th1 cytokine-dependent fashion, it has yet not been possible to test whether a complete skewing of type I NKT cells toward Th2 cytokines would lead to a suppressive phenotype. Th2 cytokines from type I NKT cells certainly do play a prominent role in asthma and the regulation of autoimmune disease. However, the more prominent subset dichotomy is between type I and type II NKT cells in which protection against tumors and the suppression of tumor immunity can be most clearly separated.

Nevertheless, it must not be forgotten that both types of NKT cells can have varying and sometimes seemingly opposite effects in different disease settings. Type I NKT cells can protect against tumors through Th1 cytokine-mediated mechanisms but can also cause asthma and suppress some autoimmune diseases through Th2 cytokine effects. Type II NKT cells can also be associated with inflammation in hepatitis and ulcerative colitis, as well as with suppression of autoimmune disease and suppression of tumor immunity. Thus, these NKT subsets are complex entities and much work remains to elucidate the mechanisms that determine their function in different settings.

It will also be important to determine the relationship between the NKT-mediated regulatory pathway and other regulatory mechanisms such as the CD4+CD25+ Treg cell. There is some evidence that Treg cells can suppress type I NKT cells (124), that type I NKT cells stimulated by αGalCer can induce Treg cells to prevent autoimmune myasthenia gravis (125), and that type I NKT protection against diabetes also requires Treg cells (126). It remains to be determined whether type II NKT cells can suppress Treg cells or synergize in mediating suppression. Although to date either NKT regulatory cells or the Treg cells seem to dominate the immunoregulation of tumor immunity in different tumor models, it is certainly possible that both may play a role concurrently in some tumor settings. Thus, it is important to determine not only how the regulatory NKT and Treg cells interact but also what determines which cell dominates in any given setting. If we can learn how tumors evade the immune system, it may be possible to remove these roadblocks to successful vaccine immunotherapy against cancer. Further, learning to alter the balance along the NKT regulatory axis may allow manipulation of immune responses to obtain favorable outcomes in multiple disease situations.

Disclosures

The authors have no financial conflict of interest.
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