NKT cells and tumor immunity—a double-edged sword

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NKT cells can rapidly produce a diversity of immunoregulatory cytokines; does this make these cells aggressors or suppressors? New experiments now reveal their immunosuppressive function in tumor control and reiterate their pivotal position.

Understanding the molecular mechanisms that regulate divergent arms of the immune response is key to developing effective immunotherapies for many diseases, including cancers. Central in the regulation of various effector arms is the relationship between the antigen presenting cells (APC) and interacting lymphocytes. One special lymphocyte population, present in both humans and rodents, that has recently received great attention from many immunologists is the paradoxical natural killer T (NKT) cell. Two papers that examine tumor immunity in this issue of *Nature Immunology* may bring us closer to understanding the role of these cells in some immune responses[1,2].

These unusual T lymphocytes, which coexpress some NK cell markers, have the capacity to recognize glycolipid antigen in the context of the major histocompatibility complex (MHC) class I–like molecule, CD1d, *via* their invariant T cell receptor (TCR) ($V_\alpha14J_\alpha281$ in the mouse and $V_\alpha24J_\alpha Q$ in human). In response to TCR ligation, NKT cells promptly produce large amounts of both pro-inflammatory T helper 1 (T_H1) cytokines, such as interferon γ (IFN-γ) and tumor necrosis factor (TNF), and anti-inflammatory T_H2 cytokines, such as interleukin 4 (IL-4) and IL-10. This has made it difficult to predict the consequences of their activation *in vivo* but has nonetheless created much speculation that they play a central role in immunoregulation. Accumulated experimental evidence supports their role in promoting anti-tumor immunity[3–5] while paradoxically suppressing autoimmunity[6], and these results have ensured continued debate.

Thirteen years after the discovery of NKT cells, we have now moved a step closer to understanding their mechanism of action in at least one type of immune response. In this issue of *Nature Immunology*, Terabe *et al.*[1] provide compelling data that defines a new function for CD4+ NKT cells as suppressors of cytotoxic T lymphocyte (CTL)-mediated anti-tumor immunity. A second, related, study in this issue[2] shows that CD4+ T cells that express the NK cell marker, DX5, play a critical role in suppressing delayed type hypersensitivity (DTH). Both of these studies highlight previously unrecognized mechanisms that regulate cell-mediated anti-tumor immunity.

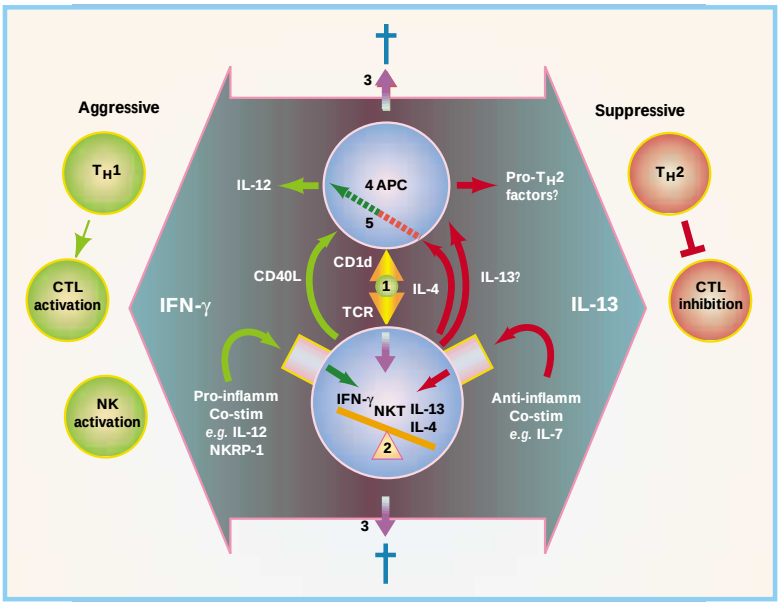

Figure 1. The influence of NKT cells on anti-tumor immunity can range from aggressive (IFN-γ–mediated) to suppressive (IL-13–mediated). This probably relates to the interactions between NKT cells and the APCs that they contact. The outcome of this interaction depends on at least several factors, designated in the figure as 1 through 5. (1) CD1d-glycolipid–TCR interaction. (2) The balance of costimulatory factors that can polarize the type of cytokines produced by NKT cells. (3) Death of either the NKT cells or the APCs. (4) The nature of the CD1d-expressing APC (DC and/or tumor). (5) Negative feedback regulation on T_H1-T_H2–type cytokine production. The consequences of this interaction can influence many cells in the local environment. On the aggressive side, IFN-γ can promote both CTL and NK activation, possibly *via* T_H1 helper cell induction. On the suppressive side, removal of T_H1 combined with potentially enhanced T_H2 responsiveness can lead to CTL inhibition and suppression of anti-tumor immunity. (Co-stim, costimulatory; Inflamm, inflammatory.)

Despite our fascination with the spontaneous regression of some human cancers and a plethora of defined tumor-escape mechanisms, investigators have never avidly dissected models in which tumors recur after spontaneous regression. Fortunately, Terabe and colleagues have persisted with their previous observation that a subset of CD4+ T cells could prevent complete CD8+ CTL-mediated eradication of an experimental tumor in mice. By further exploration of this model, Terabe *et al.* have discovered that these CD4+ T cells are in fact CD1d-restricted NKT cells that specifically prevent effective CTL-mediated tumor eradication in an IL-13–dependent manner[1]. This study is the first to demonstrate a key role for CD4+ NKT cells, and for IL-13, in the repression of CTL-mediated anti-tumor immunosurveillance. Strikingly, IL-13 mediated its effect *via* the IL-4R–STAT6 pathway, whereas IL-4–producing capacity was neither sufficient, nor necessary, for NKT cell– mediated tumor repression. A similar pattern of specific dependence on IL-13 in immune clearance of the nematode, *Nippostrongylus brasiliensis*[7], suggests this pathway may be of broader immunological significance.

Importantly, regulatory T cells may be heterogeneous and there is a potential functional diversity of T cells that carry NK cell markers. In light of this, Moodycliffe *et al.*[2] have

identified CD4+ T cells that express the NK cell marker DX5 as the key repressor cells in a well established model of ultraviolet (UV) radiation–induced immunosuppression. Although these CD4+DX5+ T cells produce T_H2 cytokines, colabeling studies have shown that CD4+NK1.1+ T cells do not typically coexpress the DX5 antigen[8,9], which raises the possibility that CD4+DX5+ T cells represent a distinct regulatory population.

The inhibition of CTL anti-tumor function described[1] supports the previously held concept that NKT cells down-regulate inflammatory cell–mediated immune responses[6]. Of particular relevance is the fact that, in an experimental animal model of anterior chamber–associated immune deviation (ACAID), a deficiency of systemic antigen-specific DTH, could not be induced in NKT cell–deficient mice. The implications for many T_H1-mediated autoimmune diseases are great. This is particularly true in the case of type 1 diabetes, where it has already been shown that NKT cells repress disease[6]. It is clear from all these studies that, although relatively rare, NKT cells are extremely potent in their ability to influence the nature of immune responses to a variety of stimuli including, but not limited to, tumors. We await a definitive demonstration that IL-13 is important in these other models of NKT cell–mediated suppression.

The studies published in this issue of *Nature Immunology* raise many new questions. As IL-13 does not directly act on T cells, the existence of indirect IL-13–mediated responses must be considered. As suggested by Terabe *et al.*, it seems plausible that NKT cell–derived IL-13 suppresses downstream CTL activity by directly acting on IL-13–responsive APCs. IL-13 is known to inhibit pro-inflammatory cytokine (such as IL-12) production and cell-mediated immune responses and is probably a key factor in promoting T_H2 immunity[10]. The examination of tumor recurrence in MHC class II–deficient mice will be important in determining whether conventional T_H2-type CD4+ T cells are intermediaries in CTL suppression by NKT cells. Other cell types could be involved: NK cells, which can be activated by NKT cells, can also produce IL-13[11] and interact with dendritic cells (DCs). Clearly, more attention should now be paid to understanding the molecular control and consequences of the APC–NKT cell interaction. When considered in light of many other studies that suggest a positive role for NKT cells in NK and CTL-mediated activities, including tumor rejection[3–5], we are reminded that NKT cells are multifunctional. Inhibition or elimination of one key effector arm of the NKT cell armament can expose a potent opposing immune reaction, which suggests that the balance of signals received by NKT cells may determine vastly different outcomes.

How might we reconcile such a diversity of outcomes in the context of an APC–NKT cell interaction? Control of the APC–NKT cell interaction is potentially complex, with many checks and balances designed to deal with non-self but minimize immunopathology. Some of the levels of regulation are outlined in **Fig. 1**. The nature of the CD1d-binding antigen (natural, altered or mimic) may be critical in regulating the role that the NKT cells play. The marine sponge glycolipid α-galactosylceramide (α-Gal-Cer) binds CD1d, rapidly stimulates secretion of IFN-γ and IL-4 from NKT cells and has a potent ability to initiate the destruction of some experimental tumors in an IFN-γ and NK cell–dependent fashion[3,5]. Evidence suggests that high affinity TCR ligands preferentially induce T_H1-type responses in conventional T cells and that CD4 signaling itself may favor T_H2-type cytokine production[12]. Therefore, one possible explanation for the immunosuppression seen is that CD1d that is complexed with natural self-ligands has a lower affinity for the NKT cell TCR than does α-Gal-Cer–CD1d, and thus preferentially induces T_H2-type cytokines. However, TCR affinity for glycolipid-CD1d cannot be the single factor that determines NKT cell function: after initial stimulation with α-Gal-Cer, polarized IL-4 production by NKT cells may result in the generation of antigen-specific T_H2 cells and a profound increase in the production of IgE[13].

Another level of regulation is by costimulatory surface molecules on NKT cells, such as NKRP1 (NK1.1), and on APCs, such as members of the TNF superfamily (CD40, OX40L, CD70 and so on). These receptors can also stimulate polarized responses by modulating TCR signals or activating NKT cells independently of CD1d restriction. Examples include the ability of IL-12 receptor or NKRP1 ligation to preferentially stimulate NKT cell IFN-γ production, or the converse stimulation of NKT cell IL-4 production by IL-7[6]. The balance of signals received by the NKT cell and the APC will determine their survival or death, and death may be another important safeguard mechanism to minimize the effector functions of these cells. Activated NKT cells undergo significant apoptosis following TCR ligation or in response to IL-12[6], and death of the interacting DC residents in T cell regions may also control CTL induction. Whether IL-13 possesses pro- or anti-apoptotic activity in this context remains to be investigated. Another level of regulation relies on the nature of MHC and CD1 expression on the APC itself (DC and/or tumor), which may have an impact on the role of NKT cells in any immune response. Our own data shows that IL-12, IFN-γ and NKT cells are critical to NK cell immunosurveillance of carcinogen-induced sarcoma[4]. In this model, NKT cells may not recognize a CD1d-restricted ligand but may simply be activated to produce IFN-γ by DC-derived IL-12. Finally, further control of APC function occurs in situations where T_H2 cytokines provide negative feedback in the generation of bioactive T_H1 cytokines[14], which suggests that the outcome will be influenced by the cytokine milieu as well as the stimulus applied.

In summary, the current report[1] is consistent with a natural role for NKT cells in preventing self-tissue destruction (including destruction of tumors) by suppressing T_H1-type immunity. Exploration of IL-13–mediated suppression of CD8+ CTL responses is now warranted to validate its role in other experimental and spontaneous tumor models. The specific role of IL-13 *versus* IL-4 in mediating various NKT cell functions also needs to be dissected, particularly in systems where it was believed that IL-4 was a key factor[6]. Distinctions between the function of NKT cells in local induction of immune responses and other emerging regulatory T cell populations that control immunologic self-tolerance[15] should soon become apparent. One hopes that, in the near future, IL-13 inhibition may prove to be a useful accessory to CTL-based anti-tumor therapies, but these studies are a timely reminder that NKT cell activation may be a double-edged sword. The excitement concerning the anti-tumor potential of activating the NKT cell must be tempered with a better understanding of the molecular controls that dictate its action.

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