Published in final edited form as:
J Cell Physiol. 2009 February ; 218(2): 246–250. doi:10.1002/jcp.21597.

Cross-regulation between distinct natural killer T cell subsets influences immune response to self and foreign antigens

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Abstract

Natural Killer T (NKT) cells generally recognize lipid-antigens presented in the context of the MHC class I-like molecule CD1d. CD1d-restricted NKT cells consist of two broad subsets: Type I, which express an invariant T cell receptor (TCR) and type II, which utilize diverse TCR gene segments. A major type II NKT subset has been shown to recognize a self-glycolipid, sulfatide. Both subsets play important roles in autoimmune diseases, tumor surveillance, and infectious diseases. While type I NKT cells protect from tumor growth by enhancing tumor surveillance, type II NKT cells may suppress anti-tumor immune responses. In a murine autoimmune hepatitis model, type I NKT cells contribute to pathogenesis, whereas activation of sulfatide-reactive type II NKT cells protects from disease. Sulfatide-mediated activation of type II NKT cells results in modification of dendritic cells and induction of anergy in type I NKT cells. Elucidation of this novel pathway of cross-regulation among NKT cell subsets will provide tools for intervention in autoimmune diseases and for designing strategies for effective anti-tumor immunity.

Keywords

NKT cells; Cancer; Tumor; Autoimmunity; Sulfatide; CD1d; Cross-regulation; Anergy

Natural Killer T (NKT) cells bridging innate and adaptive immunity have biological features of natural killer cells and conventional T cells. NKT cells express the markers NK1.1 or CD56/CD161 as well as the T cell receptor (TCR) (Bendelac et al., 2007; Gumperz et al., 2002; MacDonald, 1995; Prussin and Foster, 1997). Most NKT cells recognize antigens presented by the MHC class I-like and β_2-microglobulin-associated molecule CD1d (Bendelac et al., 1994; Brigl and Brenner, 2004; Ohteki and MacDonald, 1994). The CD1 proteins can be classified into three groups (Barral and Brenner, 2007): Group 1 comprises CD1a, CD1b and CD1c; group 2 consists of CD1d and group 3 comprises CD1e. Humans and many other mammalian species express all CD1 isoforms (CD1a-e), while mice express only CD1d (Barral and Brenner, 2007; Brigl and Brenner, 2004; Godfrey et al., 2008). Interestingly, CD1d is highly conserved among the species and between humans and mice (Brossay et al., 1998). While CD1a-c are involved in presenting (mostly microbial) lipid antigens to conventional T cells, CD1d presents lipids, glycolipids and lipoproteins, which can be of self or foreign origin, to NKT cells (Barral and Brenner, 2007). CD1e is involved in intracellular lipid trafficking, as it lacks a transmembrane domain and is exclusively found in intracellular compartments (De Libero and Mori, 2006). CD1d is expressed on dendritic cells, macrophages (including the liver resident Kupffer cells), subsets of B cells, thymocytes, hepatocytes (Bendelac et al., 2007) and tumor cells (Fais et al., 2004; Fiedler et

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al., 2002; Metelitsa et al., 2003). Hence these cells are also potentially capable of presenting lipid antigens to NKT cells.

CD1d-restricted NKT cells can be broadly categorized into two groups: Type I and type II (see figure 1). Type I NKT cells are also named invariant NKT (iNKT), because they express an invariant TCR encoded by the Vα14Jα18 gene segment paired with one of a limited number of Vβ chains (mainly Vβ8.2, Vβ7 or Vβ2) in mice (Godfrey and Kronenberg, 2004;Kronenberg and Gapin, 2002) and, correspondingly, by the Vα24Jα18 gene segments and the Vβ11 chain, in humans (Dellabona et al., 1994;Porcelli et al., 1993). Stimulation of type I NKT cells with the superantigen-like marine sponge-derived glycolipid αgalactosylceramide (αGalCer) results in a cytokine burst, TCR downregulation, and apoptosis (Crowe et al., 2003;Kawano et al., 1997;Wilson et al., 2003). Depending on their context and the antigen involved in their stimulation, they may express different cytokine secretion profiles, either a Th1-type, secreting IFN-γ and TNF-α, a Th2-type, secreting IL-4 and IL-13 (Godfrey and Kronenberg, 2004), or a combination of the two. Additionally, both the self-glycolipid isoglobotrihexosylceramide (iGb3) and bacterial-derived lipids are recognized by these cells (Kawano et al., 1997;Kinjo et al., 2005;Mattner et al., 2005;Zhou et al., 2004). Type I NKT cells can be identified using αGalCer/CD1d-tetrameric reagents. Type II NKT cells have variable TCR V-gene rearrangements that are distinct from those used by type I NKT cells (Behar et al., 1999;Cardell et al., 1995;Chiu et al., 1999). A major type II NKT cell subset recognizes the self-glycolipid 3-sulfated galactosylceramide, called sulfatide, and can be identified by sulfatide/CD1d-tetramers (Jahng et al., 2004).

In this review, we will focus on the role of both type I and type II NKT cell subsets in autoimmunity and anti-tumor immunity. We will also discuss a novel mechanism of immune regulation resulting in anergy in type I NKT cells following activation of sulfatide-reactive type II NKT cells and how this pathway can be exploited to manipulate immune responses against tumors and in autoimmune disease.

Type I NKT cells in autoimmunity and in anti-tumor immunity

In autoimmune disease models, type I NKT cells have been shown to play either a potentiating or a protective role, depending on the disease, the administration of an activating stimulus (αGalCer or its analogs), and the resulting cytokine secretion profile (Th1 vs. Th2). Thus in the absence of type I NKT cells a milder course of disease has been found in a murine allergen-induced airway hypersensitivity model for asthma (Akbari et al., 2003) and in Concanavalin A (ConA)-induced hepatitis, a model for autoimmune hepatitis (Kaneko et al., 2000; Takeda et al., 2000). Notably, adoptive transfer of type I NKT cells restores disease susceptibility in these models, and in both cases these cells have been shown to produce Th2 cytokines (Akbari et al., 2003; Kaneko et al., 2000; Takeda et al., 2000), suggesting their involvement in pathogenesis. Consistently, a high frequency of type I NKT cells secreting Th2 cytokines has been detected in the lungs of human bronchial asthma patients (Akbari et al., 2006). Protective or regulatory role for type I NKT cells has also been suggested in diabetes-prone NOD mice (Godfrey et al., 1997; Gombert et al., 1996). Following adoptive transfer of NKT cells into NOD mice, it is the Th2 cytokines which are responsible for protection from diabetes, (Hammond et al., 1998). Conversely, a skewing of type I NKT cells towards Th1 has been shown to correlate with the manifestation of type I diabetes in human (Wilson et al., 1998), although a protective role for type I NKT cells in human type I diabetes remains controversial (Lee et al., 2002).

Several studies have used administration of the foreign glycolipid αGalCer to activate type I NKT cells in order to determine their effect in disease models. It has to be kept in mind, that the immune consequences following activation by αGalCer may not represent a

physiological event, since αGalCer is not a mammalian ligand and in many ways acts as a superantigen for type I NKT cells. In some disease models, stimulation of type I NKT cells results in disease exacerbation, while in others protection from disease is observed. The role of the cytokine profile following stimulation is inconsistent, as in some cases Th2 cytokines are found to be pathogenic, although they are protective in most models. In experimental autoimmune encephalomyelitis (EAE), a murine model for multiple sclerosis, activation of type I NKT cells with αGalCer modulates the course of disease, depending on the MHC haplotype and on the timing of activation (Jahng et al., 2001; Singh et al., 2001). Coimmunization with αGalCer and disease-inducing myelin antigen potentiates disease in B10.PL mice, mediated by Th1 cytokine secretion, while it prevents EAE in C57BL/6 mice via Th2 cytokines (Jahng et al., 2001). Prior immunization (before induction of disease), however, prevents EAE in both strains, mediated by Th2 cytokines (Jahng et al., 2001). In dextran sulfate sodium-induced cholangitis, a murine model for primary sclerosing cholangitis, single administration of αGalCer results in disease exacerbation, involving Th1 cytokines, whereas repetitive stimulation is protective, skewing the cytokine profile towards Th2 (Numata et al., 2005). This could be explained by observations of Parekh et al., that repeated stimulation with αGalCer is followed by long-term anergy in type I NKT cells, which become unable to proliferate and produce IFN-γ, but which retain the ability to secrete IL-4 (Parekh et al., 2005). Protection from autoimmune disease following stimulation of type I NKT cells has further been observed following αGalCer administration in NOD mice (Hong et al., 2001; Sharif et al., 2001), although it is not clear whether protection is mediated by Th2 cytokines. Administration of OCH, a synthetic analog of αGalCer, results in Th2 cytokine release from type I NKT cells and protection from both EAE and collagen-induced arthritis (Chiba et al., 2004; Miyamoto et al., 2001).

Disease exacerbation after stimulation with αGalCer occurs in allergen-induced airway hypersensitivity (Meyer et al., 2006) and in the early stage of autoimmune cholangitis, resembling primary biliary cirrhosis (PBC) in the dominant-negative TGF-β receptor II mouse model (Chuang et al., 2008). The first involves Th2 cytokine secretion, whereas the later is associated with Th1 cytokine production. Of note, in human PBC, a role for type I NKT cells has been suggested, since their frequency is increased in livers of PBC patients, while they are decreased in the peripheral blood (Kita et al., 2002).

Type I NKT cells exert an anti-tumor effect following their activation with αGalCer (Kawano et al., 1997; Kobayashi et al., 1995; Morita et al., 1995; Motoki et al., 1995). Thus activated type I NKT cells induced rejection of several experimental tumor lines, including carcinoma, sarcoma, melanoma and thymoma (Hayakawa et al., 2004; Kawano et al., 1997; Smyth et al., 2002). Protection without exogenous stimulation has been found in the murine methylcholanthrene-induced sarcoma model (Smyth et al., 2000). Furthermore, adoptive transfer of NKT cells into type I NKT-deficient mice protects animals from sarcomas (Crowe et al., 2002).

Examinations of the mechanism involved in type I NKT cell-mediated tumor surveillance has revealed a pivotal role for IFN-γ, subsequently enhancing NK cell and cytotoxic $CD8^+$T cell activity against the tumor (Berzofsky and Terabe, 2008). Recently, an anti-tumor role for type I NKT cells has also been confirmed in a murine B cell lymphoma model (Renukaradhya et al., 2008). This protection was dependent on CD1d expression by the tumor, involved IFN-γ and IL-12 secretion and reduction of $CD11b^+Gr1^+$ myeloid suppressor cells capable of suppressing anti-tumor immunosurveillance. Anti-tumor immunity is further enforced by DC maturation and activation following stimulation of type I NKT cells with αGalCer *in vivo* (Fujii et al., 2003). Thus activated DC produce IL-12, upregulate costimulatory molecules, and induce a more effective adaptive immune response mediated by conventional class II and class I MHC-restricted $CD4^+$ and $CD8^+$ T cells,

respectively (Fujii et al., 2003). NKT and NK responses are also enhanced following intravenous injection of αGalCer–loaded tumor cells, resulting in tumor cell lysis by NK cells, subsequent uptake of αGalCer by DC and its presentation in the context of CD1d, leading to DC maturation and prolonged adaptive immunity (Fujii et al., 2007). Consistently, αGalCer-pulsed DC have been shown to expand and activate human type I NKT cells and induce their IFN-γ secretion *in vitro* (van der Vliet et al., 2003).

Collectively, these findings led to to the design of a few clinical trials in cancer patients, using either soluble αGalCer (Crul et al., 2002; Giaccone et al., 2002), *in vitro*-expanded αGalCer-pulsed dendritic cells (DCs) (Chang et al., 2005; Ishikawa et al., 2005; Nieda et al., 2004; Okai et al., 2002; Uchida et al., 2008) or adoptively transferred autologous NKT cells activated *in vitro* with αGalCer and IL-2 (Motohashi et al., 2006). Although biological effects like expansion of type I NKT cells and increased IFN-γ production were observed in the patients, none of the clinical trials so far have shown significant efficacy in terms of a partial or complete remission of tumors. This may relate to the small number of type I NKT cells in humans and insufficiency of their expansion to reach the tumor environment. For detailed information on type I NKT cells, please see comprehensive reviews on this topic by Godfrey et al..

Type II NKT cells in autoimmunity and in anti-tumor immunity

Although type II NKT cells have not been as extensively studied as type I NKT cells, these cells have been shown to play an important role in a number of autoimmune diseases. A majority of reports have revealed a potential protective role for type II NKT cells in autoimmunity. Thus, overexpression of a type II NKT cell subset, described as $V\alpha3.2^{+}V\beta9^{+}$, resulted in the protection from development of autoimmune diabetes in transgenic NOD mice (Duarte et al., 2004). Importantly, activation of a major subset of type II NKT cells by administration of the self-glycolipid sulfatide prevents EAE (Jahng et al., 2004) and Con A-induced hepatitis (Halder et al., 2007), murine models for multiple sclerosis and autoimmune hepatitis, respectively. It may be significant that during EAE, sulfatide-reactive type II NKT cells are increased several-fold in central nervous tissue, but not type I NKT cells. Furthermore, it has been shown in human active multiple sclerosis patients that NKT cells, secreting INF-γ, are significantly more frequent than in normal individuals (Shamshiev et al., 1999). In patients with autoimmune hepatitis and multiple sclerosis, the prevalence of anti-sulfatide antibodies has been demonstrated (Ilyas et al., 2003; Toda et al., 1990). Sulfatide-mediated protection from autoimmunity involves regulation of type I NKT cells, inhibition of effector functions of conventional T cells and modification of DC functions (Halder et al., 2007). We propose that anergized type I NKT cells behave like regulatory T cells and further control autoimmunity and anti-tumor immunity, as discussed below. Interestingly, a pathogenic role for type II NKT cells, involving an atypical Th2 response, has been suggested in ulcerative colitis, since these cells are present among the lamina propria T cell population from patients with ulcerative colitis, secreting high levels of IL-13 (Fuss et al., 2004).

It appears that, in contrast to the role of type I NKT cells enhancing anti-tumor responses, type II NKT cells are able to suppress tumor immunosurveillance in some tumor models (Ambrosino et al., 2007; Terabe et al., 2005). Thus, CD1d-deficient mice, lacking both type I and type II NKT cells, display enhanced anti-tumor immunity resulting in reduced tumor growth, whereas tumors in wild type mice and $J\alpha18^{-/-}$mice, lacking only type I NKT cells, developed equally (Terabe et al., 2005). These findings indicated a suppressive role for type II NKT cells in tumor surveillance and are consistent with an earlier observation that indicated enhanced anti-tumor immunity in $CD1d^{-/-}$mice vs. $J\alpha18^{-/-}$mice by CpG oligodeoxynucleotides (Sfondrini et al., 2002). A significant increase in tumor growth

following stimulation of type II NKT cells with sulfatide further indicates cross-regulation of type I NKT cells by type II NKT cells and suppression of tumor immunosurveillance (Ambrosino et al., 2007). The suppression of anti-tumor immunity by type II NKT cells appears to be associated with elevated levels of the anti-inflammatory cytokines IL-13 and TGF-β as well as $CD11b^{+}Gr1^{+}$ myeloid-derived suppressor cells (Renukaradhya et al., 2008; Terabe et al., 2003). Notably, a subset of type II NKT cells has been identified in humans with malignant disease: Chang et al. found a higher frequency of type II NKT cells reactive to lysophosphatidylcholine species and skewed towards IL-13 secretion in the plasma of multiple myeloma patients (Chang et al., 2008).

Cross-regulation among NKT cell subsets

In three different experimental models, including anti-tumor immune responses, autoimmune hepatitis, and in immunity against parasites, opposing roles for type I vs. type II NKT cells have been suggested. In murine infection with Trypanosoma cruzi, causing Chagas' disease in humans, $CD1d^{-/-}$ and wild-type mice exhibited milder disease in comparison to $J\alpha18^{-/-}$mice that developed more severe disease and died more frequently (Duthie et al., 2005). Thus, type II NKT cells seem to play a pathogenic role, involving Th1 cytokines, in this disease, while type I NKT cells might counteract by down-regulating the response. In contrast, in murine acute schistosomiasis, type I NKT cells contribute to Th1 responses and type II NKT cells might promote Th2 responses (Mallevaey et al., 2007). We have demonstrated the opposing roles for type I and type II NKT cells in autoimmunity as well as in anti-tumor immunity (Ambrosino et al., 2007; Halder et al., 2007). Thus, anergy induction in type I NKT cells occurs following activation of sulfatide-reactive type II NKT cells, resulting in subsequent protection from autoimmune disease (Halder et al., 2007). Since adoptive transfer of purified dendritic cells from sulfatide-treated animals into naïve recipients can induce anergy in type I NKT cells and prevent disease, these findings reveal a novel immune-regulatory axis in which sulfatide-reactive type II NKT cells are able to regulate type I NKT cells by modulating the function of dendritic cells (Figure 2). Currently, we are investigating the role of distinct DC subsets (e.g. plasmacytoid vs. myeloid) in this immune regulatory mechanism.

Summary and Perspectives

NKT cells appear to play an important role in autoimmune diseases, tumor surveillance, infectious diseases and several other pathological conditions. It is likely that involvement of different NKT subsets and their cytokine secretion profile (Th1-like vs. Th2-like) will account for their differential role in various disease settings. Interestingly, type I and type II NKT cell subsets seem to have opposing roles and functionally cross-regulate each other in experimental settings of autoimmunity, anti-tumor immunity and in responses to parasitic infections (Ambrosino et al., 2007; Duthie et al., 2005; Halder et al., 2007; Mallevaey et al., 2007). While type I NKT cells predominate in mice, type II NKT cells predominate in humans. Characterization of distinct type II NKT cell subsets and their self-lipid ligands will be important in understanding important biological and physiological roles of these cells in health and disease. Furthermore, a detailed understanding of the mechanisms involved in cross-regulation of type I and type II NKT cells subsets will have crucial implication for manipulating the outcome of immune responses against cancer, autoimmune and infectious diseases. Since the CD1d-dependent antigen-recognition pathway is highly conserved from mice to humans, findings in experimental models can be relatively easily translated to the clinical setting.

Acknowledgments

Contract grant sponsor: The National Institutes of Health, Contract grant number: R01 CA 100660

Contract grant sponsor: German Research Foundation, Contract grant number: AR 645/1-1

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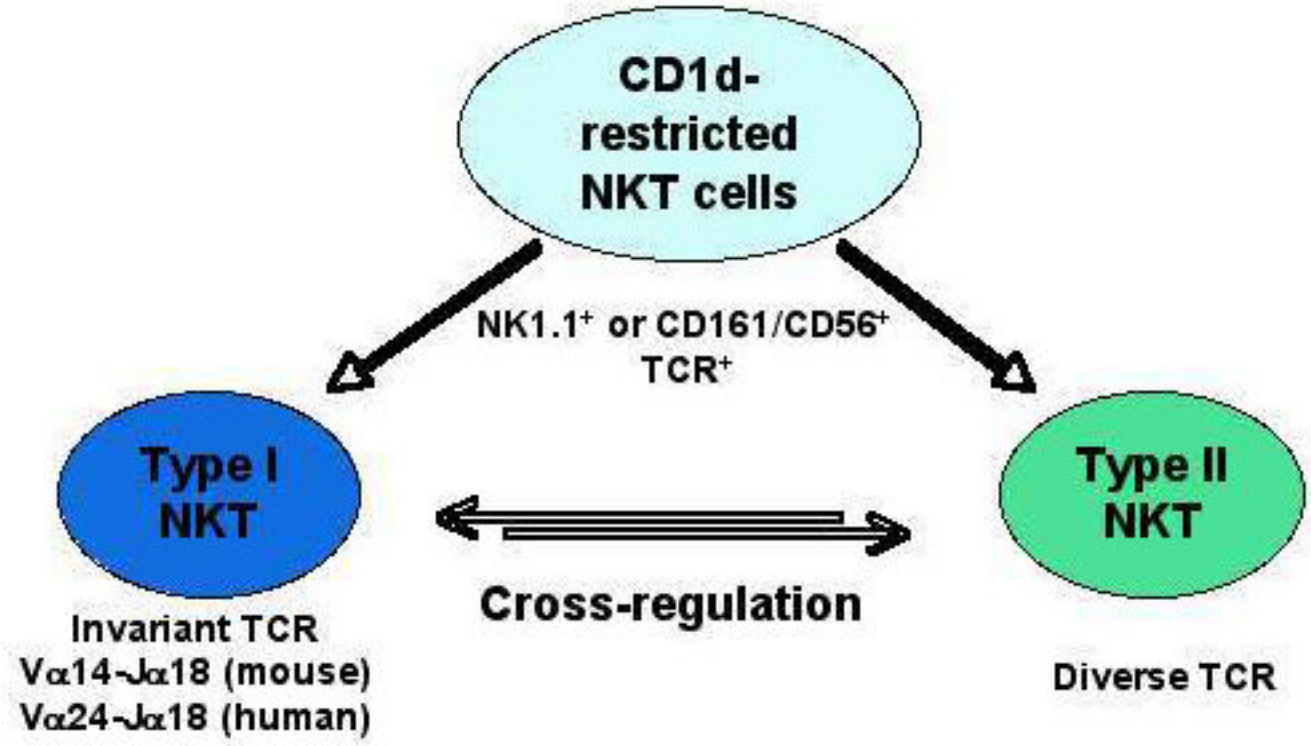

Figure 1.
A broad category of NKT cell subsets

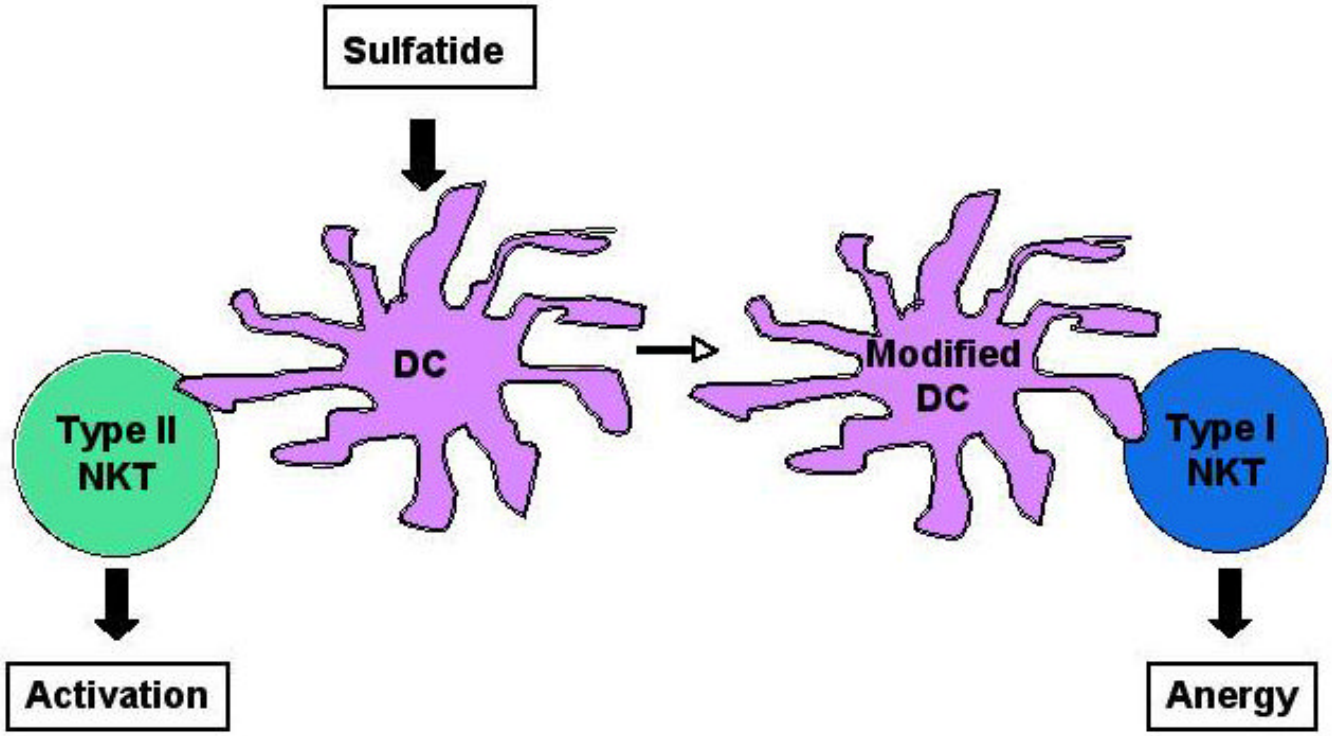

Figure 2.
A model for the type II NKT cell-mediated regulation of type I NKT cells: Following activation of sulfatide-reactive type II NKT cells, dendritic cells (DC) are modified and mediate anergy in type I NKT cells.