REVIEW

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Immunomodulatory Type II Natural Killer T Lymphocytes in Health and Disease

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Abstract

Natural killer T (NKT) lymphocytes are $\alpha\beta$ T cells activated by lipid-based ligands presented on the non-polymorphic CD1d-molecule. Type I NKT cells that carry an invariant Vα14 (in the mouse) or Vα24 (in humans) T cell receptor α-chain rearrangement have received significant attention for their involvement in a diversity of immune reactions. Their sister population, CD1d-restricted type II NKT cells, has been more difficult to study because of the lack of molecular markers that specify these cells. In the last few years, however, significant progress has been made, demonstrating that type II NKT cells have unique functions in immune responses to tumours and infections, in autoimmunity, obesity and graft-versus-host disease. Type II NKT cells appear more frequent than type I NKT cells in humans and accumulate in certain diseases such as ulcerative colitis, hepatitis and multiple myeloma. Recently, novel type II NKT cell ligands have been identified, and it is becoming clear that the type II NKT cell population may be oligoclonal. Here, we review the recent progress in the study of type II NKT cells, supporting the view that type II NKT cells may be attractive targets for immunotherapy.

Introduction

Since their discovery in 1995 [1, 2], a remarkable amount of knowledge has accumulated on the biology of CD1d-restricted natural killer T (NKT) lymphocytes. Natural killer T cells are activated by lipid-based antigens presented on the essentially non-polymorphic MHC class I like molecule CD1d. Thus, the set of ligands surveyed by NKT cells is complementary to conventional MHC-restricted T cells that are activated by foreign peptides. The term NKT cells was first used for T cell receptor (TCR) $\alpha\beta^+$ T cells expressing NK receptors but is now generally agreed to denote CD1d-restricted TCR $\alpha\beta^+$ T lymphocytes. Natural killer T cells have potent immunoregulatory functions that determine the outcome of immune reactions, including autoimmunity and immunity to infections and tumours. Promising development in the field suggests that NKT cell-directed therapy can be developed to enhance tumour immunity, prevent autoimmunity and improve vaccines. Because of the practically non-polymorphic nature of CD1d, antigen-specific approaches would be expected to be broadly applicable and not dictated by genetic variability, unlike antigen-specific responses of conventional T cells.

While conventional T lymphocytes carry highly diverse TCR, NKT cells are at least partly oligoclonal in their TCR repertoire and ligand specificity. A large proportion of CD1d-restricted T cells in the mouse, now termed type I NKT cells [3], have a semi-conserved TCR [2]. Another set of CD1d-restricted T cells, termed type II, carry different Vα and Vβ TCR segments [1] (Table 1). Type I NKT cells carry an invariant TCR α-chain with rearranged Vα14-Jα18, paired with diverse TCR β-chains using Vβ8, 7 or 2 rearrangements. The corresponding human type I NKT cell population uses the homologous Vα24-Jα18 rearrangement combined with TCR Vβ11-chains [4, 5]. Although the population of CD1d-restricted type II NKT cells comprises NKT cells with diverse TCR, there is evidence that suggests that the population includes large 'clones' with semi-variant TCR, and/or with similar ligand recognition. The term 'clone' in this context does not infer that the cells have a true clonal relationship and express identical TCR, but it is used in a functional sense for a group of cells activated by one antigen, as will be discussed further later.

The discovery of NKT cells was soon followed by the identification of a very potent artificial ligand for type I NKT cells, namely α-galactosylceramide (αGalCer) [6].

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Table 1 Characteristics of natural killer T (NKT) cell subsets.

	Type I NKT cells	Type II NKT cells
Alternative names	iNKT cells, classical NKT cells	Diverse NKT cells, non-classical NKT cells
Restriction element	CD1d	CD1d
T cell receptors	Invariant TCR α-chain (Vα14-Jα18 in mouse, Vα24-Jα18 in humans), limited β-chain	Partly diverse, partly oligoclonal
Ligands	Microbial, endogenous and artificial glycolipid ligands, including αGalCer, etc.	Sulfatide, β-GlcCer, β-GalCer, lyso-PC, pollen-derived lipids, small non-lipid molecules. Mostly unknown.
Autoreactivity	Yes	Yes
NK1.1 (CD161)	+/−	+/−
CD4/CD8	$CD4^+$ and $CD4^-CD8^-$ and some $CD8^+$ in humans	$CD4^+$ and $CD4^-CD8^-$ and some $CD8^+$ in humans
Cytokines	Diverse, including TH1 and TH2	Diverse, including TH1 and TH2

This finding was of fundamental importance for research on type I NKT cells providing a strong ligand for *in vivo* and *in vitro* functional studies. Type I NKT cells can be visualized with flow cytometry using αGalCer-loaded CD1d-tetramers providing the basis for numerous studies that demonstrate the importance of type I NKT cells in immunoregulation.

By contrast, as the defining criterium for the type II NKT population is diversity in the TCR repertoire, this mixed population remains difficult to identify with available methodology. The diverse TCR of type II NKT cells recognize different CD1d-associated ligands, discarding CD1d-tetramer technology as a simple means to define the entire type II population, and unique surface markers expressed on these cells, or on all NKT cells, are yet to be defined. Therefore, the cells can still only with certainty be defined by their CD1d-restriction or CD1d-dependent autoreactivity and absence of the invariant Vα14/Vα24 TCR α-chain. However, despite the difficulty to study these cells, progress has been made during recent years, and the findings underscore their importance and unique function in murine and human disease [7–9]. Here, we review recent progress in the study of type II NKT cells and suggest that type II NKT cells are a promising therapeutic target for immunoregulation.

Type II NKT cells have distinct immune functions in mice and humans

Studies of type II NKT cell function in mice and humans

Several studies have demonstrated the unique role of type II NKT cells in different immune reactions. By comparing mice that lack type I NKT cells (Jα18-deficient mice) and mice that lack both type I and II NKT cells (CD1d-deficient mice), it has been possible to draw conclusions regarding the contribution of type II NKT cells to immune responses. Some investigations have used blocking antibodies to CD1d to investigate the effects of type II NKT cells; however, the CD1d-molecule is not solely an antigen-presenting molecule, but cross-linking of CD1d on human and murine dendritic cells induces IL-12 production and on intestinal epithelial cells induced IL-10 production [10–12]. Experiments using antibodies that may cross-link CD1d should therefore be interpreted with caution. It has been found that type II NKT cells can act in concert with type I NKT cells, while in other situations, the two populations seem to antagonize each other. A regulatory axis also exists between the two subsets of NKT cells [13]. Studies of human type II NKT cells have mostly relied on their definition as being CD1d-autoreactive and Vα24-negative. Interestingly, such cells were more frequent than Vα24-positive type I NKT cells in human bone marrow and liver, as well as in the inflamed intestines of patients with ulcerative colitis [14–16]. The frequency of type I NKT cells is also lower in human lymphoid organs, such as liver and spleen, than in the corresponding organs in the mouse. In the mouse, type I NKT cells are likely to outnumber type II NKT cells, but the findings in humans suggest that type II NKT cells may dominate over type I NKT in this species. With the lack of specific markers that can be used to identify type II NKT cells, studies of type II NKT cells have been facilitated by the use of TCR transgenic mice, as well as when novel specific type II NKT cell ligands have been identified. Below is an overview of different human and murine immune responses in which type II NKT cells have been shown to be involved, also listed in Table 2.

Experimental autoimmune encephalomyelitis (EAE) and multiple sclerosis (MS)

The first type II NKT cell ligand to be identified was sulfatide [17]). Sulfatide is an endogenous glycolipid that is found in diverse isoforms preferentially expressed in different tissues such as the central nervous system (CNS), kidneys and β-cells in the pancreatic islets of Langerhans. Multiple sclerosis is a demyelinating autoimmune disease in which myelin-derived protein antigens are targets for autoimmune attack by conventional T cells. Several glycolipid components of the myelin

Table 2 Human and murine type 2 natural killer T (NKT) cells in disease.

Function	Species	
Experimental autoimmune encephalomyelitis (EAE)/ multiple sclerosis (MS)	Mouse	Injection of the ligand sulfatide prevents EAE in a CD1d-dependent manner. Sulfatide-specific cells infiltrate central nervous system. Type II NKT cells potentiate treatment with tolerogenic DC by increased IL-4 and IL-13 production [17, 19].
	Human	Frequency of sulfatide-specific cells increase in peripheral blood lymphocytes from patients with MS [18].
T1D	Mouse	Over expression of type II NKT cells in transgenic model prevents disease. Transgenic type II NKT cells regulate T1D through ICOS and PD-1. Sulfatide treatment of NOD mice ameliorates T1D [22, 23, 25].
Colitis	Mouse	Overexpressed CD1d promotes spontaneous colitis by IFN-γ and IL-17 producing TCR transgenic type II NKT cells [27].
	Human	IL-13 expressing type II NKT cells accumulate in lamina propria of patients with ulcerative colitis. IL-13 augments NKT cell cytotox against epithelial cells [14].
Obesity	Mouse	Type II NKT cells initiated liver and adipose tissue inflammation and exacerbated obesity [31].
Tumour immunity	Mouse	IL-13 secreting type II NKT cells induce CD11b$^+$ Gr-1$^+$ cells to produce TGF-β that suppresses CD8$^+$ anti-tumour immunity. Sulfatide administration enhances type II NKT cell-mediated suppression of tumour immunity. CpG enhances type II NKT cell-mediated suppression of tumour immunity [13, 40–42].
	Human	IL-13 producing lyso-PC-specific type II NKT cells were dramatically increased in patients with multiple myeloma [43].
Bone marrow transfer and graft-versus-host disease (GVHD)	Mouse	Type II NKT cells protect mice from GVHD through production of IFN-γ and IL-4 inducing apoptosis and TH2 skewing of donor T cells, respectively [28, 29].
	Human	Human bone marrow-derived type II NKT cells express TH2 cytokines and suppress alloreactivity *in vitro*. Type II NKT cells in G-CSF mobilized PBPC are TH1 biased and associated with positive prognosis of autologous recipients in treatment of haematological malignancies [15, 30].
Hepatitis	Mouse	Sulfatide administration protects mice from ConA-induced hepatitis mediated by type I NKT cells. Type II NKT cells mediate hepatitis in a transgenic model of hepatitis B infection [33, 34].
	Human	CD1d is upregulated in Hepatitis C-infected liver. High frequencies of IFN-γ (and some IL-13) producing type II NKT cells in hepatitis C virus-infected liver [16, 32].
Infections	Mouse	Type II NKT cells induce excessive inflammation and increased mortality after *T. cruzi* infection. Type II NKT cells skew the immune response to *S. mansoni* to the production of TH2 cytokines. Sulfatide administration ameliorates *Staphylococcus aureus* sepsis [37, 38].
Regulation of type I NKT cells	Mouse	Type II NKT cells can suppress anti-tumour effects of type I NKT cells. Type II NKT cells can suppress ConA-induced hepatitis mediated by type I NKT cells [34, 63].

sheath, including sulfatide, were found to activate CD1-restricted T cells from peripheral blood lymphocytes (PBL) of healthy controls. The frequencies of reactive cells were increased in PBL from patients with MS, suggesting that the cells may be involved in the autoimmune reaction of this disease [18]. However, in these studies, it was not addressed whether the sulfatide reactivity of these T cells was restricted by CD1d or by other human CD1 isoforms that are all known to present sulfatide to T cells.

Subsequently, Kumar *et al.* demonstrated that sulfatide was a ligand for a murine type II NKT hybridoma [17]. A significant fraction of spleen and liver T lymphocytes was identified by sulfatide-loaded CD1d-tetramers, suggesting that sulfatide reactivity is relatively common among murine type II NKT cells. It was found that sulfatide-specific type II NKT cells, but not type I NKT cells, infiltrated the CNS during the course of EAE, a murine model for MS [17], suggesting that sulfatide may act as a self ligand activating type II NKT cells during this disease. Moreover, sulfatide administration at the time of induction of EAE leads to decreased IFN-γ and IL-4 production by pathogenic autoantigen-reactive T cells and almost completely prevented the disease in WT mice, but not in CD1d-deficient mice.

A regulatory role for type II NKT cells in EAE was also suggested in a study, in which type II NKT cells were found to potentiate treatment of murine EAE with tolerogenic DC [19]. Ablation of the co-inhibitory molecule PD-L1 (B7-H1) on the administered DC released activation of type II NKT cells by the DC, followed by increased serum IL-4 and IL-13 levels thought to be derived from the activated type II NKT cells. As a consequence, the numbers of IFN-γ and IL-17 producing autoantigen-specific T cells were decreased in the CNS, and there was an increased EAE protection when PD-L1 deficient DC was injected.

Type 1 diabetes

Type 1 diabetes (T1D) results from the T cell-mediated destruction of pancreatic β-cells of the Langerhans' islets. Several studies have established a protective role of type I NKT cells in T1D in the non-obese diabetic (NOD) mouse model [20]. Using 24$\alpha\beta$ transgenic mice overexpressing a

type II NKT cell TCR on the NOD genetic background, we have performed a series of studies demonstrating that type II NKT cells also have a disease regulatory function in this model [21–23]. Mice overexpressing type II NKT cells were protected from disease, and T1D induced by diabetogenic NOD T cells in transfer models was inhibited by 24$\alpha\beta$ type II NKT cells demonstrating a dominant protection. Mechanistic studies revealed that ICOS and PD-1 interactions were required for the disease protection, while data suggested that FoxP3$^+$ Treg and an array of regulatory cytokines such as IL-4, IL-10, IL-13 and TGF-β did not play a role. 24$\alpha\beta$ type II NKT cells are not activated by sulfatide, but it is interesting to note that sulfatide is associated with insulin in the β-cells of pancreatic islets of Langerhans, suggesting that sulfatide-specific type II NKT cells might be activated by pancreas-associated sulfatide during the destruction of this tissue. Antibodies to sulfatide are found in patients with T1D but not in healthy individuals nor in patients with type 2 diabetes, suggesting that sulfatide, indeed, induces an immune response in this disease [24]. A recent publication provides data supporting that sulfatide-reactive type II NKT cells can ameliorate T1D; sulfatide treatment of NOD mice reduced T1D incidence and islet-specific T cell responses, and further, cells positive for the CD1d-tetramer loaded with sulfatide were found in pancreas-draining lymph nodes in diabetic mice [25].

Ulcerative colitis

Ulcerative colitis is an inflammatory bowel disease characterized by superficial mucosal inflammation and tissue destruction in the colon associated with a TH2-skewed cytokine profile. Using the oxazolone-induced experimental murine model for ulcerative colitis, it was found that IL-13 producing type I NKT cells were required for disease induction [26]. These findings prompted a search for NKT cells in the inflamed tissue of patients with colitis. Surprisingly, a high frequency of type II NKT cells, and not type I NKT cells, was found compared to controls [14]. The colitis-associated type II NKT cells produced high amounts of IL-13 upon activation and were cytotoxic for intestinal epithelial cells. Thus, both murine and human studies suggest a proinflammatory role for IL-13 producing NKT cells in colitis; however, different NKT subsets were involved in the two species. Recent data demonstrate that in the presence of upregulated CD1d levels, such as during inflammation, type II NKT cells can directly contribute to colitis in mice as well [27]. 24$\alpha\beta$ NKT TCR transgenic mice overexpressing CD1d spontaneously developed colitis, and transfer of transgenic NKT cells from these mice provoked the disease in recipient mice. In this model, the disease inducing transgenic type II NKT cells produced elevated levels of IFN-γ and IL-17, and reduced IL-13 and IL-4, suggesting that the mechanism of disease induction by NKT cells in this model was different from that of the IL-13 producing NKT cells described earlier.

Bone marrow transfer and graft-versus-host disease

Bone marrow transplantation is an efficient therapy for some haematological malignancies; however, graft-versus-host disease (GVHD) is a serious complication that can follow this treatment. Bone marrow, together with the liver, is the site where NKT cells are found in the highest proportion among T lymphocytes. Despite this, the role of NKT cells in this organ is poorly understood. Studies of GVHD in mice aiming to elucidate immunoregulatory mechanisms found that type II NKT cells in the bone marrow graft could protect the recipient mice from GVHD. Protection was mediated by type II NKT cell-derived IFN-γ that induced apoptosis in donor-derived T cells and IL-4 that skewed the immune response to a protective TH2 profile [28, 29]. In human bone marrow, CD1d-reactive type II NKT cells were more frequent than type I NKT cells [15]. The type II NKT cells displayed a TH2-biased cytokine profile and suppressed mixed lymphocyte reactions *in vitro*. Strikingly, G-CSF-mobilized peripheral blood progenitor cells (PBPC), used as an alternative to bone marrow transplantation for haematological malignancies, contain a high frequency of type II NKT cells just like in the bone marrow [30]. However, the PBPC-derived type II NKT cells were TH1-biased, and a high frequency of these cells was associated with a positive prognosis of autologous recipients.

Obesity

Recent studies of mice that lack type I NKT cells only, or both type I and type II NKT cells (CD1d-deficient mice), have implied type II NKT cells in diet-induced obesity. High fat diet fed CD1d-deficient mice gained less body weight and developed milder hepatosteatosis compared both to mice lacking type I NKT cells only and to wild-type mice. Further investigation suggested that type II NKT cells initiated liver and adipose tissue inflammation and exacerbated the course of obesity leading to insulin resistance [31].

Hepatitis

Human liver contains a high frequency of type II NKT cells but few type I NKT cells [16]. In chronically hepatitis C-infected liver, CD1d levels were upregulated, and the type I/II NKT cell ratio was maintained [32]. Hepatic type II NKT cells from both infected and normal donors produced large amounts of IFN-γ and some IL-13 but not IL-4. It was suggested that type II NKT cells could contribute to protection from viral infection, but

also to damage in chronic infections. In contrast to human liver, there is a very high frequency of type I NKT cells in murine liver. Type II NKT cells, including sulfatide-reactive cells, are also enriched in this organ but are present in lower numbers than type I NKT cells [17].

In a murine transgenic model of acute hepatitis B virus infection, type II NKT cells contributed to liver pathology as a result of NKG2D-dependent activation [33]. Experiments specifically targeting sulfatide-reactive type II NKT cells revealed a very different function of this subset of cells in concanavalin A–induced hepatitis. Upon intraperitoneal sulfatide administration, there was a rapid accumulation of sulfatide-reactive type II NKT cells in the liver [34]. This resulted in activation of plasmacytoid dendritic cells and the production of IL-12 and MIP-2 that imposed anergy on type I NKT cells. The anergic state was demonstrated to protect mice from concanavalin A–induced hepatitis which is mediated by type I NKT cells. Thus, in the conanavalin A–induced hepatitis model, it was shown that type II NKT cells could regulate the function of type I NKT cells.

Other infectious diseases

At present, it is well established that type I NKT cells can play major roles in the immune response to infections [35, 36], but the contribution of type II NKT cells is less well understood. It has been shown that type II NKT cells have an unique role in infections with the parasite *Trypanosoma cruzi* [37], by promoting an excessive inflammation and mortality, accompanied by decreased pathogen-specific antibody production. In contrast, type I NKT cells prevented the detrimental effects of type II NKT cells, reducing inflammation and improving mortality and antibody titres. Also during murine *Schistosoma mansoni* infection, type I and type II NKT cells had opposing roles [38]. Here, type II NKT cells skewed the immune response to the parasite towards decreased IFN-γ production and increased TH2 cytokine secretion, while type I NKT cells restored the IFN-γ response. In a different approach, we have evaluated the effect of sulfatide treatment in a murine model for *Staphylococcus aureus* sepsis (J. Kwiecinski, S. Rhost, M. Blomqvist, J.-E. Månsson, S. Cardell and T. Jin, unpublished observations). Sulfatide administration on the day of infection, and day three post-infection, reduced septic mortality and lowered serum levels of inflammatory cytokines compared to control mice. The sulfatide-mediated protection was dependent on CD1d, suggesting that type II NKT cells could ameliorate sepsis induced by systemic *S. aureus* infection.

Tumour immunity

The potent type I NKT cell ligand, αGalCer, was discovered because of its protective effects in a mouse model for lung metastasis [39]. Since then, the beneficial role of type I NKT cells in the prevention of tumour formation in a diversity of animal models of cancer has been well established. Clinical studies also support a beneficial role of type I NKT cells, as in many types of human cancers, low levels of circulating type I NKT cells correlate with a poor prognosis. Interestingly, studies of type II NKT cells in cancer immunity suggest that this population may have a predominant immunosuppressive function, preventing efficient anti-tumour immunity. In mice carrying subcutaneous melanoma, injection of the TLR9 ligand CpG-ODN inhibited the growth of the tumour, but only in the absence of type II NKT cells. Absence of type II NKT cells increased the ratio of production of IFN-γ over IL-4 [40]. The mechanisms underlying the suppression of tumour immunity by type II NKT cells were explored in a series of studies by Berzofsky and Terabe [13]. They found that type II NKT cells down-modulated tumour-specific $CD8^+$ T cells resulting in an inability to control tumour growth in several tumor models. It was disclosed that immunosuppression by type II NKT cells required IL-13 production by these cells that in turn activated myeloid Gr-1^+ $CD11b^+$ cells to produce TGF-β that suppressed tumour-specific $CD8^+$ T cells. In this model, sulfatide administration enhanced tumour growth in a CD1d-dependent manner, providing further evidence that type II NKT cells could prevent tumour immunity. In the same tumour model, activation of type I NKT cells by the administration of αGalCer prevented tumour growth demonstrating that type I NKT cell activation induced effective tumour immunity. Simultaneous treatment with sulfatide and αGalCer abolished the beneficial effects of αGalCer, suggesting that type II NKT cells may have suppressed the activation of type I NKT cells.

Opposing effects of type I/II NKT cells on tumour immunity were also described in a B lymphoma model [41]. Also in this model, type II NKT cells were found to suppress natural tumour immunity mediated by type I NKT cells, resulting in enhanced tumour growth. Suppression of tumour immunity was associated with increased production of IL-13 and TGF-β and decreased levels of IL-12 and IFN-γ, and type II NKT cells promoted the accumulation of Gr-1^+ $CD11b^+$ myeloid suppressor cells in the tumour microenvironment. Type II NKT cells were also found to suppress tumour immunity in a mouse model of CD1d-negative breast cancer [42]. Thus, data from several tumour models provide evidence that type II NKT cells, including the sulfatide-reactive subset, can prevent tumour immunity by elevated IL-13 production and promotion of myeloid-derived suppressor cells (Gr-1^+ $CD11b^+$) that suppress T cell tumour immunity by secretion of TGF-β.

Type II NKT cells may also be involved in tumour immunity in humans, as exemplified in the study by

Chang *et al.* [43] in which a subset of type II NKT cells was found to be dramatically increased among PBL from patients with multiple myeloma. By isolating CD1d-binding lipids from plasma of patients with myeloma, they identified lysophosphatidylcholine (lyso-PC) that was known previously to be increased in serum from patients with myeloma. Using lyso-PC-loaded CD1d-tetramers, they could demonstrate that non-Vα24 type II NKT cells specific for lyso-PC were several fold increased in PBL of patients with myeloma. Stimulation of the lyso-PC-specific NKT cells induced IL-13 production. Interestingly, in the same patient group, the frequency of Vα24-positive type I NKT cells was decreased in PBL compared to control PBL, and a high frequency of Vα24 type I NKT cells was associated with better prognosis. These findings suggest that type I and type II NKT cells perform different functions in multiple myeloma. Whether lyso-PC-specific type II NKT cells have a detrimental role in this disease is not known, but there are compelling similarities between the NKT cell subsets in patients with myeloma and the mouse tumour models described earlier.

Innate and adaptive activation of type II NKT cells

NKT cell activation differs from that of conventional T cells in some fundamental aspects. Natural killer T cells show an increased degree of autoreactivity to endogenous antigen-presenting cells [1, 2], and further, NKT cells seem to be more prone to innate activation that is primarily driven by inflammatory cytokines and less dependent on TCR engagement [36]. These characteristics play an important role in determining when and how NKT cells are activated. Both type I and type II NKT cells regulate autoimmunity as well as immune responses to pathogens. This means that NKT cells can be activated *in vivo* in response to microbial infections, but also that they can be activated in the absence of foreign antigens. In support of this, recognition of a number of microbial glycolipids by type I NKT cells is well documented [35], and different classes of lipid-based self antigens, such as iGb3, β-glucosylceramide (βGlcCer) and peroxisome-derived lipids, have been identified for these cells [44–47].

Data so far indicate that type II and type I NKT cells primarily recognize different antigens; type II NKT cells generally do not recognize αGalCer [48, 49]. Additional evidence that the two subsets interact differently with CD1d-ligand comes from a set of monoclonal antibodies to CD1d that differentially block CD1d-autoreactivity of type I and type II NKT cells [50]. Further, CD1d-dependent thymic selection of type I NKT cells, and their autoreactivity to CD1d, is dependent on recycling of CD1d from the cell surface to endosomal/lysosomal compartments. In contrast, type II NKT cells do not depend on this pathway, suggesting that the two NKT cell subsets are activated and selected by lipid ligands deriving from distinct intracellular compartments [51–53].

Self-lipids presented on CD1d are assumed to be required to positively select NKT cells during thymic development and are also likely to play a role when NKT cells are activated in situations of autoimmunity. Activation of dendritic cells by toll-like receptor (TLR) ligands alters glycolipid metabolism in the cells, leading to an upregulation of CD1d-associated self ligands that activate type I NKT cells [45, 54–56]. Combined with an increase in costimulatory molecules and upregulated CD1d levels on activated DC [57], this leads to an efficient activation of NKT cells by activated DC also in the absence of foreign ligands [36]. Therefore, NKT cells respond in an adoptive manner to a diversity of specific self or foreign lipid-based antigens.

In addition to this, it has been shown that cytokines such as IL-12, IL-18 and type I IFN induced during inflammation in response to infections can contribute substantially to activation of type I NKT cells [58, 59]. In fact, infection-induced IFN-γ production by type I NKT cells was only partially dependent on CD1d. Our own studies of type II NKT cells employing a TCR transgenic model [21] suggest that type II NKT cells can, similar to type I NKT cells, respond to activation in an innate-like manner S. Sedimbi and S. Cardell, unpublished S. observations. This implies that both type I and II NKT cells can respond to inflammation in an innate-like manner, independently of TCR stimulation. Interestingly, a recent study of type I NKT cells demonstrated that cytokine-driven (IL-12 and IL-18) but CD1d-independent cytokine induction may result from *in vivo* preconditioning of type I NKT cells by weak TCR-CD1d–ligand interactions [60], suggesting that an inherent weak NKT cell autoreactivity to CD1d will maintain an increased level of responsiveness to inflammatory cytokines.

Then what determines whether type I, type II or both subsets are activated in an immune response? If both types of NKT cells can be activated by dendritic cells stimulated by pathogen-associated molecules such as TLR ligands, which induce the production of stimulatory cytokines and upregulation of endogenous ligands on CD1d on the surface of the DC, it is expected that both cells become activated during infections with microbes that carry TLR ligands. On the other hand, type I and II NKT cells are known to be activated by different repertoires of CD1d-associated ligands. Therefore, it can be speculated that during infections with pathogens that lack ligands that strongly activate DC, the type I or type II NKT cells may be selectively activated by specific microbial CD1d-ligands. Similarly, when NKT cells are activated in auto- and tumour immunity, they may be selectively activated

by specific endogenous CD1d-ligands. Further studies will be necessary to resolve these issues.

The TCR repertoire of type II NKT cells and CD1d–ligand recognition

Human and mouse type II NKT populations appear to display partial oligoclonality

As mentioned previously, the division into type I and type II NKT cell subsets is based on the TCR expressed by the cells. Type II NKT cells were first defined by sets of murine T cell hybridomas that were CD1d-autoreactive and carried TCR that did not use the Vα14 segment [1, 50, 61]. The TCR repertoire of these cells was diverse, although there was some overrepresentation of certain V-segments. The size and extent of TCR and functional diversity of the type II NKT cell population are not yet known, but some conclusions can be made based on the findings so far. As summarized later, a series of reports suggest that mouse and human type II NKT cell populations are to some extent oligoclonal (Fig. 1). However, it is unclear whether all cells in the type II NKT population belong to one of several overrepresented 'clones', or whether the area depicted green in Fig. 1 covers a collection of cells that carry TCR as diverse and unique as those of conventional TCR$\alpha\beta$ cells. In this context, it is interesting to note that the TCR diversity of $\gamma\delta$Tcells is partly oligoclonal, partly diverse, reminiscent of what has been shown so far for NKT cells.

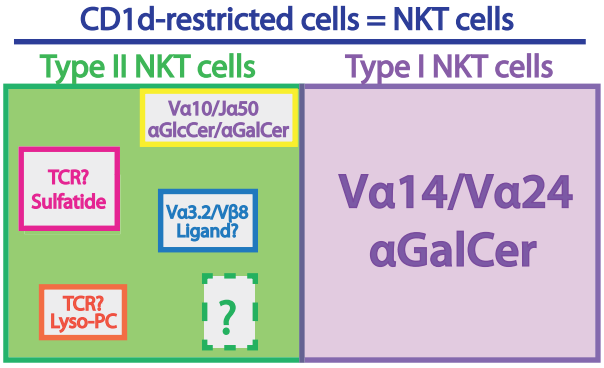

Figure 1 The CD1d-restricted natural killer T (NKT) cell population may be partially oligoclonal. Natural killer T cells are divided into type I and type II NKT cells. Type I NKT cells (the purple box) express the invariant Vα14 (mouse) or Vα24 (human) TCR α-chains and are activated by the prototypical ligand αGalCer. Type II NKT cells (green box) were initially described as having a diverse TCR repertoire; however, it has become apparent that this population contains several enlarged 'clones' of cells that share TCR and/or ligand specificity, indicated by differently coloured boxes. At this time, it is not known whether all cells among type II NKT cells belong to such an enlarged clone or whether there is a population of truly diverse type II NKT cells (green area in the figure) that have unique and different TCR just like conventional T lymphocytes.

Sulfatide-reactive NKT cells

The most well-studied type II NKT cells are the sulfatide-reactive subset. Having identified that sulfatide could serve as a ligand for the type II NKT cell hybridoma XV19 expressing a Vα1/Vβ16 TCR [1, 17], Kumar and colleagues subsequently demonstrated that sulfatide-loaded CD1d-tetramers stained approximately 5% of liver cells, and 0.2% of splenocytes, around 1/5 the size of the type I NKT cell population in these organs in mice. The TCR repertoire of the sulfatide–CD1d-tetramer-positive cells appeared to be oligoclonal and preferentially used Vα3/Vα1 and Vβ8.1/Vβ3.1 segments [62]. Unfortunately, sulfatide-loaded CD1d-tetramers appear to be more difficult to use than αGalCer-loaded CD1d-reagents, and publications using sulfatide-loaded CD1d-tetramers are limited in number. However, the concept that sulfatide-reactive type II NKT cells are relatively frequent is supported by the fact that sulfatide potently modulates several different immune responses in a CD1d-dependent manner [17, 34, 63]. Further, sulfatide reactivity has been identified with at least three different independently derived type II NKT cell hybridomas, something which would be unlikely if this reactivity was rare [17, 64, 65]. Therefore, sulfatide reactivity appears to be frequent among type II NKT cells, but it does not seem that all sulfatide-reactive cells share TCR rearrangements. Detailed analysis of XV19 reactivity using antigen-presenting cells demonstrated that lysosulfatide was the most stimulatory isoform, followed by C24:1 and C24:0 [64]. C24:1 but not lysosulfatide could induce CD1d-dependent proliferation in murine spleen cells [17]. Lysosulfatide is normally found only in very small amounts in healthy tissues, but interestingly is associated with high-density lipoprotein [66], while the C24 isoforms are enriched in the CNS. We have recently found that, besides sulfatide, also β-glucosylceramide (β-GlcCer) and β-galactosylceramide (β-GalCer), and in particular the lysoforms of these glycosphingolipids, are stimulatory for XV19 cells [67]. While sulfatide, β-GlcCer and β-GalCer could activate XV19 cells, glycosphingolipids did not seem to be required for CD1d-dependent autoreactivity of XV19 cells towards antigen-presenting cells ([64], and [67]). This shows that the XV19 TCR can be activated by distinct but structurally similar glycosphingolipids, as well as by non-glycosphingolipid antigens, presented by CD1d. The reason for this broad ligand recognition by the XV19 TCR is not clear, but it should be noted that the type I NKT TCR can be activated by several structurally different antigens presented on CD1d [47, 68]. This is possible as the type I NKT TCR is able to modulate the position of the glycolipid sugar moiety to adopt a flat conformation similar to that seen with αGalCer positioned between TCR and CD1d [69].

Crystal structure of CD1d-sulfatide shows that the sugar head group of sulfatide points upwards towards the TCR, indicating that the XV19 TCR will bind differently to CD1d-ligand than the type I TCR [70]. Sulfatide-reactive T cells have also been described in the human [18]; however, it is unclear whether these cells included CD1d-restricted type II NKT cells, as sulfatide can be presented on all CD1 isoforms expressed on the surface of human hematopoietic cells [68].

Semi-variant Vα3.2/Vβ8 type II NKT cells

The first description of an enlarged 'clone' within the type II NKT subset was of a murine CD1d-dependent T cell population expressing an invariant TCR Vα3.2-rearrangement combined with a Vβ8 TCRβ-chain [71]. These cells were not investigated in detail, but the conserved TCR rearrangement within this population suggests that cells sharing the invariant Vα3.2 rearrangement may be activated by the same ligands, in analogy with the type I NKT population.

An αGalCer-reactive NKT population expressing canonical TCR Vα10 rearrangements

Recently, a small population of αGalCer-reactive CD1d-restricted NKT cells in mice was found to use a canonical Vα10-Jα50 rearrangement [72]. The Vα10 subset had a preferential reactivity to α-glycosylceramide (αGlcCer) over αGalCer; nevertheless, these cells have a reactivity that overlaps with type I NKT cells, but the cells do not use the canonical Vα14 rearrangement which should classify the cells as type II NKT cells. Vα14/Vα24-negative αGalCer-reactive NKT cells have been described before [73, 74]. Thus, even though the αGalCer-reactive Vα10 population is several fold smaller than the Vα14 population, it should be kept in mind that αGalCer-loaded CD1d-tetramers will primarily, but not exclusively, identify NKT cells with the Vα14/Vα24 invariant TCR.

Human type II NKT cells specific for lyso-phosphatidylcholine

A certain oligoclonality is revealed also in the human type II NKT population. Natural killer T cells reactive with a lyso-phosphatidylcholine (PC)-loaded CD1d-tetramer could be expanded *in vitro* from PBL of healthy humans [43]. In PBL from healthy subjects, lyso-PC-tetramer-reactive cells were found in a low frequency, but in PBL from multiple patients with myeloma, these cells had increased several fold and made up a significant population. The TCR of these cells was primarily Vα24 negative, defining them as type II NKT cells.

Other ligands activating type II NKT cells

Additional ligands that have been described to activate human type II NKT cells are pollen-derived lipids [75] and non-lipidic small molecules [76].

Concluding remarks

Although it remains a challenge to study type II NKT cells, significant progress in the last few years demonstrates that type II NKT cells have unique and powerful functions in immune responses. The picture that emerges of type II NKT cells is complex, as for type I NKT cells; however, it seems that the two NKT populations often have opposing functions. Type II NKT cells, so far, have more often been described to downregulate autoimmunity and tumour immunity, than to contribute to antimicrobial immunity. In many situations, the activity of type II NKT cells is associated with IL-13 production, although they can produce an array of cytokines. Future studies will show whether this picture will remain. Defining new ligands and subsets of type II NKT cells that share TCR and/or ligand specificity will provide important tools that can greatly help future advances. The oligoclonal nature of type II NKT cells suggests that targeting this population with specific ligands in mice and humans can activate a significant number of NKT cells and have a decisive impact on immune responses, as shown with sulfatide-mediated immune modulation in a diversity of immune responses in the mouse. Together with the fact that CD1d is essentially non-polymorphic, this promotes type II NKT cells as a promising therapeutic target for immunomodulation.

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