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Invariant natural killer T cells: bridging innate and adaptive immunity

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Abstract

Cells of the innate immune system interact with pathogens via conserved pattern-recognition receptors, whereas cells of the adaptive immune system recognize pathogens through diverse, antigen-specific receptors that are generated by somatic DNA rearrangement. Invariant natural killer T (iNKT) cells are a subset of lymphocytes that bridge the innate and adaptive immune systems. Although iNKT cells express T cell receptors that are generated by somatic DNA rearrangement, these receptors are semi-invariant and interact with a limited set of lipid and glycolipid antigens, thus resembling the pattern-recognition receptors of the innate immune system. Functionally, iNKT cells most closely resemble cells of the innate immune system, as they rapidly elicit their effector functions following activation, and fail to develop immunological memory. iNKT cells can become activated in response to a variety of stimuli and participate in the regulation of various immune responses. Activated iNKT cells produce several cytokines with the capacity to jump-start and modulate an adaptive immune response. A variety of glycolipid antigens that can differentially elicit distinct effector functions in iNKT cells have been identified. These reagents have been employed to test the hypothesis that iNKT cells can be harnessed for therapeutic purposes in human diseases. Here, we review the innate-like properties and functions of iNKT cells and discuss their interactions with other cell types of the immune system.

Keywords

CD1d; Glycolipids; Immunomodulation; Innate immunity; Invariant natural killer T cells

Introduction

Cells of the immune system are typically grouped into cells that belong to the innate arm of the immune system and cells that belong to the adaptive arm of the immune system (Abbas et al. 2010; Murphy et al. 2007). Cells of the innate immune system express different receptors, termed pattern-recognition receptors, which react with conserved molecular patterns in microorganisms. The innate immune receptors are encoded in the germline and are present in both invertebrate and vertebrate organisms. Cells of the adaptive immune system express diverse receptors with exquisite specificity for antigens derived from particular pathogens. The adaptive immune receptors are generated by somatic DNA rearrangement and are unique to vertebrates. Another important difference between cells of the innate and adaptive immune systems is that cells of the latter but not the former are

capable of generating more robust and rapid responses upon repeated encounter with the same antigen, a property commonly referred to as immunological memory.

While most cell types can be easily categorized within the innate or adaptive arms of the immune system, this distinction is less clear for several cell types (Fig. 1) (Bendelac et al. 2001). This includes B-1 cells, marginal zone (MZ) B cells, certain subsets of $\gamma\delta$ T cells, CD8 $\alpha\alpha$ -expressing T cells in the gut, mucosal-associated invariant T (MAIT) cells, and invariant natural killer T (iNKT) cells. Each of these cell types expresses an antigen-specific receptor, either a B cell receptor or T cell receptor (TCR), that is generated by V(D)J recombination. However, the repertoire of specificities of these receptors is strongly limited, so that these cells react with a limited diversity of antigens. Hence, the receptors expressed by these so-called innate B and T lymphocytes bear similarities with the pattern-recognition receptors expressed by cells of the innate immune system (Bendelac et al. 2001). Furthermore, these cells are unable to develop immunological memory, and they have other unusual characteristics such as rapid elicitation of effector functions and a tendency for autoreactivity. This review article will focus on iNKT cells.

Definition and general properties of iNKT cells

NKT cells are a subset of T lymphocytes that express surface receptors characteristic of the T and NK cell lineages (Bendelac et al. 2007; Brigl and Brenner 2004; Godfrey et al. 2004; Kronenberg 2005; Taniguchi et al. 2003; Van Kaer 2007). Like conventional T lymphocytes, NKT cells express a TCR, but unlike conventional T cells, which react with peptide antigens in the context of major histocompatibility complex (MHC) class I or class II molecules, NKT cells react with lipid or glycolipid antigens presented by the MHC class I-related glycoprotein CD1d (Fig. 2a). CD1d is expressed predominantly by hematopoietic cells and is most abundant on antigen-presenting cells, CD4⁺CD8⁺ (double-positive) thymocytes and, in particular, MZB cells. Most NKT cells, referred to as type I or iNKT cells, express a semi-invariant TCR composed of V α 14-J α 18 and V β 8.2, -7, or -2 chains in mice or homologous V α 24-J α 18 and V β 11 chains in humans (Godfrey et al. 2004). The other subset of NKT cells, called type II or variant NKT (vNKT) cells, expresses more diverse TCRs, and these cells often play an opposite or cross-regulating role with iNKT cells (Arrenberg et al. 2009). NKT cells constitutively express surface markers such as CD25, CD69 and CD122 that are characteristic of effector or memory T cells. Furthermore, these cells express markers that are characteristic of the NK cell lineage, including the activating NK cell receptor NK1.1 (CD161 in humans) and several members of the Ly49 family of NK cell receptors, which includes mostly inhibitory receptors (Fig. 2a). In addition to type I and type II NKT cells, additional subsets of T cells that co-express a TCR and NK cell markers have been identified (Godfrey et al. 2004). These cells, referred to as NKT-like cells, represent diverse subsets but do not depend on CD1d expression for their development or reactivity. Examples of NKT-like cells include MAIT cells and a fraction of conventional CD8⁺ T cells that induce NK1.1 upon activation.

Consistent with their TCR expression, NKT cells develop in the thymus and, at least for the iNKT cell lineage, there is strong evidence that these cells undergo positive and negative selection (Godfrey et al. 2010). Interestingly, however, the positive selection of iNKT cells involves expression of CD1d on double-positive thymocytes, a phenomenon that appears to be common for T cell populations selected by non-classical MHC class I molecules, including Qa-1 and H2-M3 (Rodgers and Cook 2005). A key step in the development of iNKT cells is their acquisition of innate effector functions, which appears to be imparted by the transcription factor PLZF (promyelocytic leukemia zinc finger) (Kovalovsky et al. 2008; Savage et al. 2008). After their development in the thymus, a substantial proportion of iNKT cells remains in the thymus as a mature population and the remaining cells emigrate to the

periphery, where they represent a substantial T cell subset in the spleen, blood, liver and bone marrow, but are more rare in lymph nodes and few of these cells are found in tissues. Curiously, humans have fewer iNKT cells in most organs than mice, and the prevalence of these cells varies dramatically among distinct human subjects, for reasons that are unclear.

Stimulation of iNKT cells through their TCR leads to rapid and robust cytokine secretion and acquisition of cytotoxic activity (Matsuda et al. 2008). iNKT cells activated in this manner can produce a mixture of cytokines, including interleukin (IL)-2, -3, -4, -5, -6, -9, -10, -13, -17, and -21, interferon (IFN)- γ , tumor necrosis factor (TNF)- α , granulocyte monocyte-colony stimulating factor (GM-CSF), and several chemokines, including RANTES and MIP-1 α (Coquet et al. 2008). iNKT cells obtained from different organs produce diverse cytokine profiles, which provides a possible explanation for some of the functional differences that have been observed for iNKT cells obtained from distinct organs (Crowe et al. 2005). Differences in cytokine production have also been observed for distinct iNKT cell subsets. This is most evident for the CD4⁻ and CD4⁺ subsets of human iNKT cells, which have a preponderance for producing predominantly T helper (Th)1 cytokines, or a mixture of Th1 and Th2 cytokines, respectively (Gumperz et al. 2002; Lee et al. 2002). These differences in cytokine production are less evident for murine iNKT cells. The capacity to produce the Th17 cytokine IL-17 is largely confined to a subset of CD4⁺NK1.1⁻ iNKT cells that express the transcription factor ROR γ t (Coquet et al. 2008; Michel et al. 2007).

Modes of iNKT cell activation

iNKT cells can become activated directly via engagement of the invariant TCR with glycolipid antigens and CD1d molecules, or indirectly via activated antigen-presenting cells (Fig. 3).

Direct iNKT cell activation

A number of lipid and glycolipid antigens that can bind CD1d and activate the semi-invariant TCR on iNKT cells have been identified (Brutkiewicz 2006; Venkataswamy and Porcelli 2010). The prototypical iNKT cell antigen is KRN7000, an α -linked galactosylceramide (α -GalCer) (Fig. 2b) that was originally discovered during a search for chemicals derived from a marine sponge and with anti-metastatic activities in mice (Kawano et al. 1997; Natori et al. 1993). It is likely that KRN7000, which is usually referred to simply as α -GalCer, is derived from *Novosphingobium* (previously called *Sphingomonas*) bacteria that colonize the sponges, as multiple iNKT cell-activating glycolipids with structural similarity to α -GalCer have been identified in the cell wall of *Novosphingobium* species (Kinjo et al. 2005; Mattner et al. 2005; Sriram et al. 2005). Similar glycolipids are present in *Ehrlichia* bacteria. *Borrelia burgdorferi*, which causes Lyme disease, is another bacterium that contains cognate glycolipid antigens, i.e., diacylglycerols, for iNKT cells within its cell wall (Kinjo et al. 2006). While the majority of iNKT cells of mice and humans react with these bacterial-derived glycolipids, smaller subsets of iNKT have been shown to react with phospholipids (Gumperz et al. 2000), lysophospholipids (Fox et al. 2009), a tetramannosylated form of phosphatidylinositol termed PIM₄ derived from *Mycobacterium bovis* (Fischer et al. 2004), the tumor-derived ganglioside GD3 (Wu et al. 2003), a lipophosphoglycan from *Leishmania donovani* (Amprey et al. 2004), and a lipopeptidophosphoglycan from the membrane of *Entamoeba histolytica* (Lotter et al. 2009). Much work has been focused on identifying the endogenous glycolipid antigen (s) that is critical for development and function of iNKT cells. The main candidate is isoglobotrihexosylceramide (iGb3), a lysosomal, β -linked glycosphingolipid (Zhou et al. 2004). Although iGb3 can activate both mouse and human iNKT cells, its physiological significance for the development and function of iNKT cells is under debate (Gapin 2010).

Indirect iNKT cell activation

iNKT cells can also become activated by many microorganisms that do not contain cognate glycolipid antigens (Matsuda et al. 2008; Tupin et al. 2007). This mechanism of iNKT cell activation has been best studied for bacteria such as *Salmonella typhimurium* that contain lipopolysaccharide (LPS) in their cell wall (Brigl et al. 2003). Dendritic cells (DCs) cultured with *Salmonella* were able to induce IFN- γ production by iNKT cells. DCs cultured in the presence of *Salmonella* LPS also activated iNKT cells and this required toll-like receptor (TLR)-4 expression by the DCs. This activation also required IL-12 and CD1d expression by DCs. These findings suggested that IL-12, produced by DCs stimulated with *Salmonella* LPS, synergized with CD1d-presented self-antigens for activation of iNKT cells. This concept has been extended to other microorganisms and microbial products, with several variations on the precise mode of iNKT cell activation (Matsuda et al. 2008; Tupin et al. 2007). In the case of *Escherichia coli* LPS, both the cytokines IL-12 and IL-18 appeared to be involved, but CD1d expression was not required (Nagarajan and Kronenberg 2007). Microbes can also alter CD1d expression in a manner that results in iNKT cell activation. This has been most clearly illustrated for *Listeria monocytogenes*, which induced CD1d expression on DCs and macrophages in a manner that required IFN- β (Raghuraman et al. 2006). These changes in CD1d expression were functionally relevant because IFN- β -treated DCs were more efficient in activating iNKT cells than untreated control DCs. These studies have led to the concept that activation of antigen-presenting cells via pattern-recognition receptors can result in cytokine production, which, in concert with endogenous glycolipid antigens and CD1d, activates iNKT cells (Matsuda et al. 2008; Tupin et al. 2007). Several studies have suggested that TLR agonists can modulate lipid biosynthesis and alter the lipid antigens loaded onto CD1d, which, in turn, might influence iNKT cell activation (Muindi et al. 2010; Paget et al. 2007; Salio et al. 2007). Yet another mechanism of indirect iNKT cell activation has been observed for the response of these cells to *Schistosoma mansoni* (Faveeuw et al. 2002; Mallevaey et al. 2006). iNKT cells produced IL-4 and IFN- γ in response to DCs cultured with *S. mansoni* eggs, in a manner that required CD1d expression but was independent of IL-12 and the MyD88 signaling pathway. Collectively, these findings suggest multiple modes of indirect iNKT cell activation.

In vivo response of iNKT cells to antigenic stimulation

The dynamics of the iNKT cell population in response to antigenic stimulation has been studied most extensively for α -GalCer (Parekh et al. 2007) (Fig. 4). Quickly following in vivo treatment of mice with α -GalCer, this reagent was rapidly presented to iNKT cells in the context of DCs and/or macrophages (Barral et al. 2010; Bezbradica et al. 2005; Schmieg et al. 2005). iNKT cells became activated within hours, induced increased expression of the activation markers CD69 and CD25, and produced a variety of cytokines, including IL-2, IL-4 and IFN- γ . The capacity of these cells to rapidly produce cytokines was due, in large part, to their constitutive expression of cytokine mRNAs for IL-4 and IFN- γ (Matsuda et al. 2003; Stetson et al. 2003). iNKT cells activated in this manner produced IL-4 most abundantly during the first several hours after their activation and subsequently shifted their cytokine production profile at 8–24 h towards IFN- γ , after which cytokine production by these cells waned down. α -GalCer-activated iNKT cells transiently downregulated surface expression of the invariant TCR, which made it hard to detect these cells between 6 and 18 h after α -GalCer treatment (Crowe et al. 2003; Harada et al. 2004; Wilson et al. 2003). The NK1.1 marker also became downregulated, but this was delayed as compared with TCR downregulation, starting around 12–24 h after α -GalCer treatment and was prolonged, lasting for up to 6 months (Wilson et al. 2003). During the early time period after their activation, iNKT cells rapidly proliferated, resulting in substantial expansion of the iNKT cell population, up to approximately 10-fold in the spleen, about 5-fold in blood, bone marrow and lymph nodes, and 2- to 3-fold in liver. The peak of this expansion was around

3–4 days after the initial α -GalCer treatment, after which the iNKT cell population contracted back to pre-injection levels. Apoptosis of iNKT cells appeared to be mediated by the pro-apoptotic Bcl-2 family member Bim, as homeostatic contraction of iNKT cells was defective in Bim-deficient mice (Crowe et al. 2003). Consistent with their innate characteristics, the secondary response of iNKT cells to α -GalCer treatment did not elicit a memory response (Parekh et al. 2005, 2007; Ulrich et al. 2005). Instead, the secondary response to α -GalCer was substantially weaker as compared to the primary response to this antigen. This hyporesponsive phenotype of iNKT cells, which was manifested by a blockade in proliferation and cytokine production, was apparent as early as 3 days after the initial α -GalCer treatment and lasted for up to 2 months. The blockade in IFN- γ and IL-2 production was more profound than that in IL-4, particularly at later time points after restimulation. Additional studies revealed that this hypo-responsive phenotype of iNKT cells was largely intrinsic to these cells, and therefore represented a state of immunological anergy (Parekh et al. 2005). The blockade in proliferation (but not cytokine production) in these anergic iNKT cells could be overcome by culture with α -GalCer in the presence of IL-2, suggesting that the blockade in IL-2 production was critical for maintaining the anergic phenotype. Further studies revealed a critical role of the inhibitory, co-stimulatory molecule programmed death (PD)-1 and its ligands, PD-L1 and PD-L2, in the induction and possibly the maintenance of iNKT cell anergy (Chang et al. 2008; Parekh et al. 2009). Blockade of PD-1/PD-L interactions at the time of α -GalCer treatment of mice was able to prevent the induction of iNKT cell anergy. These findings suggested that the balance between activating and inhibitory co-stimulatory interactions during iNKT cell activation played a critical role in the outcome of the response of these cells to antigenic stimulation. Consistent with this possibility, it was shown that delivery of α -GalCer to mice in the context of DCs (expressing high levels of costimulatory molecules) did not induce anergy in iNKT cells (Fujii et al. 2002; Parekh et al. 2005), whereas α -GalCer-loaded B cells (expressing low levels of co-stimulatory molecules) induced iNKT cell anergy in mice (Parekh et al. 2005). With regard to the signaling mechanisms involved, one study identified a critical role for the ubiquitin ligase Cbl-b (Kojo et al. 2009). Cbl-b targeted the monoubiquitination of CARMA1, a crucial adaptor of the transcription factor NF- κ B, which was critical for suppressing IFN- γ production in anergic iNKT cells.

Although less well understood, studies with human subjects have suggested a similar response of human iNKT cells to α -GalCer stimulation *in vivo*. Treatment of human subjects with α -GalCer resulted in a transient “disappearance” of these cells due to TCR downregulation, followed by transient expansion and subsequent resistance to re-stimulation (Giaccone et al. 2002; Nieda et al. 2004). Consistent with the studies in mice, α -GalCer-loaded DCs were more effective than free α -GalCer in inducing iNKT cell responses and repeated injection with α -GalCer-loaded DCs resulted in profound iNKT cell expansion (Chang et al. 2005).

Hyporesponsiveness appears to be a common outcome of iNKT cell activation. iNKT cells also became anergic following challenge of mice with multiple bacterial pathogens (Chiba et al. 2008; Choi et al. 2008; Kim et al. 2008a), TLR agonists (Kim et al. 2008a), and treatment with sufatide, an agonist for a subset of type II NKT cells (Halder et al. 2007). Induction of iNKT cell anergy by these means was independent of the PD-1/PD-L pathway (Parekh et al. 2009), suggesting multiple mechanisms of anergy induction in iNKT cells. iNKT cells also became anergic in response to the lethal toxin of *Bacillus anthracis*, in a mechanism that involved inhibition of MAP kinase signaling following cleavage of the MEK-2 kinase by the bacterial toxin (Joshi et al. 2009).

Crosstalk of iNKT cells with other immune cells

α -GalCer-activated iNKT cells are capable of substantial crosstalk with other cell types of the innate and adaptive immune systems (Matsuda et al. 2008; Parekh et al. 2007). IFN- γ produced by iNKT cells, together with CD40-CD40L interactions, leads to potent activation of DCs and macrophages. DCs and macrophages activated in this manner induce co-stimulatory molecules and produce cytokines such as IL-12 and TNF- α . IFN- γ and IL-12 are potent activators of NK cells, which also become activated to secrete IFN- γ and elaborate their cytotoxic activities. Activated iNKT cells can also recruit neutrophils and induce IFN- γ production by these cells, which can then contribute to inflammatory responses. Additionally, α -GalCer-activated iNKT cells can induce expression of co-stimulatory molecules by B cells and promote plasma cell survival. Activated iNKT cells also promote the activation of conventional T lymphocytes and can influence the quality of the immune response. For example, chronic α -GalCer injection often leads to a Th2 bias in the immune response and promotes the generation of tolerogenic DCs. iNKT cells can also interact with other regulatory T cell subsets, including Foxp3-expressing Treg cells (La Cava et al. 2006) and Qa-1-restricted, regulatory CD8⁺ T cells (Varthaman et al. 2010).

Although the response of iNKT cells to glycolipids other than the prototypical α -GalCer, KRN7000, is less well-understood, several reagents that can bias Th cell responses, as compared with α -GalCer, have been identified (Parekh et al. 2007; Venkataswamy and Porcelli 2010). In particular, a structural analog of α -GalCer with a shortened sphingosine chain, called OCH, and another analog with a diunsaturated C20 fatty acid, called C20:2, elicit a Th2-biased immune response. Conversely, the C-glycoside analog α -C-GalCer and the carba-galactosyl-containing analog α -carba-GalCer, induce a Th1-biased immune response. Multiple mechanisms for the capacity of distinct α -GalCer analogs to influence the pro- and anti-inflammatory activities of iNKT cells have been identified, including their relative avidity for the invariant TCR (Oki et al. 2004), preferential presentation by distinct antigen-presenting cells (Bezbradica et al. 2005; Yu et al. 2005), requirement for intracellular loading (Bai et al. 2009; Im et al. 2009), capacity to undergo lysosomal recycling (Bai et al. 2009), and pharmacokinetic properties (Sullivan et al. 2010; Tashiro et al. 2010). Overall, it appears that complexes between Th1-biasing reagents and CD1d have an increased in vivo survival that is associated with their capacity to potentially induce IFN- γ production by NK cells (Sullivan et al. 2010).

Functions of iNKT cells

iNKT cells can exhibit both pro- and anti-inflammatory properties, depending on their mode of activation (direct or indirect), the cytokine environment, the strength and duration of their activation, as well as many other parameters (Godfrey and Kronenberg 2004). Since iNKT cells in different organs appear to exhibit slightly different effector functions (Crowe et al. 2005), the location where iNKT cells are activated can influence their contribution to an immune response. Furthermore, iNKT cell subsets distinguished by CD4 and/or NK1.1 expression can exhibit distinct effector functions, and selective activation of these subsets might therefore also impact the outcome of an immune response (Coquet et al. 2008; Crowe et al. 2005). Considering these variables, it is not always possible to predict the impact of iNKT cells on an immune response or disease, as illustrated below.

iNKT cells have been implicated in a number of infections, including infections caused by microorganisms that may or may not contain cognate iNKT cell antigens (Behar and Porcelli 2007; Tupin et al. 2007). For infections caused by *Novosphingobium*, *Ehrlichia* and *Borrelia burgdorferi*, which contain iNKT cell antigens, iNKT cells play a protective role during the infection. These cells also play a protective role in the immune response against many other

infectious agents, including bacteria, viruses, fungi, protozoa, and helminths that are not known to contain iNKT cell antigens. However, iNKT cells sometimes also play a pathogenic role, for example during the immune response against *Chlamydia muridarum* (Joyee et al. 2007). Because iNKT cells can rapidly secrete a burst of cytokines in response to activation by microorganisms, they might promote disease in some situations by contributing to the development of sepsis syndrome. For example, inoculation of mice with a high dose of *Novosphingobium* bacteria resulted in a lethal sepsis in a manner that depended on iNKT cells (Mattner et al. 2005). iNKT cells also contributed to the generation of IFN- γ and TNF- α in response to LPS treatment of mice and, consequently, iNKT cell-deficient mice were partially resistant to the generalized Shwartzman reaction (Nagarajan and Kronenberg 2007). In some cases, the impact of iNKT cells on the immune response depended on the mouse strain employed for infection, presumably reflecting strain-specific differences that have been observed in iNKT cells and their response to glycolipid antigen stimulation. An example of this is respiratory syncytial virus infection, where iNKT cells played a protective role in BALB/c mice but exacerbated disease in C57BL/6 mice (Johnson et al. 2002). Finally, a recent study provided evidence that iNKT cells can influence the colonization of the intestine of mice by commensal microorganisms (Nieuwenhuis et al. 2009), suggesting that iNKT cells can contribute to physiological processes that are impacted by commensal microorganisms.

Aged $\text{J}\alpha 18$ -deficient mice develop signs of kidney disease with an increase in anti-nuclear antibodies and activation of MZB cells, which express high levels of CD1d (Sireci et al. 2007). These findings are consistent with lupus-like autoimmunity, suggesting an anti-inflammatory role of iNKT cells in the development of autoimmunity. Studies with animal models of autoimmunity have generally confirmed this immunosuppressive role of iNKT cells (Gabriel et al. 2010). Animal models examined include the non-obese diabetic (NOD) mouse model for type 1 diabetes, the experimental autoimmune encephalomyelitis (EAE) model for multiple sclerosis in C57BL/6 and NOD mice, multiple models of systemic lupus erythematosus (SLE), and an antigen-induced, acute model of rheumatoid arthritis. However, in some models of autoimmunity, iNKT cells play a pathogenic role. During collagen-induced arthritis and antibody-mediated arthritis, iNKT cells exacerbated disease (Coppeters et al. 2007a). Likewise, iNKT cells exacerbated disease in models of primary biliary cirrhosis (Joyce and Van Kaer 2008), a disease that is associated with mitochondrial antibodies. Nevertheless, consistent with their tolerogenic properties, iNKT cells have been implicated in a variety of experimental models of tolerance induction (Nowak and Stein-Streilein 2007). This includes tolerance induced by injection of antigens in the anterior chamber of the eye, spontaneous tolerance to hepatic allografts, transplant tolerance induced by antibodies against CD4 or co-stimulatory molecules, oral tolerance in some models, and tolerance induced in the fetus, burn wounds, and skin exposed to ultraviolet light. Furthermore, iNKT cells in the host also protect against the development of graft-versus-host disease after bone marrow transplantation (Pillai et al. 2007).

The pro-inflammatory functions of iNKT cells are critical for several inflammatory diseases (Van Kaer 2007). iNKT cells appear to be critical for the development of airway hyper-responsiveness, which is one of the cardinal features of asthma. This was demonstrated in experimental models that employed model allergens such as ovalbumin, the environmental pollutant ozone, and infection with respiratory viruses. iNKT cells were also shown to play a critical role in the development of liver disease induced by the mitogen concanavalin A, which is often used as a model for acute and chronic viral hepatitis. iNKT cells play a pathogenic role in the development of contact hypersensitivity induced by multiple contact antigens, in a manner that depended on interactions with B-1 cells. iNKT cells are crucial for the development of colitis induced by another contact antigen, the hapten oxazolone, and this depended on the capacity of iNKT cells to produce IL-13. iNKT cells are also required

for acute tissue injury induced to liver or kidney in ischemia reperfusion models. Similar injury induced in blood vessels during sickle cell disease also required iNKT cells. In models of atherosclerosis, iNKT cells have been detected in atherosclerotic lesions, and these cells contributed to disease pathogenesis. In each of these examples, cytokines produced by iNKT cells likely play a key role in activating other cell types, resulting in disease pathogenesis.

The pro-inflammatory properties of iNKT cells, in particular, their capacity to produce IFN- γ and activate NK cells and CD8 T cells are critical for the anti-tumor activities of these cells (Terabe and Berzofsky 2008). These properties of iNKT cells have been demonstrated in models of tumorigenesis induced by the chemical carcinogen methylcholanthrene, transplantable tumors, and genetically engineered mice that develop oncogene-driven tumors.

Therapeutic properties of iNKT cells

The immunomodulatory activities of iNKT cells have been employed for development of immunotherapies (Cerundolo and Salio 2007; Wilson et al. 2002) and vaccine adjuvants (Cerundolo et al. 2009; Kim et al. 2008b). Many of these studies have employed α -GalCer or structural analogs of α -GalCer. In some diseases, combination therapies have also been employed. In some cases where iNKT cells play a pathogenic role, blockade of CD1d with anti-CD1d antibodies has been employed. A CD1d-dependent antagonist of iNKT cells, dipalmitoyl-phosphatidyl ethanolamine (DPPE) has also been developed (Naidenko et al. 1999).

α -GalCer has been shown to enhance clearance of a variety of infectious agents, in a manner that depended on the pro-inflammatory properties of iNKT cells (Behar and Porcelli 2007; Tupin et al. 2007). In the case of infection with malaria parasites, it was further shown that α -C-GalCer, which promotes the Th1-mediated and pro-inflammatory properties of iNKT cells, was more potent than α -GalCer in clearing the parasites (Schmieg et al. 2003). This treatment was generally only effective within a narrow time window of a few days prior to or after infection, suggesting that translation of these findings to the clinical setting will be challenging. Indeed, clinical trials of α -GalCer treatment with patients infected by hepatitis B virus or hepatitis C virus have thus far not been successful (Veldt et al. 2007; Woltman et al. 2009). The adjuvant activities of iNKT cells have also been exploited for developing vaccines against several pathogens, including malaria parasites, influenza virus, and HIV-1 (Cerundolo et al. 2009; Kim et al. 2008b).

Consistent with the general protective role of iNKT cells in autoimmunity, repeated α -GalCer treatment typically ameliorates disease (Gabriel et al. 2010; Van Kaer 2005). In most cases, the anti-inflammatory properties of iNKT cells appeared to be important, with a critical role of IL-4 and IL-10, and evidence for induction of tolerogenic DCs and/or regulatory T cells, suppression of pathogenic T cells via immune regulatory mechanisms, anergy induction, or deletion, and direct effects on the capacity of B cells to produce antibodies. Nevertheless, this type of treatment did not always protect against autoimmunity, which was dependent on a variety of variables. For example, in the EAE model induced by myelin basic protein in B10.PL mice, treatment with α -GalCer prior to EAE induction protected against disease, whereas simultaneous treatment potentiated disease (Jahng et al. 2001). Genetic effects of treatment efficacy have also been observed. For example, while α -GalCer protected BALB/c mice against development of pristane-induced lupus-like disease, similar treatment in SJL/J mice exacerbated disease (Singh et al. 2005). Similarly, in an adoptive transfer model of type 1 diabetes using diabetogenic CD8 T cells, α -GalCer protected against disease in NOD mice, but promoted disease in MHC-matched congenic

mice with a C57BL/6 background (Driver et al. 2010). Consistent with the proposed role of the Th2-promoting and anti-inflammatory properties of iNKT cells in suppressing autoimmunity, the α -GalCer analogs OCH and C20:2 demonstrated superior disease protection in some models. However, in some experimental models of autoimmunity, the Th1-promoting analog α -C-GalCer was also protective, which has led to the concept that diverse mechanisms might be involved in the immunomodulatory activities of distinct α -GalCer analogs. For example, a single treatment with α -GalCer or α -CGalCer protected DBA/1 mice against collagen-induced arthritis, with IL-10 playing a critical role in the protective effects of α -GalCer, whereas general immune suppression appeared to be associated with the protective effects of α -C-GalCer (Coppeters et al. 2007b). Likewise, α -GalCer, OCH and α -C-GalCer were all protective against ocular autoimmunity, with α -C-GalCer being most effective, which was associated with its capacity to suppress Th1 and Th17 cell responses (Grajewski et al. 2008). These examples illustrate the complex interactions between activated iNKT cells and other immune cells that will complicate translation of the preclinical findings to the clinic.

iNKT cell activation or inhibition can have varied effects on inflammatory diseases. In an allogeneic model of bone marrow transplantation where recipients were non-lethally irradiated, α -GalCer treatment of recipient mice protected against graft-versus-host disease in a manner that involved Th2 deviation in donor T cells (Haraguchi et al. 2005; Hashimoto et al. 2005). However, in a model where recipients were lethally irradiated and α -GalCer was administered at days 1 and 4 after transplantation, α -GalCer exacerbated disease (Kuns et al. 2009). Nevertheless, using the latter protocol, C20:2 potently inhibited graft-versus-host disease (Kuns et al. 2009). In models of allergic airway hyperresponsiveness, α -GalCer treatment during the sensitization phase exacerbated disease (Meyer et al. 2007). Consistent with this potentiating role of iNKT cells in airway hyperreactivity, blockade of iNKT cells with DPPE protected against allergen-induced airway hyperactivity (Lombardi et al. 2010). In agreement with the pathogenic role of iNKT cells in atherosclerosis, α -GalCer exacerbated disease in apolipoprotein E-deficient mice (Major et al. 2006). In a model of contact hypersensitivity induced by the hapten oxazolone, the CD1d-dependent antagonists α -ManCer (α -mannosylceramide) and DPPE protected against disease (Nieuwenhuis et al. 2005).

iNKT cell activation holds substantial promise for development of cancer immunotherapies (Fujii 2008; Kim et al. 2008b; Terabe and Berzofsky 2008). α -GalCer, which was discovered in the context of a search for chemicals from a marine sponge with anti-metastatic activities in mice, demonstrates anti-metastatic activities against transplantable tumors, tumors induced by the chemical carcinogen methylcholanthrene, and tumors induced in genetically engineered animals. When studied, CD1d did not need to be expressed on the tumors, indicating that α -GalCer-activated iNKT cells relied on other cell types, such as NK cells and CD8⁺ T cells, to exhibit tumor cytotoxicity. In particular, IFN- γ produced by α -GalCer-activated iNKT cells appeared to be critical for stimulating the anti-tumor activities of NK cells and CD8⁺ T cells. Furthermore, IFN- γ , produced by iNKT cells, NK cells and CD8⁺ T cells, has potent anti-angiogenic activities, and this was involved in mediating some of the anti-tumor activities of α -GalCer. Consistent with the important role of IFN- γ , α -C-GalCer had superior anti-metastatic activities as compared with α -GalCer. Furthermore, delivery of α -GalCer via DCs provided improved therapeutic efficacy. Combined treatment with α -GalCer and PD-1/PD-L blockade resulted in enhanced tumor rejection. When employed as a vaccine adjuvant, α -GalCer promoted tumor immunity against tumors expressing neoantigens or clinically relevant tumor antigens when co-injected with the respective antigen. α -GalCer was also effective when co-administered with irradiated tumor cells, or loaded onto CD1d-expressing tumor cells prior to immunization. The anti-tumor activities of iNKT cells have been tested in clinical trials, using free α -

GalCer, α -GalCer-pulsed DCs, or in vitro expanded iNKT cells (Fujii 2008; Motohashi and Nakayama 2008). These studies demonstrated that it is challenging to elicit the biological functions of iNKT cells in humans. Nevertheless, one trial, employing repeated treatment of non-small lung carcinoma patients with ex vivo expanded iNKT cells, demonstrated a promising clinical benefit (Motohashi et al. 2009).

Although iNKT cell activation holds substantial promise for developing immunotherapies and vaccine adjuvants, it should be noted that α -GalCer induces liver toxicity in mice (Osman et al. 2000). Nevertheless, no substantial adverse side effects of iNKT cell activation have been observed in clinical trials with human patients (Motohashi and Nakayama 2008).

Concluding remarks

Although iNKT cells express an antigen-specific receptor that is characteristic of the adaptive immune system, this receptor lacks diversity and therefore resembles the pattern-recognition receptors that are characteristic of the innate immune system. Many other properties of iNKT cells are more similar to cells of the innate immune system than those of the adaptive immune system. This includes an activated phenotype, rapid elicitation of effector functions, and lack of immunological memory. Activated iNKT cells can influence the behavior of many other cell types in the immune system, exhibiting anti-inflammatory properties in some situations and pro-inflammatory properties in others. These immunoregulatory functions of iNKT cells can be harnessed by glycolipids such as the marine sponge-derived α -GalCer antigen. α -GalCer and related iNKT cell antigens hold substantial promise for development of immunotherapies and vaccine adjuvants. However, the outcome of iNKT cell activation on disease processes is not always predictable and is influenced by a variety of variables. Therefore, caution is warranted before translation of some of the preclinical studies to the clinical setting.

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Fig. 1.

Invariant natural killer T cells belong to a subset of lymphocytes that straddle the innate and adaptive immune systems. *DCs* dendritic cells, *iNKT* invariant natural killer T, *MAIT* mucosal-associated invariant T, *MZB* marginal zone B, *NK* natural killer

Fig. 2.

Phenotype, specificity, effector functions and ligands of iNKT cells. **a** iNKT cells express a semi-invariant T cell receptor (TCR) together with surface markers such as NK1.1 (CD161 in humans) and Ly49 molecules that are characteristic of the NK cell lineage. Most iNKT cells also express the co-receptor CD4. The semi-invariant TCR of iNKT cells is specific for lipid or glycolipid antigens that are presented at the surface of antigen-presenting cells (APC) in the context of the MHC class I-related glycoprotein CD1d. Glycolipid-activated iNKT cells can produce a mixture of T helper (Th)1 and Th2 cytokines and several chemokines, and adopt a cytotoxic phenotype (perforin and FasL expression). **b** Structure of the prototypical iNKT cell agonist KRN7000 (α -GalCer)

Fig. 3. Pathways of iNKT cell activation. **a** Direct pathway of iNKT cell activation via lipid or glycolipid antigens that bind with *CD1d* and interact with the invariant T cell receptor (*TCR*). **b** Indirect pathway of iNKT cell activation via toll-like receptor (*TLR*) agonists and cytokines. In some cases, this pathway also involves interaction of the invariant TCR with *CD1d* and endogenous glycolipids

Fig. 4. In vivo response of iNKT cells to α -GalCer stimulation. Quickly following α -GalCer treatment, iNKT cells produce copious amounts of cytokines, induce expression of the inhibitory, co-stimulatory receptor programmed death-1 (*PD-1*), and downregulate expression of the invariant T cell receptor (*TCR*) and NK1.1. TCR downregulation is transient whereas NK1.1 downregulation is sustained. iNKT cells activated in this manner also proliferate, reaching their peak population size around 3 days after α -GalCer injection. The iNKT cell population then slowly returns to pre-injection levels and the cells adopt an anergic phenotype that is refractory to TCR restimulation