[Frontiers in Bioscience 4827-4837, May 1, 2008]

# Immune regulation by invariant NKT cells in autoimmunity

**Francesca Ronchi, Marika Falcone**

Experimental Diabetes Unit, San Raffaele Scientific Institute, Via Olgettina 60, 20132, Milan, Italy

**TABLE OF CONTENTS**



## 1. ABSTRACT

Invariant NKT cells are important regulators of T cell immunity and autoimmunity. In this review we describe evidence that supports their regulatory role in the prevention of autoimmune disease. Moreover, we will discuss the current knowledge on iNKT cell biology, antigen recognition, acquisition of a specific cytokine profile, and mechanism of action that suggest a key role for iNKT cells as negative regulators of autoimmune diseases.

## 2. INTRODUCTION

CD1d-restricted T cells that are characterized by the expression of an invariant TCR α-chain (Vα14 in mice and Vα24 in humans) and markers of natural killer (NK) cells are referred as invariant natural killer T cells (iNKT) cells, and represent a unique lymphocyte subset (1). They have a remarkable functional diversity and play various and opposite immune functions. On one hand, they actively induce T cell tolerance and are crucial for prevention of autoimmune diseases in pre-clinical models of multiple sclerosis (MS) (2, 3), type 1 diabetes (T1D) (4, 5), systemic lupus erythematosus (SLE) (6), myasthenia gravis (MG) (7) and rheumatoid arthritis (RA) (8, 9). On the other hand, iNKT cells participate in the innate immune response to promote antimicrobial (10-12) and antitumoral (13-15) immunity. The importance of iNKT cells as immune adjuvant cells have been clearly demonstrated by the findings that microbial glycolipid antigens activate iNKT cells (16-18) and that pathogens - and more specifically viruses that establish latency - have developed protective mechanism to interfere with CD1d presentation and block iNKT cell activation (19). While the adjuvant function of iNKT cells is widely recognized, the physiological role of iNKT cells as inhibitors of T cell immunity, essential to prevent autoimmune diseases, has been recently questioned (20) despite of the several lines of evidence that iNKT cells actively induce T cell tolerance and counter-regulate autoimmunity. In fact, iNKT cells not only prevent different experimental models of autoimmune diseases including MS, T1D, SLE and RA (2-8), but they are also defective both quantitatively and qualitatively in mice and

humans affected by most autoimmune diseases (6, 8, 21-32). The skepticism about the protective role of iNKT cells in autoimmune diseases is probably related to their secretion of both pro-inflammatory Th1 and protective Th2 cytokines and to the lack of knowledge about their mechanism of action. Here we will review the numerous lines of evidence that suggest that iNKT cells are important cells for the prevention of autoimmune diseases. Moreover, we will present current notions on the iNKT cell biology, antigen recognition, acquisition of a specific cytokine profile and mechanism of action that support their role as important immune regulators for prevention of autoimmune diseases.

## 3. INVARIANT iNKT CELLS: INNATE OR ADAPTIVE IMMUNE CELLS?

Since iNKT cells play an important role in the early immune response against pathogens, they are often presented as innate immune cells (33) but it would be limiting to restrict their role to such classification. Innate immune cells are, by definition, cells that are present and functional prior to the exposure to microbes and their intent is to provide a first-line defense against pathogens. iNKT cells respond to those criteria since they pre-exist in the peripheral immune system in a significant percentage (1-2% of spleen lymphocytes, 0.2-1% of lymph node cells, 10-20% of liver lymphocytes and 40% of $CD3^+$ cells in the bone marrow) (34) and are pre-committed to secrete cytokines prior to the exposure to infections (35). However, iNKT cell number is enhanced in response to pathogens and they respond with proliferation and cytokine secretion to a specific antigen, αGalactosylceramide (αGalCer) (36), thus showing the typical feature of acquired immune cells. Therefore, it is evident that iNKT cells carry features of both innate and adaptive immune cells and do not belong to a single immune compartment but rather act at the interface between innate and adaptive immunity. In this view, iNKT cells behave like other innate immune cells such as dendritic cells, macrophages and mast cells that play different and opposite functions according to the site and time of the immune response. Right after the contact with the foreign pathogen or substance they participate in and promote the inflammatory response by releasing pro-inflammatory cytokines. Later on, if T cell responses persist beyond the clearance of pathogen, they acquire a tolerant function to limit the inflammatory response and avoid destructive immunity and autoimmunity. In order to attend their dual role iNKT cells must perceive different stimuli from the environment and acquire different functional phenotype to regulate appropriately the downstream adaptive immune response. The environmental stimuli that drive the different iNKT cell functions are yet unknown but there is indication that iNKT cells can either respond directly to pathogen-induced signals, for example through stimulation of toll-like receptors (TLR) (16), or indirectly, through the presentation of self or non-self glycolipids by their antigen-presenting cells, the myeloid dendritic cells (36).

## 4. ANTIGEN SPECIFICITY OF iNKT CELLS: SELF OR NON-SELF GLYCOLIPIDS?

The invariant NKT cell receptor (NKTCR) recognizes glycolipid antigens presented by the MHC class I-like molecule CD1d (37). The invariance of the NKTCR had originally led to speculation that iNKT cells react to a single glycolipid antigen. In that view, the first NKT cell antigen identified, the marine-sponge-derived glycolipid antigen αGalCer, was thought to mimic the natural endogenous or exogenous ligand (33). Based on the assumption that iNKTCR reacts to a single antigen, for many years it was believed that the identification of such antigen could help to clarify the iNKT cell function in the immune system. The recognition of a microbial antigen could have suggested that the main function of iNKT cells is pro-inflammatory and primarily aimed to help fighting infections. On the contrary, the identification of a self-glycolipid antigen could have implied that iNKT have a predominant role in the control of T cell homeostasis and prevention of autoimmunity. However, recent studies have weakened the idea that iNKT cells recognize a single antigen. In fact, various lipids bind naturally to the CD1d molecule, and, although not all of them stimulate the NKTCR, it is clear that the iNKT cells can proliferate and secrete cytokines in response to several glycolipids derived from autologous cells, tumors, microbes and allergens and presented by the CD1d molecule (38). Two recently identified iNKT cell antigens, the bacterial α-glycuronosylceramides (16, 17, 39) and the self-glycosphingolipid, isoglobotrihexosylceramide (iGb3) (40), showed an high structural homology with αGalCer (41). Hence, the current view is that the iNKTCR does not recognize a single antigen but rather a restricted set of glycolipids that share structural homology and either derived from pathogens or self-tissues.

Although there is still no direct evidence that iNKT cells are activated by recognition of a self-antigen, it is clear that a self-glycolipid antigen is necessary for iNKT cell maturation in the thymus. The observation that mice with a deficiency in □-hexosaminidase (hexb), an enzyme required for the generation of iGb3 in lysosomes, were defective in iNKT cell development, suggested that the iGb3 self glycolipid is necessary the intrathymic iNKT cell maturation (40). However, since the glycolipid ligands for iNKT cells are loaded onto CD1d molecules in the late endosome/lysosome compartment, the iNKT cell defect in hexb-deficient mice could be related to the abnormal accumulation of glycolipids in the lysosomes rather than to a specific requirement of iGb3 for iNKT cell selection. In support of that idea, there is the observation that the iNKT cell development is defective in individuals affected by several lysosomal storage diseases since the pathological accumulation of glycosphingolipids in lysosomes influences endogenous lipid loading and/or presentation on the CD1d molecule (42). Moreover, it is in doubt that iGb3 is the physiological antigens of human iNKT cells since humans lack the iGb3 synthase (33).

While the nature of self-glycolipid antigens of iNKT cells remains elusive, important progress has been

**Table 1.** Lines of evidence for a protective role of iNKT cells in autoimmune diseases

| Autoimmune disease | Protective iNKT cell function |
|---|---|
| Multiple sclerosis | • Quantitative defect of iNKT cells mouse in MS patients (30) and SJL/J mice susceptible to EAE (the experimental model of MS) (3,21).<br>• Protective action of oral somministration of OCH that induced a Th2-type cytokines secretion by iNKT cells (2).<br>• Protection by αGalCer somministration that promoted iNKT cells expansion and cytokines secretion (IL-4, IL-10 and IFN-γ) (3). |
| Type 1 diabetes | • Quantitative and qualitative NKT cells defect in diabetes-prone mice (22-24), rats (56) and humans (28, 29) affected by T1D.<br>• Defective secretion of cytokines in NOD mice (22-24).<br>• Prevention of autoimmune diabetes in pre-clinical models of T1D (NOD mice) by activation of iNKT cells (4,5). |
| Systemic lupus erythemathosus | • Quantitative defects of Vα14 iNKT cells in the autoimmune-prone MRL/lpr mouse and NZB/NZW $F_1$ mouse, the murine models of SLE (6, 25) as well as in humans affected by SLE (8). |
| Rheumatoid arthritis | • Quantitative defect and reduced response to αGalCer antigen in patients affected by Rheumatoid Arthritis (8).<br>• Protective effect of iNKT cell activation (αGalCer administration) in collagen-induced arthritis (9). |

made in characterizing microbial iNKT cell antigens. Glycosylceramides isolated from the cell wall of Gram-negative LPS-negative bacteria *Sphingomonas* bacteria which are ubiquitously present in the environment, such as *Ehrlichia muris* and *Sphingomonas capsulata,* can activate the iNKT cells in an antigen specific manner (16, 17, 39). Interestingly, some pathogens like LPS-positive *Salmonella thyphimurium* activate iNKT cells through the recognition of an endogenous lysosomal glycosphingolipid, iGb3, presented by LPS-activated dendritic cells (16).

The current hypothesis regarding the antigen specificity of iNKT cells is that iNKT cells mature in the thymus upon recognition of a self-glycolipid and then respond peripherally with proliferation and cytokine secretion to microbial or self-glycolipid antigens. According to this view, the iNKT cell phenotype and function is not determined by the recognition of microbial or self-antigens but it is rather driven by the context in which they receive the antigenic stimulation and by the integration of different co-stimulatory signals. The recognition of a self-glycolipid in the absence of pathogens, for example during autoimmune damage of tissues, could drive iNKT cell towards a regulatory iNKT cell phenotype and function. Conversely, if iNKT cells recognize microbial or self-glycolipids in the context of an infection and in the presence of pro-inflammatory signals, they acquire an adjuvant function and contribute to the clearance of pathogens.

## 5. REGULATORY FUNCTION OF iNKT CELLS: INDUCTION OF T CELL TOLERANCE AND PREVENTION OF AUTOIMMUNITY

The hypothesis that iNKT cells could play an immune regulatory role in the immune system originated soon after their discovery. In 1994 a subset of T cells showing markers of natural killer cells together with an invariant TCR was identified (43-45) and one year later the group of W. E. Paul described a selective defect of the $CD4^+NK1.1.^+$ T cell subset in autoimmune-prone SJL/J mice, a strain highly susceptible to the induction of experimental allergic encephalomyelitis (EAE), an animal model of MS (21). Subsequently, evidence of an immune regulatory role of iNKT cells came from numerous studies showing that they are essential to protect against autoimmune diseases (table 1). On one side, iNKT cell activation through administration of their antigen, αGalCer, protected mice against several pre-clinical models of autoimmune diseases such as MS (2, 3), T1D (4, 5), SLE (6), MG (7), and collagen-induced arthritis (8, 9). On the other side, a quantitative and/or qualitative defect of iNKT cells was reported in patients affected by most autoimmune diseases including MS, T1D, SLE, Sjogren's syndrome, MG, RA, sarcoid and asthma, as well as in mouse models of T1D, MS, SLE and colitis (27-32, 46). Although there is no proof that iNKT cells directly suppress T cells *in vitro*, as conventional regulatory T cells such as $CD4^+CD25^+FoxP3^+$ or IL-10-secreting Tr1 cells do (47, 48), their capacity to down-regulate T cell immunity has been clearly demonstrated *in vivo* in several models of immune tolerance. In the model of immune privilege in the eye, the anterior chamber-associated immune deviation (ACAID), peripheral tolerance towards an antigen injected intraocularly was achieved only in the presence of iNKT cells (49). In fact, CD1d-deficient or Vα14 NKT cell-deficient mice failed to develop systemic tolerance. In addition, iNKT cells were capable to induce transplantation tolerance towards allogeneic (50) and xenogeneic (51) islet cells transplanted into the liver and towards cardiac allografts (52). In all cases, long-term survival of the grafts induced with different tolerogenic stimuli (anti-CD4, anti-CD154, anti-LFA-1 and anti-ICAM-1, anti-B7-1/2 monoclonal antibodies) was obtained in wild-type but not Vα14 iNKT cell-deficient mice (52). Also, host iNKT cells inhibited T cell immunity in Graft-versus-Host Disease (GVHD) (53). While those studies clearly proved the importance of iNKT cells in peripheral tolerance induction, the numerous reports showing a protective role of iNKT cells in autoimmune diseases probably represent the

strongest evidence of their immune regulatory role and will be described in details.

### 5.1. Multiple Sclerosis

The relevance of iNKT cells as regulatory T cells in autoimmune disease of the central nervous system (CNS) was highlighted by the finding that their percentage is reduced in SJL/J mice susceptible to EAE, the experimental model of MS (3, 21), as well as in patients with MS (30). In those studies, the iNKT cell number was assessed in SJL/J mice both by analysis of $NK1.1^+$ $CD3^+$ T cells (21) and, more recently, as CD1d-restricted iNKT cells reactive to αGalCer-loaded CD1d-dimers (3). In humans, despite of the limitation of studying iNKT cells in peripheral blood where they are underrepresented (0.1-0.5% of $CD3^+$ lymphocytes), it was demonstrated a clear reduction in the number of Vα24Jα in MS patients compared to healthy individuals or patients affected by other inflammatory disorders (30). Recently, the link between iNKT cell number and prevention of autoimmunity in the brain was further suggested by the observation that the iNKT cell percentage increased significantly in patients protected from MS relapses by the treatment with type 1 interferon-β(54).

Those studies clearly indicated a possible role of iNKT cells in the protection from MS. Additional evidence of such protective role came from the observation that iNKT cell activation prevented autoimmunity in pre-clinical models of MS, and EAE was the first autoimmune disease in which the ability of iNKT cells to prevent T cell autoimmunity was demonstrated. The oral administration of OCH, a synthetic glycolipid analogue of αGalCer, was able to prevent $MOG_{35-55}$ peptide-induced EAE in B6 mice through the regulatory action of iNKT cells (2). In fact, only wild-type B6 mice but not iNKT cell-deficient littermates were protected from EAE, thus showing that the OCH analogue specifically triggered the iNKT cell regulatory function. In that report, the protective action of the OCH analogue was linked to its ability to induce a predominant secretion of the Th2-type cytokines such as IL-4 upon iNKT cells. Subsequently, a better degree of protection against $MOG_{35-55}$ peptide-induced EAE was achieved by treatment of B6, PL/J or SJL/J mice with αGalCer, a strong NKTCR agonist that promoted iNKT cell expansion and cytokine secretion without driving them towards a specific Th2-type cytokine profile (3). The latter finding suggested that the immune regulatory role of iNKT cells in EAE did not depend upon their acquisition of a Th2 cytokine profile. In fact, the observation that αGalCer treatment did not protect from EAE neither IL-4 nor IL-10 knockout B6 mice (3) indicated that Th2 cytokines may be important for the immune regulatory pathway generated by iNKT cells but it did not necessarily imply that iNKT cells should secrete Th2 cytokines to mediate their regulatory function. In support of that view, another report demonstrated that αGalCer-activated iNKT cells must secrete different types of cytokines including IFN-□ to protect against EAE (55).

### 5.2. Type 1 diabetes

A reduced number of Vα14 iNKT cells and their abnormal cytokine secretion have been reported in animals that develop spontaneous autoimmune diabetes such as non obese diabetic (NOD) mice (22-24) and diabetes-prone BB rats (56) as well as in humans affected by T1D (27, 28). In the first study reporting an iNKT cell defect in NOD mice, the group of J-F. Bach correlated the deficit in the number of $NK1^+$-like thymocytes with the progression of the autoimmune disease (22). In different studies the restoration of a normal iNKT cell number by injection of thymic Vα14 NKT cells from non diabetic (BALB/c x NOD $F_1$) mice ameliorated autoimmune diabetes (23, 57). This observation, together with the finding that the excess of iNKT cells in Vα14 TCR transgenic NOD mice only partially prevented T1D (58, 59), demonstrated that a qualitative NKT cell defect is also present in NOD mice. Originally, it was believed that such defect regarded selectively the iNKT cell secretion of Th2 cytokines like IL-4 and IL-10 (57). The latter hypothesis was supported by the finding that in twins discordant for T1D, the diabetic sibling, together with a lower frequency of $CD4^-CD8^-$ $V\alpha24J\alpha Q^+$ T cells, also showed a reduced secretion of IL-4 by iNKT cells (27). Subsequently, the importance of Th2 cytokines secretion for the protective role of iNKT cells has been questioned by several findings. First of all, the observation that secretion of both IL-4 and IFN-□ was defective in NOD mice (24). Second, αGalCer administration was able to induce iNKT cell-mediated protection from T1D in IL-4 and IL-10 knockout NOD mice (60, 61). Finally, the selective defect of IL-4 secretion by iNKT cells in T1D patients was not confirmed by successive studies.

Although a qualitative defect of iNKT cells in autoimmune diabetes has not yet been identified, it remains unquestionable that a lower percentage of iNKT cells is present in humans and animals prone to T1D. Interestingly, a recent report has linked the reduced iNKT cell number in NOD mice to a genetic defect of the *Slamf1* gene (62). In that study, it was demonstrated that the *Slamf1* gene controls the expression of SLAM on DP thymocytes and the maturation of iNKT cells in the thymus. Hence, the genetic defect of the *Slamf1* gene in NOD mice was responsible for the reduced SLAM expression on DP thymocytes and the resulting quantitative iNKT cell defect.

As for MS, the best evidence that iNKT cells play a protective role against autoimmune diabetes was provided by the observation that iNKT cell activation resulted in prevention of T1D in NOD mice. Two studies reported that the intraperitoneal injection of αGalCer, a strong NKTCR agonist, protected NOD mice through modulation of iNKT cells (4, 5). Similarly, the activation of iNKT cells by up-regulation of the their restriction molecule, CD1d, within the pancreatic islets of insCD1d transgenic NOD mice was able to prevent autoimmune diabetes (63), while the absence of iNKT cells in CD1d knockout NOD mice significantly worsened T1D (64). Interestingly, αGalCer was effective whether administered early (4 weeks of age) or late (starting at 10 weeks of age) when the autoimmune T cell repertoire in NOD mice was already generated (5).

Hence, the regulatory effect of iNKT cells was played not only in the induction phase of the disease, altering the activation and expansion of islet-reactive T cells, but also in the effector phase with a direct inhibition of the diabetogenic potential of islet-reactive T cells. Those studies identified a protective role of iNKT cells in autoimmune diabetes, however they failed to provide a regulatory mechanism of action. They indicated that protective iNKT cells increased secretion of IL-4, a cytokine that could bias the cytokine profile of islet-reactive T cells towards a protective Th2 type. However, αGalCer-activated iNKT cells increased secretion of both IL-4 and IFN-γ (4, 5). It is possible that IFN-□ secreted by iNKT cells plays a down-regulatory effect on the diabetogenic potential of islet-reactive T cells as indicated by other studies (65). Alternatively, the regulatory iNKT cell mechanism of action does not depend on their cytokine secretion pattern.

### 5.3. Systemic lupus erythemathosus

The association between the selective reduction in numbers of Vα14 iNKT cells and autoimmune disease was demonstrated for the first time in the autoimmune-prone MRL/lpr mouse, the murine model for human systemic lupus erythematosus (SLE) (25). Such selective reduction in iNKT cell number was then confirmed in other experimental models of lupus, the NZB/NZW $F_1$ mice (6), as well as in humans affected by SLE (8). Although one study suggested that activation of iNKT cells in NZB/W $F_1$ mice could induce Th1-type immune responses exacerbating lupus, in the MRL/lpr model of lupus the reduction of Vα14 iNKT cell numbers correlated with the progression of autoimmunity, starting to decrease at 3-4 weeks of age before the onset of the autoimmune disease and completely disappearing at 10 weeks of age, when the clinical lupus manifests itself, and the injection of Vα14 TCR transgenic iNKT cells into MRL/lpr mice delayed the onset of clinical signs of lupus (25).

### 5.4. Rheumatoid arthritis

A significant decrease in the percentage of $V\alpha24^+V\beta11^+$, $CD4^-CD8^-$ iNKT cells was reported in patients affected by RA (8). Interestingly, in that report the reduced number of iNKT cells was associated with their inability to respond to antigenic stimulation with αGalCer. The latter finding suggested that iNKT cells of RA patients carry an intrinsic functional defect that rendered them unable to expand in response to antigenic stimulation.

## 6. MECHANISM OF ACTION OF REGULATORY iNKT CELLS

### 6.1. The role of cytokines

Since iNKT cells release large amounts of cytokines upon activation via their T cell receptor (TCR), it was originally believed that they mediate their regulatory or adjuvant function through secretion of cytokines. Indeed, the iNKT cell secretion of pro-inflammatory cytokines such as IFN-□ and IL-12 was linked with the adjuvant function of iNKT cells to fight infections and in anti-tumor immunity (66-68). On the other hand, early studies suggested that Th2-type cytokines could play an important role in the immunoregulatory function of iNKT cells. For example, iNKT cell-mediated protection from autoimmune diabetes induced by either injection of diabetogenic T cells or cyclophosphamide treatment was obtained only in the presence of IL-4 and/or IL-10 (5). Furthermore, iNKT cells that induced T cell tolerance and ameliorated animal models of autoimmune T1D or MS (2-5, 69) showed an increased secretion of Th2-type cytokines such as IL-4 and IL-10. In those models, the cytokine profile of self-reactive T cells was also biased towards a Th2 phenotype that is protective in T cell-mediated autoimmune diseases. Those findings together with the early reports of a selective impairment of IL-4 secretion by iNKT cells of mice and humans affected by autoimmune diseases (21, 27), suggested that the secretion of Th2 cytokines by iNKT cells were responsible for their regulatory action. Specifically, the release of IL-4 and/or IL-10 at the time and site of activation of self-reactive T cells could alter their cytokine phenotype and reduce their inflammatory potential and aggressiveness towards self-tissues. However, studies in IL-4 and IL-10 deficient mice clearly demonstrated that the secretion of Th2 cytokines was dispensable for iNKT cells to mediate their regulatory function. In fact, activation of iNKT cells by administration of αGalCer was able to protect against autoimmune diabetes and EAE both wildtype and IL-4 (60) or IL-10 deficient mice (61). In a recent work, the group of Lehuen demonstrated that iNKT cells prevented T1D without driving a Th2 shift of self-reactive T cells (70). Instead, islet-reactive $CD4^+$ T cells injected into Vα14 TCR transgenic NOD mice were primed in the pancreatic lymph nodes but rendered anergic by iNKT cells. In the same model, iNKT cell-secreted cytokines such as IL-4, IL-10, IL-13 and TGF-□ did not play any role (71). The same group reported *in vitro* experiments showing that iNKT cell modulation on self-reactive T cells required cell-cell contact (71). In that study, the authors clearly demonstrated that iNKT cells were unable to modulate T cells in a trans-well system, thus confirming that iNKT cell secretion of cytokines was not sufficient for their regulatory action. However, the experimental setting of that study did not allow identification the type of cells that were directly contacted and modulated by iNKT cells since the regulatory iNKT cells were place in contact with total splenocytes. The islet-reactive $CD4^+$T cells resulted anergic and less diabetogenic after being cultured with iNKT cells but there was no evidence that such effect was due to a direct cell-cell interaction between iNKT and T cells and it cannot be excluded the possibility that iNKT cells modulated other cells, such as antigen-presenting cells or conventional regulatory $CD4^+CD25^+FoxP3^+$ T cells or Tr1 cells that were present in culture.

### 6.2. Modulation of dendritic cells

The capacity of iNKT cells to modulate dendritic cells (DCs) in order to play their adjuvant function and boost innate immune responses has been clearly demonstrated (72). Specifically, several studies *in vivo* reported that iNKT cell activation provoked the maturation of $CD11c^+$ DCs with up-regulation of restriction molecules such as MHC class II and CD1d as well as co-stimulatory molecules such as CD80, CD86 and CD40 (73, 74).

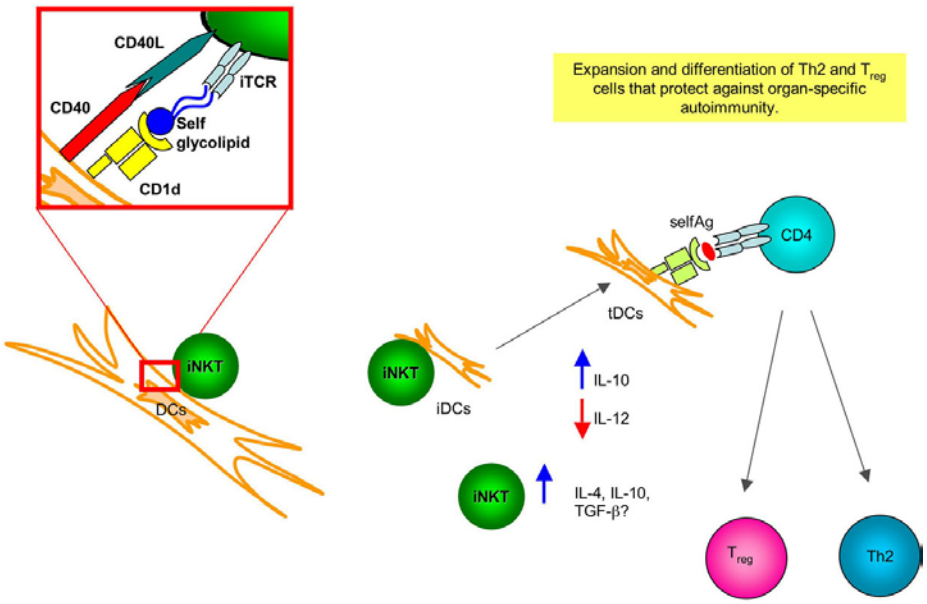


**Figure 1.** The regulatory cross-talk between iNKT cells and myeloid dendritic cells. iDCs: immature dendritic cells; tDC: tolerogenic dendritic cells; selfAg: self peptide antigens from specific organ.

Brenner and coworkers clearly demonstrated *in vitro* that DCs modulation was induced by cell-cell contact between iNKT cells and myeloid DCs (75). In that report, DCs were simultaneously stimulated via TLR with LPS and co-cultured with CD1d-restricted iNKT cell clones and, together with up-regulation of maturation markers, they also showed an increased secretion of pro-inflammatory cytokines. Those findings suggested that DCs, in the presence of pathogens, when they receive iNKT cell modulation together with other microbial signals such as LPS, secrete large amount of the pro-inflammatory cytokine IL-12 to prime inflammatory Th1 cells. Hence, adjuvant iNKT cells can respond to "danger" signals through microbial antigen recognition via TCR or stimulation of toll-like receptors and, in response, modulate DCs to increase their antigen-presenting capacity and their priming capacity on effector T cells.

The effect of iNKT cells on DCs under steady-state conditions, i.e. in the absence of pathogens during autoimmune diseases, is less clear but it may be important for the regulatory role of iNKT cells. The first report suggesting that regulatory $V\alpha14^+$ iNKT cells modulated myeloid DCs was provided by the observation that iNKT cell-mediated protection from autoimmune diabetes in NOD mice was associated with the recruitment of tolerogenic myeloid $CD8\alpha^-$ DCs within the pancreatic lymph nodes. In addition, the transfer of tolerogenic myeloid $CD8\alpha^-$ DCs isolated from PLN of iNKT cell-protected mice into NOD mice completely prevented diabetes development (76). However, a subsequent study stressed the importance of plasmacytoid rather then myeloid DCs for control of autoimmune diabetes in the NOD mice (77). Hence, although there is indication that iNKT cells could mediate their regulatory role for prevention of autoimmune diseases through modulation of DCs and up-regulation of co-stimulatory molecules (60), the functional features of those tolerogenic DCs and the molecular events operating in iNKT cell and DCs interactions were still unclear. In 2005, Taniguchi *et al.* clarified the regulatory properties of DCs modulated by Vα14 iNKT cells (78), demonstrating that stimulation of Vα14 iNKT cells *in vivo* by repeated αGalCer injections generated tolerogenic DCs characterized by up-regulation of co-stimulatory (CD80, CD86 and CD40) and MHC class II molecules and a tolerogenic cytokine profile with high IL-10 and low IL-12 secretion. Interestingly, the iNKT cell-induction of those tolerogenic DCs was associated with protection from autoimmune disease (EAE) and the acquisition of an IL-10-secreting phenotype by self-reactive $CD4^+$ T cells. Those studies clearly suggested that iNKT cell could mediate their regulatory action through modulation of myeloid DCs. However, they failed to assess whether iNKT cell directly contact DCs or rather induce a tolerogenic DCs phenotype by altering the cytokine microenvironment or through other cell intermediaries. Recently, our group collected clear evidence that iNKT cells directly interact with myeloid DCs through cell-cell contact mechanisms that involve the CD1d molecule and drive DCs towards a tolerogenic IL-10-secreting phenotype. Strikingly, iNKT cell-modulated DCs, together with tolerogenic cytokine-secreting phenotype, also showed a unique capacity to induce the differentiation of regulatory T cells when used as antigen-presenting cells (figure 1) *(Ronchi F., Caielli S., Conforti C., Baev D., Falcone M., manuscript in preparation)*.

### 6.3. Induction of $T_{reg}$ cells

iNKT cells are different from conventional regulatory T cells such as $FoxP3^+CD25^+$ $T_{reg}$ cells and IL-10-secreting Tr1 cells mostly because they do not directly suppress proliferation of effector T cells *in vitro*. However,

iNKT cells as $T_{reg}$ cells are important regulators of T cell immunity and crucial to prevent autoimmune diseases. Recent studies suggested that iNKT cells and $CD4^+CD25^+$ cooperate functionally in the counter-regulation of autoimmune diseases such as T1D (79) and experimental autoimmune MG (EAMG), the animal model of human MG (7). Specifically, in the EAMG model of autoimmune disease, it was shown that iNKT cell activation by αGalCer was able to protect mice from EAMG by favoring $T_{reg}$ expansion through IL-2-dependent mechanisms. In addition, together with a significant increase in their number, $CD4^+CD25^+$ T cells from iNKT cell-protected mice also showed a more potent suppressive capacity upon self-reactive T cells. Again, that study indicated that iNKT cells could mediate their regulatory effect through induction of conventional $T_{reg}$ able to suppress self-reactive T cells but it did not assess whether the iNKT cells interact directly with $T_{reg}$ cells. An alternative hypothesis holds that iNKT cells could favor $T_{reg}$ indirectly by contacting DCs and driving them towards a tolerogenic phenotype that, acting as APCs, promotes the expansion and the differentiation of $CD4^+CD25^+FoxP3^+$ T cells, IL-10-secreting Tr1 cells or other $T_{reg}$ cells with strong suppressive properties upon self-reactive effector T cells (figure 1).

## 7. THE THERAPEUTIC POTENTIAL OF iNKT CELLS

So far, the therapeutic use of iNKT cells to improve T cell immunity against infections and tumors or to dampen T cell immunity for prevention of autoimmune diseases has been hampered by the dual phenotype and function of iNKT cells (80). Although the mechanism of action of iNKT cells is unknown and not necessarily cytokine-mediated (71, 73 and Ronchi F. *et al.* manuscript in preparation), it is clear that a characteristic cytokine secretion pattern correlates with the iNKT cell acquisition of a specific function. For example, the secretion of pro-inflammatory cytokines such as IFN-γ and IL-12 is linked with the adjuvant function of iNKT cells to help fighting infections and in anti-tumor immunity (66-68). Conversely, iNKT cells that induce T cell tolerance and prevents or ameliorates autoimmune diseases in animal models (2-5, 69) were characterized by the release of a wide array of cytokines that included Th1 cytokines like IFN-γand Th2-type cytokines such as IL-4 and IL-10. Although it is evident that iNKT cells acquire a specific cytokine phenotype and function according to the environmental stimuli they receive, we are still unable to force the iNKT cell differentiation towards an adjuvant or regulatory phenotype. Without knowing the mechanisms that drive the iNKT cell orientation towards a specific phenotype there is no guarantee that the iNKT cells will play the required function *in vivo* to treat rather than worsen infections, tumors or autoimmune diseases. A better understanding of the different molecules and pathways involved in the differentiation of regulatory iNKT cells and of the mechanism underlying iNKT cell-regulatory function are still necessary to pave the way to exploit their therapeutic potential for prevention and/or treatment of autoimmune diseases.

## 8. REFERENCES

1. M. Kronenberg. Toward an understanding of NKT cell biology: progress and paradoxes. *Annu Rev Immunol* 23:877-900 (2005)

2. K. Miyamoto, S. Miyake, and T. Yamamura. A synthetic glycolipid prevents autoimmune encephalomyelitis by inducing TH2 bias of natural killer T cells. *Nature* 413:531-534 (2001)

3. A.K. Singh, M.T. Wilson, S. Hong, D. Olivares-Villagomez, C. Du, A.K. Stanic, S. Joyce, S. Sriram, Y. Koezuka, and L. Van Kaer. Natural killer T cell activation protects mice against experimental autoimmune encephalomyelitis. *J Exp Med* 194:1801-1811 (2001)

4. S. Hong, M.T. Wilson, I. Serizawa, L. Wu, N. Singh, O.V. Naidenko, T. Miura, T. Haba, D.C. Scherer, J. Wei, M. Kronenberg, Y. Koezuka, and L. Van Kaer. The natural killer T-cell ligand alpha-galactosylceramide prevents autoimmune diabetes in non-obese diabetic mice. *Nat Med* 7:1052-1056 (2001)

5. S. Sharif, G.A. Arreaza, P. Zucker, Q.S. Mi, J. Sondhi, O.V. Naidenko, M. Kronenberg, Y. Koezuka, T.L. Delovitch, J.M. Gombert, M. Leite-De-Moraes, C. Gouarin, R. Zhu, A. Hameg, T. Nakayama, M. Taniguchi, F. Lepault, A. Lehuen, J.F. Bach, and A. Herbelin. Activation of natural killer T cells by alpha-galactosylceramide treatment prevents the onset and recurrence of autoimmune Type 1 diabetes. *Nat Med* 7:1057-1062 (2001)

6. D. Zeng, M.K. Lee, J. Tung, A. Brendolan, and S. Strober. Cutting edge: a role for CD1 in the pathogenesis of lupus in NZB/NZW mice. *J Immunol* 164:5000-5004 (2000)

7. R. Liu, A. La Cava, X.F. Bai, Y. Jee, M. Price, D.I. Campagnolo, P. Christadoss, T.L. Vollmer, L. Van Kaer, and F.D. Shi. Cooperation of invariant NKT cells and CD4+CD25+ T regulatory cells in the prevention of autoimmune myasthenia. *J Immunol* 175:7898-7904 (2005)

8. S. Kojo, Y. Adachi, H. Keino, M. Taniguchi, and T. Sumida. Dysfunction of T cell receptor AV24AJ18+, BV11+ double-negative regulatory natural killer T cells in autoimmune diseases. *Arthritis Rheum* 44:1127-1138 (2001)

9. S. Kaieda, C. Tomi, S. Oki, T. Yamamura, and S. Miyake. Activation of invariant natural killer T cells by synthetic glycolipid ligands suppresses autoantibody-induced arthritis. *Arthritis Rheum* 56:1836-1845 (2007)

10. M. Brigl, L. Bry, S.C. Kent, J.E. Gumperz, and M.B. Brenner. Mechanism of CD1d-restricted natural killer T cell activation during microbial infection. *Nat Immunol* 4:1230-1237 (2003)

11. E.E. Nieuwenhuis, T. Matsumoto, M. Exley, R.A. Schleipman, J. Glickman, D.T. Bailey, N. Corazza, S.P. Colgan, A.B. Onderdonk, and R.S. Blumberg. CD1d-

dependent macrophage-mediated clearance of Pseudomonas aeruginosa from lung. *Nat Med* 8:588-593 (2002)
12. C. Ronet, S. Darche, M. Leite de Moraes, S. Miyake, T. Yamamura, J.A. Louis, L.H. Kasper, and D. Buzoni-Gatel. NKT cells are critical for the initiation of an inflammatory bowel response against Toxoplasma gondii. *J Immunol* 175:899-908 (2005)

13. N.Y. Crowe, J.M. Coquet, S.P. Berzins, K. Kyparissoudis, R. Keating, D.G. Pellicci, Y. Hayakawa, D.I. Godfrey, and M.J. Smyth. Differential antitumor immunity mediated by NKT cell subsets *in vivo*. *J Exp Med* 202:1279-1288 (2005)

14. D.I. Godfrey, and M. Kronenberg. Going both ways: immune regulation via CD1d-dependent NKT cells. *J Clin Invest* 114:1379-1388 (2004)

15. K. Shimizu, A. Goto, M. Fukui, M. Taniguchi, and S. Fujii. Tumor cells loaded with alpha-galactosylceramide induce innate NKT and NK cell-dependent resistance to tumor implantation in mice. *J Immunol* 178:2853-2861 (2007)

16. J. Mattner, K.L. Debord, N. Ismail, R.D. Goff, C. Cantu, 3rd, D. Zhou, P. Saint-Mezard, V. Wang, Y. Gao, N. Yin, K. Hoebe, O. Schneewind, D. Walker, B. Beutler, L. Teyton, P.B. Savage, and A. Bendelac. Exogenous and endogenous glycolipid antigens activate NKT cells during microbial infections. *Nature* 434:525-529 (2005)

17. Y. Kinjo, D. Wu, G. Kim, G.W. Xing, M.A. Poles, D.D. Ho, M. Tsuji, K. Kawahara, C.H. Wong, and M. Kronenberg. Recognition of bacterial glycosphingolipids by natural killer T cells. *Nature* 434:520-525 (2005)

18. Y. Kinjo, E. Tupin, D. Wu, M. Fujio, R. Garcia-Navarro, M.R. Benhnia, D.M. Zajonc, G. Ben-Menachem, G.D. Ainge, G.F. Painter, A. Khurana, K. Hoebe, S.M. Behar, B. Beutler, I.A. Wilson, M. Tsuji, T.J. Sellati, C.H. Wong, and M. Kronenberg. Natural killer T cells recognize diacylglycerol antigens from pathogenic bacteria. *Nat Immunol* 7:978-986 (2006)

19. D.J. Sanchez, J.E. Gumperz, and D. Ganem. Regulation of CD1d expression and function by a herpesvirus infection. *J Clin Invest* 115:1369-1378 (2005)

20. P.T. Lee, A. Putnam, K. Benlagha, L. Teyton, P.A. Gottlieb, and A. Bendelac. Testing the NKT cell hypothesis of human IDDM pathogenesis. *J Clin Invest* 110:793-800 (2002)

21. T. Yoshimoto, A. Bendelac, J. Hu-Li, and W. Paul. Defective IgE production by SJL mice is linked to the absence of CD4+NK1.1.+ T cells that promptly produce interleukin 4. *Proc Natl Acad Sci USA* 92:11931-11934 (1995)

22. J.M. Gombert, A. Herbelin, E. Tancrede-Bohin, M. Dy, C. Carnaud, and J.F. Bach. Early quantitative and functional deficiency of NK1+-like thymocytes in the NOD mouse. *Eur J Immunol* 26:2989-2998 (1996)

23. A.G. Baxter, S.J. Kinder, K.J. Hammond, R. Scollay, and D.I. Godfrey. Association between alphabetaTCR+CD4-CD8- T-cell deficiency and IDDM in NOD/Lt mice. *Diabetes* 46:572-582 (1997)

24. M. Falcone, B. Yeung, L. Tucker, E. Rodriguez, and N. Sarvetnick. A defect in interleukin 12-induced activation and interferon gamma secretion of peripheral natural killer T cells in nonobese diabetic mice suggests new pathogenic mechanisms for insulin-dependent diabetes mellitus. *J Exp Med* 190:963-972 (1999)

25. M.A. Mieza, T. Itoh, J.Q. Cui, Y. Makino, T. Kawano, K. Tsuchida, T. Koike, T. Shirai, H. Yagita, A. Matsuzawa, H. Koseki, and M. Taniguchi. Selective reduction of V alpha 14+ NK T cells associated with disease development in autoimmune-prone mice. *J Immunol* 156:4035-4040 (1996)

26. T. Sumida, A. Sakamoto, H. Murata, Y. Makino, H. Takahashi, S. Yoshida, K. Nishioka, I. Iwamoto, and M. Taniguchi. Selective reduction of T cells bearing invariant V alpha 24J alpha Q antigen receptor in patients with systemic sclerosis. *J Exp Med* 182:1163-1168 (1995)

27. S.B. Wilson, S.C. Kent, K.T. Patton, T. Orban, R.A. Jackson, M. Exley, S. Porcelli, D.A. Schatz, M.A. Atkinson, S.P. Balk, J.L. Strominger, and D.A. Hafler. Extreme Th1 bias of invariant Valpha24JalphaQ T cells in type 1 diabetes. *Nature* 391:177-181 (1998)

28. S.B. Wilson, S.C. Kent, H.F. Horton, A.A. Hill, P.L. Bollyky, D.A. Hafler, J.L. Strominger, and M.C. Byrne. Multiple differences in gene expression in regulatory Valpha 24Jalpha Q T cells from identical twins discordant for type I diabetes. *Proc Natl Acad Sci U S A* 97:7411-7416 (2000)
29. A. Kukreja, G. Cost, J. Marker, C. Zhang, Z. Sun, K. Lin-Su, S. Ten, M. Sanz, M. Exley, B. Wilson, S. Porcelli, and N. Maclaren. Multiple immuno-regulatory defects in type-1 diabetes. *J Clin Invest* 109:131-140 (2002)
30. Z. Illes, T. Kondo, J. Newcombe, N. Oka, T. Tabira, and T. Yamamura. Differential expression of NK T cell V alpha 24J alpha Q invariant TCR chain in the lesions of multiple sclerosis and chronic inflammatory demyelinating polyneuropathy. *J Immunol* 164:4375-4381 (2000)

31. R. Gausling, C. Trollmo, and D.A. Hafler. Decreases in interleukin-4 secretion by invariant CD4 (-)CD8 (-)V alpha 24J alpha Q T cells in peripheral blood of patientswith relapsing-remitting multiple sclerosis. *Clin Immunol* 98:11-17 (2001)

32. H.v.d. Vliet, B.v. Blomberg, N. Nishi, M. Reijm, A. Voskuyl, A.v. Bodegraven, C. Polman, T. Rustemeyer, P. Lips, A.v.d. Eertwegh, G. Giaccone, R. Scheper, and H. Pinedo. Circulating V (alpha24+) Vbeta11+ NKT cell

numbers are decreased in a wide variety of diseases that are characterized by autoreactive tissue damage. *Clin Immunol* 100:144-148 (2001)

33. L. Van Kaer, L. NKT cells: T lymphocytes with innate effector functions. *Curr Opin Immunol* 19:354-364 (2007)

34. M. Taniguchi, M., M. Harada, S. Kojo, T. Nakayama, and H. Wakao. The regulatory role of Valpha14 NKT cells in innate and acquired immune response. *Annu Rev Immunol* 21:483-513 (2003)

35. A. D'Andrea, D. Goux, C. De Lalla, Y. Koezuka, D. Montagna, A. Moretta, P. Dellabona, G. Casorati, and S. Abrignani. Neonatal invariant Valpha24+ NKT lymphocytes are activated memory cells. *Eur J Immunol* 30:1544-1550 (2000)

36. S. Fujii, K. Shimizu, M. Kronenberg, and R.M. Steinman. Prolonged IFN-gamma-producing NKT response induced with alpha-galactosylceramide-loaded DCs. *Nat Immunol* 3:867-874 (2002)

37. M. Brigl and M.B. Brenner. CD1: antigen presentation and T cell function. *Annu Rev Immunol* 22:817-890 (2004)

38. R.R. Brutkiewicz. CD1d ligands: the good, the bad, and the ugly. *J Immunol* 177:769-775 (2006)

39. V. Sriram, W. Du, J. Gervay-Hague, and R.R. Brutkiewicz. Cell wall glycosphingolipids of Sphingomonas paucimobilis are CD1d-specific ligands for NKT cells. *Eur J Immunol* 35:1692-1701 (2005)

40. D. Zhou, J. Mattner, C. Cantu, 3rd, N. Schrantz, N. Yin, Y. Gao, Y. Sagiv, K. Hudspeth, Y.P. Wu, T. Yamashita, S. Teneberg, D. Wang, R.L. Proia, S.B. Levery, P.B. Savage, L. Teyton, and A. Bendelac. Lysosomal glycosphingolipid recognition by NKT cells. *Science* 306:1786-1789 (2004)

41. N.A. Borg, K.S. Wun, L. Kjer-Nielsen, M.C. Wilce, D.G. Pellicci, R. Koh, G.S. Besra, M. Bharadwaj, D.I. Godfrey, J. McCluskey, and J. Rossjohn. CD1d-lipid-antigen recognition by the semi-invariant NKT T-cell receptor. *Nature* 448:44-49 (2007)

42. S.D. Gadola, J.D. Silk, A. Jeans, P.A. Illarionov, M. Salio, G.S. Besra, R. Dwek, T.D. Butters, F.M. Platt, and V. Cerundolo. Impaired selection of invariant natural killer T cells in diverse mouse models of glycosphingolipid lysosomal storage diseases. *J Exp Med* 203:2293-2303 (2006)

43. T. Yoshimoto and W.E. Paul. CD4pos, NK1.1.pos T cells promptly produce interleukin 4 in response to *in vivo* challenge with anti-CD3. *J Exp Med* 179:1285-1295 (1994)

44. T. Ohteki and H.R. MacDonald. Major histocompatibility complex class I related molecules control the development of CD4+8- and CD4-8- subsets of natural killer 1.1.+ T cell receptor-alpha/beta+ cells in the liver of mice. *J Exp Med* 180:699-704 (1994)

45. P. Dellabona, E. Padovan, G. Casorati, M. Brockhaus, and A. Lanzavecchia. An invariant V alpha 24-J alpha Q/V beta 11 T cell receptor is expressed in all individuals by clonally expanded CD4-8- T cells. *J Exp Med* 180:1171-1176 (1994)

46. A. Balandina, S. Lecart, P. Dartevelle, A. Saoudi, and S. Berrih-Aknin. Functional defect of regulatory CD4 (+)CD25+ T cells in the thymus of patients with autoimmune myasthenia gravis. *Blood* 105:735-741 (2005)

47. L. Chatenoud, B. Salomon, and J.A. Bluestone. Suppressor T cells--they're back and critical for regulation of autoimmunity! *Immunol Rev* 182:149-163 (2001)

48. H. Groux, A. O'Garra, M. Bigler, M. Rouleau, S. Antonenko, J.E. de Vries, and M.G. Roncarolo. A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* 389:737-742 (1997)

49. K.H. Sonoda, M. Exley, S. Snapper, S.P. Balk, and J. Stein-Streilein. CD1-reactive natural killer T cells are required for development of systemic tolerance through an immune-privileged site. *J Exp Med* 190:1215-1226 (1999)

50. S.H. Yang, J.Z. Jin, S.H. Lee, H. Park, C.H. Kim, D.S. Lee, S. Kim, N.H. Chung, and Y.S. Kim. Role of NKT cells in allogeneic islet graft survival. *Clin Immunol* 124:258-266 (2007)

51. Y. Ikehara, Y. Yasunami, S. Kodama, T. Maki, M. Nakano, T. Nakayama, M. Taniguchi, and S. Ikeda. CD4 (+) Valpha14 natural killer T cells are essential for acceptance of rat islet xenografts in mice. *J Clin Invest* 105:1761-1767 (2000)

52. K.I. Seino, K. Fukao, K. Muramoto, K. Yanagisawa, Y. Takada, S. Kakuta, Y. Iwakura, L. Van Kaer, K. Takeda, T. Nakayama, M. Taniguchi, H. Bashuda, H. Yagita, and K. Okumura. Requirement for natural killer T (NKT) cells in the induction of allograft tolerance. *Proc Natl Acad Sci U S A* 98:2577-2581 (2001)

53. A.B. Pillai, T.I. George, S. Dutt, P. Teo, and S. Strober. Host NKT cells can prevent graft-versus-host disease and permit graft antitumor activity after bone marrow transplantation. *J Immunol* 178:6242-6251 (2007)

54. G. Gigli, S. Caielli, D. Cutuli, and M. Falcone. Innate immunity modulates autoimmunity: type 1 interferon-beta treatment in multiple sclerosis promotes growth and function of regulatory invariant natural killer T cells through dendritic cell maturation. *Immunology* 122:409-417 (2007)

55. R. Furlan, A. Bergami, D. Cantarella, E. Brambilla, M. Taniguchi, P. Dellabona, G. Casorati, and G. Martino. Activation of invariant NKT cells by alphaGalCer administration protects mice from MOG35-

55-induced EAE: critical roles for administration route and IFN-gamma. *Eur J Immunol* 33:1830-1838 (2003)

56. N.N. Iwakoshi, D.L. Greiner, A.A. Rossini, and J.P. Mordes. Diabetes prone BB rats are severely deficient in natural killer T cells. *Autoimmunity* 31:1-14 (1999)

57. K.J. Hammond, L.D. Poulton, L.J. Palmisano, P.A. Silveira, D.I. Godfrey, and A.G. Baxter. alpha/beta-T cell receptor (TCR)+CD4-CD8- (NKT) thymocytes prevent insulin-dependent diabetes mellitus in nonobese diabetic (NOD)/Lt mice by the influence of interleukin (IL)-4 and/or IL-10. *J Exp Med* 187:1047-1056 (1998)

58. A. Lehuen, O. Lantz, L. Beaudoin, V. Laloux, C. Carnaud, A. Bendelac, J.F. Bach, and R.C. Monteiro. Overexpression of natural killer T cells protects Valpha14- Jalpha281 transgenic nonobese diabetic mice against diabetes. *J Exp Med* 188:1831-1839 (1998)

59. V. Laloux, L. Beaudoin, D. Jeske, C. Carnaud, and A. Lehuen. NK T cell-induced protection against diabetes in V alpha 14-J alpha 281 transgenic nonobese diabetic mice is associated with a Th2 shift circumscribed regionally to the islets and functionally to islet autoantigen. *J Immunol* 166:3749-3756 (2001)

60. Y.G. Chen, C.M. Choisy-Rossi, T.M. Holl, H.D. Chapman, G.S. Besra, S.A. Porcelli, D.J. Shaffer, D. Roopenian, S.B. Wilson, and D.V. Serreze. Activated NKT cells inhibit autoimmune diabetes through tolerogenic recruitment of dendritic cells to pancreatic lymph nodes. *J Immunol* 174:1196-1204 (2005)

61. Q.S. Mi, D. Ly, P. Zucker, M. McGarry, and T.L. Delovitch. Interleukin-4 but not interleukin-10 protects against spontaneous and recurrent type 1 diabetes by activated CD1d-restricted invariant natural killer T-cells. *Diabetes* 53:1303-1310 (2004)

62. M.A. Jordan, J.M. Fletcher, D. Pellicci, and A.G. Baxter. Slamf1, the NKT cell control gene Nkt1. *J Immunol* 178:1618-1627 (2007)

63. M. Falcone, F. Facciotti, N. Ghidoli, P. Monti, S. Olivieri, L. Zaccagnino, E. Bonifacio, G. Casorati, F. Sanvito, and N. Sarvetnick. Up-regulation of CD1d expression restores the immunoregulatory function of NKT cells and prevents autoimmune diabetes in nonobese diabetic mice. *J Immunol* 172:5908-5916 (2004)

64. F.D. Shi, M. Flodstrom, B. Balasa, S.H. Kim, K. Van Gunst, J.L. Strominger, S.B. Wilson, and N. Sarvetnick. Germ line deletion of the CD1 locus exacerbates diabetes in the NOD mouse. *Proc Natl Acad Sci U S A* 98:6777-6782 (2001)

65. J.A. Cain, J.A. Smith, J.K. Ondr, B. Wang, J.D. Katz. NKT cells and IFN-gamma establish the regulatory environment for the control of diabetogenic T cells in the nonobese diabetic mouse. *J Immunol* 176:1645-1654 (2006)

66. H. Kitamura, K. Iwakabe, T. Yahata, S. Nishimura, A. Ohta, Y. Ohmi, M. Sato, K. Takeda, K. Okumura, L. Van Kaer, T. Kawano, M. Taniguchi, and T. Nishimura. The natural killer T (NKT) cell ligand alpha-galactosylceramide demonstrates its immunopotentiating effect by inducing interleukin (IL)-12 production by dendritic cells and IL-12 receptor expression on NKT cells. *J Exp Med* 189:1121-1128 (1999)

67. Y.F. Yang, M. Tomura, S. Ono, T. Hamaoka, and H. Fujiwara. Requirement for IFN-gamma in IL-12 production induced by collaboration between v (alpha)14 (+) NKT cells and antigen-presenting cells. *Int Immunol* 12:1669-1675 (2000)

68. M. Tomura, W.G. Yu, H.J. Ahn, M. Yamashita, Y.F. Yang, S. Ono, T. Hamaoka, T. Kawano, M. Taniguchi, Y. Koezuka, and H. Fujiwara. A novel function of Valpha14+CD4+NKT cells: stimulation of IL-12 production by antigen-presenting cells in the innate immune system. *J Immunol* 163:93-101 (1999)

69. A.W. Jahng, I. Maricic, B. Pedersen, N. Burdin, O. Naidenko, M. Kronenberg, Y. Koezuka, and V. Kumar. Activation of natural killer T cells potentiates or prevents experimental autoimmune encephalomyelitis. *J Exp Med* 194:1789-1799 (2001)

70. L. Beaudoin, V. Laloux, J. Novak, B. Lucas, and A. Lehuen. NKT cells inhibit the onset of diabetes by impairing the development of pathogenic T cells specific for pancreatic beta cells. *Immunity* 17:725-736 (2002)

71. J. Novak, L. Beaudoin, T. Griseri, and A. Lehuen. Inhibition of T cell differentiation into effectors by NKT cells requires cell contacts. *J Immunol* 174:1954-1961 (2005)

72. S. Fujii, K. Shimizu, H. Hemmi, and R.M. Steinman. Innate Valpha14 (+) natural killer T cells mature dendritic cells, leading to strong adaptive immunity. *Immunol Rev* 220:183-198 (2007)

73. S. Fujii, K. Shimizu, C. Smith, L. Bonifaz, and R.M. Steinman. Activation of natural killer T cells by alpha-galactosylceramide rapidly induces the full maturation of dendritic cells *in vivo* and thereby acts as an adjuvant for combined CD4 and CD8 T cell immunity to a coadministered protein. *J Exp Med* 198:267-279 (2003)

74. I.F. Hermans, J.D. Silk, U. Gileadi, M. Salio, B. Mathew, G. Ritter, R. Schmidt, A.L. Harris, L. Old, and V. Cerundolo. NKT cells enhance CD4+ and CD8+ T cell responses to soluble antigen *in vivo* through direct interaction with dendritic cells. *J Immunol* 171:5140-5147 (2003)

75. M.S. Vincent, D.S. Leslie, J.E. Gumperz, X. Xiong, E.P. Grant, and M.B. Brenner. CD1-dependent dendritic cell instruction. *Nat Immunol* 3:1163-1168 (2002)

76. Y.N. Naumov, K.S. Bahjat, R. Gausling, R. Abraham, M.A. Exley, Y. Koezuka, S.B. Balk, J.L. Strominger, M. Clare-Salzer, and S.B. Wilson. Activation of CD1d-restricted T cells protects NOD mice from developing diabetes by regulating dendritic cell subsets. *Proc Natl Acad Sci U S A* 98:13838-13843 (2001)

77. V. Saxena, J.K. Ondr, A.F. Magnusen, D.H. Munn, and J.D. Katz. The countervailing actions of myeloid and plasmacytoid dendritic cells control autoimmune diabetes in the nonobese diabetic mouse. *J Immunol* 179:5041-5053 (2007)

78. S. Kojo, K. Seino, M. Harada, H. Watarai, H. Wakao, T. Uchida, T. Nakayama, and M. Taniguchi. Induction of regulatory properties in dendritic cells by Valpha14 NKT cells. *J Immunol* 175:3648-3655 (2005)

79. D. Ly, Q.S. Mi, S. Hussain, and T.L. Delovitch. Protection from type 1 diabetes by invariant NK T cells requires the activity of CD4+CD25+ regulatory T cells. *J Immunol* 177:3695-3704 (2006)

80. V. Cerundolo and M. Salio. Harnessing NKT cells for therapeutic applications. *Curr Top Microbiol Immunol* 314:325-340 (2007)

**Key Words:** Invariant Natural Killer T cells, Immune Regulation, Autoimmune Disease, Cytokines, Dendritic Cells, Regulatory T cells, Review

**Send correspondence to:** Marika Falcone, Experimental Diabetes Unit, San Raffaele Scientific Institute, Via Olgettina 60, 20132, Milan, Italy, Tel: 390226432317, Fax: 390226432914, E-mail: falcone.marika@hsr.it

**http://www.bioscience.org/current/vol13.htm**