[Frontiers in Bioscience 19, 1240-1250, June 1, 2014]

The role of invariant NKT cells in organ-specific autoimmunity

Caterina Di Pietro[1], Marika Falcone[1]

[1]Experimental Diabetes Unit, Diabetes Research Institute, Department of Immunology, Transplantation and Infectious Diseases, San Raffaele Scientific Institute, Via Olgettina 60, 20132 Milan, Italy

TABLE OF CONTENTS

1. ABSTRACT

Invariant NKT cells (iNKT) represent a unique subset of innate lymphocytes that play a dual role and exert a pro-inflammatory function and also a tolerogenic function that is crucial to maintain T cell tolerance and prevent autoimmune diseases like Multiple Sclerosis, Type 1 Diabetes, Rheumatoid Arhritis and Systemic Lupus Erithematosus (SLE). Although a large body of evidence indicated that iNKT cells are instrumental to counter-regulate T cell-mediated autoimmune diseases, there is still some controversy on whether iNKT cells can actively induce immunosuppression and directly dampen T cell autoimmunity. Moreover, the recent discovery of a distinct iNKT cell subset, the iNKT17 cells, with strong adjuvant and pro-inflammatory function raised the question on what is the role of NKT17 cells in the pathogenesis of autoimmune diseases. Here, we review the current knowledge on iNKT cell biology and focus our attention on the possible mechanism of action and final effect of the different iNKT cell subsets in the pathogenesis of T cell-mediated autoimmune diseases.

2. INTRODUCTION

CD1d-restricted T cells that are characterized by the expression of an invariant TCR alpha-chain (Valpha14 in mice and Valpha24 in humans) and markers of natural killer cells are referred as invariant natural killer T cells (iNKT) cells. iNKT cells represent a unique lymphocyte subset with remarkable functional diversity and highly diverse immune functions. On one hand, they actively induce T cell tolerance (1) and are crucial for prevention of autoimmune diseases in pre-clinical models of Multiple Sclerosis (MS), Type 1 Diabetes (T1D) and Rheumatoid Arthritis (RA). On the other hand, iNKT cells actively participate in the innate immune response to promote antimicrobial (2-4) and antitumor immunity (5-7). In fact, iNKT cells confer protective immunity against cancer and various infectious agents and also contribute as effector cells to the development of allergic airway disease, contact hypersensitivity, hepatitis, ischemia-reperfusion injury, atherosclerosis, and obesity-associated diseases (8,9). The importance of iNKT cells as immune adjuvants during infections is highlighted by the observation that several

microbial antigens can directly activate iNKT cells and pathogens escape immunity by blocking iNKT cell activation. While the effector function of iNKT cells in infections and anti-tumor immunity is widely recognized, the capacity of iNKT cells to effectively induce immune tolerance has been recently questioned and this is partially due to lack of knowledge on the mechanism of action of immune regulatory iNKT cells.

The acquisition of a specific cytokine-secretion phenotype seems to correlate with the different iNKT cell functions. For example, the secretion of pro-inflammatory cytokines such as IFN-gamma and IL-12 is linked with the adjuvant function of iNKT cells against infections and tumors (10-12), while iNKT cells that induce immune tolerance and prevent autoimmunity carry a Th2-biased cytokine phenotype. It is counter-deductive that the iNKT cell secretion of pro-inflammatory and Th1-inducing cytokines such as IFN-gamma and IL-12 can boost T cell immunity against pathogens and tumors (iNKT cell adjuvant function). However, it is less evident how iNKT cells that secrete type 2 cytokines but also significant amounts of IFN-gamma (13) can down-regulate autoimmunity. The observation that iNKT cells capable to suppress autoimmune diseases secrete a diverse array of cytokines including Th2-type cytokines like IL-4, IL-5 and IL-13 and tolerogenic cytokines like IL-10 but also type 1 inflammatory cytokines, i.e., IFN-gamma, suggests that their tolerogenic mechanism of action is not cytokine mediated. Recently, a new functional iNKT cell subset characterized by the predominant secretion of IL-17 and a differential pathway of thymic differentiation has been identified. NKT17 cells play a strong pro-inflammatory function and play a pathogenetic role in autoimmune diseases.

Hence, although the functional classification of iNKT cells in effector NKT1 cells (IFN-gamma-secreting) and regulatory NKT2 cells (IL-4-secreting) based on their cytokine-secretion profile has been accepted for many years, it is now clear that the iNKT cell role in immunity and autoimmunity is more complex and not strictly cytokine-dependent. Here, we will present current notions on the iNKT cell biology, antigen recognition, acquisition of a specific cytokine profile and mechanisms of action for tolerance induction and prevention of autoimmune diseases.

3. INVARIANT NATURAL KILLER T CELLS: MODULATORY CELLS AT THE BRIDGE BETWEEN INNATE AND ADAPTIVE IMMUNITY

Since iNKT cells play an important role in the early immune response against pathogens, they are often presented as innate immune cells but it would be limiting to restrict their role to such classification. Innate immune cells are, by definition, cells that are present and ready to function prior to the exposure to microbes and their intent is to provide first-line defense against pathogens. iNKT cells respond to such 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) and are pre-committed to secrete cytokines prior to the exposure to infections. However, iNKT cells also show typical features of adaptive immune cells such as the capacity to respond with proliferation and cytokine secretion to a specific antigenic stimulation. Those iNKT cell features indicate that they do not belong to a single immune compartment but act at the interface between innate and adaptive immunity. Importantly, like other innate immune cells such as dendritic cells, macrophages and mast cells iNKT cells 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 the inflammatory response by releasing pro-inflammatory cytokines and by cytotoxic function (14). However, they can also acquire a tolerant function to limit the inflammatory response and avoid destructive immunity and autoimmunity in steady-state or after the clearance of pathogens. 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 different iNKT cell functions are yet unknown but there is indication that iNKT cells can respond directly with a TCR-mediated mechanism to microbe-derived glycolipids presented by dendritic cells (DCs) in the context of the CD1d restriction molecule. Alternatively, pathogens trigger innate receptors (pattern recognition receptors-PPRs) within DCs, which, in turn, activate iNKT cells through the combined presentation of self-derived glycolipids and production of pro-inflammatory cytokines like IL-12 and type I interferons (IFNs) (15-17).

3.1. The antigen specificity of iNKT cells: self and non-self glycolipids

The invariant NKT cell receptor (NKTCR) recognizes glycolipid antigens presented by the MHC class I-like molecule, CD1d. The invariance of the NKTCR had originally led to speculation that iNKT cells react to a single glycolipid antigen. In that view, the first iNKT cell antigen identified, the marine-sponge-derived glycolipid antigen alphaGalCer, was thought to mimic the natural endogenous or exogenous ligand. For many years, research on iNKT cell biology was based on the assumption that iNKTCR reacts to a single glycolipid antigen and that the identification of such ligand would help to clarify the iNKT cell function in the immune system. In fact, the exclusive recognition of a microbial antigen by iNKTCR would have suggested that the main function of iNKT cells is pro-inflammatory and primarily aimed at fighting pathogens. On the contrary, the identification of a self-glycolipid antigen would have implied that iNKT have a predominant role in the steady-state for maintenance of immune tolerance towards self-antigens and prevention of autoimmune diseases. 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 both pathogen-derived as well as self-glycolipids isolated from autologous cells and tumors (18).

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 derived either from pathogens or self tissues.

Similarly to conventional T cells that need to recognize self peptides presented by MHC molecules in the thymus in order to undergo positive selection, also iNKT cells require recognition of a self glycolipid antigen for their thymic maturation. The observation that mice with a deficiency in β-hexosaminidase (hexb), an enzyme required for the generation of iGb3 in lysosomes, have a defective iNKT cell development, suggested that the iGb3 self glycolipid is necessary the intrathymic iNKT cell maturation (19). 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 the thymus. In support of that idea, there is the observation that the iNKT cell development is also defective in individuals affected by several lysosomal storage diseases in which the pathological accumulation of glycosphingolipids in the lysosomes influences endogenous lipid loading and/or CD1d restricted presentation (20).

While the nature of the self-glycolipid antigens that activate iNKT cells remain elusive, important progress has been made in characterizing microbe-derived iNKT cell antigens. Glycolipid antigens capable of triggering the iNKTCR have been isolated from different bacterial species, such as *Sphingomonas* (21, 22), *Borrelia burgdorferi* (23) and *Streptococcus pneumonia* (24). Also, evolutionary conserved structures such as glycosylceramides isolated from the cell wall of Gram-negative LPS-negative 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. Other pathogens such as the LPS-positive *Salmonella thyphimuriu, Salmonella enterica* and *Listeria monocytogenes* activate iNKT cells indirectly through the recognition of an endogenous glycolipids presented by TLR or NOD-stimulated DCs (16, 17).

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 microbe- or self-derived 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 peripheral tissues, could drive iNKT cell towards a regulatory phenotype and function suppress self-reactive T cell immunity. Conversely, when iNKT cells recognize microbial or self-glycolipids in the context of an infection and in the presence of danger signals, i.e., IL-12 secreted by PRR-stimulated DCs, they acquire an adjuvant function and critically contribute to the clearance of pathogens.

3.2. Functional iNKT cell subsets: conventional iNKT cells and NKT17 cells

Upon activation, iNKT cells rapidly secrete a broad array of cytokines belonging both to the Th1 (IFNgamma, IL-12, TNF-alpha), Th2 (IL-4, IL-5 and IL-13) and Th17 cytokine secretion pattern as well as down-modulatory cytokines such as IL-10. This highly diverse cytokine secretion capacity enable iNKT cells to modulate adaptive immunity, thus providing protection against viral and bacterial infection, as well as to regulate a large spectrum of diseases including autoimmune diseases, allergy, allograft rejection and graft versus host disease (25). Originally, iNKT cells were divided in two different functional subsets according to their cytokine secretion pattern. Pro-inflammatory iNKT cells that play adjuvant function were classified as NKT1 cells with predominant IFN-gamma and IL-12 secretion while iNKT cells with regulatory and immune suppressive function were thought to have a predominant NKT2 phenotype characterized by secretion of type 2 cytokines like IL-4 and IL-13. Those functionally distinct subsets of iNKT cells were thought to have specific surface markers. In humans, double negative ($CD4^-CD8^-$) iNKT cells exhibit a strongly biases Th1 profile while $CD4^+$ iNKT cells have a mixed Th1/Th2 cytokine secretion profile (5, 6). However, this distinction is not present in murine iNKT cells and it is now evident that iNKT cell subsets are not pre-defined but iNKT cells acquire different cytokine secretion profiles in the periphery according to the nature of activating stimuli and by the context in which those stimuli are carried out. In this view, the co-stimulatory signals provided by DCs simultaneously with antigenic stimulation such as CD40-CD40L and SLAM-SLAM interaction and cytokines like IL-12 play a central role in the iNKT cell decision to acquire a predominant IFN-gamma-secreting or IL-4-secreting phenotype (26, 27). Also, co-stimulatory signals received by other immune cell types such as NK cells, T cells and B cells are crucial to modulate the phenotype of iNKT cells through cell-surface receptors, co-stimulatory receptors and soluble mediators. For example the observation that human $CD4^+CD25^+$ Treg cells can down-regulate the activation of iNKT cell clones *in vitro* suggested that $CD4^+CD25^+$ Treg cells could directly target iNKT cells for immune suppression (28, 29).

Recently, a specialized subset of iNKT cells that releases high concentrations of IL-17 within 2–3 hour from TCR stimulation in the absence of Th17 priming cytokine such as IL-6, TGF-βor IL-23 has been identified and classified as NKT17 cells (30, 31). This iNKT cell subset is completely distinct from conventional iNKT cells (secreting different amounts of IFN-gamma, IL-4, IL-5, IL-13) and seems to have a fixed and pre-defined cytokine-secretion phenotype with release of IL-17 and IL-22 but no other Th1 or Th2 cytokines. The developmental pathway of NKT17 cells is also completely different from that of conventional iNKT cells. In the thymus, during the early phases of iNKT cell maturation (DP stage), a fraction of iNKT cell precursors maintains RORgammat expression

and undergoes NKT17 cell differentiation (32). The mechanism that regulates this process is still unclear. Different hypothesis have been suggested including the presence of specific endogenous antigens that selectively direct iNKT cell precursor cells toward a preferential NKT17 cell differentiation or a still unidentified co-stimulatory molecules that "set off" the program that favours iNKT17 cell development. NKT17 cells play a predominant effector and pro-inflammatory function and, although their role in organ-specific autoimmune diseases has not been extensively studied, there is preliminary evidence that they contribute to the pathogenesis of T cell-mediated autoimmune diseases like Type 1 Diabetes (T1D) (33).

4. THE TOLEROGENIC FUNCTION OF INKT CELLS: INDUCTION OF T CELL TOLERANCE AND PREVENTION OF AUTOIMMUNE DISEASES

The hypothesis that iNKT cells could play an immune regulatory function 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 (34) 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), the pre-clinical models of MS (35). Later on, several lines of evidence highlighted the immune regulatory role and the capacity to protect against autoimmune diseases of iNKT cells. For example, selective iNKT cell activation through administration of alphaGalCer protected mice against several pre-clinical models of autoimmune diseases including MS (EAE) (36, 37), T1D (38, 39) and collagen-induced arthritis (1, 2). Most importantly, a quantitative and/or qualitative defect of iNKT cells has been reported in patients affected by most autoimmune diseases including MS, T1D, Systemic Lupus Erithematosus (SLA), Sjogren's syndrome, Rheumatoid Arthritis (RA), sarcoid and asthma, as well as in mouse models of T1D, MS and colitis (42-47). Although there is no proof that iNKT cells directly suppress T cells *in vitro*, as conventional regulatory T cells like $CD4^+CD25^+FoxP3^+$ or IL-10-secreting Tr1 cells do, 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 (1). In fact, CD1d-deficient or Valpha14 NKT cell-deficient mice failed to develop systemic tolerance. In addition, iNKT cells were capable to induce transplantation tolerance towards allogeneic (48) and xenogeneic islet cells transplanted into the liver (49) and towards cardiac allografts (50). 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 in Valpha14 iNKT cell-deficient mice. Also, host iNKT cells inhibited T cell immunity in Graft-versus-Host Disease (GVHD). The aforementioned studies provided clear evidence that iNKT cells are crucial for induction of peripheral tolerance. The strongest evidence of a pro-tolerogenic role of iNKT cells cames from the numerous studies reporting that iNKT cells have the capacity to counter-regulate the pathogenesis of several organ-specific autoimmune diseases.

4.1. Multiple sclerosis

The importance 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 mice susceptible to EAE, the experimental model of MS (37, 51) as well as in MS patients (45). In those studies the iNKT cell number was assessed in EAE-susceptible SJL/J mice both by the analysis of $NK1.1.^+$ $CD3^+$ T cells and, more recently, of CD1d-restricted iNKT cells stained with alphaGalCer-loaded CD1d-dimers. In humans, despite of the limitation of studying iNKT cells in peripheral blood where iNKT cells are present at very low percentages (0.1.-0.5.% of $CD3^+$ lymphocytes), a clear reduction in the number of Valpha24Jalpha in MS patients compared to healthy individuals or patients affected by other inflammatory disorders was demonstrated. The link between iNKT cell number and prevention of autoimmunity in the CNS was further demonstrated by the observation that the iNKT cell percentage increased significantly in patients protected from MS relapses by the treatment with type 1 IFN-β(52). Those studies clearly suggested a possible role of iNKT cells in protection from MS. However, the final evidence of such role came from the observation that iNKT cell activation prevented autoimmunity in pre-clinical models of MS. 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 alphaGalCer, was able to prevent MOG_{35-55} peptide-induced EAE in B6 mice through the regulatory action of iNKT cells. In fact, only wild-type B6 mice but not iNKT cell-deficient littermates were protected from EAE, thus showing that the OCH analogue selectively triggered the pro-tolerogenic function of iNKT cells. 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 (36). Later on, 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 alphaGalCer, a strong NKTCR agonist that promoted iNKT cell expansion and cytokine secretion without driving them towards a specific Th2-type cytokine profile (37). The latter finding suggested that the immune regulatory role of iNKT cells in EAE did not necessarily depend upon their acquisition of a Th2 cytokine profile. The observation that alphaGalCer treatment did not protect from EAE neither IL-4 nor IL-10 knockout B6 mice indicated that Th2 cytokines may be important for the immune regulatory cascade generated by iNKT cells but it did not necessarily imply that iNKT cells mediate their pro-tolerogenic function through secretion of Th2-type cytokines. In support to the latter hypothesis, another report demonstrated thatalphaGalCer-activated iNKT cells should

secrete a diverse array of cytokines including IFN-gamma to induce protection against EAE (53).

4.2. Type 1 diabetes

A reduced number of Valpha14 iNKT cells and their abnormality in cytokine secretion have been reported in animals that develop spontaneous autoimmune diabetes such as nonobese diabetic (NOD) mice (13, 54, 55) and diabetes-prone BB rats (56) as well as in humans affected by T1D (42, 43). In the first report of iNKT cell defect in diabetes-prone NOD mice (54), the deficit in the number of $NK1^+$-like thymocytes was directly correlated with the progression of the autoimmune disease. In those studies the restoration of a normal iNKT cell number by injection of thymic Valpha14 NKT cells from nondiabetic (BALB/c X NOD F1) mice ameliorated autoimmune diabetes. This observation, together with the finding that the presence of a large number of iNKT cells in Valpha14 TCR transgenic NOD mice only partially prevented T1D, suggested that a quantitative but also qualitative NKT cell defect is present in NOD mice. Originally, it was believed that such defect regarded selectively the iNKT cell secretion of Th2 cytokines like IL-4. That idea was supported by the finding that in twins discordant for T1D, the diabetic sibling showed lower frequencies of $CD4^-CD8^-$ Valpha24JalphaQ$^+$ T cells associated with a reduced secretion of IL-4 by iNKT cells (42). Later on the importance of Th2 cytokines secretion for the protective role of iNKT cells has been strongly questioned by several findings. First of all, the observation that secretion of both IL-4 and IFN-gammawas defective in NOD mice (13). Also, the selective defect of IL-4 secretion by iNKT cells in T1D patients was not confirmed by more recent studies (57).

Although the nature of the functional defect of iNKT cells in autoimmune T1D is not yet clarified, 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 (58). 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 as well as dendritic cells (27) and the resulting quantitative iNKT cell defect.

As mentioned earlier for the role of iNKT cells in MS pathogenesis, the best evidence that iNKT cells play a protective role against autoimmune T1D was provided by the observation that iNKT cell activation prevented T1D in NOD mice. Two studies published in 2001 reported that the intraperitoneal injection of alphaGalCer, the NKTCR agonist, protected NOD mice through modulation of iNKT cells (38, 39). 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 T1D (59). Interestingly, the administration of alphaGalCer was effective when given early (4 weeks of age) and late (starting at 10 weeks of age when the autoimmune T cell repertoire in NOD mice is already generated). Hence, the regulatory effect of iNKT cells was played not only in the induction phase of the autoimmune disease for activation and/or expansion of islet-reactive T cells, but was also important in the effector phase of the disease with a direct inhibition of the diabetogenic potential of islet-reactive T cells. Those studies clearly established a protective role of iNKT cells in autoimmune T1D, however they failed to provide a mechanism of action. Preliminary evidence indicated that protective iNKT cells mainly secreted IL-4 and biased the cytokine profile of islet-reactive T cells towards a protective Th2 type. However, alphaGalCer-activated iNKT cells secrete IL-4 and IFN-gamma and it remains to be clarified whether the latter cytokine could have a direct down-regulatory effect on the diabetogenic potential of islet-reactive T cells or if a different iNKT cell-mediated mechanism of action is involved in alphaGalCer-induced T1D protection.

4.3. Systemic lupus erythematosus

The first evidence of pathogenic link between selective reduction in iNKT cell number and organ-specific autoimmune disease came from studies in the autoimmune-prone MRL/lpr mouse, the murine model for human systemic lupus erythematosus (SLE) (60). Such selective reduction in iNKT cell number was then confirmed in other experimental models of lupus, the NZB/NZW F1 mice (61), as well as in humans affected by SLE (40). In the MRL/lpr model the reduction of Valpha14 iNKT cell number correlated with the progression of autoimmunity: iNKT cells started to decrease at 3-4 weeks of age before the onset of the autoimmune disease and disappeared completely at 10 weeks of age at time of clinical manifestation of disease. In the same murine model, a further evidence of a crucial role for iNKT cells for prevention of autoimmunity, came from the observation that transfer of iNKT cells isolated from Valpha14 TCR transgenic iNKT cells and injected into MRL/lpr mice delayed onset of clinical signs of lupus.

4.4. Rheumatoid arthritis

Despite of the small percentage of iNKT cells in the PBMC, a significant decrease in the percentage of Valpha24$^+$Vβ11$^+$, $CD4^-CD8^-$ iNKT cells was reported in patients affected by RA (40). Interestingly, in that report the reduced number of iNKT cells was associated with their inability to respond to antigenic stimulation with alphaGalCer. The latter finding suggested that iNKT cells of RA patients carry an intrinsic defect of maturation that render them unable to expand in response to antigenic stimulation.

5. MECHANISM OF ACTION OF TOLEROGENIC iNKT CELLS

5.1. The role of cytokines

Since iNKT cells release massive amounts of cytokines upon activation via their TCR, it was originally believed that they mediate their regulatory or adjuvant function through secretion of cytokines. The iNKT cell secretion of pro-inflammatory cytokines such as IFN-

gamma and IL-12 was linked with the adjuvant function of iNKT cells to fight infections and in anti-tumor immunity (10, 12). On the other hand, early studies suggested that Th2-type cytokines could play an important role in the immuneregulatory function of iNKT cells. For example, 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 (39). Furthermore, iNKT cells that induced T cell tolerance and ameliorated animal models of autoimmune T1D or MS showed an increased secretion of Th2-type cytokines such as IL-4 and IL-10 (36-39, 62). 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, suggested that the secretion of Th2 cytokines by iNKT cells were responsible for their regulatory action (42, 51). 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 those cytokines was dispensable for iNKT cells to mediate their regulatory function and prevent autoimmune diseases. In fact, activation of iNKT cells by administration of alphaGalCer was able to protect against autoimmune T1D and EAE both wildtype and IL-4 (63) or IL-10 deficient mice (64). More recent reports demonstrated that iNKT cells prevented T1D without driving a Th2 shift of self-reactive T cells (65). Instead, islet-reactive $CD4^+$ T cells injected into Valpha14 TCR transgenic NOD mice were primed in the pancreatic lymph nodes but rendered anergic by iNKT cells. In that model, iNKT cell-secreted cytokines such as IL-4, IL-10, IL-13 and TGF-β did not play any role in the protective effect. The same group reported *in vitro* experiments showing that iNKT cell modulation on self-reactive T cells required cell-cell contact (66). In that experimental work, 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, that study did not clarify the type of cells that were directly contacted by iNKT cells. In fact, 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 NKT-T cell-cell interaction and it did not exclude the possibility that iNKT cells play their immunosuppressive role by modulating other immune cells, such as antigen-presenting cells, present in culture.

5.2. 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 (67). Specifically, several *in vivo* studies 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. In 2002 the group of M. Brenner clearly demonstrated *in vitro* that iNKT cell-mediated maturation of DCs was induced by cell-cell contact between iNKT cells and myeloid DCs (68). In that report, DCs co-cultured with iNKT cells and LPS up-regulated maturation markers and increased secretion of pro-inflammatory cytokines compared to LPS-stimulated DCs in the absence of iNKT cells. Those findings suggested that DCs receiving iNKT cell modulation together with other microbial signals, i.e., LPS, secrete larger amount of pro-inflammatory cytokines such as IL-12 and prime inflammatory Th1 cells. According to this model, iNKT cells recognize "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 priming of effector Th1 cells.

The effect of iNKT cells on DCs under steady-state conditions, i.e. in the absence of pathogens, for example during autoimmune diseases has been less studied but it may be similarly important for the regulatory role of iNKT cells. The first report suggesting that regulatory $Valpha14^+$ iNKT cells modulated myeloid DCs was provided by the observation that iNKT cell-mediated protection from autoimmune T1D in NOD mice was associated with the recruitment of tolerogenic myeloid $CD8alpha^-$ DCs within the pancreatic lymph nodes (69). In addition, the transfer myeloid $CD8alpha^-$ DCs isolated from PLN of iNKT cell-protected mice into NOD recipients completely prevented T1D development (69). However, a subsequent study stressed the importance of plasmacytoid rather than myeloid DCs for control of autoimmune T1D in the NOD mice (70). Hence, although there is indication that iNKT cells could mediate their regulatory role for prevention of autoimmune diseases through modulation of DCs, the functional features of those tolerogenic DCs and the molecular events operating in tolerogenic iNKT cell-DC interaction are still unclear. In 2005, the group of M. Taniguchi clarified the type of regulatory properties that iNKT cells induced on DCs (71). Specifically, in that study the Authors demonstrated that stimulation of Valpha14 iNKT cells *in vivo* by repeated alphaGalCer 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. 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 them towards a tolerogenic IL-10-secreting phenotype (72). Moreover, iNKT cell-induced DCs showed a unique capacity to activate regulatory Tr1 cells when used as antigen-presenting cells (72).

5.3. Induction of Treg 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. A recent study has suggested that iNKT cells and $CD4^+CD25^+$ cooperate functionally in the counter-regulation of experimental autoimmune diseases like T1D (28) and myasthenia gravis (EAMG), the animal model of human myasthenia (29). Specifically, it was shown that iNKT cell activation by alphaGalCer was able to protect mice from EAMG by favoring T_{reg} expansion through IL-2-dependent mechanisms. In addition to the significant increase in their number, $CD4^+CD25^+$ T cells from iNKT cell-protected mice were also more potent in inhibiting proliferation of self-reactive T cells. Although that study clearly indicated that iNKT cells mediate their regulatory effect through induction of conventional T_{reg} cells, it did not assess whether the iNKT cells interact directly with T_{reg} cells or, through cell-cell contact with DCs, favor antigen-presentation and activation of T_{reg} cells.

6. 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 (73). Although the mechanism of action of iNKT cells is unknown and not necessarily cytokine-mediated (66, 72, 74), 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-gamma and IL-12 is linked with the adjuvant function of iNKT cells to help fighting infections and in anti-tumor immunity (10-12). Conversely, iNKT cells that induce T cell tolerance and prevents or ameliorates autoimmune diseases in animal models were characterized by the release of a wide array of cytokines that included Th1 cytokines like IFN-gammaand Th2-type cytokines such as IL-4 and IL-10 (36-39, 62). Without knowing the mechanism that drives the iNKT cell orientation towards a specific cytokine 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 necessary to fully exploit their therapeutic potential for prevention and/or treatment of autoimmune diseases.

7. ACKNOWLEDGMENT

We thank Francesca Ronchi for contributing to the previously published review on “The role of iNKT cells in organ-specific autoimmunity” (Ronchi F and Falcone M, Frontiers in Bioscience 2008) whose text has been partially used for this revised version of the manuscript.

8. REFERENCES

1. K.H. Sonoda, M. Exley, S. Snapper, S.P. Balk & 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)

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

3. E.E. Nieuwenhuis, T. Matsumoto, M. Exley, R.A. Schleipman, J. Glickman, D.T. Bailey, N. Corazza, S.P. Colgan, A.B. Onderdonk, R.S. Blumberg: CD1d-dependent macrophage-mediated clearance of Pseudomonas aeruginosa from lung. *Nat Med* 8, 588-593 (2002)

4. C. Ronet, S. Darche, M. Leite de Moraes, S. Miyake, T. Yamamura, J.A. Louis, L.H. Kasper, D. Buzoni-GaTel: NKT cells are critical for the initiation of an inflammatory bowel response against Toxoplasma gondii. *J Immunol* 175, 899-908 (2005)

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

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

7. K. Shimizu, A. Goto, M. Fukui, M. Taniguchi & 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)

8. L. Wu, V.V. Parekh, C.L. Gabriel, D.P. Bracy, P.A. Marks-Shulman, R.A. Tamboli, S. Kim, Y.V. Mendez-Fernandez, G.S. Besra, J.P. Lomenick, B. Williams, D.H. Wasserman, L. Van Kaer: Activation of invariant natural killer T cells by lipid excess promotes tissue inflammation, insulin resistance, and hepatic steatosis in obese mice. *Proc Natl Acad Sci U S A* 109, E1143-1152 (2012)

9. L. Van Kaer, V.V. Parekh, & L. Wu: Invariant natural killer T cells as sensors and managers of inflammation. *Trends Immunol* 34, 50-58 (2013)

10. 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, 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)

11. Y.F. Yang, M. Tomura, S. Ono, T. Hamaoka & 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)

12. M. Tomura, W.G. Yu, H.J. Ahn, M. Yamashita, Y.F. Yang, S. Ono, T. Hamaoka, T. Kawano, M. Taniguchi, Y.

Koezuka, 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)

13. M. Falcone, B. Yeung, L. Tucker, E. Rodriguez & 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)

14. L.S. Metelitsa, O.V. Naidenko, A. Kant, H.W. Wu, M.J. Loza, B. Perussia, M. Kronenberg, R.C. Seeger: Human NKT cells mediate antitumor cytotoxicity directly by recognizing target cell CD1d with bound ligand or indirectly by producing IL-2 to activate NK cells. *J Immunol* 167, 3114-3122 (2001)

15. M. Brigl, R.V. Tatituri, G.F Watts, V. Bhowruth, E.A. Leadbetter, N. Barton, N.R. Cohen, F.F. Hsu, G.S. Besra, M.B. Brenner: Innate and cytokine-driven signals, rather than microbial antigens, dominate in natural killer T cell activation during microbial infection. *J Exp Med* 208, 1163-1177 (2011)

16. C. Paget, T. Mallevaey, A.O. Speak, D. Torres, J. Fontaine, K.C. Sheehan, M. Capron, B. Ryffel, C. Faveeuw, M. Leite de Moraes, F. Platt, F. Trottein: Activation of invariant NKT cells by toll-like receptor 9-stimulated dendritic cells requires type I interferon and charged glycosphingolipids. *Immunity* 27, 597-609 (2007)

17. T. Selvanantham, N.K. Escalante, M. Cruz Tleugabulova, S. Fiévé, S.E. Girardin, D.J. Philpott, T. Mallevaey: Nod1 and Nod2 enhance TLR-mediated invariant NKT cell activation during bacterial infection. *J Immunol* 191, 5646-5654 (2013)

18. M. Kronenberg & Y. Kinjo: Innate-like recognition of microbes by invariant natural killer T cells. *Curr Opin Immunol* 21, 391-396 (2009)

19. 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, A. Bendelac: Lysosomal glycosphingolipid recognition by NKT cells. *Science* 306, 1786-1789 (2004)

20. T. Plati, I. Visigalli, A. Capotondo, M. Buono, L. Naldini, M.P. Cosma, A. Biffi: Development and maturation of invariant NKT cells in the presence of lysosomal engulfment. *Eur J Immunol* 39, 2748-2754 (2009)

21. B. Wei, G. Wingender, D. Fujiwara, D.Y. Chen, M. McPherson, S. Brewer, J. Borneman, M. Kronenberg, J. Braun: Commensal microbiota and CD8+ T cells shape the formation of invariant NKT cells. *J Immunol* 184, 1218-1226 (2010)

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

23. 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, M. Kronenberg: Natural killer T cells recognize diacylglycerol antigens from pathogenic bacteria. *Nat Immunol* 7, 978-986 (2006)

24. Y. Kinjo, P. Illarionov, J.L. Vela, B. Pei, E. Girardi, X. Li, Y. Li, M. Imamura, Y. Kaneko, A. Okawara, Y. Miyazaki, A. Gómez-Velasco, P. Rogers, S. Dahesh, S. Uchiyama, A. Khurana, K. Kawahara, H. Yesilkaya, P.W. Andrew, C.H. Wong, K. Kawakami, V. Nizet, G.S. Besra, M. Tsuji, D.M. Zajonc, M. Kronenberg: Invariant natural killer T cells recognize glycolipids from pathogenic Gram-positive bacteria. *Nat Immunol* 12, 966-974 (2011)

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

26. S. Fujii, K. Liu, C. Smith, A.J. Bonito, R.M. & Steinman: The linkage of innate to adaptive immunity via maturing dendritic cells *in vivo* requires CD40 ligation in addition to antigen presentation and CD80/86 costimulation. *J Exp Med* 199, 1607-1618 (2004)

27. D.V. Baev, S. Caielli, F. Ronchi, M. Coccia, F. Facciotti, K.E. Nichols, M. Falcone: Impaired SLAM-SLAM homotypic interaction between invariant NKT cells and dendritic cells affects differentiation of IL-4/IL-10-secreting NKT2 cells in nonobese diabetic mice. *J Immunol* 181, 869-877 (2008)

28. D. Ly, Q.S. Mi, S. Hussain & 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)

29. R. Liu, A. La Cava, X.F. Bai, Y. Jee, M. Price, D.I. Campagnolo, P. Christadoss, T.L. Vollmer, L. Van Kaer, 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)

30. J.M. Coquet, S. Chakravarti, K. Kyparissoudis, F.W. McNab, L.A. Pitt, B.S. McKenzie, S.P. Berzins, M.J. Smyth, D.I. Godfrey: Diverse cytokine production by NKT cell subsets and identification of an IL-17-producing CD4-NK1.1.- NKT cell population. *Proc Natl Acad Sci U S A* 105, 11287-11292 (2008)

31. A.V. Rachitskaya, A.M. Hansen, R. Horai, Z. Li, R. Villasmil, D. Luger, R.B. Nussenblatt, R.R. Caspi: Cutting edge: NKT cells constitutively express IL-23 receptor and RORgammat and rapidly produce IL-17 upon receptor

ligation in an IL-6-independent fashion. *J Immunol* 180, 5167-5171 (2008)

32. M.L. Michel, D. Mendes-da-Cruz, A.C. Keller, M. Lochner, E. Schneider, M. Dy, G. Eberl, M.C. Leite-de-Moraes: Critical role of ROR-gammat in a new thymic pathway leading to IL-17-producing invariant NKT cell differentiation. *Proc Natl Acad Sci U S A* 105, 19845-19850 (2008)

33. Y. Simoni, A.S. Gautron, L. Beaudoin, L.C. Bui, M.L. Michel, X. Coumoul, G. Eberl, M. Leite-de-Moraes, A. Lehuen: NOD mice contain an elevated frequency of iNKT17 cells that exacerbate diabetes. *Eur J Immunol* 41, 3574-3585 (2011)

34. P. Dellabona, E. Padovan, G. Casorati, M. Brockhaus & 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)

35. T. Yoshimoto & 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)

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

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

38. 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, 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)

39. 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, 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)

40. S. Kojo, Y. Adachi, H. Keino, M. Taniguchi & 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)

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

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

43. S.B. Wilson, S.C. Kent, H.F. Horton, A.A. Hill, P.L. Bollyky, D.A. Hafler, J.L. Strominger, 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)

44. A. Kukreja, G. Cost, J. Marker, C. Zhang, Z. Sun, K. Lin-Su, S. Ten, M. Sanz, M. Exley, B. Wilson, S. Porcelli, N. Maclaren: Multiple immuno-regulatory defects in type-1 diabetes. *J Clin Invest* 109, 131-140 (2002)

45. Z. Illes, T. Kondo, J. Newcombe, N. Oka, T. Tabira, 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)

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

47. H.J. van der Vliet, B.M. von Blomberg, N. Nishi, M. Reijm, A.E. Voskuyl, A.A. van Bodegraven, C.H. Polman, T. Rustemeyer, P. Lips, A.J. van den Eertwegh, G. Giaccone, R.J. Scheper, H.M. 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)

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

49. Y. Ikehara, Y. Yasunami, S. Kodama, T. Maki, M. Nakano, T. Nakayama, M. Taniguchi, 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)

50. 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, 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)

51. T. Yoshimoto, A. Bendelac, J. Hu-Li & W.E. 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)

52. G. Gigli, S. Caielli, D. Cutuli & 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)

53. R. Furlan, A. Bergami, D. Cantarella, E. Brambilla, M. Taniguchi, P. Dellabona, G. Casorati, G.R. 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)

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

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

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

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

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

59. M. Falcone, F. Facciotti, N. Ghidoli, P. Monti, S. Olivieri, L. Zaccagnino, E. Bonifacio, G. Casorati, F. Sanvito, 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)

60. M.A. Mieza, T. Itoh, J.Q. Cui, Y. Makino, T. Kawano, K. Tsuchida, T. Koike, T. Shirai, H. Yagita, A. Matsuzawa, H. Koseki, 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)

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

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

63. 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, 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)

64. Q.S. Mi, D. Ly, P. Zucker, M. McGarry & 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)

65. L. Beaudoin, V. Laloux, J. Novak, B. Lucas & 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)

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

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

68. M.S. Vincent, J.E. Gumperz, M.B. Brenner: CD1-dependent dendritic cell instruction. *Nat Immunol* 3, 1163-1168 (2002)

69. Y.N. Naumov, K.S. Bahjat, R. Gausling, R. Abraham, M.A. Exley, Y. Koezuka, S.B. Balk, J.L. Strominger, M. Clare-Salzer, 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)

70. V. Saxena, J.K. Ondr, A.F. Magnusen, D.H. Munn & 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)

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

72. S. Caielli, C. Conforti-Andreoni, C. Di Pietro, V. Usuelli, E. Badami, M.L. Malosio, M. Falcone: On/off TLR signaling decides proinflammatory or tolerogenic dendritic cell maturation upon CD1d-mediated interaction with invariant NKT cells. *J Immunol* 185, 7317-7329 (2010)

73. V. Cerundolo, J.D. Silk, S.H. Masri & M. Salio: Harnessing invariant NKT cells in vaccination strategies. *Nat Rev Immunol* 9, 28-38 (2009)

74. S. Fujii, K. Shimizu, C. Smith, L. Bonifaz & 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)

Key Words: Review, Natural Killer T cells, Autoimmune diseases, Immune Tolerance, Dendritic Cells, Cytokines, Review

Send correspondence to: Marika Falcone, Experimental Diabetes Unit, Diabetes Research Institute, Department of Immunology, Transplantation and Infectious Diseases, San Raffaele Scientific Institute, Via Olgettina 60, 20132 Milan, Italy, Tel: 390226432317, Fax: 390226432914, E-mail: falcone.marika@hsr.it