[Frontiers in Bioscience 9, 2577-2587, September 1, 2004]

FUNCTIONAL ROLES OF NKT CELL IN THE IMMUNE SYSTEM

Ken-ichiro Seino and Masaru Taniguchi

Laboratory for Immune Regulation, RIKEN Research Center for Allergy and Immunology, Suehiro-cho 1-7-22, Tsurumi, Yokohama, Kanagawa 230-0045, Japan

TABLE OF CONTENTS

1. ABSTRACT

CD1d-restricted Natural Killer T cells (NKT cells), a novel lymphocyte lineage, are considered to play an intermediary role bridging innate and acquired immunity. This review discusses the characteristics of NKT cells and their biological significance in the immune system, and summarizes their *in vivo* functions observed in a number of pathological settings, including infectious diseases, cancer, autoimmunity, and transplantation. Further, we discuss recent data that have generated considerable interest in utilizing NKT cells as targets of new therapeutic interventions in various human diseases.

2. INTRODUCTION

CD1d-restricted Natural Killer T cells (hereafter: NKT cells) constitute a distinct lymphocyte subpopulation with unique characteristics. NKT cells possess several properties that distinguish them from classical MHC-restricted T cells and that may be directly related to their unique functions and significance in the immune system (reviewed in ref. 1-4). A number of studies have indicated that NKT cells are involved in various immune responses, exhibiting both protective and regulatory functions. Also, the modulation of NKT cells can lead to the amelioration of several immune-related diseases. This article first gives an outline of the properties of NKT cells compared to those of classical MHC-restricted T cells and discusses their biological significance (*Section 3*). Then, the *in vivo* functions of NKT cells are summarized and possible therapeutic applications of NKT cells are discussed (*Section 4, 5*).

3. PROPERTIES OF NKT CELLS

3.1. Invariant antigen receptor expression

NKT cells co-express a semi-invariant antigen receptor and NK cell markers, such as NK1.1 (5, 6). The antigen receptor expressed on mouse NKT cells is encoded by invariant Vα14-Jα281 gene segments and has a human homologue, Vα24-JαQ (5, 7). Both receptors recognize glycolipid antigens presented by a monomorphic MHC-like molecule, CD1d (8). These properties of NKT cells are distinct from those of conventional T cells which recognize diverse peptide antigens bound by polymorphic MHC molecules.

The Vα14-Jα281 chain is used only by the CD1d-restricted, glycolipid-reactive NKT cells, but not by classical MHC-restricted T cells, despite the fact that the Vα14 and Jα281 genes are located in the T cell antigen receptor gene cluster on the murine chromosome 14. In fact, the deletion of the Jα281 gene resulted in the loss of Vα14 antigen receptor expression and caused a complete failure of Vα14-positive, glycolipid-reactive NKT cell development while leaving other lymphoid lineages intact (9). On the other hand, the development of conventional

MHC-restricted T cells was impaired in Vα14/Vβ8.2 transgenic mice after crossing with either Cα-deficient or RAG-1-deficient mice (NKT mice) (10, 11). These observations strongly suggest that the Vα14/Vβ8.2 receptor is indispensable for the generation of CD1d-restricted, glycolipid-reactive NKT cells.

3.2. Recognition of glycolipid antigen

The CD1d molecule is highly conserved among mammalian species and has an MHC-like fold with two large hydrophobic binding grooves that are adapted to present nonpeptidic antigens (12-14). The ligand for NKT cells was found to be α-galactosylceramide (α-GalCer), a glycolipid composed of a hydrophilic carbohydrate moiety with a α-linkage to the hydrophobic ceramide portion (11). Functional analyses using various analogues of α-GalCer revealed that the length of the ceramide carbon chains is crucial; a reduction in the length of either the fatty acyl chains or the sphingosine base reduces the ability of α-GalCer to induce NKT cell proliferation (11). A structural model of the docking of α-GalCer to CD1d shows that the 2"-OH and 3"-OH on the sugar moiety and the 3-OH and the amide nitrogen on the ceramide portion are crucial for stable binding (15). Both Arg79 and Asp80 on CD1d seem to interact with the 2"-OH group, while both Asp80 and Glu83 interact with the 3"-OH group of the carbohydrate moiety. In addition, the amide nitrogen on the fatty acyl chain and the 3-OH on the sphingosine seem to interact with Asp153 and Val149, respectively. Interestingly, nonfunctional glycolipids, such as β-GalCer, α-GalCer lacking 3-OH on the sphingosine, or N-dipalmitoyl-L-α-phosphatidylethanolamine, can bind to CD1d with an affinity similar to that of the functional glycolipid (16). Thus, CD1d appears to have an ability to bind a variety of lipid-containing antigens regardless of their stimulatory activities, but only α-GalCer and α-GluCer are able to stimulate NKT cells. Obviously, α-GalCer seems not to be an endogenous ligand for NKT cells, because α-glycosphingolipids could not be detected in mammalians. Nevertheless, α-GalCer might mimic self-antigens that are recognized by NKT cells. α-GalCer has been extensively used to study the *in vivo* functions of NKT cells in various immune responses, as summarized in *Section 4* of this review.

3.3. Autoreactivity and rapid cytokine secretion

It has been well known for some time that NKT cells can rapidly (within several hours) secrete large amounts of cytokines upon ligand stimulation. Although the precise molecular mechanism enabling this quick action remains unclear, two recent studies point to a novel inter- and intra-cellular machinery responsible for this unique response of NKT cells (17, 18).

Accumulative evidence suggests that NKT cells recognize an endogenous antigen *in vivo*, although no endogenous ligand has been identified yet. In fact, freshly isolated NKT cells express activated or memory phenotypes ($CD44^{+}CD62L^{low}CD69^{+}$), indicating the *in vivo* autoreactivity of NKT cells (19). Moreover, NKT cells fail to develop in CD1d-deficient mice, suggesting that the recognition of self ligand/CD1d is essential for NKT cell development in the thymus (20-22). These results indicate NKT cells belong to the autoreactive repertoire and are always activated *in vivo*. Recent findings reported by Brenner and his colleagues (17) strongly support this notion and also provide a novel concept for the mechanisms of rapid cytokine production upon activation of NKT cells in protective immunity. According to their data (17), NKT cells are sub-optimally activated but show no functional activities (i.e. cytokine production or proliferation), under physiological condition. However, once NKT cells receive IL-12 signals through toll-like receptors, they become activated to produce cytokines, such as IFN-γ. In fact, weak responses of NKT cells to CD1d-presented self-antigens are amplified by IL-12 produced by dendritic cells (DCs) in response to microbial products sensed through Toll-like receptors. Subsequently, NKT cells produce IFN-γ by which in turn activates both innate and adaptive immune cells, such as NK cells and macrophages and pathogen-specific Th1 CD4 and cytotoxic CD8 T cells, respectively. Therefore, pathogens or their products may not activate NKT cells directly. These findings may explain the mechanism of rapid activation of NKT cells in a variety of microbial infections without specific foreign antigen recognition.

In addition to autoreactivity, NKT cells seem to use a unique transcriptional machinery to exert a quick immune response. Stetson, *et al*. recently showed that NKT cells maintain distinct patterns of constitutive cytokine mRNAs (18). Unlike classical T cells, NKT cells activate the induction of IFN-γ and IL-4 transcription during thymic development and populate the periphery with both cytokine loci previously modified by histone acetylation. These data indicate that NKT cells contain chromatin modifications at cytokine genes in a manner that promotes access by transcription factors. This induces the presence of abundant cytokine mRNAs in NKT cells that, in turn, enable the rapid secretion of cytokines upon activation. These lineage-specific patterns of cytokine transcripts predate infection and suggest an evolutionary selection for invariant but distinct types of effector responses among the earliest responding lymphocytes (18).

In any event, since mice lacking NKT cells are unable to protect themselves from various infectious diseases (see *Section 4.1.1*), NKT cells are deemed essential for protective immunity. Moreover, the findings by Brenner and his colleagues provide evidence for the important functional role of self-ligands in the activation of NKT cells and thus add to our understanding of NKT cells as a "bridging system" between innate and acquired immunity.

3.4. Anti-apoptotic nature

Quickly after the activation with α-GalCer, NKT cells become undetectable when assessed by flow cytometry with α-GalCer/CD1d tetramers or anti-NK1.1 antibody staining (23-27). This phenomenon had initially been attributed to activation-induced cell death (23, 24) mediated by up-regulated Fas-Fas ligand interaction (24). However, recent studies have demonstrated that the

activation of NKT cells induces down-regulation of the invariant Vα14 antigen receptor, rather than apoptosis, and thereafter causes a dramatic expansion of NKT cells all while preserving their ability to produce cytokines (25-27). The anti-apoptotic nature of NKT cells seems to be due to the up-regulation of several anti-apoptotic genes, such as the NAIP and MyD118 genes (27). Again, these are unique characteristics of NKT cells that are not found in classical MHC-restricted T cells. Such conventional T cells are rather sensitive to activation-induced apoptosis and become tolerant soon after down-regulation of their antigen receptors.

It is now well documented that $CD25^+CD4^+$ regulatory T cells contribute to the maintenance of self or non-self tolerance (28). Interestingly, $CD25^+CD4^+$ regulatory T (T_{reg}) cells are also reported to be relatively resistant to activation-induced apoptosis (29). This anti-apoptotic behavior of T_{reg} cells is accompanied by the up-regulation of several genes related to anti-apoptosis and cell survival. These genes include Fas-associated phosphatase 1, mitogen-activated protein kinase phosphatase 1, and members of the tumor necrosis factor receptor (TNFR)-nerve growth factor receptor superfamily, such as TNFR2, OX40, 4-1BB, and glucocorticoid-induced TNFR family-related genes (30). Furthermore, it is worthwhile to note that T_{reg} cells are also autoreactive (31, 32). It is possible that the anti-apoptotic properties and autoreactivity found in both NKT and T_{reg} cells are related to the regulatory function of these lymphocyte sub-populations and, in fact, constitute a general feature of regulatory cells. Further investigation of these properties may thus offer new clues for the understanding of the functions and homeostasis of NKT cells and T_{reg} cells, and more generally, the phenomenon of immunoregulation.

4. *IN VIVO* FUNCTIONS OF NKT CELLS

The function and importance of NKT cells in various diseases has been extensively examined over the past few years—not only in mouse models but also in humans. As mentioned above, NKT cells interact with both the innate and the acquired immune systems. NKT cells exhibit both protective and regulatory roles in the immune system, and our discussion below follows this classification.

4.1. Protective role

4.1.1. Bacterial and fungal infections

In several bacterial and fungal infection models, it has been demonstrated that NKT cells are indispensable to eliminate the pathogens and contribute to the survival of the hosts. Recently, Kawakami *et al*. designed a model to elucidate the role of NKT cells in the host defense against pulmonary infection with *Streptococcus pneumoniae* (33). In NKT cell-deficient mice, pneumococcal infection was severely exacerbated, as shown by the shorter survival time and marked increase of live bacteria in the lung compared to wild-type mice. The proportion of NKT cells, detected by α-GalCer-loaded CD1d tetramer, increased in the lung after *S. pneumoniae* infection. This increase was significantly reduced in mice with a genetic disruption of monocyte chemotactic protein (MCP)-1, which was produced in the early phase of infection in wild-type mice. In NKT cell-deficient mice, the number of neutrophils as well as macrophage inflammatory protein (MIP)-2 and TNF-α synthesis in the lungs was significantly lower than in wild-type mice. In addition, treatment of mice with α-GalCer significantly improved the outcome of this infection. Similarly, in a murine pneumonia model established by intranasal application of *Pseudomonas aeruginosa*, CD1d-deficient mice showed markedly reduced pulmonary eradication of *P. aeruginosa* compared with wild-type mice. This was associated with significantly lower amounts of MIP-2 and reduced number of neutrophils within the bronchoalveolar lavage fluid (34). Also, treatment of mice with α-GalCer increased the amount of IFN-γ; this was associated with rapid pulmonary clearance through enhanced phagocytosis of *P. aeruginosa* by alveolar macrophages. These results reveal a crucial role played by CD1d-restricted, α-GalCer-reactive NKT cells in regulating antimicrobial immune functions.

Unlike the specific recognition of mycobacterial cell-wall antigens that are presented by human CD1b and CD1c molecules (35), CD1d-mediated responses are not crucial for the resistance to *Mycobacterium tuberculosis* (36, 37). Despite this fact, Vα14 NKT cells are required for the formation of granulomatous lesions after the injection of deproteinated bacterial cell walls (mycobacterial oligomannosylated GPI, particularly PIMs) (38). This granuloma formation is an early immune response of the host during tuberculosis infection that is thought to contribute to the prevention of tuberculosis bacilli dissemination. The recruitment of NKT cells to granulomas is independent of CD1d, and does not require recognition of cognate antigen by the invariant Vα14 antigen receptor, because it can occur even when NKT cells are adoptively transferred to CD1d-deficient mice (39).

Cryptococcus neoformance is a ubiquitous fungal pathogen that causes granulomatous lesions in the lung and disseminates to the central nervous system, frequently leading to lethal meningoencephalitis, particularly in AIDS patients. IFN-γ produced by NKT cells critically controls the Th1-dependent host defense against this pathogen (40). In particular, NKT cell numbers were not increased in the lungs of MCP-1-deficient mice by Cryptococcus infection (unlike wild-type mice), suggesting that infection causes MCP-1 production followed by the recruitment of NKT cells. Also, elimination of this fungal pathogen was drastically delayed in NKT cell-deficient mice, due to the limited IFN-γ production and the failure to induce protective responses (41).

4.1.2. Parasitic infections

α-GalCer-activated NKT cells have potent anti-malaria activity, inhibiting the development of intrahepatic stages of the rodent malaria parasites *Plasmodium yoelii* and *Plasmodium berghei*. NKT cell-deficient, CD1d-deficient, and IFN-γ-deficient mice failed to show α-GalCer-mediated protection against malaria infection (42). This anti-malaria activity can be elicited in the absence of

NK, B and T cells, suggesting that NKT cells serve as effector cells to control parasite replication in the liver. Most recently, it was shown that NKT cells not only exert a direct inhibitory activity against the liver stage of malaria but also play a role in enhancing memory responses elicited by malaria vaccines, including irradiated sporozoites and a recombinant circumsporozoite protein (43). The increased protective immunity is due to the increased level of malaria-specific $CD8^+$ T cell responses by α-GalCer-activated NKT cells. The effect of α-GalCer was abolished in mice lacking CD1d, IFN-γ receptor or NKT cells, indicating that IFN-γ produced by NKT cells mediates the adjuvant activity of α-GalCer. Similar enhancing effects of activated NKT cells on antigen-specific $CD8^+$ effector T cell function have also been reported in other settings such as tumor rejection (44, 45).

4.1.3. Viral infections

It is well known that IFN-γ and IFN-α̃β inhibit hepatitis B virus (HBV) replication. Interestingly, intrahepatic Vα14 NKT cells were activated to produce IFN-γ and IFN-α/β within 24 hrs and inhibited HBV replication when administered with α-GalCer into HBV transgenic mice (46). Since the antiviral activity by α-GalCer was abolished in mice deficient for either IFN-γ- or IFN-α̃βreceptor, most of the antiviral activity was apparently mediated by these cytokines derived from NKT cells. However, another CD1d-reactive population which does not express an invariant Vα14 and recognize α-GalCer was indicated to be required in the anti-HBV response (47). In addition to virus elimination, the activation of intrahepatic NKT cells is also involved in the liver injury. In concanavalin A-induced hepatitis, NKT cells appear to be essential, because NKT cell-deficient mice do not develop the hepatocyte injury (48). Both perforin and FasL produced by NKT cells are required as effector mechanism (48). This is similar to the cytotoxic mechanism of HBV-specific CTL, in which both signaling pathways must be activated simultaneously in order to kill the hepatocytes *in vivo* (49).

Similarly, NKT cells seem to be required for virus elimination in in herpes simplex virus infection (50), but not in cytomegalovirus infection (51). But, even in the latter case, α-GalCer injections contributed to the reduction of viral replication in visceral organs (51). In this context, it should be noteworthy that human $CD4^+$Vα24 NKT cell express CCR5 and CXCR6 (HIV coreceptors), and are thus more susceptible to HIV infection than conventional $CD4^+$ T cells (52). Selective loss of $CD4^+$Vα24 NKT cells in HIV-infected individuals has been reported (52, 53).

4.1.4. Tumor defense

Many reports support the hypothesis that treatment with α-GalCer is beneficial to the prevention of the growth and metastasis of certain tumors in mice (reviewed in ref. 54). This effect occurs at least in part through NK-like direct cytotoxic action of NKT cells on tumor cells, because Vα14Vβ8 transgenic mice but not NKT cell-deficient mice were protected against experimental liver metastasis of melanoma upon stimulation with α-GalCer *in vivo* (11). However, NK and $CD8^+$ T cells also contribute to the cytotoxic effector mechanisms as a secondary effect of IFN-γproduced by activated NKT cells (45, 55). The anti-tumor immune response elicited by α-GalCer can be enhanced when administered as α-GalCer-pulsed DCs rather than by direct injection (56, 57). These observations suggest the possibility that α-GalCer-pulsed DCs might constitute an effective new tool in cancer immunotherapy and clinical studies in humans are now in progress in several centers around the world. Since 2002, and after confirming that the functions of DCs and Vα24 NKT cells in lung caner patients are preserved (58), we have undertaken a Phase I clinical trial to examine the safety of α-GalCe/DC therapy for unresectable lung cancer at Chiba University (Chiba, Japan). To date, no serious, undesirable effects have been observed and we plan to progress towards Phase II/III clinical tests in the near future.

NKT cells also play a decisive immunosurveillance role in methylcholanthrene-induced fibrosarcoma development (59, 60). This effect depends on CD1d recognition and requires the additional involvement of both NK and $CD8^+$ T cells. IFN-γ production by both NKT cells and downstream, non-Vα14 NKT cells, was essential for protection, and perforin production by effector cells was also found to be critical. These studies demonstrated that, in addition to their importance in tumor immunotherapy induced by IL-12 or α-GalCer, NKT cells can play a critical role in physiological tumor immunosurveillance, at least against methylcholanthrene-induced sarcomas, in the absence of exogenous stimulation. In this tumor immunosurveillance system, it is an important issue to determine whether NKT cells react with some glycolipid antigens derived from the tumor cells themselves or else are activated through signals such as IL-12 derived from DCs as observed in microbial infection (see *Section 3.3* and ref. 17).

4.2. Regulatory role

It has been also indicated that NKT cells can exert immuno-regulatory functions in the models such as autoimmune diseases or transplantation. The molecular mechanisms behind these regulatory functions are not yet fully understood, but the potent cytokine production by NKT cells seems to be important. Furthermore, recent data indicated that NKT cells also contribute to generating regulatory DCs.

4.2.1. Regulatory interaction between NKT cells and DCs

It has been postulated that the immune-regulatory functions of NKT cells are, at least in part, linked to the generation of regulatory DCs (61). Naumov *et al.* have reported that treatment of NOD mice with repeated injection by α-GalCer induced an accumulation of $CD8\alpha^-$ DCs in pancreatic lymph nodes, and inhibited disease development (62). These results suggest that the interaction between NKT cells and DCs is operated in the regulatory responses mediated by NKT cells. In fact, a single injection of α-GalCer into NOD mice induced rapid maturation of

DCs, manifested by up-regulation of co-stimulatory molecules and proinflammatory cytokine production. By contrast, DCs derived from mice after several injections of α-GalCer showed a non-maturated phenotype and up-regulation of the production of IL-10—a regulatory cytokines. These data suggest a novel mechanism by which NKT cells regulate the immune system through the generation of regulatory DCs (Kojo, *et al*. unpublished).

4.2.2. Autoimmunity

Since many autoimmune diseases are characterized by polarized T-helper cell responses that can be affected by NKT cells, a role for NKT cells in the regulation of autoimmune diseases has been proposed. In fact, the selective reduction in Vα24/Vβ11$^+$ NKT cell numbers has been shown in human patients with various autoimmune diseases, such as systemic sclerosis (63), SLE (64), rheumatoid arthritis (64), type 1 diabetes (65-67), and multiple sclerosis (68). Similar findings were also observed in autoimmune prone MRL/lpr or NZB/NZW F1 mice (69, 70).

4.2.3. Type 1 Diabetes

The strongest evidence in support of a role for NKT cells in the regulation of autoimmune disease is provided by studies of type 1 diabetes. NOD mice develop spontaneous autoimmune Type 1 diabetes as a result of Th1-mediated destruction of pancreatic islet cells. Several studies have shown a defect in the number and function of NKT cells in NOD mice, and it is suggested that the disease could be ameliorated by the transfer of cell populations that are enriched for NKT cells (71-73). Besides the reduced number of Vα14 NKT cells, abnormal Th2 cytokine production seems to be associated with disease development. In fact, protection against diabetes development correlated with recovered production of Th2 cytokines, such as IL-4 and IL-10 (73). Furthermore, treatment of NOD mice with the Th1 cytokine IL-12 or anti-Th2 cytokine monoclonal antibodies (mAb), such as anti-IL-4, abolished protective effects by NKT cells (74). These results indicate that diabetes development is tightly associated with the defect in Th2 polarization, while disease protection is accompanied by recovery of Th2 cytokine production. Also, the crossing of NOD mice to CD1d-deficient mice caused earlier onset and increased frequency of the disease (75, 76), further confirming the correlation between type1 diabetes and Vα14 NKT cells.

Activation of NKT cells with α-GalCer ameliorates the disease in NOD mice (62, 75, 77, 78). The prevention of autoimmune diabetes development in NOD mice has been correlated with the ability of α-GalCer to enhance Th2 cytokines (IL-4/IL-10) and suppress IFN-γ production, resulting in islet specific protective Th2 cell generation. However, it has been also indicated that recruitment of tolerogenic myeloid DCs to pancreatic lymph nodes, rather than the Th1/Th2 cytokine imbalance, is important for the protection as described above (62). Furthermore, Beaudoin *et al*. recently reported evidence for NKT cell-mediated regulation using a model of NKT cell transfer into T cell-deficient (Cα-deficient) NOD mice with injection of islet-specific BDC2.5 T cells (79). The transferred Vα14 NKT cells inhibited the differentiation of BDC2.5 T cells into IFN-γ producers by preventing their late expansion and proliferation. Therefore, in addition to the Th2 deviation, NKT cells may exhibit regulatory functions by inducing a kind of tolerogenic DCs, and by inducing anergy in effector T cells.

Numerical and functional abnormalities similar to those found in NKT cells were also found in patients with type 1 diabetes (65, 67). However, one controversial study also indicated that there was a broad range in the frequency of Vα24 NKT cells present in the blood, with no significant difference between controls and patients (80). It was argued that this discrepancy might be attributed to different methods to detect "NKT cells"; earlier works used a combination of anti-CD3/Vα24/Vβ11 monoclonal antibodies whereas more recent studies typically use α-GalCer-loaded CD1d tetramers. Furthermore, differences in patient populations, including race and age, may have been responsible for the discordant results. Considering the important role of NKT cells in type 1 diabetes, further studies with larger patient groups are needed.

4.2.4. Multiple sclerosis and experimental allergic encephalitis (EAE)

Multiple sclerosis is a chronic inflammatory disease of the central nervous system in humans. A decrease in Vα24 mRNA in the peripheral blood of patients suffering from multiple sclerosis has been demonstrated (68). Also, a Vα24 NKT cell line from multiple sclerosis patients in the remission stage showed a Th2 cytokine bias when compared to that from relapsed patients (81). These results suggested a regulatory role of Vα24 NKT cells in this disease. Murine NKT cells also correlate with the pathogenesis of EAE, a murine model of multiple sclerosis, that is well documented by the results obtained from treatment of EAE with α-GalCer or its analogue, OCH (82-84). Similar to the NOD model of diabetes, when α-GalCer was effective it usually prevented EAE by shifting the balance from a pathogenic Th1 towards a protective Th2 response to CNS antigens. However, in a recent study it was demonstrated that Vα14 NKT cell protection from EAE was conversely mediated by IFN-γ, but not IL-4, and in turn, suppressed Th1-cytokine production and fostered secretion of IL-10 from myelin oligodendrocyte glycoprotein-specific T cells (85). This study also showed that the route of administration of α-GalCer is critical for eliciting regulatory function.

4.2.5. Transplantation and other immuno-regulatory functions

The regulatory activity of NKT cells seems to be implicated in the induction or maintenance of immune tolerance. We and others have tested whether NKT cells played an important role in the induction of allo- or xeno-graft acceptance. By using a murine cardiac allograft model where costimulatory molecules are blocked, we demonstrated that NKT cells are required in the induction of allograft tolerance *in vivo* (86). Similarly, the involvement of NKT cells in transplant tolerance was demonstrated in xenogenic pancreatic islet transplantation

combined with the administration of anti-CD4 monoclonal antibody (87). In these two reports, the adoptive transfer of NKT cells restored the long-term acceptance of allo- and xeno-grafts in NKT cell-deficient mice. In both cases, however, the straightforward involvement of Th2 cytokines could not be identified, and the treatment of cardiac transplant hosts with α-GalCer failed to prolong graft survival (unpublished data). This could be due to differences in the models used, which induce different levels of immune response, and may indicate a general difficulty to overcome the allogenic barrier. Sonoda *et al*. reported that CD1d-reactive NKT cells were essential for allogenic corneal graft survival (88). Moreover, the presence of allospecific T regulatory cells (probably $CD8^+$ cells, ref. 89) correlated well with the acceptance of allogenic corneal grafts, and the adoptive transfer of these cells to $J\alpha281^{-/-}$ mice lead to an improved survival rate. The authors concluded that CD1d-reactive NKT cells are required for the induction of allospecific T regulatory cells and are thus essential for the survival of corneal allografts (88).

In a model of immune privilege in the eye, known as anterior chamber-associated immune deviation (ACAID), the involvement of NKT cells was also demonstrated (90). Tolerance was evident by a deficiency in the antigen-specific delayed-type hypersensitivity response at peripheral sites. The mechanism of immune deviation involves IL-10 produced by CD1d-reactive NKT cells as well as non-CD1d-restricted T cells with regulatory functions (91).

Down-regulation of immunity by NKT cells was also demonstrated in a model of tumor recurrence (92, 93). CTL-mediated tumor immunosurveillance of the 15-12RM tumor could be suppressed by CD1d-restricted lymphocytes, most likely NKT cells, which produced IL-13 through the IL-4α-STAT6 signaling pathway, although this negative regulation was found to be independent of IL-4. It was demonstrated that TGF-β production by $CD11b^+Gr\text{-}1^+$ cells, a mechanism that in turn is dependent on IL-13 produced by CD1d-restricted T cells, was responsible for this negative regulation. Blocking TGF-β or depleting $Gr\text{-}1^+$ cells *in vivo* prevented the tumor recurrence. These data indicate that there is an immunoregulatory circuit repressing tumor immunosurveillance in which CD1d-restricted NKT cells are involved. Further verification whether this mechanism is activated not only in a tumor system but also in other cases may contribute to a better understanding of the *in vivo* regulatory function of NKT cells.

5. PERSPECTIVES

Great progress has been made in recent years towards the elucidation of the basic immunobiology of Vα14 and Vα24 NKT cells and the NKT cell/CD1d system is now well established as a new immunological system based on glycolipid-recognition and with the capability to regulate the entire spectrum of immune responses. From a therapeutic point of view, the possibility of preventing disease development through the manipulation of Vα24 NKT cell functions with α-GalCer is of particular interest. The NKT cell/CD1d system is physiologically conserved among mammals and both human and mouse CD1d can bind α-GalCer and form complexes that can stimulate NKT cells from either species (94, 95). Therefore, some of the studies in rodents described above may have direct implications for clinical applications in humans. Clinical trials with α-GalCer to treat human cancers are well under way (96), but no clear data on efficacy are as yet available. A potential problem with the therapeutic application of NKT cells relates to the relatively small number of NKT cells in humans. But, while humans have fewer NKT cells than mice, it has been reported that human NKT cells can be expanded with cytokines such as IL-7 and IL-15 (97). But, while such approaches may solve the quantitative problem, we must realize that there is no assurance that increasing the number of NKT cells alone will prove therapeutically effective—qualitative modulations may also be needed. In the near future, more detailed investigations of the cellular and molecular mechanisms governing the *in vivo* functions of NKT cells will contribute to resolving the problems encountered with present therapeutic approaches using NKT cells and help establishing new therapeutic strategies for various human diseases, including cancer, infections, autoimmunity, transplant rejection, and allergic disorders.

6. ACKNOWLEDGEMENTS

We thank Mr. R. Trendl for reading the manuscript. This work was in part supported by The Program for Promotion for Fundamental Studies in Health Sciences of the Organization for Pharmaceutical Safety and Research, Japan, Grants-in-Aid for Scientific Research A (#13307011) and the Advanced and Innovational Research Program in Life Sciences from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and Kanae Foundation for Life & Socio-Medical Science.

7. REFERENCES

1. Bendelac, A. M. N. Rivera, S. H. Park & J. H. Roark: Mouse CD1-specific NK1 T cells: development, specificity, and function. *Annu Rev Immunol* 15, 535-562 (1997)

2. Godfrey, D.I., K. J. Hammond, L. D. Poulton, M. J. Smyth & A. G. Baxter: NKT cells: facts, functions and fallacies. *Immunol Today* 21, 573-583. (2000)

3. Kronenberg, M. & L. Gapin: The unconventional lifestyle of NKT cells. *Nat Rev Immunol* 2, 557-568 (2002)

4. Taniguchi, M., M. Harada, S. Kojo, T. Nakayama & H. Wakao: The regulatory role of Vα14 NKT cells in innate and acquired immune response. *Annu Rev Immunol* 21, 483-513 (2003)

5. Lantz, O. & A. Bendelac: An invariant T cell receptor

alpha chain is used by a unique subset of major histocompatibility complex class I-specific $CD4^+$ and $CD4^-$ 8^- T cells in mice and humans. *J Exp Med* 180, 1097-1106. (1994)

6. Makino, Y., R. Kanno, T. Ito, K. Higashino & M. Taniguchi: Predominant expression of invariant $V\alpha14^+$ TCR alpha chain in $NK1.1^+$ T cell populations. *Int Immunol* 7, 1157-1161 (1995)

7. Imai, K., M. Kanno, H. Kimoto, K. Shigemoto, S. Yamamoto & M. Taniguchi: Sequence and expression of transcripts of the T-cell antigen receptor α-chain gene in a functional, antigen-specific suppressor-T-cell hybridoma. *Proc Natl Acad Sci U S A* 83, 8708-8712 (1986)

8. Bendelac, A., O. Lantz, M. E. Quimby, J. W. Yewdell, J. R. Bennink & R. R. Brutkiewicz: CD1 recognition by mouse $NK1^+$ T lymphocytes. *Science* 268, 863-865 (1995)

9. Cui, J., T. Shin, T. Kawano, H. Sato, E. Kondo, I. Toura, Y. Kaneko, H. Koseki, M. Kanno & M. Taniguchi: Requirement for Vα14 NKT cells in IL-12-mediated rejection of tumors. *Science* 278, 1623-1626 (1997)

10. Taniguchi, M., H. Koseki, T. Tokuhisa, K. Masuda, H. Sato, E. Kondo, T. Kawano, J. Cui, A. Perkes, S. Koyasu & Y. Makino: Essential requirement of an invariant Vα14 T cell antigen receptor expression in the development of natural killer T cells. *Proc Natl Acad Sci USA* 93, 11025-11028 (1996)

11. Kawano, T., J. Cui, Y. Koezuka, I. Toura, Y. Kaneko, K. Motoki, H. Ueno, R. Nakagawa, H. Sato, E. Kondo, H. Koseki & M. Taniguchi: CD1d-restricted and TCR-mediated activation of Vα14 NKT cells by glycosylceramides. *Science* 278, 1626-1629 (1997)

12. Zeng, Z., A. R. Castano, B. W. Segelke, E. A. Stura, P. A. Peterson & I. A. Wilson: Crystal structure of mouse CD1: An MHC-like fold with a large hydrophobic binding groove. *Science* 277, 339-345 (1997)

13. Brossay, L., N. Burdin, S. Tangri & M. Kronenberg: Antigen-presenting function of mouse CD1: one molecule with two different kinds of antigenic ligands. *Immunol Rev* 163, 139-150 (1998)

14. Porcelli, S.A: Cutting glycolipids down to size. *Nat Immunol* 2, 191-192 (2001)

15. Kamada, N., H. Iijima, K. Kimura, M. Harada, E. Shimizu, S. Motohashi, T. Kawano, H. Shinkai, T. Nakayama, T. Sakai, L. Brossay, M. Kronenberg & M. Taniguchi: Crucial amino acid residues of mouse CD1d for glycolipid ligand presentation to Vα14 NKT cells. *Int Immunol* 13, 853-861 (2001)

16. Naidenko, O.V., J. K. Maher, W. A. Ernst, T. Sakai, R. L. Modlin & M. Kronenberg: Binding and antigen presentation of ceramide-containing glycolipids by soluble mouse and human CD1d molecules. *J Exp Med* 190, 1069-1080 (1999)

17. Brigl, M., 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)

18. Stetson, D.B., M. Mohrs, R. L. Reinhardt, J. L. Baron, Z. E. Wang, L. Gapin, M. Kronenberg & R. M. Locksley: Constitutive cytokine mRNAs mark natural killer (NK) and NK T cells poised for rapid effector function. *J Exp Med* 198, 1069-1076 (2003)

19. Benlagha, K. & A. Bendelac: CD1d-restricted mouse Vα14 and human Vα24 T cells: lymphocytes of innate immunity. *Semin Immunol* 12, 537-542 (2000)

20. Chen, Y.H., N. M. Chiu, M. Mandal, N. Wang & C. R. Wang: Impaired $NK1^+$ T cell development and early IL-4 production in CD1-deficient mice. *Immunity* 6, 459-467 (1997)

21. Mendiratta, S.K., W. D. Martin, S. Hong, A. Boesteanu, S. Joyce & L. Van Kaer: CD1d1 mutant mice are deficient in natural T cells that promptly produce IL-4. *Immunity* 6, 469-477 (1997)

22. Smiley, S.T., M. H. Kaplan & M. J. Grusby: Immunoglobulin E production in the absence of interleukin-4-secreting CD1-dependent cells. *Science* 275, 977-979 (1997)

23. Eberl, G. & H. R. MacDonald: Rapid death and regeneration of NKT cells in anti-CD3ε- or IL-12-treated mice: a major role for bone marrow in NKT cell homeostasis. *Immunity* 9, 345-353 (1998)

24. Leite-de-Moraes, M.C., A. Herbelin, C. Gouarin, Y. Koezuka, E. Schneider & M. Dy: Fas/Fas ligand interactions promote activation-induced cell death of NK T lymphocytes. *J Immunol* 165, 4367-4371 (2000)

25. Wilson, M.T., C. Johansson, D. Olivares-Villagomez, A. K. Singh, A. K. Stanic, C. R. Wang, S. Joyce, M. J. Wick & L. Van Kaer: The response of natural killer T cells to glycolipid antigens is characterized by surface receptor down-modulation and expansion. *Proc Natl Acad Sci U S A* 100, 10913-10918 (2003)

26. Crowe, N.Y., A. P. Uldrich, K. Kyparissoudis, K. J. Hammond, Y. Hayakawa, S. Sidobre, R. Keating, M. Kronenberg, M. J. Smyth & D. I. Godfrey: Glycolipid antigen drives rapid expansion and sustained cytokine production by NK T cells. *J Immunol* 171, 4020-4027 (2003)

27. Harada, M: Down-regulation of invariant Vα14 antigen receptor in NKT cells upon activation. *Int Immunol* (in press)

28. Sakaguchi, S., N. Sakaguchi, J. Shimizu, S. Yamazaki, T. Sakihama, M. Itoh, Y. Kuniyasu, T. Nomura, M. Toda & T. Takahashi: Immunologic tolerance maintained by $CD25^+$ $CD4^+$ regulatory T cells: their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance. *Immunol Rev* 182, 18-32 (2001)

29. Banz, A., C. Pontoux & M. Papiernik: Modulation of Fas-dependent apoptosis: a dynamic process controlling both the persistence and death of CD4 regulatory T cells and effector T cells. *J Immunol* 169, 750-757 (2002)

30. Gavin, M.A., S. R. Clarke, E. Negrou, A. Gallegos & A. Rudensky: Homeostasis and anergy of $CD4^+CD25^+$ suppressor T cells *in vivo*. *Nat Immunol* 3, 33-41 (2002)

31. Jordan, M.S., A. Boesteanu, A. J. Reed, A. L. Petrone, A. E. Holenbeck, M. A. Lerman, A. Naji & A. J. Caton: Thymic selection of $CD4^+CD25^+$ regulatory T cells induced by an agonist self-peptide. *Nat Immunol* 2, 301-306 (2001)

32. Nishikawa, H., T. Kato, K. Tanida, A. Hiasa, I. Tawara, H. Ikeda, Y. Ikarashi, H. Wakasugi, M. Kronenberg, T. Nakayama, M. Taniguchi, K. Kuribayashi, L. J. Old & H. Shiku: $CD4^+$ $CD25^+$ T cells responding to serologically defined autoantigens suppress antitumor immune responses. *Proc Natl Acad Sci U S A* 100, 10902-10906 (2003)

33. Kawakami, K., N. Yamamoto, Y. Kinjo, K. Miyagi, C. Nakasone, K. Uezu, T. Kinjo, T. Nakayama, M. Taniguchi & A. Saito: Critical role of $V\alpha14^+$ natural killer T cells in the innate phase of host protection against Streptococcus pneumoniae infection. *Eur J Immunol* 33, 3322-3330 (2003)

34. Nieuwenhuis, E.E., 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)

35. Porcelli, S.A. & R. L. Modlin: The CD1 system: antigen-presenting molecules for T cell recognition of lipids and glycolipids. *Annu Rev Immunol* 17, 297-329 (1999)

36. Sousa, A.O., R. J. Mazzaccaro, R. G. Russell, F. K. Lee, O. C. Turner, S. Hong, L. Van Kaer & B. R. Bloom: Relative contributions of distinct MHC class I-dependent cell populations in protection to tuberculosis infection in mice. *Proc Natl Acad Sci USA* 97, 4204-4208 (2000)

37. Behar, S.M., C. C. Dascher, M. J. Grusby, C. R. Wang & M. B. Brenner: Susceptibility of mice deficient in CD1D or TAP1 to infection with Mycobacterium tuberculosis. *J Exp Med* 189, 1973-1980 (1999)

38. Apostolou, I., Y. Takahama, C. Belmant, T. Kawano, M. Huerre, G. Marchal, J. Cui, M. Taniguchi, H. Nakauchi, J. J. Fournie, P. Kourilsky & G. Gachelin: Murine natural killer T (NKT) cells contribute to the granulomatous reaction caused by mycobacterial cell walls. *Proc Natl Acad Sci USA* 96, 5141-5146 (1999)

39. Mempel, M., C. Ronet, F. Suarez, M. Gilleron, G. Puzo, L. Van Kaer, A. Lehuen, P. Kourilsky & G. Gachelin: Natural killer T cells restricted by the monomorphic MHC class 1b CD1d1 molecules behave like inflammatory cells. *J Immunol* 168, 365-371 (2002)

40. Kawakami, K., Y. Kinjo, S. Yara, Y. Koguchi, K. Uezu, T. Nakayama, M. Taniguchi & A. Saito: Activation of $V\alpha14^+$ natural killer T cells by α- galactosylceramide results in development of Th1 response and local host resistance in mice infected with Cryptococcus neoformans. *Infect Immun* 69, 213-220 (2001)

41. Kawakami, K., Y. Kinjo, K. Uezu, S. Yara, K. Miyagi, Y. Koguchi, T. Nakayama, M. Taniguchi & A. Saito: Monocyte chemoattractant protein-1-dependent increase of Vα14 NKT cells in lungs and their roles in Th1 response and host defense in cryptococcal infection. *J Immunol* 167, 6525-6532 (2001)

42. Gonzalez-Aseguinolaza, G., C. de Oliveira, M. Tomaska, S. Hong, O. Bruna-Romero, T. Nakayama, M. Taniguchi, A. Bendelac, L. Van Kaer, Y. Koezuka & M. Tsuji: α-galactosylceramide-activated Vα14 natural killer T cells mediate protection against murine malaria. *Proc Natl Acad Sci USA* 97, 8461-8466 (2000)

43. Gonzalez-Aseguinolaza, G., L. Van Kaer, C. C. Bergmann, J. M. Wilson, J. Schmieg, M. Kronenberg, T. Nakayama, M. Taniguchi, Y. Koezuka & M. Tsuji: Natural killer T cell ligand α-galactosylceramide enhances protective immunity induced by malaria vaccines. *J Exp Med* 195, 617-624 (2002)

44. Fujii, S., K. Shimizu, C. Smith, L. Bonifaz & R. M. Steinman: Activation of natural killer T cells by α-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)

45. Nakagawa, R., I. Nagafune, Y. Tazunoki, H. Ehara, H. Tomura, R. Iijima, K. Motoki, M. Kamishohara & S. Seki: Mechanisms of the antimetastatic effect in the liver and of the hepatocyte injury induced by α-galactosylceramide in mice. *J Immunol* 166, 6578-6584 (2001)

46. Kakimi, G., L. G. Guidotti, Y. Koezuka & F. V. Chisari: Natural killer T cell activation inhibits hepatitis B virus replication *in vivo*. *J Exp Med* 192, 921-930 (2000)

47. Baron, J.L., L. Gardiner, S. Nishimura, K. Shinkai, R. Locksley & D. Ganem: Activation of a nonclassical NKT cell subset in a transgenic mouse model of hepatitis B virus infection. *Immunity* 16, 583-594 (2002)

48. Kaneko, Y., M. Harada, T. Kawano, M. Yamashita, Y. Shibata, F. Gejyo, T. Nakayama & M. Taniguchi: Augmentation of Vα14 NKT cell-mediated cytotoxicity by interleukin 4 in an autocrine mechanism resulting in the development of concanavalin A-induced hepatitis. *J Exp Med* 191, 105-114 (2000)

49. Nakamoto, Y., L. G. Guidotti, V. Pasquetto, R. D. Schreiber & F. V. Chisari: Differential target cell sensitivity to CTL-activated death pathways in hepatitis B virus transgenic mice. *J Immunol* 158, 5692-5697 (1997)

50. Grubor-Bauk, B., A. Simmons, G. Mayrhofer & P. G. Speck: Impaired Clearance of Herpes Simplex Virus Type 1 From Mice Lacking CD1d or NKT Cells Expressing the Semivariant Vα14-Jα281 TCR. *J Immunol* 170, 1430-1434 (2003)

51. Van Dommelen, S.L., H. A. Tabarias, M. J. Smyth & M. A. Degli-Esposti: Activation of Natural Killer (NK) T Cells during Murine Cytomegalovirus Infection Enhances the Antiviral Response Mediated by NK Cells. *J Virol* 77, 1877-1884 (2003)

52. Motsinger, A., D. W. Haas, A. K. Stanic, L. Van Kaer, S. Joyce & D. Unutmaz: CD1d-restricted human natural killer T cells are highly susceptible to human immunodeficiency virus 1 infection. *J Exp Med* 195, 869-879 (2002)

53. Sandberg, J.K., N. M. Fast, E. H. Palacios, G. Fennelly, J. Dobroszycki, P. Palumbo, A. Wiznia, R. M. Grant, N. Bhardwaj, M. G. Rosenberg & D. F. Nixon: Selective loss of innate $CD4^+$ Vα24 natural killer T cells in human immunodeficiency virus infection. *J Virol* 76, 7528-7534 (2002)

54. Smyth, M.J., N. Y. Crowe, Y. Hayakawa, K. Takeda, H. Yagita & D. I. Godfrey: NKT cells - conductors of tumor immunity? *Curr Opin Immunol* 14, 165-171 (2002)

55. Smyth, M.J., N. Y. Crowe, D. G. Pellicci, K. Kyparissoudis, J. M. Kelly, K. Takeda, H. Yagita & D. I. Godfrey: Sequential production of interferon-γ by $NK1.1^+$ T cells and natural killer cells is essential for the antimetastatic effect of α-galactosylceramide. *Blood* 99, 1259-1266 (2002)

56. Toura, I., T. Kawano, Y. Akutsu, T. Nakayama, T. Ochiai & M. Taniguchi: Cutting edge: inhibition of experimental tumor metastasis by dendritic cells pulsed with α-galactosylceramide. *J Immunol* 163, 2387-2391 (1999)

57. Fujii, S., K. Shimizu, M. Kronenberg & R. M. Steinman: Prolonged IFN-γ-producing NKT response induced with α-galactosylceramide-loaded DCs. *Nat Immunol* 3, 867-874 (2002)

58. Motohashi, S., S. Kobayashi, T. Ito, K. K. Magara, O. Mikuni, N. Kamada, T. Iizasa, T. Nakayama, T. Fujisawa & M. Taniguchi: Preserved IFN-γ production of circulating Vα24 NKT cells in primary lung cancer patients. *Int J Cancer* 102, 159-165 (2002)

59. Smyth, M.J., K. Y. Thia, S. E. Street, E. Cretney, J. A. Trapani, M. Taniguchi, T. Kawano, S. B. Pelikan, N. Y. Crowe & D. I. Godfrey: Differential tumor surveillance by natural killer (NK) and NKT cells. *J Exp Med* 191, 661-668 (2000)

60. Crowe, N.Y., M. J. Smyth & D. I. Godfrey: A critical role for natural killer T cells in immunosurveillance of methylcholanthrene-induced sarcomas. *J Exp Med* 196, 119-127 (2002)

61. Wilson, S.B. & T. L. Delovitch: Janus-like role of regulatory iNKT cells in autoimmune disease and tumour immunity. *Nat Rev Immunol* 3, 211-222 (2003)

62. Naumov, Y.N., 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)

63. Sumida, T., A. Sakamoto, H. Murata, Y. Makino, H. Takahashi, S. Yoshida, K. Nishioka, I. Iwamoto & M. Taniguchi: Selective reduction of T cells bearing invariant Vα24 JαQ antigen receptor in patients with systemic sclerosis. *J Exp Med* 182, 1163-1168 (1995)

64. Kojo, S., 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)

65. Wilson, S.B., 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 & D. A. Hafler: Extreme Th1 bias of invariant Vα24JαQ T cells in type 1 diabetes. *Nature* 391, 177-181 (1998)

66. Wilson, S.B., 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 Vα24JαQ T cells from identical twins discordant for type I diabetes. *Proc Natl Acad Sci USA* 97, 7411-7416 (2000)

67. Kukreja, A., 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)

68. Illes, Z., T. Kondo, J. Newcombe, N. Oka, T. Tabira & T. Yamamura: Differential expression of NK T cell Vα24JαQ invariant TCR chain in the lesions of multiple sclerosis and chronic inflammatory demyelinating polyneuropathy. *J Immunol* 164, 4375-4381 (2000)

69. Zeng, D., 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)

70. Mieza, M.A., 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 Vα14$^+$ NK T cells associated with disease development in autoimmune-prone mice. *J Immunol* 156, 4035-4040 (1996)

71. Lehuen, A., O. Lantz, L. Beaudoin, V. Laloux, C. Carnaud, A. Bendelac, J. F. Bach & R. C. Monteiro: Overexpression of natural killer T cells protects Vα14-Jα281 transgenic nonobese diabetic mice against diabetes. *J Exp Med* 188, 1831-1839 (1998)

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

73. Hammond, K.J., L. D. Poulton, L. J. Palmisano, P. A. Silveira, D. I. Godfrey & 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)

74. Laloux, V., L. Beaudoin, D. Jeske, C. Carnaud & A. Lehuen: NK T cell-induced protection against diabetes in Vα14-Jα281 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)

75. Wang, B., Y. B. Geng & C. R. Wang: CD1-restricted NK T cells protect nonobese diabetic mice from developing diabetes. *J Exp Med* 194, 313-320 (2001)

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

77. Hong, S., 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 α-galactosylceramide prevents autoimmune diabetes in non-obese diabetic mice. *Nat Med* 7, 1052-1056 (2001)

78. Sharif, S., 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 α-galactosylceramide treatment prevents the onset and recurrence of autoimmune Type 1 diabetes. *Nat Med* 7, 1057-1062 (2001)

79. Beaudoin, L., 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)

80. Lee, P.T., 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)

81. Araki, M., T. Kondo, J. E. Gumperz, M. B. Brenner, S. Miyake & T. Yamamura: Th2 bias of CD4$^+$ NKT cells derived from multiple sclerosis in remission. *Int Immunol* 15, 279-288 (2003)

82. Jahng, A.W., 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)

83. Singh, A.K., M. T. Wilson, S. Hong, D. Olivares-Villagomez, 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)

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

85. Furlan, R., A. Bergami, D. Cantarella, E. Brambilla, M. Taniguchi, P. Dellabona, G. Casorati & G. Martino: Activation of invariant NKT cells by αGalCer administration protects mice from MOG35-55-induced EAE: critical roles for administration route and IFN-γ. *Eur J Immunol* 33, 1830-1838 (2003)

86. Seino, K.I., 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)

87. Ikehara, Y., Y. Yasunami, S. Kodama, T. Maki, M. Nakano, T. Nakayama, M. Taniguchi & S. Ikeda: CD4$^+$ Vα14 natural killer T cells are essential for acceptance of rat islet xenografts in mice. *J Clin Invest* 105, 1761-1767 (2000)

88. Sonoda, K.H., M. Taniguchi & J. Stein-Streilein: Long-term survival of corneal allografts is dependent on intact CD1d- reactive NKT cells. *J Immunol* 168, 2028-2034 (2002)

89. Hong, S. & L. Van Kaer: Immune privilege: keeping an

eye on natural killer T cells. *J Exp Med* 190, 1197-1200 (1999)

90. Sonoda, K.H., 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)

91. Sonoda, K.H., D. E. Faunce, M. Taniguchi, M. Exley, S. Balk & J. Stein-Streilein: NK T cell-derived IL-10 is essential for the differentiation of antigen-specific T regulatory cells in systemic tolerance. *J Immunol* 166, 42-50 (2001)

92. Terabe, M., S. Matsui, N. Noben-Trauth, H. Chen, C. Watson, D. D. Donaldson, D. P. Carbone, W. E. Paul & J. A. Berzofsky: TGF-β production and myeloid cell are an effector mechanism through which CD1d-restricted T cells block CTL-mediated tumor immunosurveillance: abrogation prevents tumor recurrence. *J Exp Med* 198, 1741-1752 (2003)

93. Terabe, M., S. Matsui, N. Noben-Trauth, H. Chen, C. Watson, D. D. Donaldson, D. P. Carbone, W. E. Paul & J. A. Berzofsky: NKT cell-mediated repression of tumor immunosurveillance by IL-13 and the IL-4R-STAT6 pathway. *Nat Immunol* 1, 515-520 (2000)

94. Brossay, L., M. Chioda, N. Burdin, Y. Koezuka, G. Casorati, P. Dellabona & M. Kronenberg: CD1d-mediated recognition of an α-galactosylceramide by natural killer T cells is highly conserved through mammalian evolution. *J Exp Med* 188, 1521-1528 (1998)

95. Spada, F.M., Y. Koezuka & S. A. Porcelli: CD1d-restricted recognition of synthetic glycolipid antigens by human natural killer T cells. *J Exp Med* 188, 1529-1534 (1998)

96. Giaccone, G., C. J. Punt, Y. Ando, R. Ruijter, N. Nishi, M. Peters, B. M. von Blomberg, R. J. Scheper, H. J. van der Vliet, A. J. van den Eertwegh, M. Roelvink, J. Beijnen, H. Zwierzina & H. M. Pinedo: A phase I study of the natural killer T-cell ligand α-galactosylceramide (KRN7000) in patients with solid tumors. *Clin Cancer Res* 8, 3702-3709 (2002)

97. van der Vliet, H.J., N. Nishi, Y. Koezuka, B. M. von Blomberg, A. J. van den Eertwegh, S. A. Porcelli, H. M. Pinedo, R. J. Scheper & G. Giaccone: Potent expansion of human natural killer T cells using α-galactosylceramide (KRN7000)-loaded monocyte-derived dendritic cells, cultured in the presence of IL-7 and IL-15. *J Immunol Methods* 247, 61-72 (2001)

Key Words: CD1d, α-galactosylceramide, Vα14, Vα24, Review

Send correspondence to: Dr Ken-ichiro Seino, Laboratory for Immune Regulation, RIKEN Research Center for Allergy and Immunology, Suehiro-cho 1-7-22, Tsurumi, Yokohama, Kanagawa 230-0045, Japan, Tel: 81-45-503-7008, Fax: 81-45-503-7006, E-mail: seino@rcai.riken.jp