Role of NKT Cells in the Digestive System.

II. NKT cells and diabetes

Lan Wu and Luc Van Kaer

Department of Microbiology and Immunology, Vanderbilt University School of Medicine, Nashville, Tennessee

Submitted 30 May 2007; accepted in final form 27 June 2007

Wu L, Van Kaer L. Role of NKT Cells in the Digestive System. II. NKT cells and diabetes. Am J Physiol Gastrointest Liver Physiol 293: G919–G922, 2007. First published June 28, 2007; doi:10.1152/ajpgi.00242.2007.—Natural killer T (NKT) cells are a subset of regulatory T lymphocytes that recognize glycolipid antigens presented by the major histocompatibility complex class I-related glycoprotein CD1d. NKT cells have been implicated in regulating the progression of Type 1 diabetes (T1D) in human patients and in an animal model for T1D. In addition, glycolipid agonists of NKT cells have been successful in preventing diabetes in mice, raising enthusiasm for the development of NKT cell-based therapies for T1D.

A Primer on NKT Cell Biology

NKT cells are typically defined as T lymphocytes that coexpress characteristic surface markers of T cells and natural killer (NK) cells (15, 24) (Fig. 1A). Unlike conventional T lymphocytes, which recognize peptide antigens bound with MHC class I or class II proteins, NKT cells recognize glycolipid antigens bound with the MHC class I-related protein CD1d. One subset of NKT cells, often called invariant or type I NKT cells, expresses a semi-invariant T cell receptor (TCR), with Vα14-Jα18 and Vβ8.2/7/2 chains in mice and homologous Vα24-Jα18 and Vβ11 chains in humans. A second subset of NKT cells, called noninvariant or type II NKT cells, has a more diverse TCR repertoire. This article focuses on invariant NKT cells, which we will simply call NKT cells. NKT cells are abundant in thymus, liver, and bone marrow and represent a significant population of cells in spleen and peripheral blood but are rare in lymph nodes, the gut, and most other organs. In mice, most NKT cells express the coreceptor CD4, and the remaining cells lack CD4 and CD8 expression. In addition to these subsets, a small population of NKT cells in humans expresses CD8αα. NKT cells also express characteristic NK cell markers such as CD161 (also called NK1.1 in the mouse) and a variety of activation markers such as CD44, CD69, and CD122.

The physiological ligands that are recognized by NKT cells remain incompletely characterized (24). It is generally believed that NKT cells can react with both autologous and exogenous glycolipids. A lysosomal glycosphingolipid, isoglobotrihexosylceramide, was able to activate mouse and human NKT cells, but its importance in NKT cell functions is controversial (24). NKT cells can react with several microbial-derived antigens, including mycobacterial phosphatidylinositol tetramannosside, Sphingomonas-derived glycosylceramides, and Borrelia burgdorferi-derived diacylglycerols. Furthermore, all invariant NKT cells react with the marine sponge-derived glycolipid α-galactosylceramide (α-GalCer) (Fig. 1B), which was originally identified based on its antimetastatic activities in mice.

One hallmark of NKT cells is their capacity to rapidly produce copious amounts of both Th1 and Th2 cytokines upon TCR engagement (15, 24). Activation of NKT cells in this
NKT cells and Diabetes

Fig. 1. Natural killer T (NKT) cells and regulation of Type 1 diabetes (T1D). A: NKT cell phenotype and specificity. NKT cells express a semi-invariant T cell receptor (TCR) together with NK cell markers such as NK1.1. The TCR of NKT cells recognizes glycolipids presented by antigen-presenting cells (APC) in the context of CD1d. B: structure of the NKT cell agonist α-GalCer. C: possible mechanisms involved in the regulation of T1D by NKT cells. NKT cells promote Th2 responses, induce anergy in autoantigen-specific CD4+ and CD8+ T cells, reduce the differentiation of dendritic cells (DC) into tolerogenic DC, and promote the activities of CD4+CD25+ regulatory T cells. In concert, these mechanisms may dampen the activities of pathogenic Th1 cells and cytotoxic T lymphocytes (CTL). Circled symbols identify steps in which NKT cells are thought to directly impact immunoregulatory mechanisms.

manner also results in the transactivation of a variety of other cell types, including antigen-presenting cells (APCs), NK cells, B cells, and conventional T cells. In this context, NKT cells provide a link between the innate and adaptive immune systems and are sometimes referred to as innate T lymphocytes. Consistent with their immunoregulatory properties, NKT cells have been implicated in a variety of immune responses and diseases, including infections, tumors, allergic reactions, allografts, and autoimmunity (15, 24). The immunomodulatory properties of NKT cells have also been exploited for the development of vaccine adjuvants and for the development of immunotherapies for cancer and autoimmune and inflammatory diseases.

Regulation of T1D by NKT Cells in NOD Mice

The first indication that NKT cells might be involved in regulating T1D was based on the observation that the thymus and spleen of NOD mice have reduced numbers and functional defects in NKT cells (11). These defects were already present before birth and manifested subsequent to positive selection of CD4+CD8- double-positive thymocytes (25). This genetic control of NKT cell numbers was mapped to two loci, called Nkr1, which includes the SLAM (signal lymphocyte activation molecule) gene cluster (12, 22), and Nkr2. The proposed role of SLAM molecules in controlling NKT cell numbers in NOD mice is consistent with the critical role of the SLAM-associated adaptor protein in NKT cell development (24). Whether the NKT cell defect in NOD mice is directly involved in the susceptibility of these animals to T1D remains an issue of extensive debate. One study showed that, despite significant correction of the NKT cell defect, the Nkr1 locus did not alter the course of spontaneous diabetes in congenic mice (22). On the other hand, adoptive transfer of mature thymic CD4-CD8- NKT cells into NOD mice was able to protect these animals from the spontaneous onset of diabetes (11). NKT cells were also capable of inhibiting the onset of diabetes by adoptive transfer of transgenic T cells carrying the TCR α and β chain genes from a diabetogenic, β cell-specific CD4+ T cell clone isolated from a diabetic NOD mouse (2). Similarly, NKT cells prevented T1D in an adoptive transfer model using monoclonal CD8 T cells expressing a diabetogenic TCR (6). In sharp contrast, however, NKT cells exacerbated T1D induced by the adoptive transfer of transgenic T cells carrying the TCR from a CD8+ T cell clone specific for the influenza virus hemagglutinin into mice expressing hemagglutinin in their pancreatic β cells (10). It has been suggested that differences in the avidity of the TCRs used by the different CD8+ T cell clones for their respective antigens might play a role in the divergent effects on T1D (21). Consistent with a protective role of NKT cells in T1D, overexpression of NKT cells in Vα14-Jα18 transgenic NOD mice also protected against diabetes development (16). The effects of CD1d-deficiency on the development of diabetes in NOD mice have been controversial, with some groups reporting evidence for increased frequency and accelerated disease, whereas other groups were unable to identify differences in disease onset between wild-type and CD1d-deficient animals (21). These divergent findings may be due to differences in the number of backcrosses performed to introduce the CD1d mutation on the NOD background. Interestingly, CD1d overexpression in NOD islets under the control of the insulin promoter resulted in protection from diabetes (7). Collectively, most of these findings provide support for a suppressive role of NKT cells in the progression of diabetes in NOD mice.

Immunomodulation of T1D in NOD Mice with NKT Cell Ligands

Multiple research groups have independently demonstrated that repeated treatment of NOD mice with α-GalCer potently prevents the spontaneous development of T1D (23). This treatment only had a modest effect on the development of insulitis. Additional studies demonstrated that α-GalCer also prevents disease in the cyclophosphamide-accelerated model of T1D in NOD mice and can protect NOD mice against disease recurrence induced by adoptive transfer of diabetogenic T cells. When treatment was initiated after significant tissue destruction had already occurred, only modest effects were observed, suggesting that NKT cell activation is more effective at preventing than treating disease. Studies with structural analogs of α-GalCer have further demonstrated that a sphingosine chain-truncation variant called OCH has similar protective effects (19) and that an N-acyl variant called C20:2...
has increased efficacy against T1D (8) compared with α-GalCer.

**Regulation of T1D by NKT Cells in Humans**

Data regarding the frequency of NKT cells in human patients with T1D have been contradictory. The frequency of NKT cells has been reported to decrease, increase, or remain unaltered in peripheral blood of T1D patients compared with normal control subjects (4). Some of these inconsistencies might be due to the utility of different reagents for identifying NKT cells. Another complicating factor is that defects in NKT cells might only be apparent in certain NKT cell subsets. In this regard, one group of investigators reported selective reductions in the CD4⁺ NKT cell subset, resulting in an overall Th1 bias of NKT cells in patients with T1D (14). Of note, however, each of these studies was performed with peripheral blood, which might not accurately reflect the status of NKT cells in the environment of the pancreas. Nevertheless, one study reported that NKT cells from the pancreatic draining lymph node of T1D patients had a Th1-biased cytokine production profile (13), similar to what has been observed in some studies of NKT cells in peripheral blood (4).

**Mechanisms of NKT Cell-Mediated Regulation of T1D**

Multiple mechanisms have been proposed for the regulation of T1D by NKT cells. In many cases, NKT cell-mediated protection against T1D was associated with Th2 cytokines such as IL-4 and IL-10. Successful protection from T1D mediated by adoptive transfer of thymic NKT cells required IL-4 and IL-10 (11); protection from diabetes in Vα14-Jα18 transgenic mice was associated with attenuated Th1 responses and enhanced Th2 responses against pancreatic islets (16); and protection mediated by α-GalCer was associated with Th2 deviation (23). The possible role of Th2 deviation in disease protection is further supported by the finding that the α-GalCer analog C20:2, which was more effective in protecting NOD mice against diabetes than α-GalCer, promoted Th2 cytokine production by NKT cells (8). However, OCH, another Th2 cytokine-biasing α-GalCer analog, had similar capacity as α-GalCer in disease protection (19). One study showed that α-GalCer can protect IL-4- but not IL-10-deficient NOD mice against diabetes (18), but another study provided evidence that α-GalCer was able to protect NOD mice with a combined deficiency in IL-4 and IL-10 against diabetes (5). In addition, one study investigating the capacity of NKT cells to protect NOD mice from disease induced by adoptive transfer of transgenic, diabeticogenic CD4⁺ T cells found evidence for a role of IFN-γ in disease protection (3). A role for Th2 deviation was also suggested by some of the studies investigating NKT cells in human patients with T1D, which have provided evidence for a Th1 bias in the cytokine profile of NKT cells from diabetics compared with control subjects (4). In concert, these findings suggest a role for Th2-dependent mechanisms in the regulatory role of NKT cells in T1D (Fig. 1C) but also reveal that Th2-independent mechanisms must be involved.

NKT cells engage in intimate interactions with dendritic cells (DCs) and can influence the maturation and differentiation of DCs. As diabetes in NOD mice progresses, these animals develop an imbalance in the proportions of their DC subsets. It has been demonstrated that treatment of NOD mice with α-GalCer restores the frequency and functions of tolerogenic DCs in the pancreatic lymph nodes (6, 20). The α-GalCer analog C20:2 showed a similar and perhaps more pronounced correction of the DC imbalance in NOD mice (8). Adoptive transfer of these α-GalCer-induced, tolerogenic DC to prediabetic NOD mice was able to protect animals against disease (20).

Recent studies have provided evidence for cross talk between NKT cells and other subsets of regulatory T cells, in particular CD4⁺CD25⁺ regulatory T cells. One study has demonstrated that protection of T1D mediated by adoptive transfer of α-GalCer-activated NKT cells requires the activity of CD4⁺CD25⁺ cells (17). Furthermore, inactivation of CD25⁺-expressing cells before α-GalCer treatment abolished disease protection (17). These findings therefore suggest that protection from T1D by α-GalCer-activated NKT cells requires the activity of CD4⁺CD25⁺ regulatory T cells.

An additional mechanism that might contribute to the protective effects of NKT cells against T1D is through functional inactivation of pathogenic T cells. Such a mechanism has been implicated in the capacity of NKT cells to suppress disease induced by adoptive transfer of transgenic, diabeticogenic CD4⁺ T cells (2). NKT cells impaired the differentiation of these islet-specific T cells into Th1 effectors and instead induced unresponsiveness or anergy in these cells. The mechanisms by which NKT cells induced such anergy in pathogenic T cells remain unclear, but CD1d expression did not appear to be required, despite the need for cellular contact (2).

Collectively, these findings provide evidence that NKT cells regulate T1D by both Th2-dependent and Th2-independent mechanisms (Fig. 1C). Some of these proposed mechanisms may be linked to each other. For example, activated NKT cells might promote the development of tolerogenic DCs, which, in turn, might induce the generation of CD4⁺CD25⁺ regulatory T cells that suppress pathogenic Th1 and CD8⁺ cytotoxic T cell responses against islet autoantigens.

**Conclusions and Perspectives**

Numerous studies have provided evidence that NKT cells play a suppressive role in the development of diabetes in NOD mice. Most studies in humans have suggested a similar suppressive role of NKT cells in the development of human T1D. The finding that α-GalCer-activated NKT cells can prevent the development of diabetes in NOD mice is particularly exciting. Similar to mouse NKT cells, human NKT cells react with α-GalCer, offering the opportunity for translation of the preclinical studies to human patients. This possibility is further bolstered by clinical studies of α-GalCer in terminal cancer patients, which have shown that α-GalCer treatment of human subjects is safe, at least at the doses used and within the time frame that subjects were monitored (9). One potential limitation of α-GalCer therapy is that it may offer little protection once disease is initiated. Nevertheless, it is likely that α-GalCer analogs such as C20:2 will be able to further improve upon the therapeutic activities of α-GalCer. In this context, it will be important to obtain a better understanding of the mechanisms by which NKT cells regulate T1D, so that therapies can be designed in a rational manner. In addition, it will be worthwhile to explore combination therapies. For example, it will be interesting to combine NKT cell agonists with reagents that target antigen-specific diabeticogenic T cells. Thus, prospects for...
Themes

NKT CELLS AND DIABETES

developing NKT cell-based approaches for immunotherapy of T1D look bright.

ACKNOWLEDGMENTS

We thank members of our laboratories and many colleagues for helpful discussions. We apologize to colleagues whose work we were unable to cite due to space constraints.

GRANTS

Work of the authors was supported by a discovery grant from the Diabetes Research and Training Center at Vanderbilt University School of Medicine (to L. Van Kaer) and by grants from the National Institutes of Health (to L. Van Kaer) and the American Diabetes Association (to L. Wu).

REFERENCES