Role of NKT Cells in the Digestive System.

II. NKT cells and diabetes

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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.

Type 1 diabetes (T1D), also called insulin-dependent diabetes mellitus, is an autoimmune disease caused by the selective elimination of insulin-producing β cells in the islets of Langhans in the pancreas. This process ultimately results in the loss of glucose homeostasis and the need for insulin replacement therapy or islet transplantation. Investigations of the pathophysiology of T1D have benefited tremendously from the availability of animal models that spontaneously develop T1D. Most notably, studies with nonobese diabetic (NOD) mice have demonstrated a critical role for T lymphocytes in the pathogenesis of T1D (1). Both CD4-expressing, major histocompatibility complex (MHC) class II-restricted T lymphocytes and CD8-expressing, MHC class I-restricted T lymphocytes play a role. These T cells are directed against a variety of autoantigens. Autoreactive T cells and other bone marrow-derived cells initially infiltrate the pancreatic islets of young NOD mice without causing substantial β cell destruction in a process referred to as insulitis. After this phase of insulitis that can last for weeks or months, a phase of β cell destruction ensues, which ultimately leads to the development of frank diabetes. A similar process occurs in human patients with T1D.

While the etiology of T1D remains poorly understood, it is clear that both genetic and environmental factors play a role (1). A major factor in the development of T1D is the loss of T cell tolerance against pancreatic antigens. During normal physiological conditions, T cell tolerance is maintained by mechanisms that are active in both central and peripheral lymphoid organs. During their development in the thymus, T cells undergo an educational process that deletes cells reactive with autoantigens expressed in the thymus gland. After their exit from the thymus, T lymphocytes are subjected to additional tolerance mechanisms that can result in the elimination or functional inactivation of autoreactive T cells. In addition, autoreactive T cells that fail to be eliminated or controlled in this manner remain subject to the regulatory mechanisms mediated by several subsets of regulatory or suppressor T cells. Important subsets of regulatory T cells include CD4+CD25+ regulatory T cells, TGF-β-producing T helper type 3 cells, IL-10-producing T regulatory type 1 cells, and natural killer T (NKT) cells. Here, we review the evidence linking NKT cells to T1D in mice and humans and discuss prospects for developing effective NKT cell-based immunotherapies 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-restricted protein CD1d. One subset of NKT cells, often called invariant or type 1 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 tetramannoside, Sphingomonas-derived glycosylceramides, and Borrelia burgdorferi-derived diacylglycerols. Furthermore, all invariant NKT cells react with the marine sponge-derived glycolipid α-galactosylsphingosylceramide (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
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 (signaling 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. Successful protection from T1D mediated by adoptive transfer of transgenic, diabetogenic CD4+ 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).

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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).

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