The Review of DIABETIC STUDIES

CONFERENCE REPORT

Type 1 Diabetes and NKT Cells:

A Report on the 3rd International Workshop on NKT Cells and CD1-Mediated Antigen Presentation,

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■ **Abstract**

NKT cells play a major role in regulating the vigor and character of a broad range of immune responses. Defects in NKT cell numbers and function have been associated with type 1 diabetes, especially in the NOD mouse model. The 3rd International Workshop on NKT Cells and CD1-Mediated Antigen Presentation provided an opportunity for researchers in the field of NKT cell biology to discuss their latest results, many of which have direct relevance to understanding the etiology and pathogenesis of diabetes.

Keywords: Type 1 diabetes · IDDM · NKT cells · immunoregulation · NOD · alpha-Galactosyl Ceramide

Previous NKT Cell/CD1 Workshops

The workshops on NKT cells and CD1-mediated antigen presentation are the brainchild of Mitchell Kronenberg, from the La Jolla Institute for Allergy and Immunology in San Diego. The first meeting was held in San Diego in 1999, and set the general format for all three held to date: three or four days of talks covering structure/function issues of CD1-mediated antigen presentation, the ontogeny and characterization of NKT cell subsets, their physiological roles and involvement in tumors, infections and autoimmune disease. The workshops are fairly small, with 100-150 registrants, and provide ample opportunity for discussion and the establishment of collaborative interactions. Largely due to the sponsorship of Kirin Brewery, many speakers have received generous contributions to their travel and accommodation costs. This has played a critical role in ensuring that a broad range of views are presented - sometimes generating much controversy.

The second workshop was held at Woods Hole, Massachusetts, in 2002 and was particularly characterized by emerging data on lipid antigen processing, and CD1 assembly and trafficking. A source of especially vigorous debate at the workshop was the lack of concordance between the clinical data of Brian Wilson (Massachusetts General Hospital, Boston, USA) and those of Albert Bendelac (University of Chicago, USA), regarding the putative association between NKT cell defects and type 1 diabetes.

Background: NKT cells in Type 1 Diabetes

NKT cells are an important immunoregulatory lymphocyte population that express surface markers of both NK cells, such as NK1.1 and members of the Ly49 family, and conventional T-cells, such as the

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TCR. The best characterized subset is the invariant (i) NKT cells, which express an invariant alpha TCR chain, Vα14Jα18 in mice and Vα24Jα18 in humans, and show preferential use of the Vβ8.2, Vβ7 and Vβ2 chain in mice and the Vβ11 chain in humans [1]. They recognize a glycolipid antigen presented in the context of the β2 microglobulin associated, MHC class I-like molecule, CD1d. These cells are uniformly reactive to the marine sponge-derived glycolipid α-Galactosyl Ceramide (α-GalCer). In mice there are two major subsets based on surface expression of CD4; the $CD4^+CD8^-$ subset and the $CD4^-CD8^-$ double negative (DN) subset. These subsets also exist in humans, together with a minor $CD8^+$ population that seems to be absent from mice. NKT cells are capable of rapidly producing a large amount of cytokines, such as IFN-γ and IL-4, and are thought to regulate the character and magnitude of immune responses [1]. There are also other NKT cells present in mice that are CD1d-dependent but are not α-GalCer reactive (Type II NKT cells), and NKT-like cells that are CD1d-independent.

Invariant NKT cells appear to play a critical role in modulating the vigor and character of immune responses to pathogens, tumors and self antigens. Of particular interest is their role in the etiology and pathogenesis of type 1 diabetes. An association between defects in NKT cells and diabetes was first identified in NOD/Lt mice, which have lower numbers of NKT cells in the thymus, liver and peripheral lymphoid organs than other strains [2]. The development by Mitch Kronenberg and others of CD1d/αGalCer tetramers specific for all iNKT cells [3, 4], has enabled precise enumeration of this defect [5, 6]. In addition to numerical deficiencies, NKT cells from NOD mice produce relatively less IL-4, on a per cell basis, than non-autoimmune prone strains [6].

The association of reduced numbers of NKT cells with susceptibility to diabetes has been confirmed by: 1. Adoptive transfer of DN thymocytes enriched for NKT cells approximately 40x [7]; 2. Transgenic overexpression of the Vα14Jα18 T cell receptor to increase the proportion of NKT cells amongst thymic emigrants [8]; 3. Expansion of NKT cell numbers by administration of α-GalCer [9-13]; and 4. Elimination of NKT cells by targeted deletion of CD1d on the NOD strain background [14, 15].

The association between NKT cell deficiencies in NOD mice and type 1 diabetes prompted an investigation into the numbers of $Vα24Jα18^+$ T-cells in diabetes discordant twins. Initial studies identified a deficiency in both the number of NKT cells and their IL-4 production in human diabetic patients similar to that observed in NOD mice [16, 17]. In contrast to this, other groups found no difference in either NKT cell number or cytokine production between diabetic patients and healthy controls [18, 19]. Differences in the methods used to identify NKT cells and stimulation protocols make it difficult to compare the results. A third confounding factor in these studies is the necessity to analyze peripheral blood lymphocytes, rather than lymphocytes isolated from lymphoid organs, as is possible with mouse studies. Berzins *et al.* revisited the issue in NOD mice by examining NKT cell numbers in the peripheral blood by tetramer staining [20]. As in humans, the numbers in all mouse strains were very low, and there were no differences between diabetes-prone and diabetes-resistant strains.

Location and Facilities of the Third Workshop

The third workshop was a Boden Research Conference and was held from September 8-13, 2004 on Heron Island, Australia. It received generous support from Kirin Brewery and the Australian Academy of Science as well as contributions from Becton Dickinson and Commonwealth Serum Laboratories. Heron Island lies on the Tropic of Capricorn (23 degrees south of the Equator), 72 kilometers off the coast from Gladstone, central Queensland (about 534 kilometers north of Brisbane). It is a coral cay on the Great Barrier Reef and is one of the premier dive sites in the world. Many registrants took advantage of the recreation periods to try their hands at snorkeling or SCUBA diving around the island. The coral was in good condition, little affected by an episode of coral bleaching two years earlier, and the aquatic fauna were abundant. Large numbers of parrot fish, batfish and butterfly fish were seen, and sightings of note included a school of manta ray circling a coral pinnacle, large turtles and several 2m white tipped reef sharks. The food was excellent, and a particular highlight of the meeting was the seafood banquet, which included local shell fish and crustaceans.

News from the Workshop

In our opinion, the most exciting work presented at the workshop was that of Albert Bendelac on the natural ligand of NKT cells. Albert based his work on his observation that targeted mutant mice lacking the beta

subunit of lysosomal beta-hexosaminidase (a model of the lysosomal storage disease termed Sandhoff disease [21]) do not have NKT cells. A genetic dissection of the glycosphingolipid biosynthetic pathway in question identified the isoglobolipid isoglobotrihexosylceramide (iGb3) as a candidate. Like α-GalCer, iGb3 stimulates the vast majority of iNKT cells to produce both IL-4 and IFN-γ when presented in the context of CD1d. Although it appears to have an approximately three-fold lower affinity compared to α-GalCer, iGb3/CD1d tetramers did not work - presumably due to difficulties in loading the isoglobolipid into the pre-assembled CD1 molecules.

A number of speakers presented data on immune responses to structural analogs of α-GalCer, most of which were synthesized by Prof. Gurdyal Besra at the University of Birmingham, UK. One reasonably well-characterized derivative is OCH ((2S,3S,4R)-1-O-(-D-galactopyranosyl)-N-tetracosanoyl-2-amino-1,3,4-non-anetriol), a compound which can suppress experimental autoimmune encephalomyelitis (a model of multiple sclerosis [22]) and collagen-induced arthritis [23]. Although stimulation of iNKT cells with OCH results in brisk IL-4 production, it elicits relatively less IFN-γ than α-GalCer. Despite this, the lack of a report of efficacy in NOD mice raises the possibility that OCH does not prevent diabetes. Steven Porcelli (Albert Einstein College of Medicine, New York, USA) has tested a panel of derivatives based on modification of an azido ceramide precursor by covalent linkage of varying lipid tails. A number of C20 compounds were clearly superior to α-GalCer as they did not require endosomal loading for presentation, were therefore likely to be presented by non-professional antigen presenting cells and as a consequence not stimulate the production of IFN-γ. Steven reported that a number of these were more effective at preventing diabetes than α-GalCer. Moriya Tsuji (Aaron Diamond AIDS Research Centre, New York, USA) described a synthetic C-glycoside analog, α-C-Galactosyl Ceramide, which induces an enhanced IFN-γ response in mice, providing a 1000-fold more potent anti-malarial activity and a 100-fold more potent anti-tumor activity than α-GalCer.

Stuart Berzins (University of Melbourne, Australia) presented a talk comparing the developmental pathway of iNKT cells in humans with that developed by Dale Godfrey's laboratory in studies of mice [24]. Dr. Berzins obtained, through collaborating pediatric surgeons, paired samples of thymus and peripheral blood from infants (< 3 years of age) undergoing cardiac surgery. Proportions of human iNKT cells expressing CD161 (termed NK1.1 in mice) in the blood were twice those seen in the thymus, consistent with its status as a maturation marker. Similarly, as in mice, the majority of thymic iNKT cells were CD4-positive, while the DN population largely arose after thymic export.

Agnes Lehuen (INSERM, Paris, France) reported the results of a careful *in vivo* analysis of the requirements of protection from diabetes mediated by NKT cells in an adoptive transfer model. Relative to control strains, recipient mice expressing a Vα14Jα18 T cell receptor (see above) were relatively resistant to induction of diabetes by the adoptive transfer of naïve CD4 T-cells from BDC2.5 transgenic mice, which bear a T cell receptor from a diabetogenic CD4 T cell clone [25, 26]. In the Vα14Jα18 transgenic recipients, the BDC2.5 T-cells divided less and produced less IL-2 and IFN-γ than in control strains. In a separate approach, she cleared NKT cells from a NK1.1-expressing NOD line using a NK1.1-specific monoclonal antibody (PK136) and co-transferred both NKT cells from Vα14Jα18 transgenic mice and BDC2.5 diabetogenic T-cells from BDC2.5 transgenic mice. Surprisingly, protection occurred in the absence of IL-4, IL-10, IL-13 and TGFβ, and even when both T cell donor and recipient carried a targeted gene deletion of CD1d. *In vitro* studies suggested that cell/cell contact was required for protection. These studies suggest that cell surface receptors other than the TCR play critical roles in mediating activation and initiation of effector functions of NKT cells. Obvious candidates are the NK cell receptors.

The next workshop will be organized by Robson MacDonald (Ludwig Institute, Switzerland), Paolo Dellabona (H San Raffaele Scientific Institute, Milan, Italy) and Gennaro De Libero (Basel University Hospital, Switzerland) and held on Ile des Embiez, sea-sports resort, off Le Brusc in the south of France in 2006.

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