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Regulation of dendritic cell subsets by NKT cells

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Abstract

NKT cells expressing both invariant TCRs and NK cell receptors are an important regulatory cell subset active during initiation of innate immune responses. They are involved in a wide variety of immune responses, but the molecular details of their regulatory action are unknown. Transcriptional profiling has been used for analysis of NKT cell activation profiles, revealing that NKT cells differ from conventional T cells and would be expected to regulate immune responses by controlling dendritic cell activation. ***To cite this article: J.L. Strominger et al., C. R. Biologies 326 (2003).***

Résumé

Régulation de sous-ensembles de cellules dendritiques par des cellules NKT. Les cellules NKT exprimant à la fois des TCR invariants et des récepteurs cellulaires NK sont un sous-ensemble cellulaire régulateur important lors de l'amorçage des réponses immunitaires innées. Elles sont impliquées dans une large gamme de réponses immunes, mais les détails moléculaires de leur action régulatrice sont inconnus. L'étude des profils transcriptionnels a été utilisée pour examiner leur profil d'activation, révélant que les cellules NKT diffèrent des cellules T conventionnelles et participeraient à la régulation des réponses immunitaires en contrôlant l'activation des cellules dendritiques. ***Pour citer cet article : J.L. Strominger et al., C. R. Biologies 326 (2003).***

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Mots-clés : cellule dendritique ; cellule NKT ; cellule T ; profil d'expression ; réponse immunitaire

1. Absence of NKT cells in diabetics

The purpose of my presentation is to present an important problem and show you how transcriptional analysis employing DNA chip arrays contributed to the solution of the problem. They led to a description

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of novel mechanism for regulation of the immune response, and may have led to a potential new therapy for human diabetes.

Initially, we began to try to identify peptide epitopes derived from pancreatic autoantigens that might be important in diabetes, but were quickly diverted by the following finding. We discovered that invariant Vα24JαQT cells (NKT cells), which represent less than 0.5% of the total of peripheral T cells but nevertheless have a very important immunoregulatory function, were virtually absent in diabetic probands but were present in large numbers in long-term diabetes non-progressors [1]. Invariant Vα24JαQ T cells are called invariant NKT cells because they have an α chain which rearranges with no N-region, i.e. with no variation in the CDR3 loop of the T cell receptor (TCR) α-chain. Secondly, they express markers that are characteristic of NK cells, particularly CD161 (NKRP-1), hence the name NKT cells. Non-progressors are defined as individuals having high levels of autoantibodies against pancreatic antigens for at least 5 years.

Fortunately, the Joslin Diabetes Clinic at Harvard had four sets of twins and one triplet set in which one of the individuals was a non-progressor while the others were diabetic. In every case the frequency of the NKT cells was markedly reduced in the diabetic probands and in two cases NKT cells could neither be cloned nor found in the diabetic probands. These cells were thought to exert their immunoregulatory function by the secretion of the Th1 cytokine interferon-γ, and the Th2 cytokine IL4, that is burst production of these two cytokines was thought to regulate the deviation of the immune response toward Th1 or Th2 cells, respectively.

In the diabetes non-progressors both interferon-γ and IL4 were produced in large amounts by stimulated NKT cells. However, when cells from the diabetic patients were examined, although the Th1 cytokine interferon-γ was produced, no IL4 was detected. This discordance has been reproduced in more than 100 clones from five diabetic individuals that were examined. No NKT cells at all could be found in two of the five twin/triplet sets. Was the defect in IL4 production the only defect in the diabetic NKT cells?

2. Expression profiling of NKT cells

To examine that question, DNA microarrays were obtained from Affymetrix containing 6800 gene-specific oligonucleotide sets on each chip and used to examine the transcriptional profile in clones of each of one set of the discordant twins [2,3]. There were two possibilities: (1) The T cells from the diabetic twin expressed a subset of the transcripts expressed by those of the non-diabetic non-progressor, or (2) The two cells had differentiated in different directions and each expressed a set of genes not expressed by the other. The latter turned out to be the case. Between 1500 and 1600 transcripts were found in the resting state in both the diabetic and the non-diabetic cells, of which 900 were shared. More interestingly, on activation of these cells with anti-CD3 206 new transcripts appeared in cells from the non-progressor that were not found in cells from the diabetic proband. However, only 59 new transcripts appeared in the cell from the diabetic proband, that were not found in the non-progressor cells. The data were organized using the self organizing maps of Tomayo, Lander, Gollub, and colleagues. Six patterns emerged. A large number of genes were activated in the non-progressor cells that could not be activated in the diabetic cells. One of these was the defect in IL-4 production which led us to this study, but it was only the tip of the iceberg. Many other genes were not expressed in the diabetic cells.

3. Identification of dendritic cells as targets of NKT cells

The data were further organized using the grouping of genes according to their function as defined in the TIGR database. Two groups of particular interest are cytokine genes (of which IL4 is one) and chemokine genes that are activated in the non-diabetic cell but are not activated in the diabetic cells. The activation of IL-4 was small compared to many other cytokine genes that are produced upon activation of the non-diabetic, non-progressor cell. None of these genes was expressed in the diabetic cell and two of them in fact were in reduced amounts in the diabetic cells.

Two groups of transcripts were particularly interesting. One comprises a group of cytokines that are involved in the recruitment and differentiation

of myeloid dendritic cells. Another group contained the two main components of cytotoxic granules, perforin and granzymes. NKT cells are restricted by the unusual non-polymorphic class 1b molecule CD1d which is encoded on chromosome 1 (i.e. outside of the MHC on human chromosome 6) [4]. All of the NKT clones from the diabetes non-progressors were restricted by CD1d and they produced both Interferon-γ and IL-4 and proliferated in response to CD1d. Moreover, they were cytotoxic for transfectants expressing CD1d. Not only did they secrete immunoreactive cytokines, but they were also cytotoxic to cells expressing CD1d.

The next question was which cell in the myeloid lineage expresses CD1d and is the target for these invariant NKT cells. A very small set of myeloid dendritic cells (called DC1 cells) express CD1d both by FACS analysis and by immunoprecipitation and western blot. So invariant NKT cells may regulate the immune response not by burst production of interferon-γ and IL-4, but by a feedback mechanism that involves DC1 cells. On contact with CD1d, they secrete a group of cytokines which are involved in the recruitment and differentiation of DC1 cells, and they also carry out cytolysis of their targets [5]. The result is the depletion of DC1 cells, expressing CD1d. DC1 cells are the major source of IL-12, the cytokine that drives the expansion of Th1 cells.

The next set of experiments was carried out in the NOD mouse (non-obese diabetic mouse), the best animal model of type I diabetes. The frequency of NKT cells in the islets of NOD mice and in the islets of NOR mice (a diabetes resistant strain of mice that are congenic at the MHC locus and share $>90\%$ genetic identity with the NOD mouse) was determined. Female NOD mice developed diabetes at a high frequency (75–90%) between ten and twenty-six weeks of age. At ten weeks, a dramatic *decrease* in the numbers of NKT cells in the islets was observed and in the NOR mice a corresponding *increase* in the NKT cells in the islets was seen.

4. Stimulation of NKT cells by α-galactosylceramide

The CD1d molecule has a single unusual pocket in the MHC groove, which is highly hydrophobic. A group in Japan at the Kirin brewery has identified a ligand for this pocket. It was isolated from sea sponge and identified as α-galactosylceramide. The two acyl side chains attached to the polar carbohydrate head bind in the hydrophobic pocket; its sugar moiety points upward and is presented to T cells. Other CD1 molecules (CD1a, CD1b and CD1c that are found in man, but not in the mouse) also bind and present various glycosylated lipids. Interestingly, α-galactosylceramide is not a natural lipid for vertebrates. The natural lipid that binds to CD1d and stimulates NKT cells has not yet been identified.

Next, stimulation of NKT cell formation *in vivo* was attempted by administering α-galactosylceramide to NOD mice. The appearance of diabetes in control females began at 15 weeks of age and reached 70–80% by thirty weeks. If α-galactosylceramide was used, the frequency of diabetes was reduced to 40% and the onset was delayed by about two weeks. α-mannosylceramide, an antagonist of α-galactosylceramide, administered in the same way increased the frequency of appearance of diabetes and accelerated its appearance. Again, all of these changes were highly significant statistically. So far, only one dosage schedule has been attempted, so even better results might be obtained by changing the amount and/or frequency of administration.

How does α-galactosylceramide function? Or how does CD1d function to ameliorate diabetes? The frequency of NKT cells in peripancreatic lymph nodes was examined in controls either untreated or treated with vehicle. A striking increase in the numbers of NKT cells in peripancreatic lymph nodes was seen. No change in the numbers in neighboring inguinal lymph nodes was found. That is, immunoregulation was localized to the region of the affected tissue in which insulitis (without cell destruction) had begun to appear as the first sign of disease. The importance of CD1d in this process has recently been highlighted by the use of NOD/CD1d-/- mice. The NOD/CD1d-/- mice develop diabetes at both an accelerated pace and with increased frequency [6].

A striking increase in the number of tolerogenic DC2-like dendritic cells in the pancreatic lymph nodes was also evident while no change in their numbers in inguinal lymph nodes was seen [7]. The appearance of these dendritic cells could be easily observed historically using a well-known cytochemical marker, CD11c. Peripancreatic lymph nodes

from mice, treated with α-galactosylceramide, were loaded with DC2 cells while animals treated with α-manosylceramide had decreased numbers of these cells. Lastly, in a recall experiment, peripancreatic lymph node cells were removed from animals that had been treated with α-galactosylceramide and challenged *in vitro* either with vehicle control or α-galactosylceramide. These peripancreatic lymph nodes produced large amounts of IL-4 and interferon-γ on stimulation but inguinal lymph node cells on rechallenge *in vitro* produced only very small levels of either cytokine.

Thus, α-galactosylceramide ameliorated diabetes in the NOD mouse by producing a local immigration of NKT cells and dendritic cells to the peripancreatic lymph nodes. Since new onset diabetes patients still have about 10–20% of residual islet function this kind of substance may have potential in treatment of new onset diabetes in man. Of course, we were led to these findings by transcriptional analysis using Affymetrix DNA chips. They opened up the whole problem.

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