# The role of the T cells in autoimmune type 1 diabetes mellitus

PhD. Thesis

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## INTRODUCTION

Type 1 diabetes mellitus (T1DM) affects 1 in 400-600 people; the incidence of the disease is rising worldwide. The T1DM is caused by the chronic autoimmune destruction of the insulin producing β-cells. The exact etiology and the primary autoantigen is not known. The autoimmune, chronic and progressive nature of the disease raised the possibility of intervention, which can slow down or stop the destruction of the β-cells preferably as early as possible in prediabetic stage.

A lot of proofs were gathered about, that the T cells, mainly the regulatory T cells and the natural killer cells have fundamental role in regulating the autoimmune process. The most often used regulatory markers on the CD4+ T cells is the high expression of the CD25 protein (a subunit of the IL-2 receptor), and a transcriptional factor, called FoxP3.

The iNKT cells are a special group of thymus derived T cells, which are expressing both the NK (natural killer) markers and the T cell receptor (TCR). Most of the NKT cells have an invariant TCR, which means that between the Vα24 and JαQ (the alfa chain 24$^{th}$ variable region and the junction Q region -Vα14-Jα18 in mice) there is no nucleotide insertion. By the expression of the CD4 and CD8 these cells can be positive for each or none of

them (double negative iNKT cells). After the TCR stimulation the iNKT cells rapidly can produce high amount of Th1 (T helper, like IFN-γ) or Th2 type (IL-4) cytokines. The produced cytokines can influence the differentiation of the naive T cells, the inflammatory responses and they can have a role either in the acquired or the inborn immunity.

AIMS

Based on the published data we supposed the function of the CD4+ human T cells is impaired in T1DM. To study these changes we used microarray technique for analyzing the gene expression of the CD4+ T cells in newly diagnosed T1DM patients (ndT1DM), in long-term T2DM (ltT2DM) and in healthy (H) subjects. We also aimed to confirm the gene expression data on protein level (Western blot, flow cytometry)

In the differentiation of the CD4+ T cells, the iNKT cells and other regulatory cells have fundamental role. Because the detection of the extremely rare iNKT cells is technically difficult, we had to find a method, which can reliably and reproductively identify the iNKT cells. Using this technique we aimed to compare the frequency of the iNKT cells in H, ndT1DM, ltT1DM and ltT2DM groups, to characterize the subgroups, to analyze

their functions and cytokine products. The regulatory function of the iNKT cells is referred by several publications, but beside the cytokine production it was not proved. We intend to study the regulatory markers and use the iNKT cells in mixed lymphocyte stimulation assays.

Our aims in points:

- to analyze the gene expressions of CD4+ T cells with DNA microarray and comparing it among H, ndT1DM and ltT1DM patients;
- to confirm the results at protein level as well;
- to find a method, which can reliably and reproducibly detect the invariant NKT cells;
- to compare the frequency of the iNKT cells among H, ndT1DM, ltT1DM, ltT2DM patients groups;
- to characterize the subgroups, and to compare them;
- to analyze the cell function, mainly the cytokine production;

- to study the accepted regulatory markers;

- to run in vitro stimulations T cells assays using iNKT cells and their cytokine products to check their regulatory effects.

METHODS

Using magnetic beads, we isolated CD4+ T cells from healthy (H), from newly diagnosed T1DM (less than 4 months-ndT1DM), from long term T2DM (more than one year-ltT2DM) patients. We studied the gene expression of the CD4+ T cells with affymetrix gene chip. We used a multi-step comparing method to exclude the effect of the glucose homeostasis on our data. Then we confirmed the results with other genetic studies (PCR) and with we also checked it in protein level (Western blot and FACS analysis), as well as with functional assays.

The iNKT cells can potentially regulate the all CD4+ T cell population. Thus we measured the frequency of these cells; we isolated (from H, ndT1DM, ltT1DM and ltT2DM patients) and cloned them. For detecting the iNKT cells antibodies against the invariant TCR were used. After single cell sorting we expand the iNKT cells, characterized the clones by surface markers. The results were confirmed with PCR and Western blott as well. The cloning can

potentially change some features of the cells, so we also performed these studies with unstimulated iNKT cells.

## RESULTS

The component of the immune signal, the adhesions molecules, the chemokines, the cytokines, their receptors and the immune-transcriptional factors have fundamental role in the pathogenesis of T1DM. We have found that the function of all CD4+ T cell population impaired in T1DM. We studied almost 23000 sequences and 143 genes were T1DM specific among them. The expression pattern showed significant differences, mainly in the regulatory genes of the immune signal and cell cycle and the cell surface linked signal transduction. The gene chip results were confirmed with genetic studies (PCR) and on protein level (Western blot, FACS analysis), and with functional assays.

The iNKT cells detecting method based on the staining of the invariant TCR is reliable and reproductive (correlation r=0.97). The frequency of the iNKT cells in the different patient groups was 0.055-0.061% among the lymphocytes without significant difference between the groups. The ratio of the CD4+ iNKT cells significantly decreased among the ltT1DM (45%) patients comparing to the H (88%) and ltT2DM (91%) patient groups. The

cytokine production of the iNKT cells shifted to the harmful direction in ndT1DM and in ltT1DM groups. We proved the presence of the regulatory markers (CD25high+, FoxP3) on iNKT cells. In the in vitro mixed lymphocytes assays the iNKT cells and their products showed strong, dose dependent activation effect.

## CONCLUSION

The investigation of the CD4+ T cells and iNKT cells links to each other at several points. The high cytokine production capacity of the iNKT cells can make them possible to conduct the immunological responses, so the impaired CD4+ T cell function in T1DM can be affected by them. We did not study the exact interaction between the two cell populations. The antigen specific T cells were not examined by these studies; nevertheless it is surprising that we have found significant differences in the all CD4+ T cell population. Due to analyzing the iNKT cells and their subgroups in more details and using more independent confirmation method, than the previous publications, we found new results.

The differences in the all CD4+ T cell population and the CD4+ iNKT cell subgroup, which has more potent regulatory function and the changed cytokine production can be helpful to understand the immune-pathogenesis of

type 1 diabetes mellitus. The clinical studies modulating the T cells as well as the iNKT cells have already started. We hope that our results can help to these intervention trials.

PUBLICATIONS

Publications connected to the thesis

Articles:

**Kis J**, Engelmann P, Farkas K, Richman G, Eck S, Lolley J, Jalahej H, Borowiec M, Kent SC, Treszl A, Orban T. (2006) Reduced CD4+ subset and Th1 bias of the human iNKT cells in Type 1 diabetes mellitus. *J Leukoc Biol.* Mar;81(3):654-62. *impact factor: 4,57*

**Kis János**, Engelmann Péter, Heyam Jalahej, Orbán Tihamér (2006) 1-es típusú diabetes mellitus, az immunológiai prevenció lehetősége *LAM.* 16(8-9): 771–773.

Orban T, **Kis J,** Szereday L, Engelmann P, Farkas K, Jalahej H, Treszl A. (2007) Reduced CD4+ T-cell-specific gene expression in human type 1 diabetes mellitus. *J Autoimmun*. Jun;28(4):177-87. *impact factor: 2,15*

**Lectures:**

**Kis J**. Characterization of natural killer T cells. *Joslin Diabetes Center*, *Immunology data club*. Boston, USA 2005.12.12.

**Kis J**. Natural killer T cells in type 1 diabetes. *Joslin Diabetes Center, Harvard*, Boston, USA, 2006.02.14.

Posters/ Abstracts:

**Janos Kis**, Peter Engelmann, James Lolley, Geoff Richman, Heyam Jalahej, Tihamer Orban, (2005) Characterization of Human NKT Cell Clones Identified by Vα24 and 6B11 Double Staining in Type 1 Diabetes poster: *ADA San Diego*, *65th Scientific Sessions, 43-LB*

Peter Engelmann, **Janos Kis**, Geoffrey Richman, Shawn Eck, Heyam Jalahey, Tihamer Orban (2006) The Regulatory Function of the Invariant Natural Killer T Cells, poster: *ADA Washington, DC 66th Scientific Sessions, 1179-P*, abstract: *Diabetes.* Jun; 55(Suppl.:1):A278

**Janos Kis**, Peter Engelmann, Geoffrey Richman, Shawn Eck, Heyam Jalahey, Tihamer Orban. (2006) Human Invariant Natural Killer T Cells in Type 1 Diabetes Mellitus, poster: *ADA Washington, DC 66th Scientific Sessions, 1187-P*, abstract: *Diabetes.* Jun; 55(Suppl.:1):A279

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Publications not connected to the thesis

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Posters/abstracts:

**Kis J**, Rédei Cs, Nemesánszky E. (2002) Az oesophagus pH monitorozás szerepe a gastrooesophagealis reflux betegség atípusos formáinak diagnosztikájában, poster: *A Magyar Belgyógyász Társaság XXXIX. Nagygyűlése, nov. 21-23.,* abstract: *MBA;*LV(Suppl.:3):82

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**Janos Kis**, Antal Csepregi, C. P. Strassburg, P. Obermayer-Straub, S. Kneip, A. Kayser, E. Nemesanszky and M. Manns (2002) Antimitochondrial antibodies against dihydrolipoamide acyltransferases (PDC-E2 and BCKADCE2) in immunofluorescent AMA-negative rheumatoid arthritis sera, poster: *37th Annual Meeting of the European Association for the Study of the Liver Madrid, Spain, 17-21 April, 610,* abstract: *J. Hepatol;*36(Suppl:1):153-154

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