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Altered immune profile from pre-diabetes to manifestation of type 1 diabetes

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ABSTRACT

Background: While the mechanisms leading to β-cell destruction and clinical onset of T1D are still unclear, the composition of the immune profile is probably important for the outcome of immune activity.

The aim of this study was to investigate the composition and possible changes of the immunological profile, spontaneously and following stimulation with the autoantigens GAD_{65}, and HSP60, at high-risk and T1D onset and further to 8 months post diagnosis.

Methods: Fifteen first-degree relatives of T1D patients with a high risk of developing the disease within five years, 25 children approximately four days and 8 months after diagnosis of T1D and 16 healthy children were included in the study. Cytokines (IL-1β, -6, -7, -10, -13, -17, IFN-γ and TNF-α) and chemokines (CCL2, -3, -4, -5 and CXCL10) associated with Th1, Th2, Tr1 and inflammatory cells were detected in cell culture supernatants by Luminex-technique, and markers associated with regulatory T-cells (FOXP3, CTLA-4 and TGF-β) by real-time RT-PCR.

Results: High-risk individuals differed in immunity from that seen in healthy and T1D children. High-risk individuals had a low TNF-α response and fewer responders from mitogen exposure as well as low spontaneous secretions of IL-13 compared to healthy children. High-risk individuals that later developed T1D, had a lower FOXP3 and CTLA-4 mRNA expression, following stimulation with GAD_{65}, in combination with higher secretion of the pro-inflammatory chemokine CCL4.

Conclusion: Changes in immunity seen in individuals with high risk of developing T1D points to alterations/actions in the immune system already early in the pre-diabetic phase.

1. Introduction

An autoimmune attack, leading to the destruction of the insulin secreting pancreatic β-cells, is the causative factor for clinical onset of type 1 diabetes (T1D).

Insulitis, the infiltration of $CD8^+$ and $CD4^+$ T-cells, macrophages and B-cells, is considered pathognomonic for T1D in children with recent onset [1,2]. The T-cell mediated immunity in T1D involves $CD4^+$ T helper (Th) cells as well as $CD8^+$ cytotoxic T (Tc) cells [3,4] and mediates their β-cell destructive

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Abbreviations: CXCL, chemokines with an amino acid between the two N-terminal cysteine residues; CCL, chemokines with an amino acid neighbouring the two N-terminal cysteine residues; CT, threshold cycle; GAD, glutamic acid decarboxylase; HSP60, heat-shock protein 60; IFN-γ, interferon-γ; IL-, interleukin; T1D, type 1 diabetes; TNF-α, tumour necrosis factor-α; Tr1, T regulatory 1.

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functions through the release of e.g. cytokines and chemokines. However, as the mechanisms leading to β-cell destruction are still unclear, the composition of the secreted profile is probably important for the outcome of immune activity.

Th1-like lymphocytes contribute to cell-mediated immunity, e.g. cytotoxic and inflammatory responses, by production of interferon (IFN)-γ and tumour necrosis factor (TNF)-α, enhanced Tc activity and activation of macrophages, leading to increased levels of inflammatory cytokines such as interleukin (IL)-1β and IL-6 [5].

Humoral immunity connected to antibody production and enhanced eosinophil proliferation is, however, considered to be the activity of the Th2-like lymphocytes that produces e.g. IL-4, -5 and -13. The Th1- and Th2 subsets express different sets of chemokine receptors which allow them to respond and migrate to different tissues [6].

The cytokines characteristically produced by Th1- or Th2 cells are strongly cross-regulated between the phenotypes that inhibit the differentiation and effector functions of the reciprocal phenotype; e.g. IFN-γ inhibits proliferation of Th2 cells [5,6]. In addition, IL-10 secreted from T-cells of regulatory feature (Tr1), and transforming growth factor (TGF)-β secreted by Th3 cells, inhibits Th1 cytokine synthesis [7]. Cytokine patterns can be altered by strong stimuli or other cytokines, which might be a way to allow short term modulation of cytokine responses without altering the T-cell populations permanently.

Moreover, the concept of Th1- or Th2 immunity can also be extrapolated to chemokine secretions and the expression of their receptors. Chemokines allow cells with an appropriate receptor to migrate to the site of infection or tissue damage [8]. Th cells express different sets of chemokine receptors. The CXCL10 receptor CXCR3 is expressed by Th1 cells, while CCR3 binding CCL5 is foremost expressed by Th2 cells. CCL5, however also binds to the more promiscuous receptor CCR5 found to be expressed on both Th1 and Th2 cells [9,10]. CCL3 and CCL4 also binds to the receptor CCR5 [9]. Despite the reported promiscuity of the CCR5 receptor, CCL3, CCL4 and CCL5 are often discussed in relation to Th1 immunity. Further, CCL2 has been suggested to be produced by Th1 cells [11].

Regulatory T-cells (Tregs) are thought to be responsible for maintenance of self-tolerance and immune homeostasis and can be divided into naturally occurring and induced phenotypes. Natural Tregs express CD4, CD25, cytotoxic T-lymphocyte associated protein 4 (CTLA-4) and Forkhead box protein P3 (FOXP3). However, all of these markers are also up-regulated upon stimulation of effector CD4+ T-cells [12].

Induced Tregs, in contrast to CD4 + CD25+ Tregs, include Tr1-cells, secreting IL-4 and IL-10 and Th3-cells secreting TGF-β. TGF-β is an important marker of Tregs of an adaptive nature, expressed after activation [13].

In the pathogenesis of T1D, several autoantigens, such as glutamic acid decarboxylase (GAD_{65}), tyrosine phosphatase-like protein (IA-2) and the peptide antigen, corresponding to a conserved region of the heat-shock protein (HSP)60 (amino acids (a.a) 437–460), have been indicated due to the presence of autoantibodies and T-cells reactive against them [14,15].

This study aimed at investigating the composition and possible changes of the immunological profile, spontaneously and following stimulation with putative autoantigens, in a cohort constitutive of first-degree relatives of T1D patients and T1D children at onset and 8 months post diagnosis, in order to reveal any immunological changes from pre-diabetes to manifestation of type 1 diabetes.

2. Materials and methods

2.1. Protocol design

The study was conducted according to the following protocol (Fig. 1).

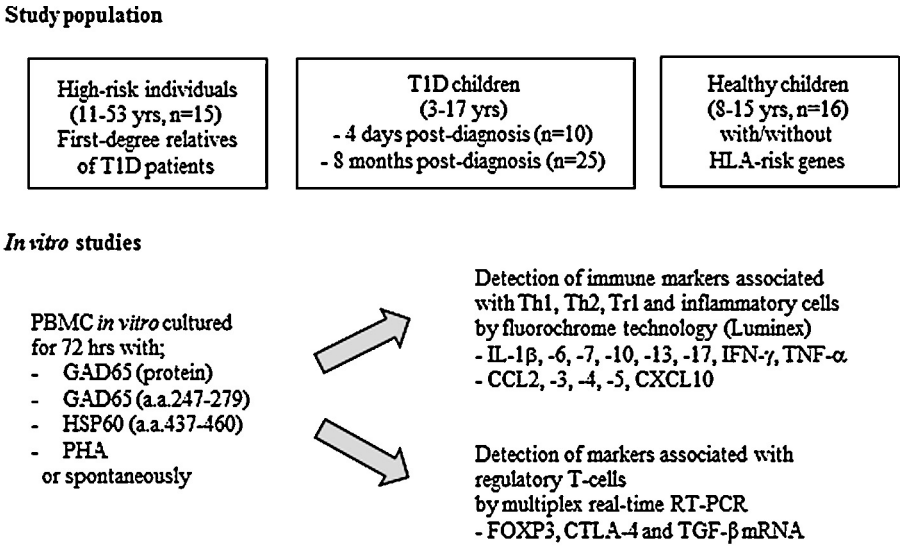

Fig. 1 – Description of study population; high-risk individuals, T1D children and healthy children and, study design including in vitro culture and detection of markers associated with regulatory T-cells (FOXP3, CTLA-4 and TGF-β mRNA) and immune markers associated with Th1, Th2, Tr1 and inflammatory cells (IL-1β, -6, -7, -10, -13, -17, IFN-γ, TNF-α, CCL2, -3, -4, -5 and CXCL10).