Clinical Immunology 174 (2017) 18–23

Contents lists available at ScienceDirect

Clinical Immunology

journal homepage: www.elsevier.com/locate/yclim

Neonatal levels of adiponectin, interleukin-10 and interleukin-12 are associated with the risk of developing type 1 diabetes in childhood and adolescence: A nationwide Danish case-control study

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ARTICLE INFO

Article history:
Received 6 July 2016
Received in revised form 11 November 2016
accepted with revision 13 November 2016
Available online 15 November 2016

Keywords:
Diabetes Mellitus, Type 1
Cytokines/immunology
TREM1, human
C-reactive protein
Mannose-binding lectin

ABSTRACT

Background/aim: An in-depth understanding of the early phase of type 1 diabetes (T1D) pathogenesis is important for targeting primary prevention. We examined if 14 preselected mediators of immune responses differed in neonates that later developed T1D compared to control neonates.
Methods: The study is a case-control study with a 1:2 matching. The individuals were born between 1981 through 2002. Cases were validated using the National Patient Register and the Danish Childhood Diabetes Register. Interleukin(IL)-1β, IL-4, IL-6, IL-8, IL-10, IL-12p70, interferon gamma, tumor necrosis factor alpha, transforming growth factor beta 1 (active form), leptin, adiponectin, c-reactive protein, mannose-binding lectin and soluble triggering receptor expressed on myeloid cells-1 were measured by using a flowmetric Luminex xMAP® technology. We tested two models both including a number of possible confounders. In the first model (model 1) we also adjusted for HLA-DQB1 genotype. A total of 1930 groups of assay-matched cases and controls (4746 individuals) were included in the statistical analyses.
Results: Adiponectin was negatively associated with later risk of T1D in both models (relative change (RC), model 1: 0.95, $P = 0.046$ and model 2: 0.95, $P = 0.006$). IL-10 and IL-12 were both positively associated with T1D risk in the model 2 (RC, 1.19, $P = 0.006$ and 1.07, $P = 0.02$, respectively)—these results were borderline significant in model 1, but showed the same direction as the results from model 2.
Conclusions: Our results indicate that specific immunological signatures are already present at time of birth in children developing T1D before the age of 18 years.

1. Introduction

Type 1 diabetes (T1D) is a T-cell-mediated autoimmune disease, where loss of self-tolerance towards the insulin-producing pancreatic β-cells leads to their destruction [1,2]. This breach in self-tolerance is believed to develop in the interface of environmental factors, genetic susceptibility, and an imbalanced microbiome, and may be initiated already in early-life [3–7]. An increase in incidence of T1D is observed worldwide—including Denmark—especially in children diagnosed before the age of 5 years (early-onset) [8–11]. Early-onset suggests a more aggressive autoimmune response, though it remains to be examined at which time-point the autoimmune response is initiated.

Immune system maturation is a dynamic physiological process, which is initiated in utero and complete maturation is gained within the first 5 years of life [12], the autoimmune response may already be triggered in utero or around time of birth [13]. A successful self-tolerant maturation is dependent on a balanced interaction between different immune cells either by cell-to-cell contact or by cross-talking via signaling proteins, i.e. cytokines [14]. Twin studies have shown that genetic factors influence an individual's cytokine production, but environmental factors are the main reason for differences in the cytokine signatures seen between individuals [15,16]. Such cytokine signatures may already be present at birth as a result of gene-environmental interactions in the neonate, and distinct signatures may mirror subtle immunological imbalances, which may result in an altered risk of developing autoimmune diseases, e.g. T1D, later in life.

Certain cytokines and other mediators of immune responses—hereafter referred to as immune mediators—have been associated with

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http://dx.doi.org/10.1016/j.clim.2016.11.007
1521-6616/

T1D pathogenesis [17–21]. Most of the studies are in vitro or have been conducted after T1D onset whereas the pre-diabetic and neonatal periods largely remain unexplored, though activation of specific cytokine pathways have been shown to precede autoantibody development in children genetically predisposed to T1D [22,23].

To our knowledge, no studies have examined if neonatal circulating levels of immune mediators are associated with later risk of developing T1D. Identification of such associations would provide new information to the understanding of the earliest T1D etio-pathogenesis, and open up for optimal timing of selective blockade of immune mediator pathways as a component of preventive T1D immunotherapy [24,25].

We investigated if circulating levels of immune mediators in neonates that later developed T1D compared to healthy controls differed. The Danish Neonatal Screening Biobank offers a unique opportunity to examine this question by using neonatal dried blood spots (DBS)—taken by a heel prick within one week after birth [26]. A priori, we selected 14 immune mediators associated with T1D and/or the innate immune system to be quantified on the DBS.

2. Methods

2.1. Data sources

The Danish Civil Registration System, established in 1968, registers all persons in Denmark alive from April 2, 1968 and born thereafter [27]. Unique personal identification numbers (CPR number) from the Danish Civil Registration System can be used for linking individual information from large clinical databases in the country. Through Statistics Denmark, which maintains a large number of national registers, we gained access to variables in the Medical Birth Registry, the National Patient Register and the Danish Civil Registration System using the CPR number as the key variable [28].

2.2. Study design, sample population, data sources and variables

Our study is a case-control study with a 1:2 matching.

Cases born from January 1, 1981 through December 31, 2002 and diagnosed with T1D before May 1, 2004 were identified from the National Patient Register. Cases were furthermore validated against the Danish Childhood Diabetes Register. Controls were selected by pulling out the neighbouring DBS cards when cases were identified in the Danish Newborn Screening Biobank [26]. Cases and controls were therefore individually matched on date of birth. Since 1981 the DBS have been stored at −20 °C/−4 °F in the Danish Newborn Screening Biobank and this biobank covers close to 100% of the Danish population born since 1982 [29].

We considered the following variables as either basic or as possible confounders: gender, birth weight (BW) (coding: ≤2499 g, 2500–4499 g and ≥4500 g), gestational age (GA) (weeks), mother's age at delivery (years), season (spring (March through May), summer (June through August), autumn (September through November) and winter (December through February)) and HLA-DQB1 genotype (HLA-risk) (genotyping has been described elsewhere [30] and HLA-risk categorization is specified in Table 1).

A total of 4746 individuals (1600 cases and 3146 controls) with complete recordings of all 14 immune mediators and the aforementioned variables were eligible for the statistical analyses. These individuals were grouped into 1930 groups of assay-matched cases and controls. Three hundred and thirty groups are comprised of controls alone due to missing complete cases.

Table 1
Descriptive characteristics of the sample population.

Variables	Case-control study		
	Case (n = 1600)	Control (n = 3146)	*P*-value[a]
Basic characteristics			
Gender			
Female, n/% of total	758/47.4	1504/47.8	0.8
Male, n/% of total	842/52.6	1642/52.2	
Age at onset			
Median/Q1–Q3, years	7.8/4.8–11.3	–	–
Mother's age at child's birth			
Median/Q1–Q3, years	28/25–31	28/25–31	0.6
Pregnancy and birth			
Gestational age			
Median/Q1-Q3, weeks	40/39–40	40/39–40.4	<0.0001
Birth weight (g), n/% of total			
<2500	74/4.6	145/4.6	0.9
2500–4500	1489/93.1	2933/93.2	
>4500	37/2.3	68/2.2	
Season at birth, n/% of total			
Winter	339/21.2	623/19.8	0.3
Spring	394/24.6	741/23.6	
Summer	426/26.6	917/29.2	
Autumn	441/27.6	865/27.5	
HLA-risk groups			
High[b], n/% of total	1011/63.2	447/14.2	<0.0001
Moderate[c] n/% of total	331/20.7	756/24.0	
Low/protective[d] n/% of total	258/16.1	1943/61.8	
Diabetes status during pregnancy[e]			
Mothers' diabetes diagnosis, n/% of total			
Yes	84/5.2	55/1.7	<0.0001
No	1516/94.8	3091/98.3	
Autoantibody status at birth[f]			
Child's autoantibody status, n/% of total			
Yes	101/6.3	16/0.5	<0.0001
No	1499/93.7	3130/99.5	

[a] Chi-square test for categorical, and Mann-Whitney *U* test for numerical variables.
[b] HLA-DQB1 *allele_1/*allele_2: 03:02/99:99, 03:02/02, 06:04/03:02.
[c] HLA-DQB1 *allele_1/*allele_2: 03:01/02, 06:03/03:02, 02/99:99, 06:04/02, 06:04/99:99, 03:01/03:02, 06:04/03:04.
[d] HLA-DQB1 *allele_1/*allele_2: 06:02/03:02, 06:02/99:99, 06:02/02, 06:03/99:99, 03:01/99:99, 06:02/03:01, 06:03/03:01, 06:04/03:01, 06:03/02, 03:04/99:99, 03:04/02, 06:02/03:04, 99:99/99:99. 99:99 = remaining alleles.
[e] All diabetes diagnoses, excluding gestational diabetes, given in a hospital setting before the individual's birth.
[f] Positive for glutamic acid decarboxylase-65 antibodies, islet antigen-2 antibodies, or both.

2.3. Assessment of biomarkers on DBS

By using a multiplexed sandwich immunoassays, based on flowmetric Luminex xMAP® technology, which can measure up to 25 inflammatory markers simultaneously on DBS (3.2 mm diameter), we were able to quantify the following cytokines: interleukin(IL)-1β, IL-4, IL-6, IL-8, IL-10, IL-12(p70), interferon gamma (IFNγ), tumor necrosis factor alpha (TNFα), transforming growth factor beta 1 (active form) (TGFβ), leptin, and adiponectin. Furthermore, we also quantified c-reactive protein (CRP), mannose-binding lectin (MBL) and soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) [31]. In all assays, matched pairs were run together to avoid batch effects/interassay variation [32]. Biomarker analyses are described in detail elsewhere [31].

Quality control of the analysis were made using mouse IL-6 as an internal analyte added to the extraction buffer to detect pipetting errors, and biotinylated beads to detect signal errors (more thoroughly described in Skogstrand [33]). Calibration curves were used on each plate together with one high and two low controls. Samples, calibrators, and controls were analyzed in duplicates.

2.4. Assessment of autoantibodies on DBS

Autoantibodies against glutamic acid decarboxylase-65 (GAD65Ab) and insulinoma-associated protein 2 (IA-2Ab) were determined in a standard radioligand binding assay, details are described elsewhere