Charles University

2nd Faculty of Medicine

Program: Immunology

Mgr. Zuzana Paračková

Immune system dysregulation in type 1 diabetes

Dysregulácia imunitnej odpovede u diabetu mellitu 1. typu

Doctoral thesis

Supervisor: Prof. MUDr. Anna Šedivá, DSc.

Prague, 2020

DECLARATION

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IDENTIFICATION RECORD

PARAČKOVÁ, Zuzana. *Dysregulácia imunitnej odpovede u diabetu mellitu 1. typu [Immune system dysregulation in type 1 diabetes]*. Prague, 2020, 69p, 8 appendixes, Ph.D. Thesis (Ph.D.). Charles University, Second Faculty of Medicine, Department of Immunology. Supervisor: Šedivá Anna.

Identifikační záznam:

PARAČKOVÁ, Zuzana. *Dysregulácia imunitnej odpovede u diabetu mellitu 1. typu [Immune system dysregulation in type 1 diabetes]*. Praha, 2020, 69s, 8 příl., Dizertační práce (Ph.D.). Univerzita Karlova, 2. lékařská fakulta, Ústav imunologie. Vedúca práce: Šedivá Anna.

ACKNOWLEDGEMENTS

I would like to thank my supervisor, prof. MUDr. Anna Šedivá, DSc., for her professional advice, her trust in me and for giving me the opportunity to work on this project and to evolve to an independent researcher capable of leading scientific projects. I also thank all employees of the Department of Immunology for creating a friendly environment, where I enjoy working and spending time. I specifically thank my colleagues and friends Markéta Bloomfield, Petra Vrabcová and Irena Zentsová for their support, help and great time we spent together, not only in the lab. My deepest gratitude belongs to my mother Anna Paračková for her endless support, kindness and sense of humour.

I would like to dedicate this thesis to my father and uncle.

ABSTRAKT

Diabetes mellitus 1. typu (DM1) je multifaktorálne autoimunitné ochorenie, ktoré spočíva v napadnutí inzulín produkujúcich beta buniek v pankrease autoreaktívnymi cytotoxickými CD8 lymfocytmi. Vďaka kooperácii rôznych zložiek vrodenej aj získanej imunity dochádza k rozvoji zápalu a následnej autoimunitnej reakcii. Autoreaktívne T lymfocyty sa podieľajú na priamom aj nepriamom ničení beta buniek, B lymfocyty sú producentmi autoprotilátok a bunky vrodenej imunity sú považované za iniciátorov autoimunitných pochodov vedúcim k aktivácii T a B buniek.

V tejto dizertačnej práci poukazujeme na viaceré dysregulované zložky vrodenej aj adaptívnej imunity u pacientov s DM1. Tieto zmeny v imunitnom systéme sa často odohrávajú už pred samotným objavením sa prvých syndrómov a teda nie sú len dôsledkom hyperglykémie, ktorá typicky sprevádza DM1. Zmeny v množstve a aj v niektorých základných funkciách T regulačných lymfocytov (Treg) a B lymfocytov sa objavujú už u asymptomatických príbuzných pacientov s DM1. Počas prvého roka od objavenia sa príznakov dochádza k postupnému znižovaniu počtu neutrofilov v periférii, ktoré pravdepodobne infiltrujú pankreas. Podrobnejšie sme sa zamerali na výskum zložiek vrodenej imunity a ich spoluúčasť k patogenéze DM1. Ukázali sme, že produkty neutrofilov, ktoré sa nazývajú neutrofilné extracelulárne pasce (NET), sú schopné indukovať IFNγ-produkujúce T lymfocyty skrz aktiváciu dendritických buniek (DC). NETy sa skladajú najmä z vlastnej DNA a antimikrobiálnych proteínov a sú význačným mechanizmom antiinfekčnej imunity. Avšak v posledných rokoch si NETy získali nemalú pozornosť v oblasti autoimunitných ochorení, pretože sami o sebe predstavujú potenciálny zdroj autoantigénov. V našej ďalšej práci ukazujeme ako monocyty a DC od DM1 pacientov prehnane reagujú na prítomnosť DNA bez ohľadu na jej pôvod, vrátane mikrobiálnej aj vlastnej DNA, čo naznačuje, že aberantné rozoznávanie DNA v NEToch sa tiež podieľa na zápale spojenom s autoimunitných procesom. Naša ďalšia štúdia venovaná biológii DC u DM1 pacientov popisuje signalizáciu IL-27 cytokínu. Výsledky z RNA mikroarrayí odhalili zvýšenú expresiu podjednotky IL-27 receptora na myeloidných DC u DM1 pacientov. Toto zvýšené množstvo receptorovej podjednotky sa odzrkadľovalo zvýšenou fosforyláciou STAT3 molekuly a expresii PD-L1, čo naznačovalo kompenzačnú snahu buniek DM1 pacientov voči prebiehajúcemu zápalu spojenému s autoimunitným procesom.

Táto dizertačná práca poukazuje na viaceré dysregulované aspekty imunity u pacientov s DM1 a naznačuje, že k týmto poruchám dochádza už pred vznikom ochorenia. Dysregulácia vrodenej imunity je naďalej zjavná aj u chronických pacientov a nie je asociovaná so zmenami v metabolizme, z čoho vyplýva že ide o potenciálne geneticky podmienené faktory. Pretože presymptomatickí pacienti by mali veľký úžitok z včasnej identifikácie nástupu patologických procesov vedúcich k symptomatickému DM1, domnievame sa, že informácie získané v tejto práci podkladajú pevné zázemie pre budúci výskum.

Kľúčové slová: diabetes mellitus 1. typu; neutrofily; neutrofilné extracelulárne pasce; dendritické bunky; rozoznávanie DNA; IL-27; T regulačné lymfocyty; B lymfocyty

ABSTRACT

Type 1 diabetes (T1D) is an autoimmune disease with multifactorial aetiology that involves an attack of self-reactive cytotoxic CD8 lymphocytes on insulin-producing beta cells in the pancreas. In the T1D pathophysiology, both innate and adaptive immunity mechanisms cooperate in the development of inflammation leading to autoimmune destruction. Autoreactive T lymphocytes are the canonical destructors of the beta cells, and B cells produce autoantibodies; the innate immunity cells are considered the initiators of the pathological autoimmune reaction by promoting T and B cell activation.

Here, we provide evidence of both innate and adaptive immunity cell types dysregulation in patients with T1D, and that these changes occur before the onset of the disease. The changes in T regulatory lymphocytes (Tregs) and B cell subpopulations occur already in asymptomatic T1D first-degree relatives. During the first year after the onset of the disease, there is a gradual decrease in the neutrophil numbers in the periphery, which probably infiltrate the pancreas. We have focused more closely on the innate immunity dysregulation and its contribution to T1D pathogenesis. Initially, we describe that neutrophil products called neutrophil extracellular traps (NETs) are able to induce IFNγ-producing T cells through activation of dendritic cells (DCs). NET structures are predominantly composed of neutrophil DNA and antimicrobial proteins and are an important mechanism of antimicrobial defence; however, in recent years, NETs gained considerable attention in the field of autoimmune diseases as a source of potential autoantigens. Then, we show that T1D monocytes and DCs inappropriately react to the presence of DNA regardless of the origin, including microbial or endogenous sources, suggesting that aberrant recognition of DNA contained in NETs also participates in the inflammation associated with T1D. Our another study of DC biology in T1D patients focused on IL-27 signalling because the results of the RNA microarray assays revealed a profound increase in the IL-27 receptor subunit expression on myeloid DCs. The increased receptor expression was mirrored by an increase in STAT3 signalling and PD-L1 expression, suggesting compensatory mechanisms of ongoing inflammation in long-term treated T1D patients.

Overall, this doctoral thesis provides evidence of impaired aspects of immunity in T1D patients, including adaptive and innate immunity, and suggests that this orchestrated dysregulation occurs before the onset of the disease. Innate immunity dysregulation is also apparent in long-term treated T1D patients and is not associated with metabolic changes, suggesting that the changes are intrinsic and potentially determined genetically. Since presymptomatic patients benefit from early identification of the onset of the pathological processes leading to symptomatic T1D, we believe that information obtained in this work defines a solid background for future research.

Key words: type 1 diabetes; neutrophils, neutrophil extracellular traps; dendritic cells, DNA recognition, IL-27; T regulatory lymphocytes, B cells

TABLE OF CONTENTS

Declaration - 2 -
Identification record - 3 -
Acknowledgements - 4 -
Abstrakt - 5 -
Abstract - 6 -
TABLE OF CONTENTS - 7 -
Abbreviations - 9 -
Literature overview - 11 -
1.1 Type 1 diabetes - 11 -
1.1.1 Factors contributing to T1D - 12 -
1.1.1.1 Genetic susceptibility - 12 -
1.1.1.2 Environmental determinants - 14 -
1.1.2 Stages of T1D pathogenesis - 16 -
1.1.3 Islet autoantibodies as T1D predictors - 17 -
1.1.4 Prevention of T1D and prevention trials - 18 -
1.1.5 Insulitis - 20 -
1.2 Immune system dysregulation in T1D - 21 -
1.2.1 Antigen-presenting cells - 21 -
1.2.1.1 Monocytes and macrophages in T1D - 22 -
1.2.1.2 Dendritic cells - 23 -
1.2.1.3 APC as a therapeutic target - 25 -
1.2.2 Neutrophils - 26 -
1.2.2.1 Neutrophils as a therapeutic target - 28 -
1.2.3 T cells - 28 -
1.2.3.1 Loss of immunological tolerance to beta cells - 29 -
1.2.3.2 T cell autoantigens and neoantigens - 29 -
1.2.3.3 CD4 and CD8 T cells - 30 -
1.2.3.4 Regulatory T cells - 31 -
1.2.3.5 T cells and Tregs as a therapeutic target - 34 -
1.2.4 B cells - 35 -
1.2.4.1 B cells as autoantibody and cytokine producers - 35 -
1.2.4.2 B cells as antigen-presenting cells - 36 -
1.2.4.3 B cell subpopulations - 36 -

1.2.4.4 B cells as a therapeutic target ..... - 38 -
1.2.4.5 A novel type of immune cells ..... - 38 -
Aims of the thesis ..... - 40 -
Results ..... - 41 -
1.1 Neutrophil extracellular trap induced dendritic cell activation leads to Th1 polarization in type 1 diabetes ..... - 43 -
1.2 Monocytes contribute to DNA sensing through the TBK1 signalling pathway in type 1 diabetes patients ..... - 44 -
1.3 Enhanced STAT3 phosphorylation and PD-L1 expression in myeloid dendritic cells indicate impaired IL-27Ralpha signalling in type 1 diabetes ..... - 45 -
1.4 Changes in innate and adaptive immunity over the first year after the onset of type 1 diabetes ..... - 46 -
1.5 T regulatory lymphocytes in type 1 diabetes: Impaired CD25 expression and IL-2 induced STAT5 phosphorylation in paediatric patients ..... - 47 -
1.6 Alteration of B cell subsets and the receptor for B cell activating factor (BAFF) in paediatric patients with type 1 diabetes ..... - 48 -
1.7 Neutrofilní extracelulární pasti – záchranné sítě imunitního systému (Neutrophil extracellular traps – immune system' s safety net) ..... - 49 -
1.8 Patogény v pasci DNA (Pathogens in the DNA trap) ..... - 49 -
Conclusions ..... - 50 -
Summary ..... - 51 -
References ..... - 52 -
Appendix - Attached manuscripts ..... - 70 -

ABBREVIATIONS

ANCA	antineutrophil cytoplasmic antibody
APC	antigen-presenting cells
APRIL	a proliferation-inducing ligand
ATG	anti-thymocyte globulin
BAFF	B-cell activating factor
BCR	B cell receptor
Blys	B cell-activating factor
CAR	chimeric antigen receptor
CTLA-4	cytotoxic T-lymphocyte antigen 4
ČENDA	The Czech national childhood diabetes register
DAISY	Diabetes autoimmunity study in the young
DAMP	danger-associated molecular patterns
DC	dendritic cells
DIPP	Diabetes prediction and prevention
FINDIA	Finnish dietary intervention trial for the prevention of type 1 diabetes
FoxP3	forkhead box P3
GAD65	glutamic acid decarboxylase 65
GADA	glutamic acid decarboxylase 65 antibodies
G-CSF	granulocyte colony-stimulating factor
HEV	human enteroviruses
HLA	human leukocyte antigen
IA-2A	islet antigen antibodies
IAA	antibodies against insulin
IAbs	Islet cell autoantibodies
IAPP	islet amyloid peptide
IDO	indolamine dioxygenase
IFN	interferon
IGPR	islet-specific glucose-6-phosphatase catalytic subunit related protein
IL	interleukin
INNODIA	Innovative approach to understanding type 1 diabetes
INS	insulin
IPEX	immunodysregulation polyendocrinopathy enteropathy X-linked syndrome
JDRF	Juvenile diabetes research foundation
LFA-1	lymphocyte function-associated antigen 1
mDC	myeloid dendritic cells
NET	neutrophil extracellular traps
NO	nitric oxide
NOD	non-obese diabetic
nPOD	Network of pancreatic organ donors with diabetes
PD-1	programmed cell death 1
pDC	plasmacytoid dendritic cells
PD-L1	programmed cell death ligand 1

PTM	post-translational modifications
PTPN22	lymphocyte protein tyrosine phosphatase
ROS	reactive oxygen species
SLE	systemic lupus erythematosus
STAT	signal transducer and activator of transcription
STING	stimulator of interferon genes
T1D	type 1 diabetes
TBK1	TANK-binding kinase 1
TCR	T cell receptor
tDC	tolerogenic DCs
TEDDY	the Environmental determinants of diabetes in young
Teffs	effector T cells
TLR	Toll-like receptors
TNF	tumor necrosis factor
Tr1	T regulatory lymphocytes type 1
Treg	T regulatory lymphocytes
TRIGR	Trial to reduce IDDM in the genetically at risk
VLA-4	very late antigen 4
VNTR	variable number of tandem repeats
ZnT8A	zinc transporter 8 antibodies

LITERATURE OVERVIEW

1.1 TYPE 1 DIABETES

Type 1 diabetes (T1D) is a chronic autoimmune disease characterized by the destruction of insulin-producing beta cells in the pancreas. T1D is one of the most common endocrine and metabolic conditions occurring in childhood (1). The precise aetiology and pathology of T1D remains unclear; however, observations of the natural history of human T1D, including its incidence in twins, suggest that the disease results from an attack of the immune system on the beta cells of the pancreas due to multiple genetic and environmental factors and subsequent impairment of insulin secretion and hyperglycaemia. Long-term hyperglycaemia may frequently lead to a number of serious chronic diabetic complications: diabetic retinopathy leading to blindness, diabetic nephropathy leading to chronic renal failure and diabetic neuropathy leading to several consequences, such as diabetic foot. Despite the improvement in T1D management, patient suffering from this disease are reliant on lifelong treatment with exogenous insulin therapy (2).

T1D prevalence and incidence have an increasing trend worldwide. The data of epidemiologic studies showed a 3.4% increase *per annum* in the incidence rate, suggesting doubling of the incidence rate within approximately 20 years in Europe (3). Meta-analysis studies showed that incidence in 2020 is 15 per 100,000 people and prevalence is 9.5% with the highest values in North America and Europe (4).

The peak age of T1D incidence is in the age group of 10-14 years (3, 5). More than 1,110,000 children and adolescents younger than 20 years of age are estimated to have T1D in the world with the highest incidence and prevalence in North America, Australia and Europe (Figure 1).

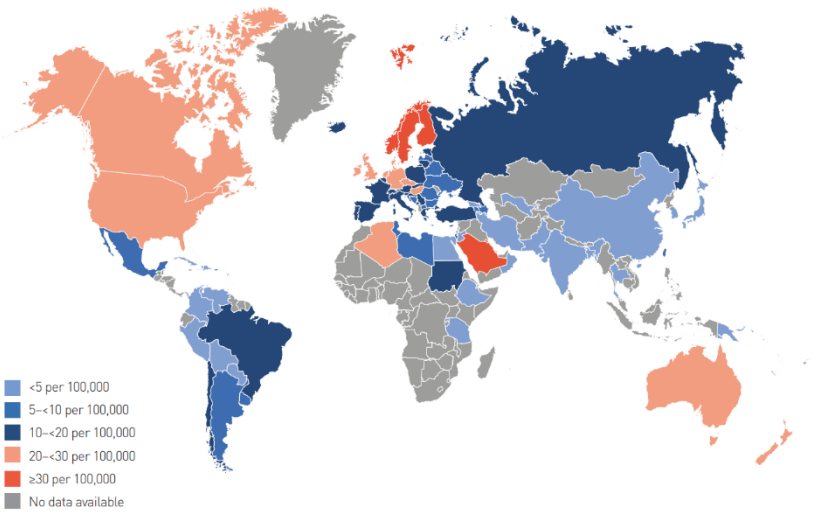

Figure 1. The Incidence rates (per 100,000 *per annum*) of T1D in children and adolescents
(diabetesatlas.org)

The T1D incidence rates substantially vary in various countries. The regions with the highest incidence rate are Scandinavian countries (more than 60 new case per 100,000), Northern America, Saudi Arabia and Kuwait (30 new cases per 100 000). Certain countries, such as Venezuela or China, are on the opposite side of the T1D incidence spectrum (0.1 case per 100,000) (2, 3, 6). In Europe, a study from 26 countries showed a 3.4% increase in T1D incidence in children despite slowing of the childhood incidence rates reported in some high-incidence areas, such as Scandinavian countries or UK. The highest incidence increase was estimated in Poland (6.6%) and the lowest incidence increase was estimated in Spain in Catalonia (0.8%)(3). The observed slowing of incidence increase in several high-incidence countries occurs after a relatively long period of an increase in the incidence rates (1995–2001), which can potentially reflect a re-established balance between environmental exposure and the genetic background of the population (7).

The Czech National Childhood Diabetes Register (ČENDA) is a web-based nationwide database that stores and processes anonymized data from paediatric patients with diabetes and contains the data from 4,361 children aged 0-19 years; 94% of these children had T1D, 1.5% had T2D and 4.5% had genetically proven diabetes. ČENDA was established in 2013 and is a system allowing online comparison of selected key attributes of diabetes control in diabetes clinics in the Czech Republic (8). In CR, an annual increase in the incidence ratio was estimated to be 4.7% (3). In 2009-2013, the incidence rate was estimated to be 21.8 per 100,000 (3) and is increasing (unpublished data).

1.1.1 Factors contributing to T1D

The accepted model of T1D development considers that T1D is due to an interplay between genetic predisposition and environmental determinants. Due to increasing prevalence, the environmental factors and modern lifestyle associated with excessive calorie intake, physical inactivity, excessive hygiene, etc. appear to have a greater influence on the risk of T1D at present than that in the past. Multiple evidence based on *in vivo* observations, epidemiological analysis, animal studies and *in vitro* models support this hypothesis (9, 10).

1.1.1.1 Genetic susceptibility

T1D is a polygenic disorder, and more than 60 genetic variants were described to contribute to the risk of T1D development. Polymorphism in human leukocyte antigen (HLA) class II largely defines the hereditary risk of T1D development. The most important loci include HLA-DR and HLA-DQ. The highest risk haplotypes are *DRB1*04-DQA1*03:01-DQB1*03:02* (known as DR4-DQ8) and

*DRB1*03:01-DQA1*05:01-DQB1*02:01* (DR3-DQ2). In the general population, carriers of these variants have a 30-fold higher risk of T1D development. First-degree relatives carrying this variant have an additional risk of T1D development (11). On the other hand, the *DRB1*15-DQA1*01-DQB1*06:02, DRB1*14-DQA1*01-DQB1*05:03* and *DRB1*07*-DQA1*02*:01-DQB1*03:03* haplotypes are associated with protection (11).

Moreover, polymorphisms of the genes localized outside of the HLA region encoding insulin (*INS*) and *PTPN22* (lymphocyte protein tyrosine phosphatase) are associated with higher T1D susceptibility (12, 13). Polymorphism of the insulin gene associated with T1D is located in the promoter region of the gene. The risk for T1D development is determined by the size of the variable number tandem repeats (VNTRs) in the noncoding region. The size of VNTRs in the insulin promoter region probably influences the binding of the transcriptional factor AIRE in the thymus and thereby governs the insulin expression level. Low insulin expression due to shorter VNTRs may be associated with subsequent insufficient elimination of autoreactive T cells during negative selection and may lead to generally impaired peripheral tolerance to insulin (12). Additional genes outside of the HLA regions are associated with T1D development and are listed in Table 1. Genome-wide association studies identified several loci associated with enhanced risk of T1D; however, the effect on the disease is lower. In most cases, identified genes are associated with immune regulation or beta cell functions (14).

Gene	Protein	Polymorphism associated with T1D	Affected function
PTPN22	Protein tyrosine phosphatase	rs2476601	Response to T and B cell activation. Survival of autoreactive cells in thymus
CTLA4	Cytotoxic T lymphocyte associated protein 4	rs3087243	Negative regulation of autoreactive T cells
IL2RA	IL-2 receptor subunit α	rs12722495	Response to IL-2 by regulatory T cells and memory T cells
IFIH1	Interferon induced with helicase C domain 1	rs1990760	Receptor for dsRNA, induces IFN production
ERBB3	Erb-b2 receptor tyrosine kinase 3	rs2292239	Regulator of beta cell apoptosis, modulates antigen presentation
BACH2	BTB domain and CNC homologue 2	rs3757247	Regulator of beta cell apoptosis

***Table 1: Selected genes associated with increased T1D susceptibility**. Modified from Ilonen, 2019 (15).*

1.1.1.2 Environmental determinants

An increase in T1D incidence in children from genetically stable populations and less than 50% concordance in T1D development in monozygotic twins (16) evidence the major role of environmental factors in the T1D aetiology. This increase has started in the middle of the 20th century (3). The number of patients with a low level of HLA-defined genetic risk (17) and increased T1D incidence trend in people migrating from low-incidence to high-incidence countries, especially in children born in the new countries or who moved at young age (18, 19), also support environmental influence on T1D development. The time of residency of the mother in a new country is also associated with enhanced risk of T1D in the children born in the new country, indicating an important role of foetal period in the subsequent risk of T1D (20). Evidence from longitudinal cohort studies of at-risk children indicates that environmental exposure in early life contributes to T1D risk and is related to maternal influence on the foetus during pregnancy, neonatal factors or later effects during infancy and early childhood. Various environmental factors acting at a various time points integrally involve an interaction between the immune system and beta cells (10). Potential early-life environmental triggers associated with T1D progression and initiation are summarized in Figure 2.

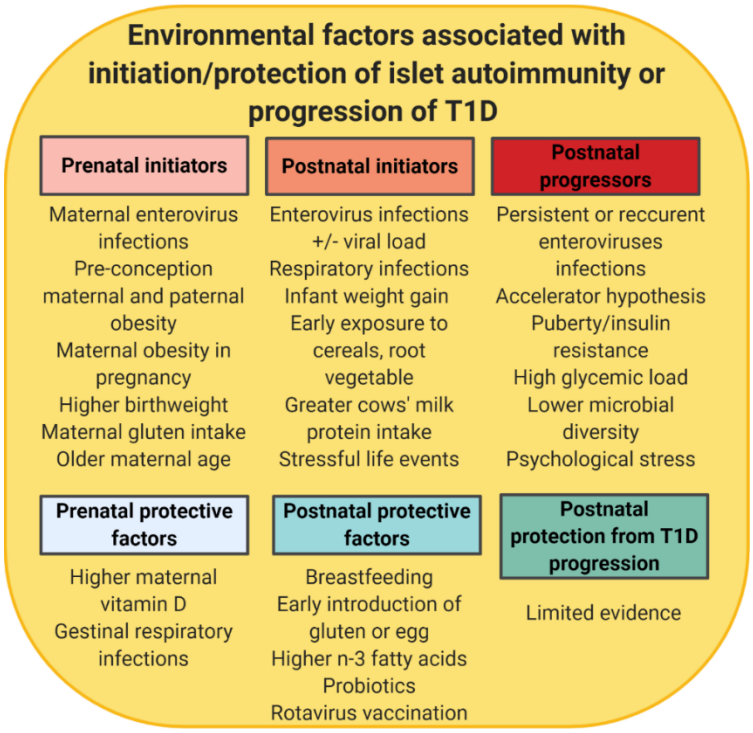

Figure 2: Early-life environmental factors contributing to T1D development. *Modified from Craig, 2019. Created with Biorender.*

Many studies have shown associations between various infections and T1D susceptibility. Viral infections, such as human enteroviruses (HEV) (21), rotavirus (22), mumps virus (23), rubella virus (24) and cytomegalovirus (25), may induce or accelerate the autodestructive process (26). The pathogenic mechanism of viral infections can be based on direct toxicity to beta cells, antigen mimicry or immune system hyperactivity. This cross-reactivity has been recognized many years ago and has been detected in the case of the pathogens and autoantigens recognized by antibodies or/and T cells of patients in a broad variety of autoimmune diseases (27). Strongest evidence of viral involvement in T1D was obtained in the case of HEVs, which are also frequently detected in peripheral blood and pancreas of T1D patients and in recent-onset T1D patients (28–31). Importantly, a large meta-analysis of 33 T1D prevalence studies involving almost 2,000 T1D patients and 2,500 control individuals confirmed a significant association between HEV infection and the development of T1D (32). In addition to the role of viruses as inducers or accelerators of T1D, it is important to acknowledge that viruses also can reduce the incidence rate of T1D according to published animal studies (33, 34) thus supporting the so-called "hygiene hypothesis" (35). Viral inflammation can function as an "immune-tuning" event leading to the bystander demise of aggressive T cells or induction of counteracting regulatory mechanisms (26, 36).

Intensive studies focused on investigation of the role of gut microbiota and its association with T1D susceptibility have not provided conclusive results. A growing number of studies have shown various differences in gut microbiome composition between T1D patients, patients at-risk and healthy donors (37). These changes may influence T1D susceptibility by affecting intestinal permeability, molecular mimicry and modulation of the innate and adaptive immunity. Children with T1D have increased intestinal permeability, also called leaky gut, probably due to the aberrant gut microbiota (38), and increased intestinal permeability can cause the activation and proliferation of diabetogenic $CD8^+$ T cells thus promoting insulitis (39).

Diet is another environmental factor influencing the development and progression of the disease. For example, gluten-free diet was shown to delay the progression of diabetes (40). Vitamin D is considered to protect against T1D; however, no definitive effect was proven (41). Original studies also indicated that early exposure to cow milk proteins accelerated T1D (42); however, TRIGR (Trial to Reduce IDDM in the Genetically at Risk) clinical study showed no effect (43). Similarly, the role of antibiotic abuse as T1D trigger is controversial. Certain studies proposed that the use of antibiotics in early life increased T1D susceptibility; however, other studies showed that antibiotics reduce the T1D risk in the animal models (44–46). In children, antibiotics used within 1 year are not associated

with the higher risk of T1D development (47). Low carbohydrate diets (less than 130 g/day or 26% of total energy intake from carbohydrates according to the American Diabetes Association) have been associated with lower levels of HbAc1 in a large observational study of 1,020 patients with T1D; however, the definitive effect was not demonstrated, and more primary studies are needed (48, 49).

1.1.2 Stages of T1D pathogenesis

The ability to screen for risk and to stage T1D provides a chance to interfere, delay and ultimately prevent the onset of the clinical symptoms (50). Prospective longitudinal studies of individuals at risk for T1D development have demonstrated that T1D develops over time and that the disease is a continuum that progresses sequentially at variable but predictable rates through distinct identifiable stages prior to its symptomatic manifestations.

According to a T1D model suggested in 1986, T1D was divided into 6 distinct stages (51); however, a current model of T1D acknowledged by ADA, TrialNet and other diabetes organizations includes 3 stages (Figure 3) (50).

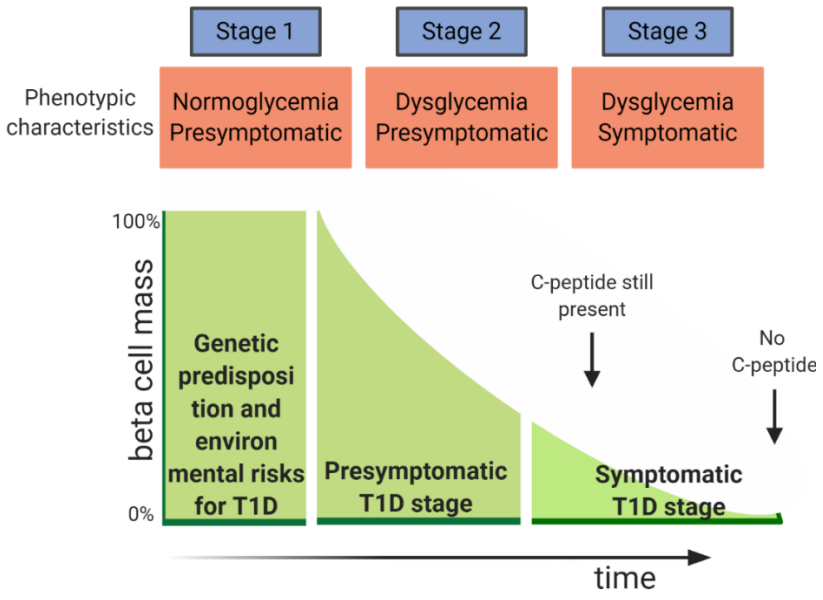

Figure 3: Stages of T1D development. *The stages are listed according to the level of beta cell mass. Adapted from Insel, 2015 (50). Created with Biorender.*

Stage 1 represents individuals who have developed two or more T1D-associated autoantibodies but have normal glycaemia. The risk of T1D development at this stage is similar in genetically at-risk

children and first-degree relatives. The 5-year and 10-year risks of symptomatic disease in children who reach this stage are approximately 44% and 70%, respectively, and the lifetime risk approaches 100% (52–54).

Stage 2 also includes persons with two or more islet autoantibodies whose disease has progressed to the loss of the functional β-cell mass resulting in elevated blood glucose. The 5-year risk of symptomatic disease at this stage is approximately 75%, and the lifetime risk approaches 100% (55).

Stage 3 represents manifestations of the typical clinical symptoms and signs of diabetes, which may include polyuria, polydipsia, weight loss, fatigue, diabetic ketoacidosis and others. Metabolic markers appear after sufficient depletion of the beta cell mass, and critically low numbers of insulin-producing cells result in hyperglycaemia in combination with other symptoms. At the time of metabolic diagnosis, most individuals have residual production of insulin reflected by detectable levels of C-peptide in the blood. With time, beta cells are completely destroyed, and C-peptide becomes undetectable (50).

1.1.3 Islet autoantibodies as T1D predictors

Islet autoantibodies (IAbs) are strongly associated with the T1D development, and their presence is currently the most reliable biomarker of islet autoimmunity. IAbs usually appear years before the first T1D symptoms, and children with 2 and more IAbs have a 70% risk of the disease development in 10 years (52). Autoantibodies that bind to specific proteins found in the pancreas were described for the first time almost 40 years ago (56). The main IAbs include antibodies against insulin (IAA), GAD65 (glutamic acid decarboxylase 65) (GADA), islet antigen (IA-2A) and zinc transporter 8 (ZnT8A). IAbs usually develop sequentially, and IAA is often the first to develop in children after birth; however, GADA is often detected as the first in older children (57).

Several screening studies recruited T1D patients, their relatives and general population to better understand the natural history of T1D, define the T1D onset and its potential triggers and identify the individuals at risk (58). These clinical trials have been focused on the prevention or at least a delay in T1D development for as long as 50 years (Table 2). Large international cohorts have been studied from birth in relatives of the patients with T1D and more recently in the general population with high genetic risk. Investigations of these individuals over time identified the most pronounced risk predictors of T1D progression, i.e., islet autoantibodies. IAbs develop in 90–95% of the subjects

destined to develop T1D (59). Large birth cohorts, including BABYDIAB in Germany (started in 1989), Diabetes Prediction and Prevention in Finland (DIPP; started in 1994), and Colorado Diabetes Autoimmunity Study in the Young (DAISY; started in 1993), demonstrated a peak in islet autoimmunity development within the first 2–5 years of life. T1D then more rapidly progresses in the subjects with autoantibodies in the early years compared to the subjects that develop antibodies later in the childhood and adulthood (52, 57, 60, 61).

Study name	Initiation year	Participant characteristics
Barts-Windsor-Middlesex Family Study	1978	Siblings and parents of T1D patients diagnosed at age <21 and had 1 or more siblings without T1D
Barts-Oxford Study	1985	Siblings and parents of T1D patients diagnosed at age <15
BABY-DIAB		Newborn offspring of parents with T1D
DAISY (Diabetes Autoimmunity Study in the Young)	1993	Newborn offspring with high risk HLA or with a first-degree relative with T1D
DIPP (T1D Prediction and Prevention Project)	1994	Newborn offspring with high risk HLA
TEDDY (The Environmental Determinants of Diabetes in Young)	2004	Newborn offspring with high risk HLA or with a first-degree relative with T1D regardless of genetic risk
TialNet Pathways to Prevention Study	2004	Phase 1: First-degree, second-degree and third-degree relatives of T1D patients; Phase 2: positive for 1 autoantibody; Phase 3: completed phase 2
INNODIA (An Innovative Approach to Understanding Type 1 Diabetes)	2017	New onset in various age groups with first-degree relatives

Table 2: T1D natural history studies

A new and important area of research concentrates on identification of autoantibodies to neoantigens or neoepitopes created by various posttranslational modifications, which are likely to improve the understanding of the mechanisms of T1D autoimmunity. Additional biomarkers, such as cellular immune markers, metabolic changes and genetic background, are likely to enhance the prediction of the rate of T1D progression (58).

1.1.4 Prevention of T1D and prevention trials

The main goal of the medical care of T1D children shifted from the symptomatic treatment to disease-modifying interventions. Children included in the prospective T1D studies often have better

metabolic indicators, which facilitate the management of the disease, decrease hypoglycaemic episodes and delay the development of long-term complications. Furthermore, probability of a honeymoon phase is increased if intensive treatment, which helps patients to maintain higher C-peptide levels, is initiated as soon as possible after the diagnosis of symptomatic T1D, suggesting that patients who are treated as early as possible have improved long-term outcomes (50, 62, 63).

Primary prevention studies begin prior to the autoimmunity development, typically in children at increased T1D genetic risk. Prevention trials (Table 2 and 3) aim to slow down or halt the destruction of the beta cells using multiple approaches; unfortunately, the success of the interventions remains limited. The various studies use different approaches such as dietary changes, antigen-based therapy, immunomodulatory and immunosuppression therapies. Primary dietary prevention strategies have been used starting from the 90s to evaluate the role of hydrolysed cow milk formula without antigenic compounds (free from intact bovine insulin) (43); the Finnish Dietary Intervention Trial for the Prevention of Type 1 Diabetes (FINDIA) used insulin-free bovine formula, BABYDIET analysed a gluten-free diet in the first year of life, and the TrialNet Nutritional Intervention to Prevent Type 1 diabetes study used docosahexaenoic acid. The results of all studies have been disappointing and failed to show efficacy, except FINDIA that demonstrated a delay in the development of autoantibodies (64, 65).

The main role of the antigen therapy is to induce peripheral tolerance by exposure of the immune system to the antigens in the target organ or by induction of anergy of already present autoreactive T cells (66). In the DIPP study in Finland, children were treated with intranasal insulin or placebo, and the outcomes in the groups were similar. In Germany, a similar Pre-POINT study was performed; the results demonstrated immunological effects, including elevated serum IgG and salivary IgA binding to insulin and an increase in regulatory T cells (64). Another study using antigen therapy with GAD65 (alum-formulated GAD65 in combination with vitamin D) showed promising results and demonstrated partial preservation of the residual beta cell function (DIAGNODE study) (64, 67).

Immunologic modulation in the prevention and intervention studies gained recent visibility after encouraging results demonstrating efficacy in the new-onset studies. Attempts to restore self-tolerance, promote regulatory T cells and reduce effector T cells have been evaluated using several different classes of drugs, including anti-CD3 (teplizumab and otelixizumab). Primary promising results demonstrated greater beta cell mass preservation and delayed clinical T1D progression in

high-risk participants (68, 69). Moreover, administration of abatacept/CTLA-Ig demonstrated a slower rate of beta cell decline maintained for 1 year after termination of the a therapy (64). Another ongoing study in Australia is using a cellular therapy approach with regulatory T cells and autologous cord blood (The CoRD Study) (70). Different approaches targeting immune system are discussed in more detail in following sections.

Name	Intervention	Number of participants	Primary Outcome Results
Finnish TRIGR Study	Bovine milk protein avoidance	230	Decreased autoantibody
TRIGR	Bovine milk protein avoidance	5156	Decreased autoantibody but no T1D prevention
FINDIA	Bovine insulin avoidance	1104	Decreased incidence of autoantibody
BABYDIET	Delayed gluten exposure	150	No benefit
denis	Nicotinamide	55	No benefit
endit	Nicotinamide	552	No benefit
Belgian Diabetes Registry	Parental insulin	25	No benefit
dpt-1	Parental insulin	339	No benefit
dpt-1	Oral insulin	372	No benefit
TrialNet oral insulin	Oral insulin	560	No benefit
dipp	Nasal insulin	264	No benefit
DIAGNODE	GAD-alum	50	Safe
tn-10	Teplizumab	76	Delay of diagnosis

Table 3: Prevention and intervention studies in type 1 diabetes

After efficacy, safety and feasibility (and hopefully a mechanism) are demonstrated in new-onset type 1 diabetes patients, the therapies are moved into the prevention trials. Due to the number of potential therapeutic targets, a combination of both immune and nonimmune therapies targeting multiple aspects of this disease is likely to be needed. The timing of the initiation and duration of the treatment are very important areas of study; however, the timing is sometimes difficult to determine (70).

1.1.5 Insulitis

In 2007, the Juvenile Diabetes Research Foundation (JDRF) launched a programme called the Network of Pancreatic Organ Donors with Diabetes (nPOD). The main aim of this project is to collect and distribute the pancreatic and related tissues of cadaveric organ donors with prediabetes or ongoing diabetes to study the mechanisms of organ-specific autoimmunity in humans. Insulitis is an inflammatory lesion of the islets associated with the loss of beta cells and was not detected in

individuals with a single autoantibody thanks to nPOD initiative. Moreover, beta cells are not completely destroyed at the time of diagnosis and can persist for a long period of time. Inflammatory cells are usually observed in the islet periphery or within the parenchyma, and only 10-30% of the islets show insulitis at any time point (71, 72). In 80 studied T1D donors, 17 donors had insulitis even after 12 years of the disease, suggesting that autoimmunity persists for years after diagnosis (73). Residual beta cells are present in patients with insulitis, and the beta cell mass in these patients is higher than that in patients without insulitis. T1D patients who had the disease with residual insulin secretion for 50 years also had insulitis (74). T1D is an autoimmune disease with complex aetiology and undeniable contribution of the immune system at every stage of the disease development. In the next section of this thesis, distinct parts of the immune system and their participation in T1D pathology are described in detail.

1.2 IMMUNE SYSTEM DYSREGULATION IN T1D

T1D is an autoimmune disorder characterized by the destruction of beta cells by self-reactive lymphocytes. However, adaptive immunity is not the only factor involved in the destruction of the insulin-producing cells. Components of innate immunity also participate in the initiation of the damage process. Identification of molecular pathways implicated in T1D pathogenesis may lead to therapy that can prevent and cure T1D. Details about the adaptive and innate immunity components engaged in the disease and their potential use for therapy are discussed below.

1.2.1 Antigen-presenting cells

Monocytes, macrophages and dendritic cells are the main populations involved in antigen presentation. These cells are the key components of innate immunity included in the regulation of the initiation, development and resolution of inflammation. In addition, these populations participate in tissue repair and regeneration. They produce a wide range of cytokines and chemokines, which influence and recruit other cell types. These antigen-presenting cells (APC) can contribute to several autoimmune diseases, and their infiltration into the diseased tissues is a hallmark of the disorders. However, exact contribution of APCs and whether APCs actually initiate the inflammation or are a part of already ongoing dysregulation is unknown (75, 76). Other cell populations, such as B cells, mTECs and stromal cells, also act as APCs. However, we will discuss in detail only the main APC populations, including monocytes, macrophages and DCs, in the next chapters.

1.2.1.1 Monocytes and macrophages in T1D

Currently, a variety of observations suggest that monocyte activities are altered in T1D. Altered monocyte activities may be a response to the inflammatory and metabolic changes associated with T1D or may be intrinsic and potentially genetically determined. Monocytes are useful for the generation and study of monocyte-derived antigen-presenting cells, but they also act as contributors to the pathological processes by themselves. In T1D patients, reports about monocyte numbers in circulation are contradictory. Both decreased (77) and increased (78) monocyte numbers in the periphery were described. Prior to the T1D onset, patients have elevated serum monocyte-derived cytokines, such as tumour necrosis factor (TNFα), IL-1α, IL-10 and others (79). Another study showed that T1D monocytes displayed inflammatory phenotype and enhanced production of IL-1β and IL-6 resulting in the induction of Th17 lymphocytes (80). Purified $CD14^+$ monocytes from children with newly diagnosed T1D had different gene expression patterns compared to that from the healthy subjects. The changes in several pathways involved in adhesion, NFκB signalling, regulation of apoptosis, IL-1β processing and other inflammatory functions were detected (81). Several studies demonstrated aberrant expression and signalling of the Toll-like receptors (TLR) in monocytes from T1D patients, such as TLR2 that recognizes the components of gram-positive bacteria and TLR4 that recognizes lipopolysaccharide (82, 83). Accordingly, our group have studied TLR9 signalling involved in nucleic acid recognition in T1D patients; the data indicated that T1D monocytes also express TLR9 (84). Our observation was unexpected since TLR9 is expressed almost prominently on plasmacytoid dendritic cells and B cells (85). In addition to TLR9-dependent DNA sensing, we showed that monocytes from T1D patients can recognize nucleic acids that reach the cytosolic compartment and sense DNA via cytosolic DNA sensors (86). Indeed, monocytes from T1D patients triggered the production of proinflammatory cytokines mediated by cytosolic molecules STING, TBK1, and other sensors involved in aberrant DNA sensing, suggesting the monocyte contribution in the autoimmunity inflammation (84).

Macrophages are detected in the islets in the pancreatic postmortem sections obtained from patients with T1D and at the time of onset (87, 88). Animal models of T1D showed compromised phagocytosis in macrophages, suggesting that insufficient clearance of apoptotic cells and consequential generation of self-antigens contribute to the T1D development (89). In addition, macrophages from non-obese diabetic (NOD) mice are abnormally activated and have enhanced cytolytic activity towards beta cells (90). Notably, depletion of macrophages effectively abolished T1D in NOD mice. In general macrophages represent one of the main APC population and can effectively trigger adaptive immune responses; in T1D, macrophages present antigens to

autoreactive $CD4^+$ T cells, resulting in the activation of these cells (91). Moreover, the data obtained in a T1D mouse model showed that macrophages are involved in the recruitment of autoreactive $CD8^+$ T cells into the islets, and this trafficking apparently depends on type I IFN signalling. Depletion of islet-resident macrophages resulted in reduced $CD4^+$ T cell infiltration and severity of the disease (92).

The proinflammatory serum milieu in T1D patients promotes M1 polarization of macrophages (93), which results in elevated secretion of IL-6 and IL-1β by macrophages and expression of costimulatory markers thus enhancing the inflammation. M1 macrophages also produce bactericidal mediators, such as reactive oxygen species (ROS) and nitric oxide (NO), which are toxic to beta cells. The data obtained in a mouse model of T1D showed that transfer of immunosuppressive alternatively activated M2 macrophages reduced the onset of T1D, hyperglycaemia and kidney injury (94).

1.2.1.2 Dendritic cells

Dendritic cells (DC) are a heterogeneous group of innate immunity cells. DCs have two general functions in controlling T cell immunity. First, DCs act as APCs and present the antigens to T cells and activate them; second, DCs secrete cytokines that shape the T cell response. Moreover, DCs play an undeniable role in the maintenance of the central and peripheral tolerance. Impairment of these activities contribute to the T1D pathogenesis (95, 96).

Studies of pancreatic biopsies have revealed that activated DCs are present at the islets where they contribute to the proinflammatory environment by producing inflammatory cytokines, such as TNFα (88, 97). Several reports have analysed the DC numbers in the periphery in newly diagnosed T1D patients; however, the data are inconclusive. The levels of plasmacytoid dendritic cells (pDC) were found to be decreased (98) or increased (99) at the time of diagnosis. Our group has reported a decrease in the number of pDC and myeloid DC (mDCs) in T1D patients and their first-degree relatives (100). Additionally, in another study, we have shown that originally low numbers of pDCs are increased in the first year after T1D diagnosis (101).

Similar to macrophages, DCs participate in T1D pathogenesis by impairing the activation of T cells due to abnormal antigen presentation, enhanced cytokine production and defective maintenance of tolerance mechanisms (95). Investigations in animal models have shown that DCs produce higher levels of IL-12, a cytokine necessary for Th1 polarization, and demonstrate enhanced expression of costimulatory molecules CD80/86, which resulted in aberrant induction of IFNγ-producing T cells

(102, 103). Moreover, pancreas-infiltrating DCs phagocyte apoptotic beta cells and process and present an autoantigen on their MHC molecules to autoreactive $CD4^+$ T cells or to $CD8^+$ T cell in cross-presentation and thus initiate autoimmune response (104). Different APC functions and processes involved in T1D immunopathology are summarized in Figure 4.

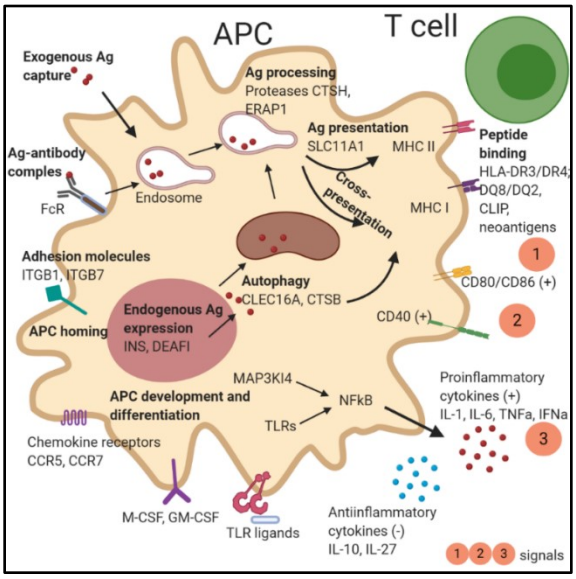

Figure 4: Summary of APC biological processes affected in T1D. *APC development, cell numbers and yields; antigen capture, processing and presentation; APC activation and costimulation; cytokine production; chemotaxis and cell adhesion are the main APC alterations in T1D. Created with Biorender. Adapted from Creusot at el.* (105)*. Ag – antigen, FcR – Fc receptors, VDR – vitamin D receptor*

Our group focused on studies of DCs for several years. Using gene arrays, we have identified several genes dysregulated in T1D DCs. A receptor subunit of IL-27, *IL-27Ralpha,* was identified as one of the candidate genes elevated in mDCs. We have performed detailed investigation that demonstrated aberrant reactivity of mDCs to IL-27 involving enhanced signalling through STAT3 and increased PD-L1 expression on the surface of mDCs (106). IL-27 plays a dual role due to the induction of proinflammatory Th1 and regulatory Tr1 cells. Our findings suggested compensatory anti-inflammatory mechanisms of ongoing inflammation. The expression of PD-L1, PD-L2 and PD-1 are essential for the regulation of T cell activation and promotion of immune tolerance. The PD-1/PD-L1 pathway regulates the induction and maintenance of the central and peripheral immune tolerance and protects the tissues against autoimmune attacks (107). Increasing evidence has shown that impaired function of PD-1/PD-L1 signalling plays an important role in several autoimmune diseases, including psoriasis, inflammatory bowel disease, SLE or T1D (108–112).

IFNα-producing pDCs are involved in the pathogenesis of T1D. Diana et al. showed that self-DNA released upon physiological pancreatic beta cell death activates pDCs through TLR9 to induce type I IFN production, which consequently leads to the initiation of autoimmune T cell response (104). Notably, $TLR9^{-/-}$ NOD mice were protected against the development of T1D (113). Our investigation that focused on microbial and self-nucleic acid sensing by T1D monocytes and DCs demonstrated

that T1D pDCs displayed more mature phenotype and higher production of IFNα and other proinflammatory cytokines in reactivity to DNA in a TLR9-dependent manner (84).

1.2.1.3 APC as a therapeutic target

Crucial role of DCs in the induction and maintenance of self-tolerance has made them an attractive target for therapeutic interventions. The main strategies include adoptive transfer of tolerogenic DCs, *in vitro* expansion of T regulatory lymphocytes (Tregs) by specific DC subsets and *in vivo* targeting of DC subsets or their products (95).

Intervening proinflammatory cytokines produced by APCs can result in preservation of beta cell functions. IL-1α and IL-1β are important cytokines produced mostly by monocytes and are involved in T1D pathology by activating T helper cells and increasing the numbers of circulating memory T cells. The results of a clinical trial suggested that blocking these cytokines by the corresponding antagonists may be beneficial for preservation of the beta cells (114). Similarly, blocking TNFα with a fusion protein etanercept or the IL-6 and IL-12/23 cytokine pathways improved the protection of beta cell functions (115, 116).

Tolerogenic DCs (tDCs) are DC with so-called semi-mature phenotype that produces IL-10 and low amounts of proinflammatory cytokines. tDCs attenuate T cell stimulatory capacity, induce Tregs and may be the most effective means for T1D intervention therapy. However, one of the major concerns associated with *in vivo* administration of *in vitro* prepared tDCs is the stability of their tolerogenic activities in the proinflammatory environment (117). The results of the clinical studies with tDCs showed that the treatment is safe and result in an increase in Tregs; however, no significant differences in glycaemia were observed (118, 119). Most likely, co-administration of tDCs and Tregs allows stabilization of $FoxP3^+$ population and elevates IL-10, retinoic acid and TGFβ levels by tDCs thus reducing proinflammatory milieu.

Several approaches to targeting DCs *in vivo* have been tested in animal models. For example, use of microparticles loaded with oligonucleotides specific for CD40, CD80 and CD86 resulted in an increase in Treg levels and a delay in the onset of the disease (120). Treatment of NOD mice with soluble CTLA4-Ig also resulted in T1D prevention by inducing immunosuppressive mechanism of tryptophan catabolism through indolamine dioxygenase (IDO) activation (121). Moreover, administration of the low doses of high affinity antigens, which cause *in vivo* induction of Tregs by antigen presentation via DCs, was effective in slowing the disease progression (122, 123).

1.2.2 Neutrophils

The classical view of neutrophils as short-lived cells possessing only a limited capacity for biosynthetic activity and releasing granules and reactive oxygen species (ROS) has changed over the past years. Neutrophils have a longer circulatory life span (up to 5 days) and they act as a crucial element of the inflammatory response to direct and guide the innate immunity response by engaging in complex interactions with macrophages, natural killer cells, dendritic cells and T cells through soluble mediators or by direct cell-cell contacts (124, 125). Upon activation, neutrophils release soluble pattern recognition molecules, promote phagocytosis, stimulate complement and modulate inflammation (126, 127). These cells produce a wide variety of cytokines, antimicrobial proteins and peptides and neutrophil extracellular traps (NETs). NETs are extracellular web-like structures composed of cytosolic and granule proteins assembled on decondensed chromatin (128) and are released to trap, neutralize and kill bacteria (128), viruses (129) and fungi (130).

Studies on the neutrophil functions and phenotype in T1D patients started in the 70s. The obtained data have indicated defective chemotaxis, phagocytosis, release of lysosomal content and microbicidal activities, impaired ROS production and other abnormalities that was usually attributed to hyperglycaemia (131–133). However, the significance of these early studies and used technologies has remained questionable for a long time. Currently, the studies of the samples from the subjects with or without normoglycaemia enable to explore the role of intrinsic factors versus hyperglycaemia in defective neutrophil functions. It is not known whether neutrophils are active players in T1D pathogenesis or an indirect marker of distinct pathological mechanisms (134).

The role of neutrophils in T1D has gained renewed interest in 2013 when Diana et al. published their work in NOD mice that for the first time identified the earliest key immunological events of T1D pathogenesis. The authors demonstrated that physiological beta cell death promotes infiltration of B lymphocytes, pDCs and neutrophils to the pancreas (104). Around the same time, another group reported that circulating neutrophils are reduced in T1D patients and in T1D autoantibody-positive at-risk individuals (135). Importantly, neutrophils were detected in pancreases prior to the onset of T1D and were absent in patients with T2D; these pancreas-resident neutrophils extrude NETs (136). Notably, NETs represent endogenous danger signals that are sufficient to initiate inflammation in the absence of microbial priming and can potentiate the inflammation through the regulation of inflammatory cytokines directly or indirectly by modulating and activating other immune cells (137, 138). Moreover, NETs are also a source of autoantigens and can contribute to the pathogenesis of certain autoimmune diseases, particularly those associated

with autoantibodies against neutrophil-derived proteins, such as an antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (139). In systemic lupus erythaematosus (SLE), NETs can trigger the production of type I IFNs by activating pDCs to drive autoimmune pathology (140, 141). The proposed role of neutrophils in T1D is illustrated in Figure 5.

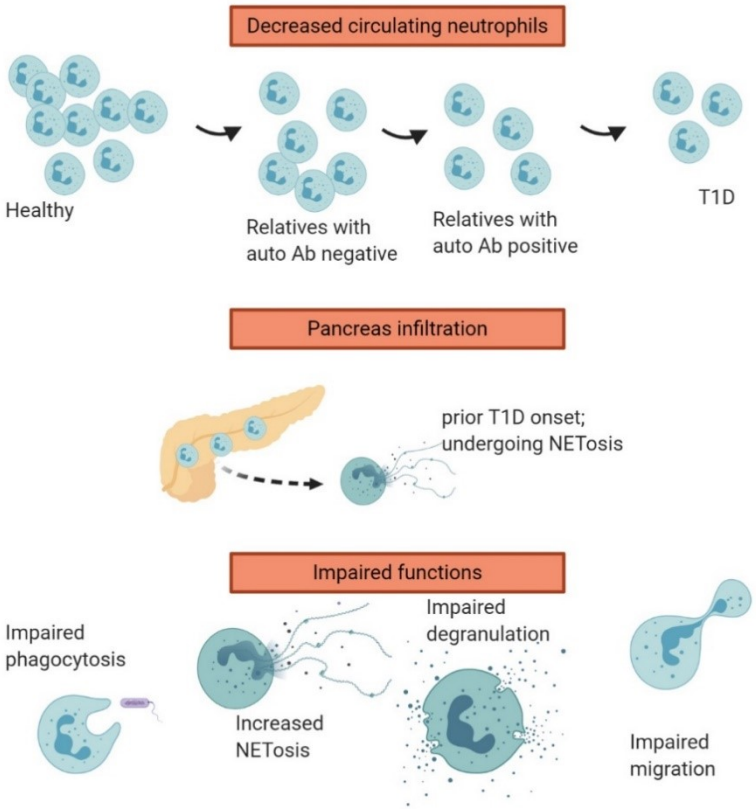

Figure 5: Proposed role of neutrophils in T1D. *Decreased neutrophil numbers in the peripheral blood, impaired phagocytosis, degranulation and migration are altered in T1D patients. Neutrophils infiltrate pancreases prior T1D onset, where they undergo NETosis. The inappropriate elimination of NET-associated products leads to the stimulation of APCs and consequently to activation of autoreactive T and B lymphocytes. Created with Biorender.*

In the pancreas, residing NETs may play an active role in disease development by presenting modified autoantigens or directly damaging the tissue. Since it is almost impossible to access the pancreas in the living subjects, it is important to determine whether the markers of NETosis can be detected in the periphery and whether their amounts correlate with pancreatic functions. The data of the literature are inconclusive since both increased and decreased NET-related biomarkers have been found in T1D patients (142, 143). The discrepancy may results from the selection and grouping of the samples (134).

The studies of our group also focused on the functions of neutrophil and NETs in T1D patients. Our present study showed a continuous decrease in the numbers of neutrophils in the first year after T1D onset in addition to other immunological parameters, i.e., cell population and levels of antibodies (101). Moreover, our data indicated that only NETs from T1D patients activated DCs resulting in enhanced cytokine production, phenotype changes and induction of IFNγ-producing T cells. Notably, the transcriptomic analysis revealed that T1D NETs were able to induce pro-T1D signature in healthy DCs, suggesting their pathological role in inflammation (144). Moreover, our work revealed that T1D monocytes and DCs reacted to NETs by engaging the intracellular DNA sensors (TBK1-STING), which was mirrored by releasing proinflammatory cytokines (84), supporting the impaired sensing of DNA by innate immune cells in T1D.

1.2.2.1 Neutrophils as a therapeutic target

Neutrophils may be also a candidate target for therapeutic approaches due to their involvement in T1D pathology. Neutrophil activation, binding to the endothelium, transendothelial migration, migration into pancreatic islets and release of cytotoxic products are potential targets (145). Studies in mouse models have shown that blocking neutrophil recruitment to the pancreas by a CXCR2 agonist delayed T1D progression (146). Similarly, inhibition neutrophil adhesion to endothelial venules within the pancreas may be an effective approach. Antibodies against L-selectin and VLA-4 (very late antigen 4) and administration of anti-α4-integrin and anti-LFA-1 antibodies were reported to successfully in depress insulitis and reduce the destruction of the beta cells (147).

1.2.3 T cells

In healthy individuals, self-reactive lymphocytes are strictly regulated through the mechanisms of the central and peripheral tolerance that prevent their activation and accumulation. However, in T1D patients, a combination of genetic and environmental factors allows autoreactive clones to escape the tolerance checkpoints, leading to autoimmunity. Autoreactive T cells target a broad repertoire of the antigens and are considered to be the main destructors of the beta cells. nPOD studies showed that $CD8^+$ T cells are the predominant T cell population and the most abundant inflammatory cell type in the pancreas in T1D (73). Moreover, autoreactive T cells are also present in the peripheral blood of the patients (148, 149), and antigen specificity of this self-reactive population increases with the duration of the disease, suggesting that epitope spreading occurs after the diagnosis (73). Interestingly, transplantation of a pancreatic segment from a healthy non-diabetic twin to a diabetic twin was followed by recurrent beta cell destruction, suggesting islet-specific immunological memory (150). Similarly, T1D can be transferred after bone marrow

transplantation, which was not depleted of T cells, from diabetic donors (151). Importantly, therapies against T cells have been proven beneficial since they slow down the disease progression and prevent recurrent beta cell destruction after islet transplantation (152, 153).

1.2.3.1 Loss of immunological tolerance to beta cells

Immunological tolerance is the prevention of an immune response against particular antigens. Immunological tolerance is an active, complex and carefully regulated mechanism especially important for the prevention of an autoimmune disease. Tolerance can be central or peripheral, depending on where the processes are originally established, i.e., thymus and bone marrow (central) or tissues and lymph nodes (peripheral). The autoreactive T cell clones are depleted before they develop into fully immunocompetent cells in the thymus, where they are exposed to self-autoantigens. Most self-molecules, including those with tissue-specific expression, such as insulin, are expressed in the thymus early in life to establish central tolerance. However, several mechanisms may be impaired causing flawed self-tolerance. A polymorphic variable nucleotide tandem repeat (VNTR) sequence at the insulin gene influences the level of insulin expression in the thymus. Alleles with predisposing T1D variants are associated with lower insulin expression in the thymus (12). An additional mechanism involves epigenetic suppression of the expression of a parental copy of insulin (154). HLA molecules associated with T1D predisposition weakly interact with proinsulin peptides leading to insufficient presentation to TCR thus enabling the survival of thymic selection (155). These mechanisms permit autoreactive clones to escape thymic selection.

Central tolerance is not the only type of tolerance influenced by T1D. Peripheral tolerance develops after T cells mature and enter peripheral tissues and lymph nodes, and its role is to ensure that self-reactive T and B cells, which escaped central tolerance selection, do not react to autoantigens. The main mechanisms involved in peripheral tolerance are direct inactivation of effector T cells by clonal depletion, anergy induction or conversion into regulatory T cells (Tregs), which suppress the effector functions of other T cells. T1D patients demonstrate defective peripheral tolerance, including impaired Treg functions or enhanced effector T cell (Teffs) resistance to Treg suppression. Certain risk alleles are associated with immune regulation, for example, CTLA-4 and IL-2RA, which control important T cell functions (156).

1.2.3.2 T cell autoantigens and neoantigens

Identification of autoreactive T cells involved in the pathogenesis is one of the major objectives for T1D research enables the development of antigen-specific methods to prevent and/or reverse the

disease (157). Self-reactive CD4 and CD8 T cell clones are present in the T1D pancreas and are also detected in the periphery; however, peripheral clones are characterized by exceptionally low amounts making their detection difficult. However, the ability to detect and subsequently phenotype autoreactive T cells in the periphery has been improved. For example, HLA I monomers/multimers or HLA II tetramers can be used to detect autoreactive T cells without *in vitro* amplification, which can influence the phenotypes of these cells (158, 159).

Analysis of antigen specificity of islet-infiltrating T cells from 9 donors 2-20 years after the diagnosis revealed a broad repertoire and heterogeneity in T cell responses, which may reflect the differences in HLA restriction, environmental exposure, and disease stage. More than 250 T cell lines and clones with distinct specificities were obtained, and only a small fraction (19 out of 50 studied) of them reacted to known antigens (160).

Self-reactive CD4 and CD8 T cell clones recognize several autoantigens, including native proteins and epitopes of proinsulin, GAD65, tyrosine-phosphatase-like insulinoma associated antigen 2 (IA-2), islet-specific glucose-6-phosphatase catalytic subunit related protein (IGPR), cation efflux transporter ZNT8, chromogranin, islet amyloid peptide (IAPP), etc. (156). However, increasing number of studies suggest that islet autoimmunity also targets neoepitopes, which may be unavailable for negative thymic selection. Immune system cannot recognize alternatively spliced variants expressed in the pancreas that are not expressed in thymus, which may be the reason for promotion of autoimmunity against various epitopes of IA-2 and IGRP (161, 162). Moreover, inflammation and stress often cause posttranslational modifications (PTM) of many epitopes. For example, T cells are able to recognize oxidized cysteine residues of the insulin A chain (163). Citrullination or transglutamination enhance GAD65 binding to a specific HLA allele (164). Peptide fusions of C-peptide and other beta cell secretory granule proteins, such as chromogranin, IAPP and neuropeptide Y, act as high-affinity T cell targets (157). Finally, predisposing HLA types are crucial for neoepitope presentation to T cells (165).

1.2.3.3 CD4 and CD8 T cells

CD4 T cells in T1D patients assist effector CD8 T cells, stimulate antibody secretion by B cells and stimulate islet-resident macrophages. These cells are defined as proinflammatory cells capable of producing IFNγ, IL-17 and other mediators (93). CD4 and CD8 T cells represent the dominant immune cell types in pancreatic islets and play the key role in beta cell killing. In T1D, CD4 T cell responses play a central role in beta cell autoimmunity; however, the exact epitopes recognized by these cells remain unknown (157). Autoreactive CD4 T cells are activated in the pancreatic lymph

nodes by APCs that present beta cell antigens. These cells are required for efficient activation of autoreactive CD8 T cells and macrophages, which destroy beta cells. Moreover, CD4 T cells can directly kill beta cells in an antigen-dependent manner (166).

Autoreactive T cells are present before the clinical onset of T1D. Naïve $CD4^+CD45RA^+$ T cells with beta cell reactivity can be detected in the cord blood of healthy neonates with genetic risk (167). Presence of naïve $CD4^+$ T cells responsive to a beta cell antigen suggests that T cell autoimmune reactivity occurs prior to the development of beta cell-specific memory $CD4^+$ T cell responses and seroconversion, which can be beneficial as an early diagnosis biomarker helpful for the prevention of metabolic complications often associated with the clinical onset of T1D (168).

$CD8^+$ T cells are the predominant population of T cells infiltrating the pancreas in T1D during insulitis. nPOD studies showed that beta cell destruction is mediated in part by direct contact of CD8 T cells with beta cells via perforin and granzyme B (73, 169). Moreover, nPOD studies proved broad antigen specificity of infiltrated CD8 T cells using HLA multimers. The spectrum of recognized autoantigens increases with longer disease duration (73). Autoreactive CD8 T cells are activated through MHC I, which consequently leads to contact-dependent beta cell death. Hyperexpression of HLA I molecules by endocrine cells in insulin-containing islets explains predominance $CD8^+$ T cells in insulitis (170). HLA I expression may be triggered by viral infections associated with T1D risk factors (171) or by the effects of proinflammatory cytokines, such as IFNγ or TNFα, on beta cells (172).

1.2.3.4 Regulatory T cells

A specialized population called regulatory T cells (Tregs) functions as suppressors and regulators of immune responses and is the major component of the peripheral tolerance mechanisms. A defect in these functions has been shown in many autoimmune diseases, including T1D. A population expressing FoxP3 and high level of the IL-2R alpha subunit (CD25) can develop in the thymus (tTregs or nTregs) and in the periphery (pTregs). Generation of Tregs depends on their encounter with an antigen and IL-2 signalling. IL-2 is a crucial cytokine important for Treg generation, survival, proliferation and functions (173). Tregs function via several mechanisms, which can be contact-dependent or -independent (Figure 6). For example, Tregs deprive effector T cells from IL-2 due to the expression of a high-affinity receptor for IL-2 or from tryptophan due to IDO (indolamine dioxygenase) production. Tregs also release anti-inflammatory cytokines, such as TGFβ, IL-35 or adenosine, and Tregs can directly reduce T cell activation by expressing CTLA-4 (174). These and several other mechanisms are involved in Treg-mediated suppression (Figure 6).

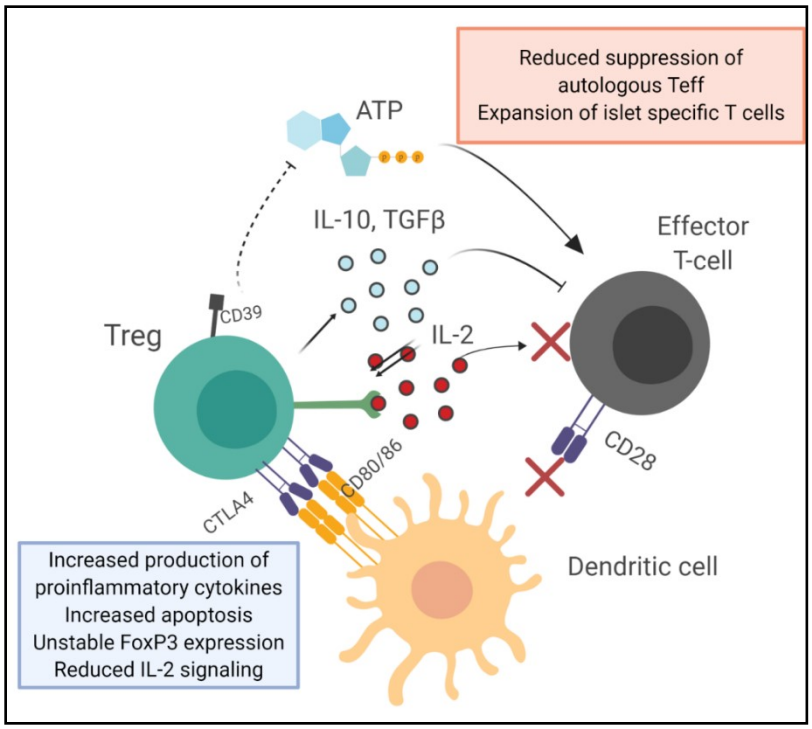

Figure 6: Treg functions and their alterations in T1D patients. *Both contact-dependent and -independent (immunosuppressive cytokine production, IL-2 adsorption) mechanisms of Tregs are affected in T1D. Created with Biorender.*

The strongest link between Tregs and autoimmunity is observed in the disorder called IPEX (immunodysregulation polyendocrinopathy enteropathy X-linked syndrome) caused by loss-of-function mutations in the *FoxP3* gene. Affected individuals develop a wide variety of pathologies and autoimmune diseases, including T1D. T1D develops in more than 80% of IPEX patients under 2 years of age (175), which means FoxP3 deficiency can provoke T1D regardless of T1D-associated genetic and environmental factors. These observations indicate a key role of Tregs in prevention of T1D development. Conversely, therapies based on increasing the numbers or functional capacity of Tregs can delay T1D progression (176).

Certain alterations in the functions of Tregs in T1D partially clarify the aspects of T1D pathology and provide an opportunity for novel therapeutic interventions or adoptive transfer strategies. Many identified loci (*IL2RA, IL2, CTLA4, PTPN2* and *IL10*) (177) associated with T1D risks are involved in the functional properties of Tregs.

In general, the peripheral frequency of Tregs in T1D is unaltered compared to that in healthy individuals; however, studies focused on Treg numbers in T1D showed variable results (178–181). Studies defining only $CD4^{+}CD25^{hi}$ T cells as Treg showed a decrease in Treg numbers (182), whereas studies using more accurate markers, FoxP3 and CD127, reported unaltered numbers. Our group has reported quantification of Tregs using a more accurate gating strategy based on the expression of CD25, FoxP3 and CD127; however, we have demonstrated a decrease in the numbers of Tregs in

circulation in a large cohort of long-term treated patients (183). Treg identification in humans is not easy because FoxP3 is transiently expressed also in effector T cells (Teffs) and high expression of CD25 is also detected on activated T cells, meaning that the population identified as $CD4^+CD25^+FoxP3^+$ may be a mixture of regulatory and non-regulatory T cells (184). Treg definition is also hindered by the fact that Treg population is a heterologous group of cells with distinct phenotypic subtypes of the cells that reflect various stages of maturation and activation or use distinct types of suppression (185).

In T1D, Tregs demonstrate clearly impaired suppressive properties. In T1D patients, Tregs are less able to control the proliferation of Teffs; this phenomenon was reported for the first time in 2005 (178). Moreover, cytokine analysis revealed a Treg trend towards proinflammatory cytokine production in individuals with T1D, whereas Tregs from healthy control subjects also produce IL-10. The reduced suppressive ability of Tregs appears to be consistent because it is still present in patients 20 years after the diagnosis. Additionally, an increase in the population of Tregs producing IFNγ and IL-17 was reported in T1D (186, 187). Several groups reported an increase in Treg apoptosis close to diagnosis, and Treg apoptosis starts to decrease 6 months after the diagnosis (188, 189).

Considering the key role of IL-2 signalling in all important Treg functions, Treg intrinsic defects were postulated to be associated with reduced IL-2 signalling (190, 191). If the source of IL-2 is limited, Tregs cannot maintain FoxP3 expression and display reduced suppressive functions; however, the precise molecular mechanism remains unknown (192). Our group also reported impaired IL-2 signalling in Tregs of T1D patients. Tregs of T1D patients expressed lower level of CD25, and reactivity of these Tregs to IL-2 was diminished based on a reduction in STAT5 phosphorylation (183).

Interestingly, studies in human cadavers revealed that pancreas barely contains infiltrating $FoxP3^+$ T cells (87), suggesting that regulation of the immune response is located elsewhere. An investigation of human lymph nodes of T1D individuals showed diminished suppression by Tregs and increased production of IL-17 (193).

In addition to $FoxP3^+$ T cells, other $CD4^+$ T cell subsets display regulatory functions mediated by characteristic high IL-10 production. These T regulatory type 1 (Tr1) Tregs can inhibit T cell responses and modulate the functions of antigen-presenting cells (APC) via several mechanisms, such as expression of inhibitory molecules, cytolytic activity and IL-10 production (194). In T1D,

exposure to islet autoantigens results in generation of a population of $CD4^+$ T cells with potent suppressive properties and high IL-10 secretion. This population of Tr1 is enriched in individuals at risk of T1D which lack islet autoimmunity. These cells are also associated with a less aggressive form of T1D and Tr1 cells of first-degree relatives secrete more IL-10 compared with that in T1D patients, suggesting that this population is associated with protection from islet autoimmunity (93).

1.2.3.5 T cells and Tregs as a therapeutic target

Current strategies for immunotherapies can be classified as antigen-independent and antigen-dependent. Non-antigen specific interventions include general immunosuppression, antibody-based therapies that allow polyclonal depletion of T and B cells, cytokine-based strategies and an increase in the numbers of polyclonal Tregs (195). Teplizumab and otelixizumab are antibody-based therapies comprising anti-CD3 monoclonal antibodies targeting the CD3/T cell receptor (TCR) complex to convert T cells into an anergic stage; the drugs have been tested and clinically approved in T1D patients for C-peptide preservation (152). Abatacept is a fusion protein composed of the Fc region of human IgG1 fused with the extracellular domain of CTLA4 and a costimulatory molecule inducing anergy in T cells; the drug has demonstrated potential against T1D in a recent clinical trial (196). The elimination of autoreactive memory T cells is necessary to obtain lasting results. This goal can be achieved by blocking CD2 signalling by alefacept, a fusion protein that promotes apoptosis in memory and effector T cells. However, a clinical trial utilizing alefacept achieved only modest preservation of beta cell functions (197). Anti-thymocyte globulin (ATG) in combination with granulocyte colony-stimulating factor (G-CSF) was able to deplete activated T cells and induce the protection of the beta cell mass (198).

Natural polyclonal expansion of autologous Tregs was suggested as a promising therapy for T1D patients. A clinical trial has shown that adoptive transfer of Tregs is safe and feasible (199). Another clinical trial was testing low doses of IL-2 to selectively promote Treg activity with the rationale that Tregs respond to lower doses of IL-2. Although low dose IL-2 therapy was able to increase the Treg numbers, it did not result in better glycaemic control (200).

To prevent beta cell destruction, the most relevant T effector clones have to be deleted by antigen-dependent treatment strategies, which allow preservation of normal immune homeostasis. The main objectives include induction of tolerance of self-reactive T cells and expansion of autoantigen-specific Tregs. Success in tolerization of T effector cells depends on various factors, especially identification of the autoantigens that drive the process (195). Identification can be achieved by beta cell-autoantigen vaccination strategies. Well-known T cell epitopes against insulin and glutamic

acid decarboxylase have been investigated. The administration of C19-A2 proinsulin peptide resulted in the higher C peptide levels in recently diagnosed T1D patients (201).

In recent years, immunotherapy using engineered T cells expressing chimeric antigen receptor (CAR) specific against CD19 emerged as a breakthrough in cancer immunotherapy of B cell leukaemia (202). It is logical to hypothesize that improving Tregs with beta cell-specific CARs may enhance Treg functions to protect islet cells from destruction. Several studies demonstrated considerable potential for CAR-Tregs therapy in autoimmune and other disorders, such as EAE, haemophilia A or asthma (203–206). In T1D, the lack of specific autoantigens to generate islet-protective CAR-Tregs remains the biggest challenge (207).

1.2.4 B cells

Islet antigen-specific T cells are believed to be the main destructors of the beta cells; however, increasing evidence shows that islet-specific B lymphocytes are also involved in TD pathogenesis. However, precise role of these cells is unclear. The depletion of this population delays the disease progression in newly diagnosed patients, and the numbers of pancreatic CD20 B cells correlate with beta cell loss (208, 209). B cells are present in small numbers in early insulitis and are recruited as beta cell death progresses, suggesting that they are a consequence rather than the cause of insulitis (87). B lymphocytes are capable of autoantigen presentation to T cells and production of cytokines and autoantibodies in humans and mice (54, 210, 211) (Figure 7). In 2001, a study reported an individual deficient in B cells who developed T1D (212), indicating that B cells are not necessary for the development of the disease; however, other studies have shown that individuals with lymphopaenia may accumulate autoreactive T cells through homeostatic expansion that does not involve B cells (213). The exact role of B cells in T1D pathogenesis remains a subject of investigation.

1.2.4.1 B cells as autoantibody and cytokine producers

The presence of islet autoantibodies is one of the T1D predictors and supports the pathogenic role of B cells. Individuals with more than two islet autoantibodies are almost guaranteed to develop T1D. However, the autoantibodies do not have pathogenic potential, and their production is probably a byproduct of anergic B cell activation (214). All known B cell epitopes are intracellular, preventing antibody-dependent cell-mediated cytotoxicity and eliminating the cytolytic function of the autoantibodies. A negative correlation between the level of autoantibodies and T cell proliferation has been observed (215, 216), suggesting that autoantibodies may function in the

immune regulation of ongoing inflammation and that B cells display impaired regulation and not pathogenic activities. B cells must be initially activated by T cells and cannot prime naïve T cells, signifying that autoantibodies are a consequence of a loss of tolerance towards beta cells, which is T cell-mediated. Therefore, it is unlikely that B cells initiate the disease pathology (217). Interestingly, prevalence of diabetes-associated autoantibodies in the general population is 3% versus 0.3% prevalence of these antibodies in T1D, and 90% of seropositive individuals remain healthy (218).

B cells can be activated by sensing danger-associated molecular patterns (DAMPs), such as extracellular DNA, RNA or others molecules, via TLR (Toll-like receptors) with subsequent release of proinflammatory cytokines, such as IL-6 or TNFα (219, 220), that in turn accelerate proinflammatory environment and contribute to the initiation of diabetic kidney disease (221).

1.2.4.2 B cells as antigen-presenting cells

Several studies suggested that B cells contribute to the T1D pathology by presenting islet autoantigens to autoreactive T cells. One of the studies on cadaveric pancreatic tissues focused on the infiltrating cell population revealed that B cells accumulate in the pancreas during the development of insulitis together with CD8 lymphocytes, supporting an idea about B cell cross-presentation to CD8 T cells (87). However, B cells are less efficient that DCs if they do not express B cell receptor (BCR) for a certain islet autoantigen (74, 75). In NOD mice, inhibition of antigen presentation activity of B cells prevented T1D development demonstrating the importance of B cells in antigen presentation to $CD4^+$ T cells and in cross-presentation to $CD8^+$ T cells (210, 222). The ability to present autoantigens likely results from compromised B cell anergy, which can result from the effects of genetic and/or environmental factors (214). B cells contribute to the later stages of the disease by increasing the rate and range of islet destruction through epitope spreading (217).

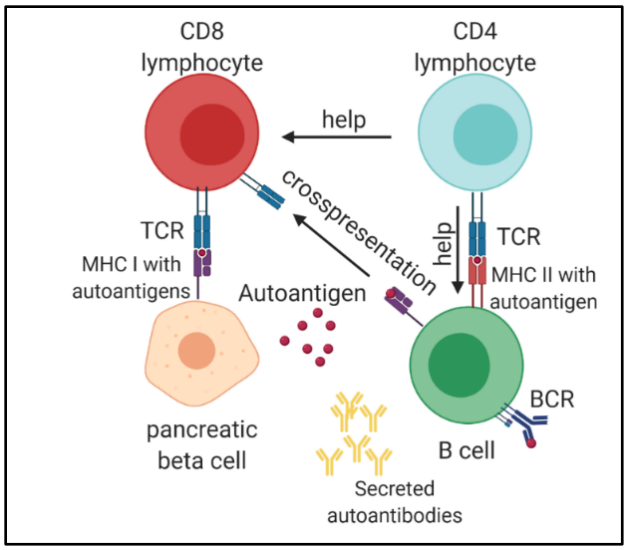

Figure 7: Contribution of B cells in T1D

1.2.4.3 B cell subpopulations

T1D is an organ-specific autoimmune disease; ideally, the studies should focus on the cells localized in targeted tissues; however, many studies have been performed on peripheral blood presuming

that changes in the pancreas are mirrored in the periphery. Several studies attempted to identify the changes in the B cell compartment during the disease progression. Autoreactive B cells at both stages, including new emigrants and mature naïve cells, have been found in the circulation in T1D patients suggesting a deficiency of the tolerance mechanisms (223).

B cells undergo complex development from the bone marrow as "transitional" B cells to the periphery and particularly to the spleen. In the spleen, transitional B cells are rescued from the negative selection by apoptosis induced by self-reactivity under the influence of BAFF, also known as BlyS (B cell-activating factor). BAFF also drives the transitional B cells towards differentiation into long-lived and T cell-independent IgM-producing "marginal zone-like" B cells or "naïve" follicular B lymphocytes. Upon encountering their antigens, naïve B cells mature and enter into the peripheral lymphoid organs, where they cooperate with T cells and become memory B cells and eventually plasmablasts and plasma cells (224). B cell development is illustrated in Figure 8.

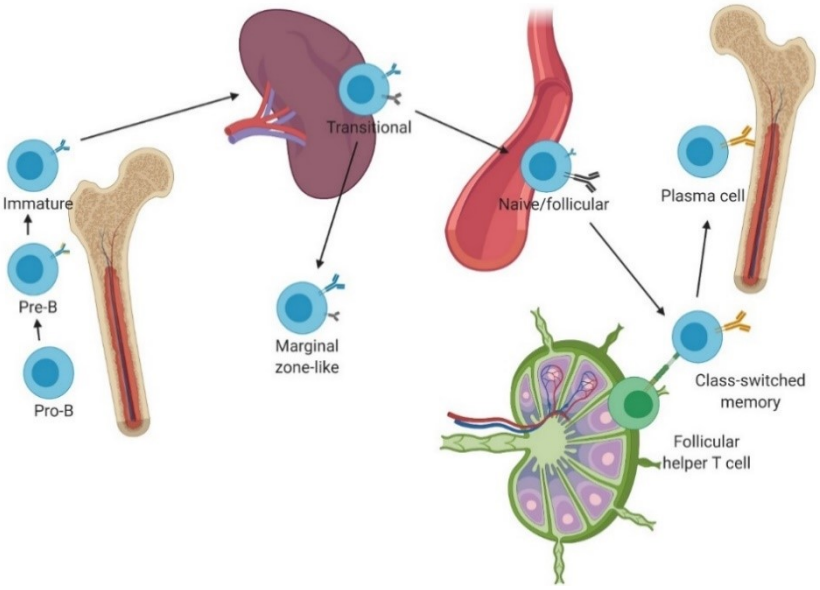

Figure 8: B cell development. *Created with Biorender.*

The transition of B cells from the bone marrow to the circulation represents a crucial step mostly due to the presence of tolerance checkpoints establishing subsequent antigen-driven development of B cells in the peripheral lymphoid tissues. Several studies focused on the alterations in B cell subpopulations in T1D with inconclusive outcomes. A study showed elevated percentages of MZ-like B cells in adult patients with T1D (225). Another group reported a lack of differences between patients and controls; however, the study was limited to a small cohort (226). Our group focused on the B cell compartment in the large cohorts of T1D patients and their relatives and demonstrated

that B cells are skewed in T1D due to a decrease in the number of cells at earlier stages and a shift towards antibody-secreting plasmablasts (227). The differences between the studies are predominantly caused by various gating strategies and material used for the analysis.

B cell at the periphery display disrupted maturation in long-standing T1D patients, who expressed considerably lower levels of the BAFF receptor (TACI) compared to the levels of other maturation molecules (228). BAFF can rescue and promote the survival of low-affinity autoreactive clones of B cells (229) and has thus gained considerable attention, especially in the field of autoimmune diseases (230). In our work, we show lower expression of BAFF receptor (BAFFR) on B cells and impaired reactivity to BAFF of B cells and T cells (227).

1.2.4.4 B cells as a therapeutic target

Current B cell therapies have focused on depletion of B cells in general and have not considered antigen specificity, which results in issues due to increased risks of infections and hypogammaglobulinaemia. Rituximab is an anti CD20 monoclonal antibody that targets pre-B cells and mature B cells and causes cell lysis by inducing antibody-dependent cell-mediated cytotoxicity, which reduces the activation of self-reactive T cells. A patient with newly diagnosed T1D receiving this treatment demonstrated an increase in the levels of C-peptide and required lower doses of insulin 1 year after the treatment. However, 30 months after the treatment, the effect was abolished following B cell recovery, which probably mirrored the return of autoreactive B lymphocytes (231, 232). In an animal model, the treatment also targeted BAFF and a proliferation-inducing ligand (APRIL) molecules that play important roles in B cell development and maturation (233).

1.2.4.5 A novel type of immune cells

Recently, in 2019, Ahmed et al. described a hybrid lymphocyte, which bears the characteristics of both B and T cells and plays a role in T1D autoimmunity; however, the mechanisms and development of this population are unknown. Using flow cytometry, the group has identified a cell population expressing both TCR and BCR on the surface. The cells express genes specific to T and B cells, and the population had higher frequency in T1D patients. In this dual population, BCR is expressed as a specific sequence present in the majority of the cells of the population, which is unusual because this region is usually very diverse. Notably, the sequence was detected only in T1D patients and was not detected in healthy individuals. The authors suggested that the sequence of the peptide, which can tightly bind to HLA-DQ8, is a variant that also binds insulin and plays a major

role during the initial phase of the disease; the authors suggested that this sequence can be used as a marker for early diagnosis (234).

In summary, the mechanisms of T1D immunopathology are overly complex and involve various cell populations and their altered functions. The main cell populations and products involved in the autoimmune reactions in T1D are illustrated in Figure 9.

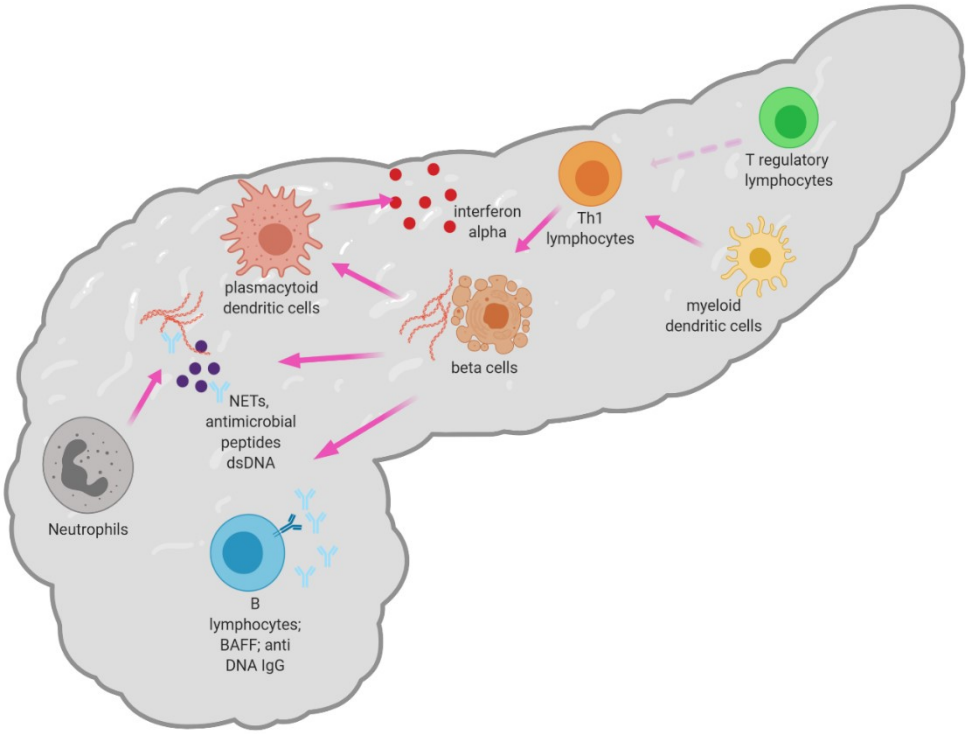

Figure 9: Complex immune reactions involved in T1D immunopathology. *Both innate and adaptivity immunity cells are involved in T1D pathogenesis. Enhanced NET formation and/or insufficient NET elimination lead to inappropriate activation of APC, which consequently activate autoreactive T and B lymphocytes. Created with BioRender*

AIMS OF THE THESIS

Efforts to understand the mechanisms of the type 1 diabetes (T1D) pathology continue. The number of tentative clinical T1D therapies is increasing every year; however, widely available and effective therapies for T1D patients are unavailable at this moment. Investigation of the immune system dysregulation in T1D will provide further opportunity for the therapeutic approach based on the prevention or reversal of immune-mediated damage to insulin-producing cells. In this thesis, we have focused mainly on the role of dendritic cells, which is the crucial population shaping the adaptive T and B cell responses, T cell tolerogenicity and often ignored neutrophils.

The specific objectives of this thesis are:

- To assess the role of neutrophil extracellular traps in shaping dendritic cell response in T1D
- To evaluate the aberrant sensing of microbial and self-DNA by T1D monocytes and dendritic cells in T1D and its potential impact on pathogenesis of the disease
- To explore the effect of IL-27 on dendritic cells within the context of T1D
- To monitor the role of T regulatory lymphocytes in prevention or acceleration of T1D
- To map the alteration of B cell subpopulations in T1D patients and their relatives, especially concerning the presence or absence of diagnostic autoantibodies

RESULTS

The list of publications with direct connection to the presented thesis:

Parackova, Z., I. Zentsova, P. Vrabcova, A. Klocperk, Z. Sumnik, S. Pruhova, L. Petruzelkova, R. Hasler, and A. Sediva. 2020. Neutrophil Extracellular Trap Induced Dendritic Cell Activation Leads to Th1 Polarization in Type 1 Diabetes. *Front. Immunol.* 11: 661. (**IF=5.05**)

Parackova, Z., P. Vrabcova, I. Zentsova, J. Kayserova, I. Richtrova, L. Sojka, K. Stechova, Z. Sumnik, and A. Sediva. 2020. Enhanced STAT3 phosphorylation and PD-L1 expression in myeloid dendritic cells indicate impaired IL-27Ralpha signaling in type 1 diabetes. *Sci. Rep.* 10: 8–15. (**IF=4.11**)

Zentsova, I., **Z. Parackova**, J. Kayserova, L. Palova-Jelinkova, P. Vrabcova, N. Volfova, Z. Sumnik, S. Pruhova, L. Petruzelkova, and A. Sediva. 2019. Monocytes contribute to DNA sensing through the TBK1 signaling pathway in type 1 diabetes patients. *J. Autoimmun.* . (**IF=6.65**)

Klocperk, A., L. Petruzelkova, M. Pavlikova, M. Rataj, J. Kayserova, S. Pruhova, S. Kolouskova, J. Sklenarova, **Z. Parackova**, A. Sediva, and Z. Sumnik. 2019. Changes in innate and adaptive immunity over the first year after the onset of type 1 diabetes. *Acta Diabetol.* . (**IF=3.42**)

Parackova, Z., J. Kayserova, K. Danova, K. Sismova, E. Dudkova, Z. Sumnik, S. Kolouskova, J. Lebl, K. Stechova, and A. Sediva. 2016. T regulatory lymphocytes in type 1 diabetes: Impaired CD25 expression and IL-2 induced STAT5 phosphorylation in pediatric patients. *Autoimmunity* 1–9. (**IF=2.63**)

Parackova, Z., A. Klocperk, M. Rataj, J. Kayserova, I. Zentsova, Z. Sumnik, S. Kolouskova, J. Sklenarova, S. Pruhova, B. Obermannova, L. Petruzelkova, J. Lebl, T. Kalina, and A. Sediva. 2017. Alteration of B cell subsets and the receptor for B cell activating factor (BAFF) in paediatric patients with type 1 diabetes. *Immunol. Lett.* 189: 94–100. (**IF=3.01**)

Paračková, Z., J. Kayserová, and A. Šedivá. 2017. Neutrofilní extracelulární pasti – záchranné sítě imunitního systému Neutrophil extracellular traps – immune system ' s safety net. *Alergie*. 17–22. (**without IF, article written in Czech language**)

Paračková, Z. 2019. Patogény v pasci DNA. *Vesmír.* 516-517. (**without IF, popularisation science article written in Slovak language)**

I also participated in the studies of primary immunodeficiencies and authored several publications focused on the impact of various inherited mutations influencing a variety of immunodeficiencies. These projects involved cooperation with a group from the CLIP, Department of Paediatric Haematology and Oncology, Childhood Leukaemia Investigation Prague.

Parackova, Z*., M. Bloomfield*, P. Vrabcova, I. Zentsova, A. Klocperk, T. Milota, M. Svaton, J.-L. Casanova, J. Bustamante, E. Fronkova, and A. Sediva. 2019. Mutual alteration of NOD2-associated Blau syndrome and IFNγR1 deficiency. *J. Clin. Immunol.* . (**IF=6.78**)

*Authors contributed equally

Parackova, Z., T. Milota, P. Vrabcova, J. Smetanova, M. Svaton, T. Freiberger, V. Kanderova, and A. Sediva. 2020. Novel XIAP mutation causing enhanced spontaneous apoptosis and disturbed NOD2 signalling in a patient with atypical adult-onset Crohn's disease. *Cell Death Dis.* 11. (**IF=6.3**)

Bloomfield*, M., **Z. Parackova***, T. Cabelova, I. Pospisilova, P. Kabicek, H. Houstkova, and A. Sediva. 2019. Anti-IL6 Autoantibodies in an Infant With CRP-Less Septic Shock. *Front. Immunol.* 10: 1–6. (**IF=5.05**)

*Authors contributed equally

Bloomfield, M., V. Kanderová, **Z. Paračková**, P. Vrabcová, M. Svatoň, E. Froňková, M. Fejtková, R. Zachová, M. Rataj, I. Zentsová, T. Milota, A. Klocperk, T. Kalina, A. Šedivá, Z. Paračková, P. Vrabcová, M. Svatoň, E. Froňková, M. Fejtková, R. Zachová, M. Rataj, I. Zentsová, T. Milota, A. Klocperk, T. Kalina, A. Šedivá, Z. Paračková, P. Vrabcová, M. Svatoň, E. Froňková, M. Fejtková, R. Zachová, M. Rataj, I. Zentsová, T. Milota, A. Klocperk, T. Kalina, and A. Šedivá. 2018. Utility of ruxolitinib in a child with chronic mucocutaneous candidiasis caused by a novel STAT1 gain-of-function mutation. *J. Clin. Immunol.* 38: 589–601. (**IF=4.85**)

Moreover, during the 2020 pandemic, I was involved in research concerning innate immunity in COVID-19 patients, where I become a primary investigator and authored 2 publications as the first author and 2 manuscripts as a contributing author.

Parackova Z, Zentsova I, Bloomfield M, Vrabcova P, Smetanova J, Klocperk A, Mesežnikov G, Casas Mendez LF, Vymazal T, Sediva A. Disharmonic Inflammatory Signatures in COVID-19 : Augmented Neutrophils' but Impaired Monocytes' and Dendritic Cells' Responsiveness. *Cells* (2020) **9**:1–19. doi:10.3390/cells9102206

Parackova Z, Bloomfield M, Klocperk A, Sediva A. Neutrophils mediate Th17 promotion in COVID-19 patients. *J. Leukoc. Biol.* (2020) doi: 10.1002/JLB.4COVCRA0820-481RRR

Klocperk A, **Parackova Z**, Dissou J, Malcova H, Pavlicek P, Vymazal T, Dolezalova P, Sediva A. Case Report : Systemic Inflammatory Response and Fast Recovery in a Pediatric Patient With COVID-19. (2020) **11**:1–5. doi:10.3389/fimmu.2020.01665

Klocperk A, Bloomfield M, **Parackova Z**, Zentsova I, Vrabcova P, Balko J, Meseznikov G, Casas Mendez LF, Grandcourtova A, Sipek J, et al. Complex Immunometabolic Profiling Reveals the Activation of Cellular Immunity and Biliary Lesions in Patients with Severe COVID-19. *J Clin Med* (2020) **9**:3000. doi:10.3390/jcm9093000

1.1 Neutrophil extracellular trap induced dendritic cell activation leads to Th1 polarization in type 1 diabetes

Neutrophil extracellular traps (NETs) are a source of autoantigens and danger-associated molecules that contribute to the pathogenesis of several autoimmune diseases. The aim of this paper was to determine whether NETs are an active player in type 1 diabetes (T1D) pathology. We showed that NETs induce a shift towards IFNγ-producing T cells, canonical inducers of T1D, through activation of dendritic cells (DCs). This activation was manifested as increased expression of maturation markers, production of proinflammatory cytokines and metabolic switch towards glycolysis. Moreover, T1D-NETs were able to induce a similar proinflammatory phenotype in healthy DCs, while healthy NETs had no effect on DCs. Interestingly, RNA-seq analysis revealed that T1D-NETs were able to create a pro-T1D signature in healthy DCs. Our data indicated that NETs and neutrophils play an important role in T1D pathogenesis and identify another critical connection between innate and adaptive immunity in T1D pathogenesis.

This was my longest project; the methodology and pipeline for this project was established from the ground up, including the techniques of neutrophil isolation, NET generation, microscopy, coculture with DCs and T cells, ImageStream and RNA-seq. The project ended up as a part of 2 publications in international peer-reviewed journals and 2 Czech articles and was presented as an oral presentation at several conferences (FOCIS 2019, Boston USA; EFIS on tour 2019, Prague, CR; and CSAKI 2020, Prague, CR). Interestingly, this project was a part of a grant project supported by Czech Ministry of Health, which was nominated for the Ministry of Health Award.

My contribution: experimental design, generation of NETs and monocyte-derived dendritic cells (moDCs), functional tests of moDCs, including phenotype, cytokine production, DC and T cell cocultures, analysis of T cell subpopulations, data analysis and interpretation, RNA seq analysis, figures and manuscript preparation

1.2 MONOCYTES CONTRIBUTE TO DNA SENSING THROUGH THE TBK1 SIGNALLING PATHWAY IN TYPE 1 DIABETES PATIENTS

In this project, we focused on aberrant recognition of both self and nonself nucleic acids by the innate immune system in T1D patients. Abnormal sensing of microbial or self-DNA and consequent activation of the immune system are known mechanisms associated with immunopathology of several autoimmune diseases; however, these issues have been rarely investigated in T1D. Thus, in a cohort of T1D patients, we have investigated the role of Toll-like receptor 9 (TLR9), the most potent receptor of microbial DNA, and participation of cytosolic sequence-nonspecific DNA sensors in DNA sensing of microbial and host DNA. Neutrophil extracellular traps (NETs) were used as a model of self-DNA.

The data demonstrated the prominent production of proinflammatory cytokines by T1D monocytes and plasmacytoid DCs (pDCs) upon DNA exposure, which was not mirrored in healthy cells. pDCs recognized DNA mostly through TLR9; however, monocytes frequently engaged cytosolic DNA receptors other than TLR9. Image cytometry was used to demonstrate colocalization of DNA with STING, a cytosolic DNA receptor, in monocytes. Then, we demonstrated the activation of TBK1, a kinase facilitating the signalling of these cytosolic DNA sensors. Therefore, monocytes trigger the STING and TBK1 signalling pathways to increase the production of proinflammatory cytokines and type I interferons, thus contributing to the pathology of T1D.

We have also shown that this proinflammatory loop involving TBK1 is active upon stimulation with artificial ligands and by direct stimulation with diabetic NETs. This work showed that aberrant DNA recognition mostly by monocytes in T1D patients is involved in the proinflammatory environment associated with the autoimmune processes.

My contribution: in general part about self-DNA recognition, NET generation, cytokine production upon NET exposure, inhibition assays, data analysis and interpretation, consultations, figure and manuscript preparation

1.3 Enhanced STAT3 phosphorylation and PD-L1 expression in myeloid dendritic cells indicate impaired IL-27Ralpha signalling in type 1 diabetes

The study of dendritic cells in T1D pathology has a rich history at the workplace where I have been working during my Ph.D. studies. RNA microarray assays of sorted T1D and healthy DCs were performed in cooperation with a biotech company, Sotio, ac. The results of the array-based assays revealed significantly higher expression of IL-27 receptor alpha subunit (IL-27Ralpha) in a population of myeloid DCs (mDCs). IL-27 has both pro- and anti-inflammatory features and is known as an important factor for T cell differentiation; however, its effect on DC remains unknown. Therefore, we aimed to determine whether higher expression of the receptor subunit results in impaired IL-27 signalling and what are the effects of these changes on DC biology. We have analysed the signalling through the IL-27 receptor in mDCs, leading to STAT1 and STAT3 phosphorylation. An increase in STAT3 phosphorylation was mirrored by enhanced PD-L1, PD-L2 and PD-1 expression, suggesting the presence of certain compensatory mechanisms of mDCs in T1D against ongoing inflammation. The results of a similar set of experiments on a group of T2D patients revealed that our observations were specific for T1D patients.

This project resulted in a publication in Cell Reports and in a review article published in Alergie and helped to establish cooperation with our fellows from the Sotio, ac.

My contribution: experimental design, set up of phosphoflow, DC phenotype, data interpretation, figures and manuscript preparation

1.4 CHANGES IN INNATE AND ADAPTIVE IMMUNITY OVER THE FIRST YEAR AFTER THE ONSET OF TYPE 1 DIABETES

This long-term multiparametric study was focused on the changes in the innate and adaptive immune system parameters in paediatric type 1 diabetic (T1D) patients in the first year following the onset of the disease. The patients were tested at 3 time points (0, 6 and 12 months after the onset of the disease) to determine a set of immunological (leukocyte subsets, immunoglobulins, complement, autoantibodies, T and B cell subpopulations and DCs) and metabolic (mixed meal tolerance test and C peptide area under the curve) parameters. Robust statistical analysis of the data demonstrated considerable changes in innate and adaptive immunity during the first year after the T1D diagnosis. We documented a gradual decrease in leukocytes, especially neutrophils. On the other hand, the levels of Tregs and pDC were increased over time. pDCs have been implicated in the stimulation of cytotoxic T cells through IFNα production after recognition of the neutrophil-associated products in prediabetic pancreas. We proposed that pDCs migrate from the pancreas to the peripheral blood after the disease has been manifested. Increased Treg numbers during the first year after manifestation may represent a tolerogenic response to pancreatic inflammation. Interestingly, immunological parameters did not correlate with residual beta cell activity, suggesting that the changes in the immune system parameters are not associated with the metabolic changes during the first year after the onset of the disease.

My contribution: discussion regarding innate immunity parameters, particularly neutrophils and dendritic cells

1.5 T REGULATORY LYMPHOCYTES IN TYPE 1 DIABETES: IMPAIRED CD25 EXPRESSION AND IL-2 INDUCED STAT5 PHOSPHORYLATION IN PAEDIATRIC PATIENTS

T regulatory lymphocytes (Tregs) play a well-known and crucial role in the prevention autoimmune diseases mediated by several immunosuppressive functions. In this project, we have investigated the numbers and functions of Tregs in a large cohort of T1D patients and their first-degree relatives. We have observed a decrease in Treg numbers in the periphery in patients and altered functionality of Tregs. In T1D, Tregs expressed lower levels of CD25 (IL-2Ralpha), an essential molecule for Treg survival and appropriate functions. In T1D patients, Tregs had impaired IL-2 signalling. A decrease in CD25 expression was mirrored by lower STAT5 phosphorylation in Tregs upon IL-2 stimulation. Our data demonstrate functional impairment of Tregs in T1D, which may contribute to the autoimmune inflammatory response facilitating the destruction of pancreatic beta cells and the progression of the disease.

My contribution: analysis of Treg population, IL-2 signalling, phosphoflow, data analysis and interpretation, figure and manuscript preparation

1.6 ALTERATION OF B CELL SUBSETS AND THE RECEPTOR FOR B CELL ACTIVATING FACTOR (BAFF) IN PAEDIATRIC PATIENTS WITH TYPE 1 DIABETES

B cells are producers of T1D autoantibodies, which serve as an essential diagnostic marker with a potential pathogenic role, and attracted attention as a prospective therapeutic target in various autoimmune diseases; thus, we have analysed alterations in B cell subsets in a large cohort of T1D patients and their first-degree relatives. Moreover, we have focused on B cell-activating factor (BAFF); BAFF is a cytokine crucial for B cell development associated with autoimmunity progression since BAFF can promote the survival of autoreactive B cell clones. We have measured the expression of B cell-activating factor receptor (BAFFR) on B and T cells in these cohorts and the effect of the activating cytokine BAFF on T and B cells. The B panel included naïve, transitional, MZ-like and switched memory cells and plasmablasts. T1D patients were characterized by significantly reduced numbers of early developmental stages and a shift towards antibody-secreting plasmablasts in the B cell compartment. Interestingly, we showed that impaired BAFF signalling may play a role in the alterations in the B cell compartment in T1D. In T1D, naïve and transitional B cells had higher proliferation after BAFF exposure than that in healthy cells.

My contribution: B panel analysis, BAFF effect on T and B cells, data interpretation, figure and manuscript preparation

1.7 NEUTROFILNÍ EXTRACELULÁRNÍ PASTI – ZÁCHRANNÉ SÍTĚ IMUNITNÍHO SYSTÉMU (NEUTROPHIL EXTRACELLULAR TRAPS – IMMUNE SYSTEM' S SAFETY NET)

In this literature review published in Czech language, I have described neutrophil extracellular traps (NETs), their composition, physiological role, effect on various cell types and their immunopathological contribution to immunodeficiencies, autoimmune diseases and cancer. This review was recognized as the best review published in Alergie in 2017.

My contribution: the literature review, manuscript and figure preparation

1.8 PATOGÉNY V PASCI DNA (PATHOGENS IN THE DNA TRAP)

In this popular science article written in Slovak language, I described innate immunity mechanisms used in the fight against infections with special focus on neutrophils and theirs NETs.

My contribution: literature review, figure and article preparation

CONCLUSIONS

Based on a series of our findings concerning the innate and adaptive immune responses in T1D patients, we conclude:

- adaptive and innate immunity changes occur before the onset of T1D
- neutrophil numbers gradually decrease after the onset of T1D
- neutrophil extracellular traps shape T cell polarisation through DC activation and are able to induce the pro-T1D profile in healthy DCs
- monocytes and dendritic cells of patients aberrantly recognize, both self- and nonself-DNA
- T1D monocytes engage intracellular sensor in DNA recognition
- enhanced IL-27 signalling in myeloid DCs in long-term treated T1D patients may serve as a compensatory mechanism of ongoing inflammation
- impaired IL-2 signalling in Tregs in T1D represents a crucial point in T regulatory impairment in T1D
- a shift towards mature forms of B lymphocytes in T1D patients

All observed changes reflect a complex network of immune reactions of the innate and adaptive immunity in a T1D setting, which finally leads to a loss of immune tolerance and ongoing autoimmune process with a clinical presentation of T1D.

SUMMARY

Type 1 diabetes (T1D) is an autoimmune disease with increasing prevalence. Epidemiologic studies suggest that T1D incidence is increasing by 3% every year (3); there is no effective therapy that prevents the disease, and only symptomatic treatment is available. The detailed understanding of the disease pathology will provide new therapeutic approaches.

The main contribution of this thesis to the current knowledge is that we were the first to demonstrate that aberrant DNA recognition by innate immunity cells plays an important role in inflammation associated with T1D pathology. Neutrophils and their products, neutrophil extracellular traps composed predominantly from self-DNA, have an immense effect on the immune response pattern in T1D, particularly on dendritic cells and consequently T lymphocytes, which are the canonical T1D inducers. The changes in the innate immune system result in the downstream changes in the adaptive immune system components demonstrated by us as the alterations in the numbers and functions of Tregs and B cells. In summary, we encourage further studies because the findings directly translate to potentially useful diagnostic and therapeutic approaches.

REFERENCES

1. Katsarou, A., S. Gudbjörnsdottir, A. Rawshani, D. Dabelea, E. Bonifacio, B. J. Anderson, L. M. Jacobsen, D. A. Schatz, and A. Lernmark. 2017. Type 1 diabetes mellitus. *Nat. Rev. Dis. Prim.* 3: 1–17.

2. Atkinson, M. A., G. S. Eisenbarth, and A. W. Michels. 2014. Type 1 diabetes. *Lancet* 383: 69–82.

3. Patterson, C. C., V. Harjutsalo, J. Rosenbauer, A. Neu, O. Cinek, T. Skrivarhaug, B. Rami-Merhar, G. Soltesz, J. Svensson, R. C. Parslow, C. Castell, E. J. Schoenle, P. J. Bingley, G. Dahlquist, P. K. Jarosz-Chobot, D. Marčiulionytė, E. F. Roche, U. Rothe, N. Bratina, C. Ionescu-Tirgoviste, I. Weets, M. Kocova, V. Cherubini, N. Rojnic Putarek, C. E. deBeaufort, M. Samardzic, and A. Green. 2019. Trends and cyclical variation in the incidence of childhood type 1 diabetes in 26 European centres in the 25 year period 1989–2013: a multicentre prospective registration study. *Diabetologia* 62: 408–417.

4. Mobasseri, M., M. Shirmohammadi, T. Amiri, N. Vahed, H. H. Fard, and M. Ghojazadeh. 2020. Prevalence and incidence of type 1 diabetes in the world: A systematic review and meta-analysis. *Heal. Promot. Perspect.* 10: 98–115.

5. Mayer-Davis, E. J., J. M. Lawrence, D. Dabelea, J. Divers, S. Isom, L. Dolan, G. Imperatore, B. Linder, S. Marcovina, D. J. Pettitt, C. Pihoker, S. Saydah, L. Wagenknecht, and SEARCH for Diabetes in Youth Study. 2017. Incidence Trends of Type 1 and Type 2 Diabetes among Youths, 2002–2012. *N. Engl. J. Med.* 376: 1419–1429.

6. Harjutsalo, V., L. Sjöberg, and J. Tuomilehto. 2008. Time trends in the incidence of type 1 diabetes in Finnish children: a cohort study. *Lancet* 371: 1777–1782.

7. Cinek, O., M. Kulich, and Z. Sumnik. 2012. The incidence of type 1 diabetes in young Czech children stopped rising. *Pediatr. Diabetes* 13: 559–563.

8. Šumník, Z., J. Venháčová, J. Škvor, R. Pomahačová, P. Konečná, D. Neumann, J. Vosáhlo, J. Strnadel, J. Čížek, B. Obermannová, L. Petruželková, Š. Průhová, M. Pavlíková, and O. Cinek. 2020. Five years of improving diabetes control in Czech children after the establishment of the population-based childhood diabetes register ČENDA. *Pediatr. Diabetes* 21: 77–87.

9. Fourlanos, S., M. D. Varney, B. D. Tait, G. Morahan, M. C. Honeyman, P. G. Colman, and L. C. Harrison. 2008. The rising incidence of type 1 diabetes is accounted for by cases with lower-risk human leukocyte antigen genotypes. *Diabetes Care* 31: 1546–9.

10. Craig, M. E., K. W. Kim, S. R. Isaacs, M. A. Penno, E. E. Hamilton-Williams, J. J. Couper, and W. D. Rawlinson. 2019. Early-life factors contributing to type 1 diabetes. *Diabetologia* 62: 1823–1834.

11. Erlich, H., A. M. Valdes, J. Noble, J. A. Carlson, M. Varney, P. Concannon, J. C. Mychaleckyj, J. A. Todd, P. Bonella, A. L. Fear, E. Lavant, A. Louey, P. Moonsamy, and Type 1 Diabetes Genetics Consortium. 2008. HLA DR-DQ Haplotypes and Genotypes and Type 1 Diabetes Risk: Analysis of the Type 1 Diabetes Genetics Consortium Families. *Diabetes* 57: 1084–1092.

12. Pugliese, A., M. Zeller, A. Fernandez, L. J. Zalcberg, R. J. Bartlett, C. Ricordi, M. Pietropaolo, G. S. Eisenbarth, S. T. Bennett, and D. D. Patel. 1997. The insulin gene is transcribed in the human thymus and transcription levels correlate with allelic variation at the INS VNTR-IDDM2 susceptibility locus for type 1 diabetes. *Nat. Genet.* 15: 293–297.

13. Bottini, N., L. Musumeci, A. Alonso, S. Rahmouni, K. Nika, M. Rostamkhani, J. MacMurray, G. F. Meloni, P. Lucarelli, M. Pellecchia, G. S. Eisenbarth, D. Comings, and T. Mustelin. 2004. A functional

variant of lymphoid tyrosine phosphatase is associated with type I diabetes. *Nat. Genet.* 36: 337–338.

14. Pociot, F., and Å. Lernmark. 2016. Genetic risk factors for type 1 diabetes. *Lancet* 387: 2331–2339.

15. Ilonen, J., J. Lempainen, and R. Veijola. 2019. The heterogeneous pathogenesis of type 1 diabetes mellitus. *Nat. Rev. Endocrinol.* 15: 635–650.

16. Redondo, M. J., L. Yu, M. Hawa, T. Mackenzie, D. A. Pyke, G. S. Eisenbarth, and R. D. G. Leslie. 2001. Heterogeneity of Type I diabetes: analysis of monozygotic twins in Great Britain and the United States. *Diabetologia* 44: 354–362.

17. Gillespie, K. M., S. C. Bain, A. H. Barnett, P. J. Bingley, M. R. Christie, G. V Gill, and E. A. Gale. 2004. The rising incidence of childhood type 1 diabetes and reduced contribution of high-risk HLA haplotypes. *Lancet* 364: 1699–1700.

18. Oilinki, T., T. Otonkoski, J. Ilonen, M. Knip, and P. Miettinen. 2012. Prevalence and characteristics of diabetes among Somali children and adolescents living in Helsinki, Finland. *Pediatr. Diabetes* 13: 176–180.

19. Peled, A., B. Gordon, G. Twig, J. Mendlovic, E. Derazne, M. Lisnyansky, I. Raz, and A. Afek. 2017. Immigration to Israel during childhood is associated with diabetes at adolescence: a study of 2.7 million adolescents. *Diabetologia* 60: 2226–2230.

20. Hussen, H. I., T. Moradi, and M. Persson. 2015. The Risk of Type 1 Diabetes Among Offspring of Immigrant Mothers in Relation to the Duration of Residency in Sweden: Table 1. *Diabetes Care* 38: 934–936.

21. Tracy, S., K. M. Drescher, and N. M. Chapman. 2011. Enteroviruses and type 1 diabetes. *Diabetes. Metab. Res. Rev.* 27: 820–823.

22. Honeyman, M. C., B. S. Coulson, N. L. Stone, S. A. Gellert, P. N. Goldwater, C. E. Steele, J. J. Couper, B. D. Tait, P. G. Colman, and L. C. Harrison. 2000. Association between rotavirus infection and pancreatic islet autoimmunity in children at risk of developing type 1 diabetes. *Diabetes* 49: 1319–1324.

23. Hyöty, H., P. Leinikki, A. Reunanen, J. Ilonen, H. M. Surcel, A. Rilva, M. L. Käär, T. Huupponen, A. Hakulinen, and A. L. Mäkelä. 1988. Mumps infections in the etiology of type 1 (insulin-dependent) diabetes. *Diabetes Res.* 9: 111–6.

24. Gale, E. A. M. 2008. Congenital rubella: citation virus or viral cause of type 1 diabetes? *Diabetologia* 51: 1559–1566.

25. Pak, C. Y., H. M. Eun, R. G. McArthur, and J. W. Yoon. 1988. Association of cytomegalovirus infection with autoimmune type 1 diabetes. *Lancet (London, England)* 2: 1–4.

26. Christen, U., C. Bender, and M. G. von Herrath. 2012. Infection as a cause of type 1 diabetes? *Curr. Opin. Rheumatol.* 24: 417–423.

27. Srinivasappa, J., J. Saegusa, B. S. Prabhakar, M. K. Gentry, M. J. Buchmeier, T. J. Wiktor, H. Koprowski, M. B. Oldstone, and A. L. Notkins. 1986. Molecular mimicry: frequency of reactivity of monoclonal antiviral antibodies with normal tissues. *J. Virol.* 57: 397–401.

28. Richardson, S. J., A. Willcox, A. J. Bone, A. K. Foulis, and N. G. Morgan. 2009. The prevalence of

enteroviral capsid protein vp1 immunostaining in pancreatic islets in human type 1 diabetes. *Diabetologia* 52: 1143–1151.

29. Richardson, S. J., A. Willcox, D. A. Hilton, S. Tauriainen, H. Hyoty, A. J. Bone, A. K. Foulis, and N. G. Morgan. 2010. Use of antisera directed against dsRNA to detect viral infections in formalin-fixed paraffin-embedded tissue. *J. Clin. Virol.* 49: 180–185.

30. Al-Hello, H., A. Paananen, M. Eskelinen, P. Ylipaasto, T. Hovi, K. Salmela, A. N. Lukashev, S. Bobegamage, and M. Roivainen. 2008. An enterovirus strain isolated from diabetic child belongs to a genetic subcluster of echovirus 11, but is also neutralised with monotypic antisera to coxsackievirus A9. *J. Gen. Virol.* 89: 1949–1959.

31. Yoon, J.-W., M. Austin, T. Onodera, and A. L. Notkins. 1979. Virus-Induced Diabetes Mellitus. *N. Engl. J. Med.* 300: 1173–1179.

32. Yeung, W.-C. G., W. D. Rawlinson, and M. E. Craig. 2011. Enterovirus infection and type 1 diabetes mellitus: systematic review and meta-analysis of observational molecular studies. *BMJ* 342: d35–d35.

33. Filippi, C. M., E. A. Estes, J. E. Oldham, and M. G. von Herrath. 2009. Immunoregulatory mechanisms triggered by viral infections protect from type 1 diabetes in mice. *J. Clin. Invest.* .

34. Diana, J., V. Brezar, L. Beaudoin, M. Dalod, A. Mellor, A. Tafuri, M. von Herrath, C. Boitard, R. Mallone, and A. Lehuen. 2011. Viral infection prevents diabetes by inducing regulatory T cells through NKT cell–plasmacytoid dendritic cell interplay. *J. Exp. Med.* 208: 729–745.

35. Ehlers, S., and S. H. E. Kaufmann. 2010. 99th Dahlem Conference on Infection, Inflammation and Chronic Inflammatory Disorders: Lifestyle changes affecting the host-environment interface. *Clin. Exp. Immunol.* 160: 10–14.

36. Christen, U., and M. G. von Herrath. 2005. Infections and Autoimmunity—Good or Bad? *J. Immunol.* 174: 7481–7486.

37. Zheng, P., Z. Li, and Z. Zhou. 2018. Gut microbiome in type 1 diabetes: A comprehensive review. *Diabetes. Metab. Res. Rev.* 34: e3043.

38. Maffeis, C., A. Martina, M. Corradi, S. Quarella, N. Nori, S. Torriani, M. Plebani, G. Contreas, and G. E. Felis. 2016. Association between intestinal permeability and faecal microbiota composition in Italian children with beta cell autoimmunity at risk for type 1 diabetes. *Diabetes. Metab. Res. Rev.* 32: 700–709.

39. Li, X., and M. A. Atkinson. 2015. The role for gut permeability in the pathogenesis of type 1 diabetes - a solid or leaky concept? *Pediatr. Diabetes* 16: 485–492.

40. Neuman, V., S. Pruhova, M. Kulich, S. Kolouskova, J. Vosahlo, M. Romanova, L. Petruzelkova, B. Obermannova, D. P. Funda, O. Cinek, and Z. Sumnik. 2020. Gluten-free diet in children with recent-onset type 1 diabetes: A 12-month intervention trial. *Diabetes, Obes. Metab.* 22: 866–872.

41. Du, T., Z.-G. Zhou, S. You, J. Lin, L. Yang, W.-D. Zhou, G. Huang, and C. Chao. 2009. Regulation by 1, 25-dihydroxy-vitamin D3 on altered TLRs expression and response to ligands of monocyte from autoimmune diabetes. *Clin. Chim. Acta* 402: 133–138.

42. Luopajärvi, K., E. Savilahti, S. M. Virtanen, J. Ilonen, M. Knip, H. K. Åkerblom, and O. Vaarala. 2008. Enhanced levels of cow's milk antibodies in infancy in children who develop type 1 diabetes later in childhood. *Pediatr. Diabetes* 9: 434–441.

43. Knip, M., H. K. Åkerblom, E. Al Taji, D. Becker, J. Bruining, L. Castano, T. Danne, C. de Beaufort, H.-M. Dosch, J. Dupre, W. D. Fraser, N. Howard, J. Ilonen, D. Konrad, O. Kordonouri, J. P. Krischer, M. L. Lawson, J. Ludvigsson, L. Madacsy, J. L. Mahon, A. Ormisson, J. P. Palmer, P. Pozzilli, E. Savilahti, M. Serrano-Rios, M. Songini, S. Taback, O. Vaarala, N. H. White, S. M. Virtanen, R. Wasikowa, and R. Wasikowa. 2018. Effect of Hydrolyzed Infant Formula vs Conventional Formula on Risk of Type 1 Diabetes. *JAMA* 319: 38.

44. Candon, S., A. Perez-Arroyo, C. Marquet, F. Valette, A.-P. Foray, B. Pelletier, C. Milani, M. Ventura, J.-F. Bach, L. Chatenoud, and L. Chatenoud. 2015. Antibiotics in Early Life Alter the Gut Microbiome and Increase Disease Incidence in a Spontaneous Mouse Model of Autoimmune Insulin-Dependent Diabetes. *PLoS One* 10: e0125448.

45. Brugman, S., F. A. Klatter, J. T. J. Visser, A. C. M. Wildeboer-Veloo, H. J. M. Harmsen, J. Rozing, and N. A. Bos. 2006. Antibiotic treatment partially protects against type 1 diabetes in the Bio-Breeding diabetes-prone rat. Is the gut flora involved in the development of type 1 diabetes? *Diabetologia* 49: 2105–2108.

46. Hansen, C. H. F., L. Krych, D. S. Nielsen, F. K. Vogensen, L. H. Hansen, S. J. Sørensen, K. Buschard, and A. K. Hansen. 2012. Early life treatment with vancomycin propagates Akkermansia muciniphila and reduces diabetes incidence in the NOD mouse. *Diabetologia* 55: 2285–2294.

47. Mikkelsen, K. H., F. K. Knop, T. Vilsbøll, M. Frost, J. Hallas, and A. Pottegård. 2017. Use of antibiotics in childhood and risk of Type 1 diabetes: a population-based case-control study. *Diabet. Med.* 34: 272–277.

48. Buyken, A. E., M. Toeller, G. Heitkamp, K. Irsigler, C. Holler, F. Santeusanio, P. Stehle, J. H. Fuller, G. John, G. C. Viberti, M. Mattock, A. Collins, A. Dredge, R. Sharp, B. Karamanos, C. Tountas, A. Kofinis, K. Petrou, N. Katsilambros, D. Roussi-Penessi, M. Cignarelli, R. Giorgino, M. L. De Geco, I. Ramunni, C. Ionescu-Tirgoviste, R. Strachinariu, A. Nicolau, G. Tamas, Z. Kerenyi, A. M. Ahmed, J. Toth, P. Kempler, S. Muntoni, M. Songini, M. Stabilini, M. Fossarello, S. Pintus, B. Ferriss, C. C. Cronin, M. Humphreys, M. Toeller, A. Klischan, T. Forst, F. A. Gries, W. Schumacher, R. Rottiers, H. Priem, M. J. Deschoolmeester, P. Ebeling, M. Sinisalo, V. A. Koivisto, B. Idzior-Walus, B. Solnica, L. Szopinska-Ciba, K. Solnica, H. M. J. Krans, H. H. P. J. Lemkes, J. J. Jansen, B. M. Eltede Wever, J. Nunes-Correa, J. Boavida, R. Carvalho, M. J. Afonso, M. Monteiro, R. David, E. Jepson, S. McHardy-Young, D. J. Betteridge, M. Milne, T. Thompson, G. Michel, R. Wirion, S. Paquet, H. Hornick, A. J. M. Boulton, H. Ashe, D. J. S. Fernando, J. Curwell, G. Pozza, G. Slaviero, G. Comi, B. Fattor, F. Bandello, M. Marchi, H. Mehnert, A. Nuber, H. Janka, M. Nichting, E. Standl, G. Crepaldi, R. Nosadini, G. Cathelineau, B. Villatte Cathelineau, M. Jellal, N. Grodner, P. Gervais Feiss, N. Baclet, F. Santeusanio, and G. Rosi. 2000. Carbohydrate sources and glycaemic control in type 1 diabetes mellitus. *Diabet. Med.* 17: 351–359.

49. Turton, J. L., R. Raab, and K. B. Rooney. 2018. Low-carbohydrate diets for type 1 diabetes mellitus: A systematic review. *PLoS One* 13.

50. Insel, R. A., J. L. Dunne, M. A. Atkinson, J. L. Chiang, D. Dabelea, P. A. Gottlieb, C. J. Greenbaum, K. C. Herold, J. P. Krischer, Å. Lernmark, R. E. Ratner, M. J. Rewers, D. A. Schatz, J. S. Skyler, J. M. Sosenko, and A.-G. Ziegler. 2015. Staging presymptomatic type 1 diabetes: a scientific statement of JDRF, the Endocrine Society, and the American Diabetes Association. *Diabetes Care* 38: 1964–74.

51. Flier, J. S., L. H. Underhill, and G. S. Eisenbarth. 1986. Type I Diabetes Mellitus. *N. Engl. J. Med.* 314: 1360–1368.

52. Ziegler, A. G., M. Rewers, O. Simell, T. Simell, J. Lempainen, A. Steck, C. Winkler, J. Ilonen, R. Veijola, M. Knip, E. Bonifacio, and G. S. Eisenbarth. 2013. Seroconversion to Multiple Islet

Autoantibodies and Risk of Progression to Diabetes in Children. *JAMA* 309: 2473.

53. Steck, A. K., K. Vehik, E. Bonifacio, A. Lernmark, A.-G. Ziegler, W. A. Hagopian, J. She, O. Simell, B. Akolkar, J. Krischer, D. Schatz, M. J. Rewers, and the T. S. TEDDY Study Group. 2015. Predictors of Progression From the Appearance of Islet Autoantibodies to Early Childhood Diabetes: The Environmental Determinants of Diabetes in the Young (TEDDY). *Diabetes Care* 38: 808–13.

54. Orban, T., J. M. Sosenko, D. Cuthbertson, J. P. Krischer, J. S. Skyler, R. Jackson, L. Yu, J. P. Palmer, D. Schatz, G. Eisenbarth, and for the D. P. T. 1 S. Diabetes Prevention Trial-Type 1 Study Group. 2009. Pancreatic islet autoantibodies as predictors of type 1 diabetes in the Diabetes Prevention Trial-Type 1. *Diabetes Care* 32: 2269–74.

55. Krischer, J. P., and Type 1 Diabetes TrialNet Study Group. 2013. The use of intermediate endpoints in the design of type 1 diabetes prevention trials. *Diabetologia* 56: 1919–1924.

56. Bottazzo, G. F., A. Florin-Christensen, and D. Doniach. 1974. ISLET-CELL ANTIBODIES IN DIABETES MELLITUS WITH AUTOIMMUNE POLYENDOCRINE DEFICIENCIES. *Lancet* 304: 1279–1283.

57. Krischer, J. P., K. F. Lynch, D. A. Schatz, J. Ilonen, Å. Lernmark, W. A. Hagopian, M. J. Rewers, J.-X. She, O. G. Simell, J. Toppari, A.-G. Ziegler, B. Akolkar, E. Bonifacio, and TEDDY Study Group. 2015. The 6 year incidence of diabetes-associated autoantibodies in genetically at-risk children: the TEDDY study. *Diabetologia* 58: 980–987.

58. Yu, L., Z. Zhao, and A. K. Steck. 2017. T1D Autoantibodies: Room for improvement? *Curr. Opin. Endocrinol. Diabetes Obes.* 24: 285–291.

59. Wenzlau, J. M., and J. C. Hutton. 2013. Novel Diabetes Autoantibodies and Prediction of Type 1 Diabetes. *Curr. Diab. Rep.* 13: 608–615.

60. Achenbach, P., E. Bonifacio, K. Koczwara, and A.-G. Ziegler. 2005. Natural history of type 1 diabetes. *Diabetes* 54 Suppl 2: S25-31.

61. Nokoff, N., and M. Rewers. 2013. Pathogenesis of type 1 diabetes: lessons from natural history studies of high-risk individuals. *Ann. N. Y. Acad. Sci.* 1281: 1–15.

62. LUDVIGSSON, J., L. G. HEDING, Y. LARSSON, and E. LEANDER. 1977. C-PEPTIDE IN JUVENILE DIABETICS BEYOND THE POSTINITIAL REMISSION PERIOD Relation to Clinical Manifestations at Onset of Diabetes, Remission and Diabetic Control. *Acta Paediatr.* 66: 177–184.

63. Steffes, M. W., S. Sibley, M. Jackson, and W. Thomas. 2003. Beta-cell function and the development of diabetes-related complications in the diabetes control and complications trial. *Diabetes Care* 26: 832–6.

64. Jacobsen, L., and D. Schatz. 2016. Current and future efforts toward the prevention of type 1 diabetes. *Pediatr. Diabetes* 17: 78–86.

65. Ziegler, A. G., T. Danne, D. B. Dunger, R. Berner, R. Puff, W. Kiess, G. Agiostratidou, J. A. Todd, and E. Bonifacio. 2016. Primary prevention of beta-cell autoimmunity and type 1 diabetes – The Global Platform for the Prevention of Autoimmune Diabetes (GPPAD) perspectives. *Mol. Metab.* 5: 255–262.

66. Peakman, M., and M. von Herrath. 2010. Antigen-Specific Immunotherapy for Type 1 Diabetes: Maximizing the Potential. *Diabetes* 59: 2087–2093.

67. Ludvigsson, J., J. Wahlberg, and R. Casas. 2017. Intralymphatic Injection of Autoantigen in Type 1

Diabetes. *N. Engl. J. Med.* 376: 697–699.

68. Sherry, N., W. Hagopian, J. Ludvigsson, S. M. Jain, J. Wahlen, R. J. Ferry, B. Bode, S. Aronoff, C. Holland, D. Carlin, K. L. King, R. L. Wilder, S. Pillemer, E. Bonvini, S. Johnson, K. E. Stein, S. Koenig, K. C. Herold, A. G. Daifotis, and Protégé Trial Investigators. 2011. Teplizumab for treatment of type 1 diabetes (Protégé study): 1-year results from a randomised, placebo-controlled trial. *Lancet* 378: 487–497.

69. Herold, K. C., B. N. Bundy, S. A. Long, J. A. Bluestone, L. A. DiMeglio, M. J. Dufort, S. E. Gitelman, P. A. Gottlieb, J. P. Krischer, P. S. Linsley, J. B. Marks, W. Moore, A. Moran, H. Rodriguez, W. E. Russell, D. Schatz, J. S. Skyler, E. Tsalikian, D. K. Wherrett, A.-G. Ziegler, and C. J. Greenbaum. 2019. An Anti-CD3 Antibody, Teplizumab, in Relatives at Risk for Type 1 Diabetes. *N. Engl. J. Med.* 381: 603–613.

70. Jacobsen, L. M., M. J. Haller, and D. A. Schatz. 2018. Understanding Pre-Type 1 Diabetes: The Key to Prevention. *Front. Endocrinol. (Lausanne).* 9: 70.

71. Campbell-Thompson, M., A. Fu, J. S. Kaddis, C. Wasserfall, D. A. Schatz, A. Pugliese, and M. A. Atkinson. 2016. Insulitis and β-Cell Mass in the Natural History of Type 1 Diabetes. *Diabetes* 65: 719–731.

72. Pugliese, A., M. Yang, I. Kusmarteva, T. Heiple, F. Vendrame, C. Wasserfall, P. Rowe, J. M. Moraski, S. Ball, L. Jebson, D. A. Schatz, R. Gianani, G. W. Burke, C. Nierras, T. Staeva, J. S. Kaddis, M. Campbell-Thompson, and M. A. Atkinson. 2014. The Juvenile Diabetes Research Foundation Network for Pancreatic Organ Donors with Diabetes (nPOD) Program: Goals, operational model and emerging findings. *Pediatr. Diabetes* 15: 1–9.

73. Coppieters, K. T., F. Dotta, N. Amirian, P. D. Campbell, T. W. H. Kay, M. A. Atkinson, B. O. Roep, and M. G. von Herrath. 2012. Demonstration of islet-autoreactive CD8 T cells in insulitic lesions from recent onset and long-term type 1 diabetes patients. *J. Exp. Med.* 209: 51–60.

74. Keenan, H. A., J. K. Sun, J. Levine, A. Doria, L. P. Aiello, G. Eisenbarth, S. Bonner-Weir, and G. L. King. 2010. Residual insulin production and pancreatic ß-cell turnover after 50 years of diabetes: Joslin Medalist Study. *Diabetes* 59: 2846–53.

75. Ma, W.-T., F. Gao, K. Gu, and D.-K. Chen. 2019. The Role of Monocytes and Macrophages in Autoimmune Diseases: A Comprehensive Review. *Front. Immunol.* 10: 1140.

76. Navegantes, K. C., R. de Souza Gomes, P. A. T. Pereira, P. G. Czaikoski, C. H. M. Azevedo, and M. C. Monteiro. 2017. Immune modulation of some autoimmune diseases: the critical role of macrophages and neutrophils in the innate and adaptive immunity. *J. Transl. Med.* 15: 36.

77. Bulum, T., B. Kolarić, and L. Duvnjak. 2014. Decreased serum monocytes and elevated neutrophils as additional markers of insulin resistance in type 1 diabetes. *Int. J. Diabetes Dev. Ctries.* 34: 150–155.

78. Min, D., B. Brooks, J. Wong, R. Salomon, W. Bao, B. Harrisberg, S. M. Twigg, D. K. Yue, and S. V. McLennan. 2012. Alterations in Monocyte CD16 in Association with Diabetes Complications. *Mediators Inflamm.* 2012: 1–10.

79. Hussain, M. J., M. Peakman, H. Gallati, S. S. Lo, M. Hawa, G. C. Viberti, P. J. Watkins, R. D. Leslie, and D. Vergani. 1996. Elevated serum levels of macrophage-derived cytokines precede and accompany the onset of IDDM. *Diabetologia* 39: 60–9.

80. Bradshaw, E. M., K. Raddassi, W. Elyaman, T. Orban, P. A. Gottlieb, S. C. Kent, and D. A. Hafler.

2009. Monocytes from Patients with Type 1 Diabetes Spontaneously Secrete Proinflammatory Cytokines Inducing Th17 Cells. *J. Immunol.* 183: 4432–4439.

81. Irvine, K. M., P. Gallego, X. An, S. E. Best, G. Thomas, C. Wells, M. Harris, A. Cotterill, and R. Thomas. 2012. Peripheral Blood Monocyte Gene Expression Profile Clinically Stratifies Patients With Recent-Onset Type 1 Diabetes. *Diabetes* 61: 1281–1290.

82. Dasu, M. R., S. Devaraj, L. Zhao, D. H. Hwang, and I. Jialal. 2008. High Glucose Induces Toll-Like Receptor Expression in Human Monocytes: Mechanism of Activation. *Diabetes* 57: 3090–3098.

83. Meyers, A. J., R. R. Shah, P. A. Gottlieb, and D. Zipris. 2010. Altered toll-like receptor signaling pathways in human type 1 diabetes. *J. Mol. Med.* 88: 1221–1231.

84. Zentsova, I., Z. Parackova, J. Kayserova, L. Palova-Jelinkova, P. Vrabcova, N. Volfova, Z. Sumnik, S. Pruhova, L. Petruzelkova, and A. Sediva. 2019. Monocytes contribute to DNA sensing through the TBK1 signaling pathway in type 1 diabetes patients. *J. Autoimmun.* .

85. Barbalat, R., S. E. Ewald, M. L. Mouchess, and G. M. Barton. 2011. Nucleic Acid Recognition by the Innate Immune System. *Annu. Rev. Immunol.* 29: 185–214.

86. Hornung, V., and E. Latz. 2010. Intracellular DNA recognition. *Nat. Rev. Immunol.* 10: 123–130.

87. Willcox, A., S. J. Richardson, A. J. Bone, A. K. Foulis, and N. G. Morgan. 2009. Analysis of islet inflammation in human type 1 diabetes. *Clin. Exp. Immunol.* 155: 173–181.

88. Uno, S., A. Imagawa, K. Okita, K. Sayama, M. Moriwaki, H. Iwahashi, K. Yamagata, S. Tamura, Y. Matsuzawa, T. Hanafusa, J. Miyagawa, and I. Shimomura. 2007. Macrophages and dendritic cells infiltrating islets with or without beta cells produce tumour necrosis factor-α in patients with recent-onset type 1 diabetes. *Diabetologia* 50: 596–601.

89. O'Brien, B. A., X. Geng, C. H. Orteu, Y. Huang, M. Ghoreishi, Y. Zhang, J. A. Bush, G. Li, D. T. Finegood, and J. P. Dutz. 2006. A deficiency in the in vivo clearance of apoptotic cells is a feature of the NOD mouse. *J. Autoimmun.* 26: 104–115.

90. Calderon, B., A. Suri, and E. R. Unanue. 2006. In CD4+ T-Cell-Induced Diabetes, Macrophages Are the Final Effector Cells that Mediate Islet β-Cell Killing. *Am. J. Pathol.* 169: 2137–2147.

91. Vomund, A. N., B. H. Zinselmeyer, J. Hughes, B. Calderon, C. Valderrama, S. T. Ferris, X. Wan, K. Kanekura, J. A. Carrero, F. Urano, and E. R. Unanue. 2015. Beta cells transfer vesicles containing insulin to phagocytes for presentation to T cells. *Proc. Natl. Acad. Sci.* 112: E5496–E5502.

92. Marro, B. S., S. Legrain, B. C. Ware, and M. B. A. Oldstone. 2019. Macrophage IFN-I signaling promotes autoreactive T cell infiltration into islets in type 1 diabetes model. *JCI Insight* 4.

93. Arif, S., T. I. Tree, T. P. Astill, J. M. Tremble, A. J. Bishop, C. M. Dayan, B. O. Roep, and M. Peakman. 2004. Autoreactive T cell responses show proinflammatory polarization in diabetes but a regulatory phenotype in health. *J. Clin. Invest.* 113: 451–463.

94. Parsa, R., P. Andresen, A. Gillett, S. Mia, X. M. Zhang, S. Mayans, D. Holmberg, and R. A. Harris. 2012. Adoptive transfer of immunomodulatory M2 macrophages prevents type 1 diabetes in NOD mice. *Diabetes* 61: 2881–2892.

95. Morel, P. A. 2013. Dendritic cell subsets in type 1 diabetes: friend or foe? *Front. Immunol.* 4: 415.

96. Szablewski, L. 2014. Role of immune system in type 1 diabetes mellitus pathogenesis. *Int. Immunopharmacol.* 22: 182–191.

97. Aida, K., Y. Nishida, S. Tanaka, T. Maruyama, A. Shimada, T. Awata, M. Suzuki, H. Shimura, S. Takizawa, M. Ichijo, D. Akiyama, F. Furuya, A. Kawaguchi, M. Kaneshige, J. Itakura, H. Fujii, T. Endo, and T. Kobayashi. 2011. RIG-I– and MDA5-Initiated Innate Immunity Linked With Adaptive Immunity Accelerates β-Cell Death in Fulminant Type 1 Diabetes. *Diabetes* 60: 884–889.

98. Vuckovic, S., G. Withers, M. Harris, D. Khalil, D. Gardiner, I. Flesch, S. Tepes, R. Greer, D. Cowley, A. Cotterill, and D. N. J. Hart. 2007. Decreased blood dendritic cell counts in type 1 diabetic children. *Clin. Immunol.* 123: 281–288.

99. Allen, J. S., K. Pang, A. Skowera, R. Ellis, C. Rackham, B. Lozanoska-Ochser, T. Tree, R. D. G. Leslie, J. M. Tremble, C. M. Dayan, and M. Peakman. 2009. Plasmacytoid Dendritic Cells Are Proportionally Expanded at Diagnosis of Type 1 Diabetes and Enhance Islet Autoantigen Presentation to T-Cells Through Immune Complex Capture. *Diabetes* 58: 138–145.

100. Kayserova, J., J. Vcelakova, K. Stechova, E. Dudkova, H. Hromadkova, Z. Sumnik, S. Kolouskova, R. Spisek, and A. Sediva. 2014. Decreased dendritic cell numbers but increased TLR9-mediated interferon-alpha production in first degree relatives of type 1 diabetes patients. *Clin. Immunol.* 153: 49–55.

101. Klocperk, A., L. Petruzelkova, M. Pavlikova, M. Rataj, J. Kayserova, S. Pruhova, S. Kolouskova, J. Sklenarova, Z. Parackova, A. Sediva, and Z. Sumnik. 2019. Changes in innate and adaptive immunity over the first year after the onset of type 1 diabetes. *Acta Diabetol.* .

102. Marleau, A. M., and B. Singh. 2002. Myeloid Dendritic Cells in Non-Obese Diabetic Mice have Elevated Costimulatory and T Helper-1-Inducing Abilities. *J. Autoimmun.* 19: 23–35.

103. Poligone, B., D. J. Weaver, P. Sen, A. S. Baldwin, and R. Tisch. 2002. Elevated NF-κB Activation in Nonobese Diabetic Mouse Dendritic Cells Results in Enhanced APC Function. *J. Immunol.* 168: 188–196.

104. Diana, J., Y. Simoni, L. Furio, L. Beaudoin, B. Agerberth, F. Barrat, and A. Lehuen. 2012. Crosstalk between neutrophils, B-1a cells and plasmacytoid dendritic cells initiates autoimmune diabetes. *Nat. Med.* 19: 65–73.

105. Creusot, R. J., J. Postigo-Fernandez, and N. Teteloshvili. 2018. Altered Function of Antigen-Presenting Cells in Type 1 Diabetes: A Challenge for Antigen-Specific Immunotherapy? *Diabetes* 67: 1481–1494.

106. Parackova, Z., P. Vrabcova, I. Zentsova, J. Kayserova, I. Richtrova, L. Sojka, K. Stechova, Z. Sumnik, and A. Sediva. 2020. Enhanced STAT3 phosphorylation and PD-L1 expression in myeloid dendritic cells indicate impaired IL-27Ralpha signaling in type 1 diabetes. *Sci. Rep.* 10: 8–15.

107. Chen, L., and D. B. Flies. 2013. Molecular mechanisms of T cell co-stimulation and co-inhibition. *Nat. Rev. Immunol.* 13: 227–242.

108. Guleria, I., M. Gubbels Bupp, S. Dada, B. Fife, Q. Tang, M. J. Ansari, S. Trikudanathan, N. Vadivel, P. Fiorina, H. Yagita, M. Azuma, M. Atkinson, J. A. Bluestone, and M. H. Sayegh. 2007. Mechanisms of PDL1-mediated regulation of autoimmune diabetes. *Clin. Immunol.* 125: 16–25.

109. Wang, J., T. Yoshida, F. Nakaki, H. Hiai, T. Okazaki, and T. Honjo. 2005. Establishment of NOD-Pdcd1-/- mice as an efficient animal model of type I diabetes. *Proc. Natl. Acad. Sci.* 102: 11823–11828.

110. Yadav, D., N. Hill, H. Yagita, M. Azuma, and N. Sarvetnick. 2009. Altered availability of PD-1/PD

ligands is associated with the failure to control autoimmunity in NOD mice. *Cell. Immunol.* 258: 161–171.

111. Kadri, N., E. Korpos, S. Gupta, C. Briet, L. Löfbom, H. Yagita, A. Lehuen, C. Boitard, D. Holmberg, L. Sorokin, and S. L. Cardell. 2012. CD4 + Type II NKT Cells Mediate ICOS and Programmed Death-1–Dependent Regulation of Type 1 Diabetes. *J. Immunol.* 188: 3138–3149.

112. Qin, W., L. Hu, X. Zhang, S. Jiang, J. Li, Z. Zhang, and X. Wang. 2019. The Diverse Function of PD-1/PD-L Pathway Beyond Cancer. *Front. Immunol.* 10: 2298.

113. Liu, M., J. Peng, N. Tai, J. A. Pearson, C. Hu, J. Guo, L. Hou, H. Zhao, F. S. Wong, and L. Wen. 2018. Toll-like receptor 9 negatively regulates pancreatic islet beta cell growth and function in a mouse model of type 1 diabetes. *Diabetologia* 61: 2333–2343.

114. Sumpter, K. M., S. Adhikari, E. K. Grishman, and P. C. White. 2011. Preliminary studies related to anti-interleukin-1β therapy in children with newly diagnosed type 1 diabetes. *Pediatr. Diabetes* 12: 656–667.

115. Mastrandrea, L., J. Yu, T. Behrens, J. Buchlis, C. Albini, S. Fourtner, and T. Quattrin. 2009. Etanercept Treatment in Children With New-Onset Type 1 Diabetes: Pilot randomized, placebo-controlled, double-blind study. *Diabetes Care* 32: 1244–1249.

116. Hundhausen, C., A. Roth, E. Whalen, J. Chen, A. Schneider, S. A. Long, S. Wei, R. Rawlings, M. Kinsman, S. P. Evanko, T. N. Wight, C. J. Greenbaum, K. Cerosaletti, and J. H. Buckner. 2016. Enhanced T cell responses to IL-6 in type 1 diabetes are associated with early clinical disease and increased IL-6 receptor expression. *Sci. Transl. Med.* 8: 356ra119-356ra119.

117. Dáňová, K., A. Klapetková, J. Kayserová, A. Šedivá, R. Špíšek, and L. P. Jelínková. 2015. NF-κB, p38 MAPK, ERK1/2, mTOR, STAT3 and increased glycolysis regulate stability of paricalcitol/dexamethasone- generated tolerogenic dendritic cells in the inflammatory environment. 6.

118. Giannoukakis, N., B. Phillips, D. Finegold, J. Harnaha, and M. Trucco. 2011. Phase I (Safety) Study of Autologous Tolerogenic Dendritic Cells in Type 1 Diabetic Patients. *Diabetes Care* 34: 2026–2032.

119. Coppieters, K. T., L. C. Harrison, and M. G. von Herrath. 2013. Trials in type 1 diabetes: Antigen-specific therapies. *Clin. Immunol.* 149: 345–355.

120. Phillips, B., K. Nylander, J. Harnaha, J. Machen, R. Lakomy, A. Styche, K. Gillis, L. Brown, D. Lafreniere, M. Gallo, J. Knox, K. Hogeland, M. Trucco, and N. Giannoukakis. 2008. A Microsphere-Based Vaccine Prevents and Reverses New-Onset Autoimmune Diabetes. *Diabetes* 57: 1544–1555.

121. Fallarino, F., R. Bianchi, C. Orabona, C. Vacca, M. L. Belladonna, M. C. Fioretti, D. V. Serreze, U. Grohmann, and P. Puccetti. 2004. CTLA-4–Ig Activates Forkhead Transcription Factors and Protects Dendritic Cells from Oxidative Stress in Nonobese Diabetic Mice. *J. Exp. Med.* 200: 1051–1062.

122. Turner, M. S., L. P. Kane, and P. A. Morel. 2009. Dominant Role of Antigen Dose in CD4 + Foxp3 + Regulatory T Cell Induction and Expansion. *J. Immunol.* 183: 4895–4903.

123. Long, S. A., M. Rieck, M. Tatum, P. L. Bollyky, R. P. Wu, I. Muller, J.-C. Ho, H. G. Shilling, and J. H. Buckner. 2011. Low-Dose Antigen Promotes Induction of FOXP3 in Human CD4 + T Cells. *J. Immunol.* 187: 3511–3520.

124. Mantovani, A., M. A. Cassatella, C. Costantini, and S. Jaillon. 2011. Neutrophils in the activation

and regulation of innate and adaptive immunity. *Nat. Rev. Immunol.* 11: 519–531.

125. Mayadas, T. N., X. Cullere, and C. A. Lowell. 2014. The Multifaceted Functions of Neutrophils. *Annu. Rev. Pathol. Mech. Dis.* 9: 181–218.

126. Mocsai, A. 2013. Diverse novel functions of neutrophils in immunity, inflammation, and beyond. *J. Exp. Med.* 210: 1283–1299.

127. Nathan, C. 2006. Neutrophils and immunity: challenges and opportunities. *Nat. Rev. Immunol.* 6: 173–82.

128. Brinkmann, V., U. Reichard, C. Goosmann, B. Fauler, Y. Uhlemann, D. S. Weiss, Y. Weinrauch, and A. Zychlinsky. 2004. Neutrophil extracellular traps kill bacteria. *Science* 303: 1532–5.

129. Saitoh, T., J. Komano, Y. Saitoh, T. Misawa, M. Takahama, T. Kozaki, T. Uehata, H. Iwasaki, H. Omori, S. Yamaoka, N. Yamamoto, and S. Akira. 2012. Neutrophil extracellular traps mediate a host defense response to human immunodeficiency virus-1. *Cell Host Microbe* 12: 109–16.

130. Urban, C. F., U. Reichard, V. Brinkmann, and A. Zychlinsky. 2006. Neutrophil extracellular traps capture and kill Candida albicans yeast and hyphal forms. *Cell. Microbiol.* 8: 668–76.

131. Mowat, A. G., and J. Baum. 1971. Chemotaxis of Polymorphonuclear Leukocytes from Patients with Diabetes Mellitus. *N. Engl. J. Med.* 284: 621–627.

132. Tan, J. S., J. L. Anderson, C. Watanakunakorn, and J. P. Phair. 1975. Neutrophil dysfunction in diabetes mellitus. *J. Lab. Clin. Med.* 85: 26–33.

133. BAGDADE, J. D., K. L. NIELSON, and R. J. BULGER. 1972. Reversible abnormalities in phagocytic function in poorlycontrolled diabetic patients. *Am. J. Med. Sci.* 263: 451–456.

134. Battaglia, M., A. Petrelli, and F. Vecchio. 2019. Neutrophils and type 1 diabetes. *Curr. Opin. Endocrinol. Diabetes Obes.* 26: 201–206.

135. Valle, A., G. M. Giamporcaro, M. Scavini, A. Stabilini, P. Grogan, E. Bianconi, G. Sebastiani, M. Masini, N. Maugeri, L. Porretti, R. Bonfanti, F. Meschi, M. De Pellegrin, A. Lesma, S. Rossini, L. Piemonti, P. Marchetti, F. Dotta, E. Bosi, and M. Battaglia. 2013. Reduction of Circulating Neutrophils Precedes and Accompanies Type 1 Diabetes. *Diabetes* 62: 2072–2077.

136. Vecchio, F., N. Lo Buono, A. Stabilini, L. Nigi, M. J. Dufort, S. Geyer, P. M. Rancoita, F. Cugnata, A. Mandelli, A. Valle, P. Leete, F. Mancarella, P. S. Linsley, L. Krogvold, K. C. Herold, H. E. Larsson, S. J. Richardson, N. G. Morgan, K. Dahl-Jørgensen, G. Sebastiani, F. Dotta, E. Bosi, M. Battaglia, Type 1 Diabetes TrialNet Study Group, and M. Battaglia. 2018. Abnormal neutrophil signature in the blood and pancreas of presymptomatic and symptomatic type 1 diabetes. *JCI Insight* 3.

137. Warnatsch, A., M. Ioannou, Q. Wang, and V. Papayannopoulos. 2015. Neutrophil extracellular traps license macrophages for cytokine production in atherosclerosis. *Science (80-.).* 349.

138. Qiu, S.-L., H. Zhang, Q. Tang, J. Bai, Z.-Y. He, J.-Q. Zhang, M.-H. Li, J.-M. Deng, G.-N. Liu, and X.-N. Zhong. 2017. Neutrophil extracellular traps induced by cigarette smoke activate plasmacytoid dendritic cells. *Thorax* 72: 1084–1093.

139. Tang, S., Y. Zhang, S.-W. Yin, X.-J. Gao, W.-W. Shi, Y. Wang, X. Huang, L. Wang, L.-Y. Zou, J.-H. Zhao, Y.-J. Huang, L.-Y. Shan, A. S. Gounni, Y.-Z. Wu, and J.-B. Zhang. 2015. Neutrophil extracellular trap formation is associated with autophagy-related signalling in ANCA-associated vasculitis. *Clin. Exp. Immunol.* 180: 408–418.

140. Garcia-Romo, G. S., S. Caielli, B. Vega, J. Connolly, F. Allantaz, Z. Xu, M. Punaro, J. Baisch, C. Guiducci, R. L. Coffman, F. J. Barrat, J. Banchereau, and V. Pascual. 2011. Netting neutrophils are major inducers of type I IFN production in pediatric systemic lupus erythematosus. *Sci. Transl. Med.* 3: 73ra20.

141. Lande, R., J. Gregorio, V. Facchinetti, B. Chatterjee, Y.-H. Wang, B. Homey, W. Cao, Y.-H. Wang, B. Su, F. O. Nestle, T. Zal, I. Mellman, J.-M. Schröder, Y.-J. Liu, and M. Gilliet. 2007. Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. *Nature* 449: 564–9.

142. Qin, J., S. Fu, C. Speake, C. J. Greenbaum, and J. M. Odegard. 2016. NETosis-associated serum biomarkers are reduced in type 1 diabetes in association with neutrophil count. *Clin. Exp. Immunol.* 184: 318–22.

143. Wang, Y., Y. Xiao, L. Zhong, D. Ye, J. Zhang, Y. Tu, S. R. Bornstein, Z. Zhou, K. S. L. Lam, and A. Xu. 2014. Increased neutrophil elastase and proteinase 3 and augmented NETosis are closely associated with β-cell autoimmunity in patients with type 1 diabetes. *Diabetes* 63: 4239–48.

144. Parackova, Z., I. Zentsova, P. Vrabcova, A. Klocperk, Z. Sumnik, S. Pruhova, L. Petruzelkova, R. Hasler, and A. Sediva. 2020. Neutrophil Extracellular Trap Induced Dendritic Cell Activation Leads to Th1 Polarization in Type 1 Diabetes. *Front. Immunol.* 11: 661.

145. Huang, J., Y. Xiao, A. Xu, and Z. Zhou. 2015. Neutrophils in type 1 diabetes. *J. Diabetes Investig.* 7: n/a-n/a.

146. Diana, J., and A. Lehuen. 2014. Macrophages and β-cells are responsible for CXCR2-mediated neutrophil infiltration of the pancreas during autoimmune diabetes. *EMBO Mol. Med.* 6: 1090–1104.

147. Yang, X. D., N. Karin, R. Tisch, L. Steinman, and H. O. McDevitt. 1993. Inhibition of insulitis and prevention of diabetes in nonobese diabetic mice by blocking L-selectin and very late antigen 4 adhesion receptors. *Proc. Natl. Acad. Sci. U. S. A.* 90: 10494–8.

148. Roep, B. O., S. D. Arden, R. R. P. de vries, and J. C. Hutton. 1990. T-cell clones from a type-1 diabetes patient respond to insulin secretory granule proteins. *Nature* 345: 632–634.

149. Hawkes, C. J., N. C. Schloot, J. Marks, S. J. Willemen, J. W. Drijfhout, E. K. Mayer, M. R. Christie, and B. O. Roep. 2000. T-cell lines reactive to an immunodominant epitope of the tyrosine phosphatase-like autoantigen IA-2 in type 1 diabetes. *Diabetes* 49: 356–366.

150. Sibley, R. K., D. E. Sutherland, F. Goetz, and A. F. Michael. 1985. Recurrent diabetes mellitus in the pancreas iso- and allograft. A light and electron microscopic and immunohistochemical analysis of four cases. *Lab. Invest.* 53: 132–44.

151. Lampeter, E. F., M. Homberg, K. Quabeck, U. W. Schaefer, P. Wernet, J. Bertrams, H. Grosse-Wilde, F. A. Gries, and H. Kolb. 1993. Transfer of insulin-dependent diabetes between HLA-identical siblings by bone marrow transplantation. *Lancet (London, England)* 341: 1243–4.

152. Hagopian, W., R. J. Ferry, N. Sherry, D. Carlin, E. Bonvini, S. Johnson, K. E. Stein, S. Koenig, A. G. Daifotis, K. C. Herold, J. Ludvigsson, and Protégé Trial Investigators. 2013. Teplizumab Preserves C-Peptide in Recent-Onset Type 1 Diabetes: Two-Year Results From the Randomized, Placebo-Controlled Protege Trial. *Diabetes* 62: 3901–3908.

153. Herold, K. C., J. A. Bluestone, A. G. Montag, A. Parih, A. Wiegner, R. E. Gress, and R. Hirsch. 1992. Prevention of Autoimmune Diabetes With Nonactivating Anti-CD3 Monoclonal Antibody. *Diabetes* 41: 385–391.

154. Vafiadis, P., H. Ounissi-Benkalha, M. Palumbo, R. Grabs, M. Rousseau, C. G. Goodyer, and C. Polychronakos. 2001. Class III Alleles of the Variable Number of Tandem Repeat Insulin Polymorphism Associated with Silencing of Thymic Insulin Predispose to Type 1 Diabetes. *J. Clin. Endocrinol. Metab.* 86: 3705–3710.

155. Bulek, A. M., D. K. Cole, A. Skowera, G. Dolton, S. Gras, F. Madura, A. Fuller, J. J. Miles, E. Gostick, D. A. Price, J. W. Drijfhout, R. R. Knight, G. C. Huang, N. Lissin, P. E. Molloy, L. Wooldridge, B. K. Jakobsen, J. Rossjohn, M. Peakman, P. J. Rizkallah, and A. K. Sewell. 2012. Structural basis for the killing of human beta cells by CD8+ T cells in type 1 diabetes. *Nat. Immunol.* 13: 283–289.

156. Pugliese, A. 2017. Autoreactive T cells in type 1 diabetes. *J. Clin. Invest.* 127: 2881–2891.

157. Delong, T., T. A. Wiles, R. L. Baker, B. Bradley, G. Barbour, R. Reisdorph, M. Armstrong, R. L. Powell, N. Reisdorph, N. Kumar, C. M. Elso, M. DeNicola, R. Bottino, A. C. Powers, D. M. Harlan, S. C. Kent, S. I. Mannering, and K. Haskins. 2016. Pathogenic CD4 T cells in type 1 diabetes recognize epitopes formed by peptide fusion. *Science (80-.).* 351: 711–714.

158. Unger, W. W., J. Velthuis, J. R. F. Abreu, S. Laban, E. Quinten, M. G. D. Kester, S. Reker-Hadrup, A. H. Bakker, G. Duinkerken, A. Mulder, K. L. M. C. Franken, R. Hilbrands, B. Keymeulen, M. Peakman, F. Ossendorp, J. W. Drijfhout, T. N. Schumacher, and B. O. Roep. 2011. Discovery of low-affinity preproinsulin epitopes and detection of autoreactive CD8 T-cells using combinatorial MHC multimers. *J. Autoimmun.* 37: 151–159.

159. Velthuis, J. H., W. W. Unger, J. R. F. Abreu, G. Duinkerken, K. Franken, M. Peakman, A. H. Bakker, S. Reker-Hadrup, B. Keymeulen, J. W. Drijfhout, T. N. Schumacher, and B. O. Roep. 2010. Simultaneous Detection of Circulating Autoreactive CD8+ T-Cells Specific for Different Islet Cell-Associated Epitopes Using Combinatorial MHC Multimers. *Diabetes* 59: 1721–1730.

160. Babon, J. A. B., M. E. DeNicola, D. M. Blodgett, I. Crèvecoeur, T. S. Buttrick, R. Maehr, R. Bottino, A. Naji, J. Kaddis, W. Elyaman, E. A. James, R. Haliyur, M. Brissova, L. Overbergh, C. Mathieu, T. Delong, K. Haskins, A. Pugliese, M. Campbell-Thompson, C. Mathews, M. A. Atkinson, A. C. Powers, D. M. Harlan, and S. C. Kent. 2016. Analysis of self-antigen specificity of islet-infiltrating T cells from human donors with type 1 diabetes. *Nat. Med.* 22: 1482–1487.

161. Diez, J., Y. Park, M. Zeller, D. Brown, D. Garza, C. Ricordi, J. Hutton, G. S. Eisenbarth, and A. Pugliese. 2001. Differential Splicing of the IA-2 mRNA in Pancreas and Lymphoid Organs as a Permissive Genetic Mechanism for Autoimmunity Against the IA-2 Type 1 Diabetes Autoantigen. *Diabetes* 50: 895–900.

162. Dogra, R. S., P. Vaidyanathan, K. R. Prabakar, K. E. Marshall, J. C. Hutton, and A. Pugliese. 2006. Alternative splicing of G6PC2, the gene coding for the islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP), results in differential expression in human thymus and spleen compared with pancreas. *Diabetologia* 49: 953–957.

163. Doyle, H. A., and M. J. Mamula. 2012. Autoantigenesis: the evolution of protein modifications in autoimmune disease. *Curr. Opin. Immunol.* 24: 112–118.

164. McGinty, J. W., I.-T. Chow, C. Greenbaum, J. Odegard, W. W. Kwok, and E. A. James. 2014. Recognition of Posttranslationally Modified GAD65 Epitopes in Subjects With Type 1 Diabetes. *Diabetes* 63: 3033–3040.

165. Ferris, S. T., J. A. Carrero, and E. R. Unanue. 2016. Antigen presentation events during the initiation of autoimmune diabetes in the NOD mouse. *J. Autoimmun.* 71: 19–25.

166. Friedman, R. S., R. S. Lindsay, J. K. Lilly, V. Nguyen, C. M. Sorensen, J. Jacobelli, and M. F. Krummel. 2014. An evolving autoimmune microenvironment regulates the quality of effector T cell restimulation and function. *Proc. Natl. Acad. Sci. U. S. A.* 111: 9223–9228.

167. Heninger, A. K., P. Monti, C. Wilhelm, P. Schwaiger, D. Kuehn, A. G. Ziegler, and E. Bonifacio. 2013. Activation of islet autoreactive naïve T cells in infants is influenced by homeostatic mechanisms and antigen-presenting capacity. *Diabetes* 62: 2059–2066.

168. Heninger, A. K., A. Eugster, D. Kuehn, F. Buettner, M. Kuhn, A. Lindner, S. Dietz, S. Jergens, C. Wilhelm, A. Beyerlein, A. G. Ziegler, and E. Bonifacio. 2017. A divergent population of autoantigen-responsive CD4+ T cells in infants prior to b cell autoimmunity. *Sci. Transl. Med.* 9.

169. Sarikonda, G., J. Pettus, S. Phatak, S. Sachithanantham, J. F. Miller, J. D. Wesley, E. Cadag, J. Chae, L. Ganesan, R. Mallios, S. Edelman, B. Peters, and M. Von Herrath. 2014. CD8 T-cell reactivity to islet antigens is unique to type 1 while CD4 T-cell reactivity exists in both type 1 and type 2 diabetes. *J. Autoimmun.* 50: 77–82.

170. Richardson, S. J., T. Rodriguez-Calvo, I. C. Gerling, C. E. Mathews, J. S. Kaddis, M. A. Russell, M. Zeissler, P. Leete, L. Krogvold, K. Dahl-Jørgensen, M. von Herrath, A. Pugliese, M. A. Atkinson, and N. G. Morgan. 2016. Islet cell hyperexpression of HLA class I antigens: a defining feature in type 1 diabetes. *Diabetologia* 59: 2448–2458.

171. Krogvold, L., B. Edwin, T. Buanes, G. Frisk, O. Skog, M. Anagandula, O. Korsgren, D. Undlien, M. C. Eike, S. J. Richardson, P. Leete, N. G. Morgan, S. Oikarinen, M. Oikarinen, J. E. Laiho, H. Hyöty, J. Ludvigsson, K. F. Hanssen, and K. Dahl-Jørgensen. 2015. Detection of a Low-Grade Enteroviral Infection in the Islets of Langerhans of Living Patients Newly Diagnosed With Type 1 Diabetes. *Diabetes* 64: 1682–1687.

172. Campbell, lain L., L. Oxbrow, J. West, and L. C. Harrison. 1988. Regulation of MHC Protein Expression in Pancreatic β-Cells by Interferon-γ and Tumor Necrosis Factor-α. *Mol. Endocrinol.* 2: 101–107.

173. Malek, T. R., and A. L. Bayer. 2004. Tolerance, not immunity, crucially depends on IL-2. *Nat. Rev. Immunol.* 4: 665–674.

174. Sojka, D. K., Y.-H. Huang, and D. J. Fowell. 2008. Mechanisms of regulatory T-cell suppression – a diverse arsenal for a moving target. *Immunology* 124: 13–22.

175. Wildin, R. S., F. Ramsdell, J. Peake, F. Faravelli, J.-L. Casanova, N. Buist, E. Levy-Lahad, M. Mazzella, O. Goulet, L. Perroni, F. Dagna Bricarelli, G. Byrne, M. McEuen, S. Proll, M. Appleby, and M. E. Brunkow. 2001. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nat. Genet.* 27: 18–20.

176. Grinberg-Bleyer, Y., A. Baeyens, S. You, R. Elhage, G. Fourcade, S. Gregoire, N. Cagnard, W. Carpentier, Q. Tang, J. Bluestone, L. Chatenoud, D. Klatzmann, B. L. Salomon, and E. Piaggio. 2010. IL-2 reverses established type 1 diabetes in NOD mice by a local effect on pancreatic regulatory T cells. *J. Exp. Med.* 207: 1871–1878.

177. Todd, J. A., N. M. Walker, J. D. Cooper, D. J. Smyth, K. Downes, V. Plagnol, R. Bailey, S. Nejentsev, S. F. Field, F. Payne, C. E. Lowe, J. S. Szeszko, J. P. Hafler, L. Zeitels, J. H. M. Yang, A. Vella, S. Nutland, H. E. Stevens, H. Schuilenburg, G. Coleman, M. Maisuria, W. Meadows, L. J. Smink, B. Healy, O. S. Burren, A. A. C. Lam, N. R. Ovington, J. Allen, E. Adlem, H.-T. Leung, C. Wallace, J. M. M. Howson, C. Guja, C. Ionescu-Tîrgovişte, M. J. Simmonds, J. M. Heward, S. C. L. Gough, D. B. Dunger, L. S. Wicker, D. G. Clayton, L. S. Wicker, and D. G. Clayton. 2007. Robust associations of four new chromosome regions

from genome-wide analyses of type 1 diabetes. *Nat. Genet.* 39: 857–864.

178. Lindley, S., C. M. Dayan, A. Bishop, B. O. Roep, M. Peakman, and T. I. M. Tree. 2004. Defective Suppressor Function in CD4+CD25+ T-Cells From Patients With Type 1 Diabetes. *Diabetes* 54: 92–99.

179. Brusko, T. M., C. H. Wasserfall, M. J. Clare-Salzler, D. A. Schatz, and M. A. Atkinson. 2005. Functional Defects and the Influence of Age on the Frequency of CD4+CD25+ T-Cells in Type 1 Diabetes. *Diabetes* 54: 1407–1414.

180. Brusko, T., C. Wasserfall, K. McGrail, R. Schatz, H. L. Viener, D. Schatz, M. Haller, J. Rockell, P. Gottlieb, M. Clare-Salzler, and M. Atkinson. 2007. No Alterations in the Frequency of FOXP3+ Regulatory T-Cells in Type 1 Diabetes. *Diabetes* 56: 604–612.

181. Putnam, A. L., F. Vendrame, F. Dotta, and P. A. Gottlieb. 2005. CD4+CD25high regulatory T cells in human autoimmune diabetes. *J. Autoimmun.* 24: 55–62.

182. Kukreja, A., G. Cost, J. Marker, C. Zhang, Z. Sun, K. Lin-Su, S. Ten, M. Sanz, M. Exley, B. Wilson, S. Porcelli, and N. Maclaren. 2002. Multiple immuno-regulatory defects in type-1 diabetes. *J. Clin. Invest.* 109: 131–140.

183. Parackova, Z., J. Kayserova, K. Danova, K. Sismova, E. Dudkova, Z. Sumnik, S. Kolouskova, J. Lebl, K. Stechova, and A. Sediva. 2016. T regulatory lymphocytes in type 1 diabetes: Impaired CD25 expression and IL-2 induced STAT5 phosphorylation in pediatric patients. *Autoimmunity* 49: 523–531.

184. Wang, J., A. Ioan-Facsinay, E. I. H. van der Voort, T. W. J. Huizinga, and R. E. M. Toes. 2007. Transient expression of FOXP3 in human activated nonregulatory CD4+ T cells. *Eur. J. Immunol.* 37: 129–138.

185. Mason, G. M., K. Lowe, R. Melchiotti, R. Ellis, E. de Rinaldis, M. Peakman, S. Heck, G. Lombardi, and T. I. M. Tree. 2015. Phenotypic Complexity of the Human Regulatory T Cell Compartment Revealed by Mass Cytometry. *J. Immunol.* 195: 2030–2037.

186. McClymont, S. A., A. L. Putnam, M. R. Lee, J. H. Esensten, W. Liu, M. A. Hulme, U. Hoffmüller, U. Baron, S. Olek, J. A. Bluestone, and T. M. Brusko. 2011. Plasticity of Human Regulatory T Cells in Healthy Subjects and Patients with Type 1 Diabetes. *J. Immunol.* 186: 3918–3926.

187. Marwaha, A. K., S. Q. Crome, C. Panagiotopoulos, K. B. Berg, H. Qin, Q. Ouyang, L. Xu, J. J. Priatel, M. K. Levings, and R. Tan. 2010. Cutting Edge: Increased IL-17–Secreting T Cells in Children with New-Onset Type 1 Diabetes. *J. Immunol.* 185: 3814–3818.

188. Glisic-Milosavljevic, S., T. Wang, M. Koppen, J. Kramer, S. Ehlenbach, J. Waukau, P. Jailwala, S. Jana, R. Alemzadeh, and S. Ghosh. 2007. Dynamic changes in CD4+ CD25+ high T cell apoptosis after the diagnosis of type 1 diabetes. *Clin. Exp. Immunol.* 150: 75–82.

189. Glisic-Milosavljevic, S., J. Waukau, P. Jailwala, S. Jana, H.-J. Khoo, H. Albertz, J. Woodliff, M. Koppen, R. Alemzadeh, W. Hagopian, and S. Ghosh. 2007. At-Risk and Recent-Onset Type 1 Diabetic Subjects Have Increased Apoptosis in the CD4+CD25+high T-Cell Fraction. *PLoS One* 2: e146.

190. Long, S. A., K. Cerosaletti, J. Y. Wan, J.-C. Ho, M. Tatum, S. Wei, H. G. Shilling, and J. H. Buckner. 2011. An autoimmune-associated variant in PTPN2 reveals an impairment of IL-2R signaling in CD4+ T cells. *Genes Immun.* 12: 116–125.

191. Garg, G., J. R. Tyler, J. H. M. Yang, A. J. Cutler, K. Downes, M. Pekalski, G. L. Bell, S. Nutland, M.

Peakman, J. A. Todd, L. S. Wicker, and T. I. M. Tree. 2012. Type 1 diabetes-associated IL2RA variation lowers IL-2 signaling and contributes to diminished CD4+CD25+ regulatory T cell function. *J. Immunol.* 188: 4644–53.

192. Yang, J. H. M., A. J. Cutler, R. C. Ferreira, J. L. Reading, N. J. Cooper, C. Wallace, P. Clarke, D. J. Smyth, C. S. Boyce, G.-J. Gao, J. A. Todd, L. S. Wicker, and T. I. M. Tree. 2015. Natural Variation in Interleukin-2 Sensitivity Influences Regulatory T-Cell Frequency and Function in Individuals With Long-standing Type 1 Diabetes. *Diabetes* 64: 3891–3902.

193. Ferraro, A., C. Socci, A. Stabilini, A. Valle, P. Monti, L. Piemonti, R. Nano, S. Olek, P. Maffi, M. Scavini, A. Secchi, C. Staudacher, E. Bonifacio, and M. Battaglia. 2011. Expansion of Th17 Cells and Functional Defects in T Regulatory Cells Are Key Features of the Pancreatic Lymph Nodes in Patients With Type 1 Diabetes. *Diabetes* 60: 2903–2913.

194. Roncarolo, M. G., S. Gregori, R. Bacchetta, and M. Battaglia. 2014. Tr1 Cells and the Counter-Regulation of Immunity: Natural Mechanisms and Therapeutic Applications. In 39–68.

195. Cabello-Olmo, M., M. Araña, I. Radichev, P. Smith, E. Huarte, and M. Barajas. 2019. New Insights into Immunotherapy Strategies for Treating Autoimmune Diabetes. *Int. J. Mol. Sci.* 20: 4789.

196. Orban, T., B. Bundy, D. J. Becker, L. A. DiMeglio, S. E. Gitelman, R. Goland, P. A. Gottlieb, C. J. Greenbaum, J. B. Marks, R. Monzavi, A. Moran, M. Peakman, P. Raskin, W. E. Russell, D. Schatz, D. K. Wherrett, D. M. Wilson, J. P. Krischer, J. S. Skyler, and Type 1 Diabetes TrialNet Abatacept Study Group. 2014. Costimulation Modulation With Abatacept in Patients With Recent-Onset Type 1 Diabetes: Follow-up 1 Year After Cessation of Treatment. *Diabetes Care* 37: 1069–1075.

197. Rigby, M. R., L. A. DiMeglio, M. S. Rendell, E. I. Felner, J. M. Dostou, S. E. Gitelman, C. M. Patel, K. J. Griffin, E. Tsalikian, P. A. Gottlieb, C. J. Greenbaum, N. A. Sherry, W. V Moore, R. Monzavi, S. M. Willi, P. Raskin, A. Moran, W. E. Russell, A. Pinckney, L. Keyes-Elstein, M. Howell, S. Aggarwal, N. Lim, D. Phippard, G. T. Nepom, J. McNamara, M. R. Ehlers, and T1DAL Study Team. 2013. Targeting of memory T cells with alefacept in new-onset type 1 diabetes (T1DAL study): 12 month results of a randomised, double-blind, placebo-controlled phase 2 trial. *Lancet Diabetes Endocrinol.* 1: 284–294.

198. Haller, M. J., S. E. Gitelman, P. A. Gottlieb, A. W. Michels, S. M. Rosenthal, J. J. Shuster, B. Zou, T. M. Brusko, M. A. Hulme, C. H. Wasserfall, C. E. Mathews, M. A. Atkinson, and D. A. Schatz. 2015. Anti-thymocyte globulin/G-CSF treatment preserves β cell function in patients with established type 1 diabetes. *J. Clin. Invest.* 125: 448–455.

199. Bluestone, J. A., J. H. Buckner, M. Fitch, S. E. Gitelman, S. Gupta, M. K. Hellerstein, K. C. Herold, A. Lares, M. R. Lee, K. Li, W. Liu, S. A. Long, L. M. Masiello, V. Nguyen, A. L. Putnam, M. Rieck, P. H. Sayre, and Q. Tang. 2015. Type 1 diabetes immunotherapy using polyclonal regulatory T cells. *Sci. Transl. Med.* 7: 315ra189-315ra189.

200. Hartemann, A., G. Bensimon, C. A. Payan, S. Jacqueminet, O. Bourron, N. Nicolas, M. Fonfrede, M. Rosenzwajg, C. Bernard, and D. Klatzmann. 2013. Low-dose interleukin 2 in patients with type 1 diabetes: a phase 1/2 randomised, double-blind, placebo-controlled trial. *Lancet Diabetes Endocrinol.* 1: 295–305.

201. Alhadj Ali, M., Y.-F. Liu, S. Arif, D. Tatovic, H. Shariff, V. B. Gibson, N. Yusuf, R. Baptista, M. Eichmann, N. Petrov, S. Heck, J. H. M. Yang, T. I. M. Tree, I. Pujol-Autonell, L. Yeo, L. R. Baumard, R. Stenson, A. Howell, A. Clark, Z. Boult, J. Powrie, L. Adams, F. S. Wong, S. Luzio, G. Dunseath, K. Green, A. O'Keefe, G. Bayly, N. Thorogood, R. Andrews, N. Leech, F. Joseph, S. Nair, S. Seal, H. Cheung, C. Beam, R. Hills, M. Peakman, and C. M. Dayan. 2017. Metabolic and immune effects of immunotherapy

with proinsulin peptide in human new-onset type 1 diabetes. *Sci. Transl. Med.* 9: eaaf7779.

202. Maude, S., and D. M. Barrett. 2016. Current status of chimeric antigen receptor therapy for haematological malignancies. *Br. J. Haematol.* 172: 11–22.

203. Pierini, A., B. P. Iliopoulou, H. Peiris, M. Pérez-Cruz, J. Baker, K. Hsu, X. Gu, P.-P. Zheng, T. Erkers, S.-W. Tang, W. Strober, M. Alvarez, A. Ring, A. Velardi, R. S. Negrin, S. K. Kim, and E. H. Meyer. 2017. T cells expressing chimeric antigen receptor promote immune tolerance. *JCI insight* 2.

204. Fransson, M., E. Piras, J. Burman, B. Nilsson, M. Essand, B. Lu, R. A. Harris, P. U. Magnusson, E. Brittebo, and A. S. Loskog. 2012. CAR/FoxP3-engineered T regulatory cells target the CNS and suppress EAE upon intranasal delivery. *J. Neuroinflammation* 9: 576.

205. Skuljec, J., M. Chmielewski, C. Happle, A. Habener, M. Busse, H. Abken, and G. Hansen. 2017. Chimeric Antigen Receptor-Redirected Regulatory T Cells Suppress Experimental Allergic Airway Inflammation, a Model of Asthma. *Front. Immunol.* 8.

206. Kim, Y. C., A.-H. Zhang, Y. Su, S. A. Rieder, R. J. Rossi, R. A. Ettinger, K. P. Pratt, E. M. Shevach, and D. W. Scott. 2015. Engineered antigen-specific human regulatory T cells: immunosuppression of FVIII-specific T- and B-cell responses. *Blood* 125: 1107–1115.

207. Hull, C. M., L. E. Nickolay, M. Estorninho, M. W. Richardson, J. L. Riley, M. Peakman, J. Maher, and T. I. M. Tree. 2017. Generation of human islet-specific regulatory T cells by TCR gene transfer. *J. Autoimmun.* 79: 63–73.

208. Hu, C., D. Rodriguez-Pinto, W. Du, A. Ahuja, O. Henegariu, F. S. Wong, M. J. Shlomchik, and L. Wen. 2007. Treatment with CD20-specific antibody prevents and reverses autoimmune diabetes in mice. *J. Clin. Invest.* 117: 3857–3867.

209. Xiu, Y., C. P. Wong, J.-D. Bouaziz, Y. Hamaguchi, Y. Wang, S. M. Pop, R. M. Tisch, and T. F. Tedder. 2008. B Lymphocyte Depletion by CD20 Monoclonal Antibody Prevents Diabetes in Nonobese Diabetic Mice despite Isotype-Specific Differences in FcγR Effector Functions. *J. Immunol.* 180: 2863–2875.

210. Noorchashm, H., A. J. Reed, S. Y. Rostami, R. Mozaffari, G. Zekavat, B. Koeberlein, A. J. Caton, and A. Naji. 2006. B Cell-Mediated Antigen Presentation Is Required for the Pathogenesis of Acute Cardiac Allograft Rejection. *J. Immunol.* 177: 7715–7722.

211. Silveira, P., E. Johnson, H. Chapman, T. Bui, R. Tisch, and D. Serreze. 2002. The preferential ability of B lymphocytes to act as diabetogenic APC in NOD mice depends on expression of self-antigen-specific immunoglobulin receptors. *Eur. J. Immunol.* 32: 3657–3666.

212. Martin, S., D. Wolf-Eichbaum, G. Duinkerken, W. A. Scherbaum, H. Kolb, J. G. Noordzij, and B. O. Roep. 2001. Development of Type 1 Diabetes despite Severe Hereditary B-Cell Deficiency. *N. Engl. J. Med.* 345: 1036–1040.

213. Jones, J. L., S. A. J. Thompson, P. Loh, J. L. Davies, O. C. Tuohy, A. J. Curry, L. Azzopardi, G. Hill-Cawthorne, M. T. Fahey, A. Compston, and A. J. Coles. 2013. Human autoimmunity after lymphocyte depletion is caused by homeostatic T-cell proliferation. *Proc. Natl. Acad. Sci.* 110: 20200–20205.

214. Smith, M. J., K. M. Simmons, and J. C. Cambier. 2017. B cells in type 1 diabetes mellitus and diabetic kidney disease. *Nat. Rev. Nephrol.* 13: 712–720.

215. Roep, B. O., G. Duinkerken, G. M. T. Schreuder, H. Kolb, R. R. P. de Vries, and S. Martin. 1996. HLA-associated inverse correlation between T cell and antibody responsiveness to islet autoantigen

in recent-onset insulin-dependent diabetes mellitus. *Eur. J. Immunol.* 26: 1285–1289.

216. Harrison, L. C., M. C. Honeyman, H. J. DeAizpurua, R. S. Schmidli, P. G. Colman, B. D. Tait, and D. S. Cram. 1993. Inverse relation between humoral and cellular immunity to glutamic acid decarboxylase in subjects at risk of insulin-dependent diabetes. *Lancet (London, England)* 341: 1365–9.

217. Bloem, S. J., and B. O. Roep. 2017. The elusive role of B lymphocytes and islet autoantibodies in (human) type 1 diabetes. *Diabetologia* 60: 1185–1189.

218. Bingley, P. J., E. Bonifacio, and E. A. M. Gale. 1993. Can We Really Predict IDDM? *Diabetes* 42: 213–220.

219. Tian, J., D. Zekzer, L. Hanssen, Y. Lu, A. Olcott, and D. L. Kaufman. 2001. Lipopolysaccharide-Activated B Cells Down-Regulate Th1 Immunity and Prevent Autoimmune Diabetes in Nonobese Diabetic Mice. *J. Immunol.* 167: 1081–1089.

220. Jagannathan, M., M. McDonnell, Y. Liang, H. Hasturk, J. Hetzel, D. Rubin, A. Kantarci, T. E. Van Dyke, L. M. Ganley-Leal, and B. S. Nikolajczyk. 2010. Toll-like receptors regulate B cell cytokine production in patients with diabetes. *Diabetologia* 126: 690–701.

221. Agrawal, S., and S. Gupta. 2011. TLR1/2, TLR7, and TLR9 Signals Directly Activate Human Peripheral Blood Naive and Memory B Cell Subsets to Produce Cytokines, Chemokines, and Hematopoietic Growth Factors. *J. Clin. Immunol.* 31: 89–98.

222. Marino, E., B. Tan, L. Binge, C. R. Mackay, and S. T. Grey. 2012. B-Cell Cross-Presentation of Autologous Antigen Precipitates Diabetes. *Diabetes* 61: 2893–2905.

223. Menard, L., D. Saadoun, I. Isnardi, Y.-S. Ng, G. Meyers, C. Massad, C. Price, C. Abraham, R. Motaghedi, J. H. Buckner, P. K. Gregersen, and E. Meffre. 2011. The PTPN22 allele encoding an R620W variant interferes with the removal of developing autoreactive B cells in humans. *J. Clin. Invest.* 121: 3635–3644.

224. Mackay, F., and P. Schneider. 2009. Cracking the BAFF code. *Nat. Rev. Immunol.* 9: 491–502.

225. Deng, C., Y. Xiang, T. Tan, Z. Ren, C. Cao, G. Huang, L. Wen, and Z. Zhou. 2016. Altered Peripheral B-Lymphocyte Subsets in Type 1 Diabetes and Latent Autoimmune Diabetes in Adults. *Diabetes Care* 39: 434–440.

226. Thompson, W. S., M. L. Pekalski, H. Z. Simons, D. J. Smyth, X. Castro-Dopico, H. Guo, C. Guy, D. B. Dunger, S. Arif, M. Peakman, C. Wallace, L. S. Wicker, J. A. Todd, and R. C. Ferreira. 2014. Multi-parametric flow cytometric and genetic investigation of the peripheral B cell compartment in human type 1 diabetes. *Clin. Exp. Immunol.* 177: 571–585.

227. Parackova, Z., A. Klocperk, M. Rataj, J. Kayserova, I. Zentsova, Z. Sumnik, S. Kolouskova, J. Sklenarova, S. Pruhova, B. Obermannova, L. Petruzelkova, J. Lebl, T. Kalina, and A. Sediva. 2017. Alteration of B cell subsets and the receptor for B cell activating factor (BAFF) in paediatric patients with type 1 diabetes. *Immunol. Lett.* 189: 94–100.

228. Hanley, P., J. A. Sutter, N. G. Goodman, Y. Du, D. R. Sekiguchi, W. Meng, M. R. Rickels, A. Naji, and E. T. Luning Prak. 2017. Circulating B cells in type 1 diabetics exhibit fewer maturation-associated phenotypes. *Clin. Immunol.* 183: 336–343.

229. Thien, M., T. G. Phan, S. Gardam, M. Amesbury, A. Basten, F. Mackay, and R. Brink. 2004. Excess BAFF Rescues Self-Reactive B Cells from Peripheral Deletion and Allows Them to Enter Forbidden

Follicular and Marginal Zone Niches. *Immunity* 20: 785–798.

230. Garcia, A., and J. B. De Sanctis. 2016. A Review of Clinical Trials of Belimumab In The Management of Systemic Lupus Erythematosus. *Curr. Pharm. Des.* .

231. Pescovitz, M. D., C. J. Greenbaum, H. Krause-Steinrauf, D. J. Becker, S. E. Gitelman, R. Goland, P. A. Gottlieb, J. B. Marks, P. F. McGee, A. M. Moran, P. Raskin, H. Rodriguez, D. A. Schatz, D. Wherrett, D. M. Wilson, J. M. Lachin, J. S. Skyler, and Type 1 Diabetes TrialNet Anti-CD20 Study Group. 2009. Rituximab, B-Lymphocyte Depletion, and Preservation of Beta-Cell Function. *N. Engl. J. Med.* 361: 2143–2152.

232. Pescovitz, M. D., C. J. Greenbaum, B. Bundy, D. J. Becker, S. E. Gitelman, R. Goland, P. A. Gottlieb, J. B. Marks, A. Moran, P. Raskin, H. Rodriguez, D. A. Schatz, D. K. Wherrett, D. M. Wilson, J. P. Krischer, J. S. Skyler, and Type 1 Diabetes TrialNet Anti-CD20 Study Group. 2014. B-Lymphocyte Depletion With Rituximab and -Cell Function: Two-Year Results. *Diabetes Care* 37: 453–459.

233. Zekavat, G., S. Y. Rostami, A. Badkerhanian, R. F. Parsons, B. Koeberlein, M. Yu, C. D. Ward, T.-S. Migone, L. Yu, G. S. Eisenbarth, M. P. Cancro, A. Naji, and H. Noorchashm. 2008. In Vivo BLyS/BAFF Neutralization Ameliorates Islet-Directed Autoimmunity in Nonobese Diabetic Mice. *J. Immunol.* 181: 8133–8144.

234. Ahmed, R., Z. Omidian, A. Giwa, B. Cornwell, N. Majety, D. R. Bell, S. Lee, H. Zhang, A. Michels, S. Desiderio, S. Sadegh-Nasseri, H. Rabb, S. Gritsch, M. L. Suva, P. Cahan, R. Zhou, C. Jie, T. Donner, and A. R. A. Hamad. 2019. A Public BCR Present in a Unique Dual-Receptor-Expressing Lymphocyte from Type 1 Diabetes Patients Encodes a Potent T Cell Autoantigen. *Cell* 177: 1583-1599.e16.

APPENDIX - ATTACHED MANUSCRIPTS