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From immune suppression to immune modulation in type 1 diabetes patients

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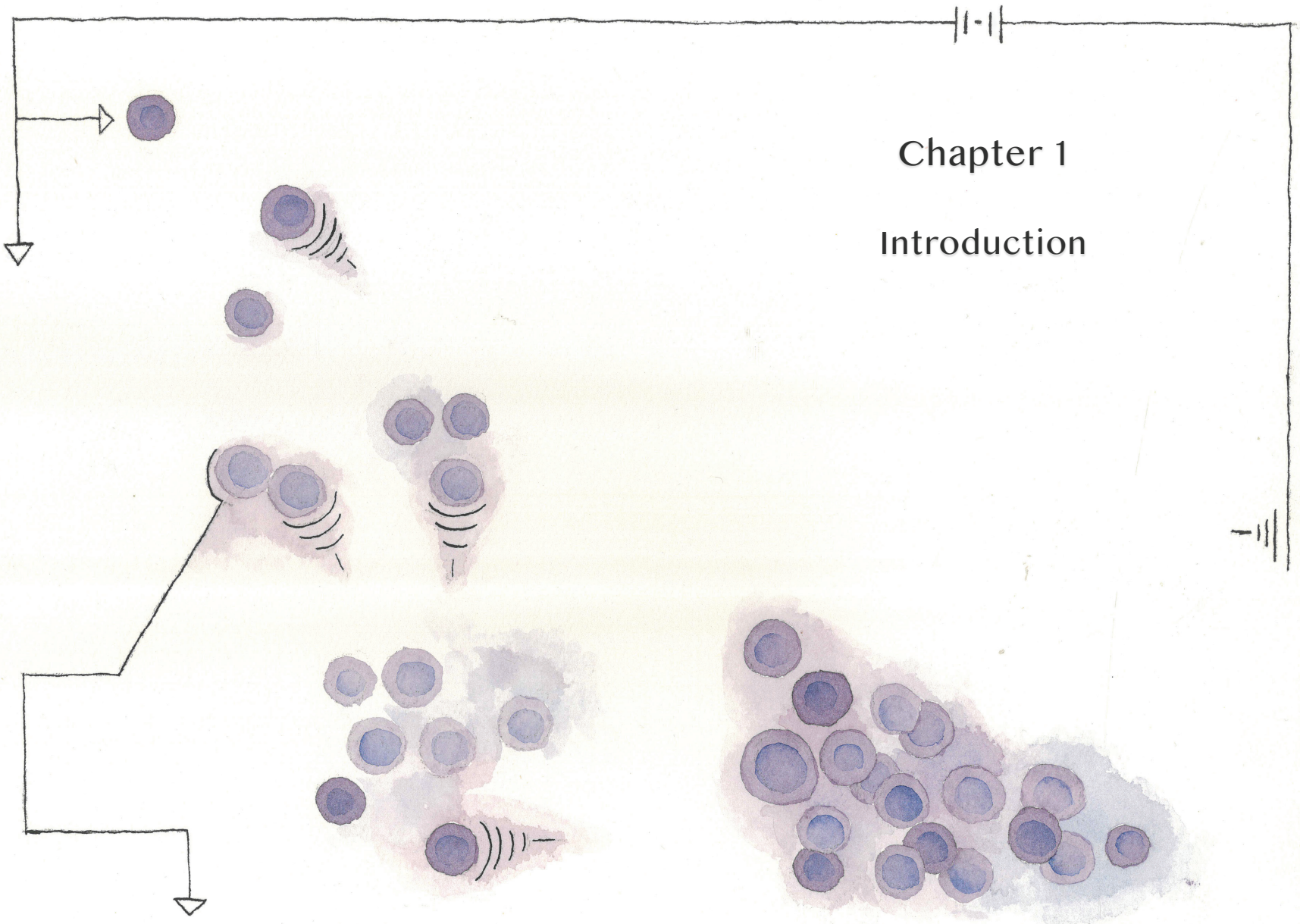

Chapter 1

Introduction

1. Type 1 diabetes

Type 1 diabetes (T1D) is an auto-immune disease characterized by the destruction of the insulin-producing β cells in the pancreas. Insulin is a hormone that lowers blood glucose levels by facilitating the uptake of glucose in peripheral tissues. Therefore, T1D patients present with high blood glucose levels at diagnosis (1).

1.1. Clinical diagnosis of type 1 diabetes

The clinical diagnosis of diabetes is made by various laboratory tests, namely a fasting blood glucose higher than 7 mmol/L (126 mg/dL), symptoms of hyperglycemia with any blood glucose of 11.1 mmol/dL (200 mg/dL) or higher, or a 2 hour oral glucose tolerance test of more than 11.1 mmol/dL. More recently, glycated hemoglobulin (HbA1c) of 6.5% or higher has been added as an independent diagnostic criterion, which reflects glucose control in the previous eight to twelve weeks (2). A new staging system for T1D was proposed in 2015, which allows for diagnosis before the presence of clinical symptoms (**Figure 1A**). Stage 1 T1D includes patients with two or more diabetes associated auto-antibodies; stage 2 requires the presence of dysglycemia on top of islet autoimmunity; and stage 3 is considered as the classical T1D diagnosis; whereas stage 4 is long-standing disease (3). The presentation of T1D differs significantly between patients. The assumption that T1D is a typical children's disease proved to be wrong; the disease is diagnosed at any age at the same rate (4). Yet, children and adolescents present more often with full-blown ketoacidosis, whereas disease presentation in the adult population can be much more moderate, which could mislead to diagnosis of type 2 diabetes (T2D) (2). Serum c-peptide, a measure of endogenous insulin production, also widely varies between patients depending on the age and timeliness of diagnosis as an exponential drop is observed in the first 7 years after diagnosis, after which c-peptide levels remain stable over time (5). Diagnosis of T1D prompts the start of insulin therapy, which is injected by a pump or manually to manage blood glucose levels and, ultimately, for survival (2).

1.2. The burden of living with type 1 diabetes

T1D could pose a burden on patients, as managing glycemic control with insulin therapy is troublesome. Indeed, one in four adult patients feel a moderate-to-high emotional burden from diabetes (6), whereas in adolescents one in three are affected by diabetes-related distress (7). These studies conclude that diabetes-related stress could be associated with poor glycemic control as indicated by higher HbA1c (8). In turn, poor glycemic control could negatively impact academic achievements (9), whereas hypoglycemic episodes were associated with reduced verbal IQ in youth with T1D (10). This touches upon the conundrum of T1D care, namely that insulin is at the same time the best friend and foe of a T1D patient. Yet, even intensive glycemic control cannot always

prevent development of diabetic complications (11, 12). A better, safer, and stress-relieving therapy is needed that targets the cause of the disease instead of merely the symptoms.

1.3. Epidemiology

The sense of urgency for finding a cure for T1D has increased, since T1D incidence worldwide increased annually by 1.8% between 2002-2012 (13). Although T1D is historically known as a childhood disease, it can actually be diagnosed at any age (14). Still, an increased incidence is noted between the ages five and seven and at puberty (13, 15). In addition, incidence is higher in autumn and winter months and in countries with higher latitudes, such as Finland (16, 17). One common denominator of these risk factors is low sun exposure. Indeed, endogenous production of vitamin D3 is dependent upon ultraviolet B (UVB) radiation from the sun and a lack of vitamin D3 (VD3) and variations in the genes involved in the VD3 pathway have been associated with T1D development (18-20).

1.4. Genetics

Besides polymorphisms in the VD3 pathway, several other gene polymorphisms are associated with an increased risk of developing T1D (21). A common misconception regarding T1D, however, is that it is a heritable disorder that runs in families. In reality, T1D is a disease with polygenic predisposition and less than 10-20% of new cases have a family history of T1D (22, 23). Most of the genetic susceptibility is determined by the human leukocyte antigen (HLA) region on chromosome 6. HLA class II is expressed on antigen-presenting cells and functions as the carrier in which antigen is presented to T cells. Both susceptible HLA haplotypes (for instance DRB1*0401-DQB1*0302 and DRB1*0301-DQB1*0201) and protective HLA haplotypes (such as DRB1*1501-DQA1*0102-DQB1*0602) exist (24). The majority of other susceptibility genes are related to modulating the immune response (25). Therapies that could decrease the expression of these genetic risk markers, at least in some cell types, may be successful in treating or reducing the risk of developing T1D. Yet, a profound role for environmental and/or epigenetic factors in the development of T1D next to genetics should not be overlooked, as a study showed that there is 30-65% concordance between monozygotic twins after long term follow-up (26).

1.5. Epigenetics

Not solely are genes important, but also how they are regulated. Gene expression can be influenced by epigenetics. Epigenetics is a relatively new field which studies the heritable changes in gene expression that are not due to changes in the DNA sequence. Examples of epigenetic modifications are methylation of cytosines at CpG dinucleotides, histone

modifications and microRNAs that can all affect gene expression (27). It is not inconceivable that epigenetics could play a role in T1D, as T1D cannot fully be explained by genetics, and causative environmental factors are still elusive (28). Indeed, DNA methylation variability was increased in cord blood of newborns that would later develop T1D, compared to newborns that did not, suggesting that these epigenetic changes could contribute to T1D disease onset (29). In addition, epigenetic modifications were found in promotor regions of T1D risk genes in T1D patients compared to healthy controls (30, 31). Currently we are only scratching the surface of the implications of epigenetics on T1D disease onset and progression, as is exemplified by the paucity of literature on this subject. Besides, epigenetics could prove to be important in determining the stability of cellular therapies, as epigenetics has been implicated in establishing stable cellular phenotypes (32, 33).

1.6. Pathophysiology

T cells

Studies on the pathophysiology of T1D have historically focused on the immune system as the causative agent behind the destruction of β cells in the pancreas. Indeed, autoreactive CD8+ T cells are the most abundant immune cell type found in inflamed islets, followed by macrophages, CD4+ T cells, and B cells (**Figure 1A**) (34-37). Once CD4+ T cells are activated by presentation of antigen on HLA class II on antigen presenting cells, CD4+ T cells activate CD8+ T cells that kill insulin-producing β cells by recognizing islet antigens on HLA class I (35, 38, 39). Healthy individuals also have autoreactive T cells, but they are held in check by immune regulation by for instance T regulatory cells (Tregs) (40). The level of Tregs in T1D patients is similar to healthy individuals, but they are less capable of suppressing T cells, while effector autoreactive T-cells of T1D patients are more resistant to suppression, which may contribute to the progression of autoimmunity (41, 42).

The death of a β cell: revisiting the homicide / suicide model

At disease onset, 50-70% of islets are deprived of insulin staining, while inflammation is almost exclusively limited to insulin-containing islets, suggesting a targeted immune-mediated β cell attack (43, 44). According to the conventional model, islet autoreactive T cells target β cells and commit homicide of 'innocent' β cells, while an alternative model adds β cell suicide to the story (45, 46). This homicide/suicide model was first coined by Bottazzo in 1986, but since then many discoveries have shed a slightly different light on this scenario (47). It seems that β cells initiate interactions with T cells and T cells are merely acting on these requests, which would suggest more dialogue between the two parties rather than one-sided homicide or suicide. To illustrate this, β cells attract immune cells into the islet by secreting CXCL10 and expose themselves to T cells by

hyperexpressing HLA class I (**Figure 1A**) (48-50). Moreover, β cells present modified peptides which activate the immune system, as central tolerance in the thymus has not deleted T cells responsive to these "neo-antigens'' (51). In a similar way, cancer cells express mutated antigens, which allows the immune system to remove the cancer (52). It is not yet clear what exactly triggers β cells to express these immune-activating neo-antigens. The prevailing hypothesis suggests a stress response of β cells, which induces the unfolded protein response and consequently post-translational modifications and defective ribosomal products (53-55). Proposed β cell stressors are cytokine-induced endoplasmic reticulum stress and hyperglycemia (56, 57).

In this sense, β cell death in T1D is not a case of homicide or suicide, but rather of T cell-assisted euthanasia of a stressed β cell calling for attention. Beta cell destruction is incomplete, however, as remaining insulin-positive β cells are found even in long-standing T1D (58). These β cells seem to be functionally impaired or hibernating, as they do not secrete insulin in response to hyperglycemia (59). This is an encouraging insight, as new therapies targeting β cell function may potentially wake up these hibernating β cells to secrete insulin again.

Stromal cells in the islet of Langerhans

The function of β cells could be supported by neighboring cells in the islet of Langerhans. Stromal cells, for instance, are embedded in the islets. Mesenchymal stromal cells (MSCs) are within the islets (**Figure 1A**) (60), whereas myofibroblasts surround the islets (61). In 1979 it was already known that fibroblasts promote the survival and function of β cells, although stromal cells have not received much attention up until recently (62). Besides the potential of MSCs to differentiate into β cells, MSCs improved the islet environment by secreting several growth factors such as vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) that could promote angiogenesis and β cell regeneration, respectively (63-65). In this regard, MSCs may be beneficial for β cell function, while at the same time they could contribute to maintaining immune balance in the islets (66). Thus, these on first sight innocuous cells may be used therapeutically in T1D to improve the islet environment.

Monocytes and dendritic cells

The destruction of β cells is set in motion by presentation of β cell-specific antigens to T cells by antigen presenting cells (APCs) (**Figure 1A**). Indeed, APCs are the true directors of the immune system orchestra. Conceivably, aberrant APC function may be implicated in the pathophysiology of T1D. Several cell types have antigen presenting capacities, but dendritic cells (DCs) are professional antigen-presenting cells, which could be derived from monocytes (67). Monocyte-derived DCs from T1D patients indeed showed

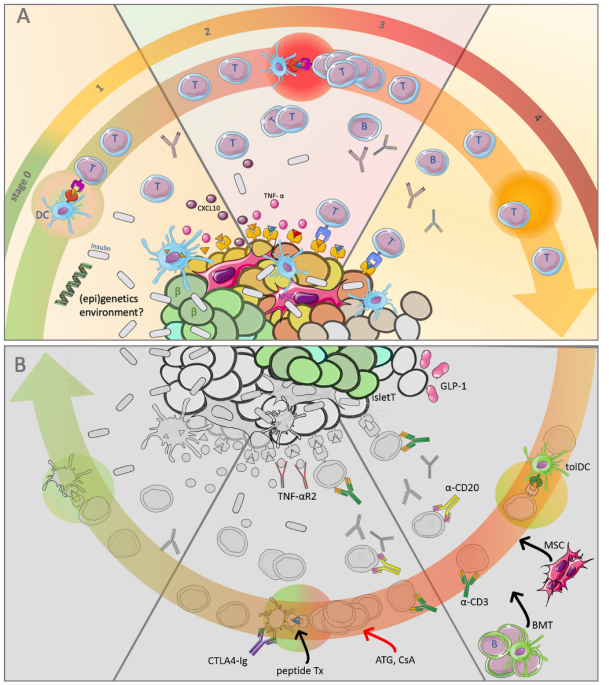

Figure 1: Natural History and Therapeutic Strategies in Type 1 Diabetes. (A) *The natural history and stages of Type 1 Diabetes. It is yet unclear which environmental triggers cause the onset of islet autoreactivity in genetically susceptible T1D patients. This onset is characterized by beta cell-antigen uptake and presentation by dendritic cells to autoreactive T cells. T cells then activate B cells to produce autoantibodies, which are detected in the blood. Once two autoantibodies are detected, a diagnosis of stage 1 T1D is prompted. Beta cells, in their turn, secrete the chemokine CXCL10 that attracts more immune cells into the islets. This causes more insulitis, which results in more dysfunctional beta cells and the initiation of dysglycemia and the start of stage 2 T1D. Consequently, cytokine production of infiltrating immune cells and antigen-specific cytotoxicity causes more beta cell death, which ultimately results in stage 3 T1D, necessitating exogenous insulin administration. In long-standing stage 4 T1D, beta cell mass is critically decreased, and what beta cells are still present are mostly in a dormant state not secreting insulin.* ***(B)*** *Therapies in T1D aim to reverse this vicious cycle of autoreactive T cell cytotoxicity and beta cell apoptosis by either targeting the immune system or the islets of Langerhans. In this animation, cellular, antigen-specific, and antibody therapies are depicted, next to drugs. CXCL10 is C-X-C motif chemokine ligand 10; ATG is anti-thymocyte globulin; CsA is cyclosporine A; peptide Tx is peptide therapy; MSC is mesenchymal stromal cell; BMT is bone marrow transplantation; tolDC is tolerogenic dendritic cell; GLP-1 is glucoagon-like peptide 1. Created in Biorender.com.*

differences compared to healthy subjects. Mainly decreased DC maturation and decreased capacity to stimulate autologous and allogeneic T cells was seen (68). Other studies corroborated that monocyte-derived DCs from T1D patients had abnormal NF-kB signaling and were less mature with low levels of activating molecules CD83, CD80, and CD86 (39, 68-70). These results seem counterintuitive as decreased DC maturation would impede activation of the immune system. Tolerance, however, is an active process, so these DCs with decreased maturation may still be able to activate T cells but not to regulate them. Besides functional differences, the frequencies of DCs differ, with higher levels of DCs at T1D diagnosis (39) and lower levels in new and recent-onset (71, 72) and established T1D, compared to healthy controls (73). Monocyte frequencies, however, were similar in T1D compared to healthy controls (72). In conclusion, both the function and frequencies of at least a subset of DCs have been claimed to be altered in T1D and modulating these cells may direct the immune system towards regulation.

B cells and antibodies

Although T cell-mediated β cell destruction is held to be the main cause of T1D, B cells and humoral autoimmunity should be considered as well. Several studies found that B cells infiltrate the islets in T1D (**Figure 1A**), which is even more prominent in patients diagnosed before the age of 7 (34, 74). Yet, a causal role for B cells and antibodies is still lacking (75). In fact, T1D was diagnosed in a patient with severe hereditary B-lymphocyte deficiency, illustrating that T1D can develop without the presence of B cells and antibodies (76). Nonetheless, β cell auto-antibodies have been found useful for diagnostic purposes and prediction of T1D development, even though 10% of T1D patients are negative (77, 78). If B cells do not cause T1D, why do they infiltrate the islets of T1D patients? One explanation could be that B cells are recruited secondarily by activated CD4 T cells and exacerbate T1D progression (34). Alternatively, B cells and the humoral response might regulate T cells in T1D, rather than contributing to β cell destruction. Several studies showed that islet auto-antibodies actually correlated inversely with T cell proliferation or activated CD8 T cell counts in T1D, corroborating this hypothesis (72, 79, 80). Furthermore, T cells secreted the inhibitory cytokine IL-10, but not the inflammatory cytokine IFN-γ, when recognizing an epitope that was shared with B cells (81). Thus far, however, no therapies have been successful in exploiting this postulated regulatory role of humoral immunity in T1D.

2. Therapies for type 1 diabetes

2.1. Rationale for curative type 1 diabetes therapies

After T1D diagnosis, insulin replacement therapy is started. Unfortunately, exogenous insulin is not a cure for T1D. Excessive amounts of insulin causes life-threatening hypoglycemia, whereas insufficient insulin subjects the patient to complications (82, 83). Retinopathy, neuropathy and nephropathy are long-term complications that are caused by periods of hyperglycemia. Although the incidence of these complications is reduced with intensive insulin treatment, there is no effective therapy today to prevent these (11, 12). Furthermore, meeting the HbA1c target of <7% remains a struggle for patients with 70% failing to achieve this and in a clinical trial this target was not even met despite strict intensive insulin therapy (83-85). Thus, mainstay insulin therapy does not satisfy the unmet need to improve glycemic control and decrease long-term complications in T1D patients. The rationale for curative T1D therapies shifts together with our understanding of the complexity and heterogeneity of the disease. Whereas the first T1D clinical trials primarily focused on suppressing the immune system, new strategies target multiple immune pathways, utilize antigen-specific strategies or cells as a vehicle and, finally, include β cells in the equation as well.

2.2. Immunotherapies for type 1 diabetes

Mono immunotherapies

The first immunotherapy trials assessed the effect of immune suppression by cyclosporine A that blocks T cell activity (**Figure 1B**). Two independent studies indeed showed that cyclosporine A reduced exogenous insulin needs for over 1 year. However, no lasting effect was obtained after cessation of therapy (86, 87), while cyclosporine A comes with the risk of nephro- and β cell-toxicity (88-90). Anti-CD3 antibodies such as teplizumab and otelixizumab also target the T cell (**Figure 1B**). Both antibodies improved c-peptide temporarily in a subgroup of patients with better baseline glycemic control, but not in the overall study population (91-93). Furthermore, in a preventative study, a two-week course of teplizumab was sufficient to delay the onset of T1D in high-risk individuals by two years (94). T-cell activation could also be blocked by preventing co-stimulation with the CTLA-4-Ig abatacept (**Figure 1B**). Abatacept delayed c-peptide decline in recent-onset T1D by approximately 10 months, but sustained treatment could not prevent subsequent loss in c-peptide. The authors concluded that T cell activation might be less prominent over time, as six months after start of abatacept the rate of decline was similar in the treatment group as control (95). Similarly, rituximab, an anti-CD20 antibody targeting B cells (**Figure 1B**), delayed c-peptide decline in a small subset of patients but was unable to result in sustained remission (96, 97). Treatment with alefacept, a drug that inhibits activated T-cells, resulted in sustained preservation of c-peptide secretion up to 15 months after

cessation of therapy (98, 99). Other therapies, such as the TNF-α inhibitor etanercept and Bacillus Calmette-Guerin (BCG) vaccination, have shown improvements in c-peptide levels at least in some subjects (100, 101), whereas anakinra, an IL-1 receptor agonist, and intravenous immunoglobulin (IVIG) did not (102, 103).

Together, these trials emphasize the notion of heterogeneity between T1D patients in terms of response to treatment, as only subgroups of patients responded to many of these targeted mono therapies. Nonetheless, all patients could conceivably be subject to side effects posed by these drugs, as most of them cause nonspecific immune suppression. The abatacept trial illustrated that the optimal time to interfere might be earlier in the disease process and this could be dependent upon the intervention used. Thus, it is crucial to identify the right patient population that would benefit from the treatment as well as the right timing and length of intervention for each drug regimen separately. A way to possibly circumvent these problems is to target several pathways at once, so that more patients will experience efficacy for a longer period.

Combination immunotherapies

After the somewhat disheartening results from monotherapy trials, a change of tack was needed. The facts were obvious: T1D is a complex, multi-system disease that is heterogenous between patients. The belief to cure or counter this disease with a monotherapy in all patients was perhaps wishful thinking. Nonetheless, subgroup effectivity of monotherapies should not be disregarded, but combining therapies that target multiple pathways may broaden the scope of effectivity to more patients and may empirically reduce dosing and side effects (104).

Unfortunately, the first combination trials were unsuccessful and even resulted in increased c-peptide decline in the case of rapamycin and interleukin-2 (IL-2) or adverse events in the case of mycophenolate mofetil (MMF) with daclizumab (DZB) (105, 106). Although low-dose anti-thymocyte globulin (ATG) reduced c-peptide decline and improved HbA1c, the combination of ATG with granulocyte colony-stimulating factor (GCSF) did not reduce c-peptide decline compared to placebo after 2-year follow-up (107, 108). A more drastic approach relied on a modified autologous hematopoietic stem cell transplantation using GCSF and cyclophosphamide to mobilize cells and cyclophosphamide and ATG to ablate the immune system (**Figure 1B**). This method had the unprecedented result of achieving insulin independence in the majority of patients after more than 2 years follow-up with even longer lasting insulin independence in a subgroup with low autoimmunity at baseline (109, 110).

Theoretically, combination therapies seem sensible in the context of T1D, but there is much to learn. These trials emphasize, once again, that the timing, patient population,

and the specific combination of therapies matter. What the magical combination of therapies would be is still unclear, but combining antigen nonspecific drugs that attack a similar pathway warrants increased side effects. Indeed, the future might be in combining immunomodulatory drugs with antigen-specific drugs.

Antigen-specific immunotherapies

Antigen-specific immunotherapies could be one of the most promising strategies to treat T1D, as this disease is characterized by a very specific attack on β cells by an autoimmune insult targeted at their autoantigens (**Figure 1B**) (111). In general, antigen-specific therapies aim to induce an immune response to specific antigens, instead of suppressing immunity as a whole and in the latter case, risking infections and impaired cancer surveillance. In immune activating therapies, the antigen is conventionally given with an adjuvant, which could either be a cell (discussed in the next paragraph) or another type of immune activator or engager (112). Adjuvant optimization is key to the success of any antigen-specific therapy and could determine whether the therapy is immune activating or inducing tolerance to the antigen, as is desired in T1D. Trials with oral insulin in this regard showed beneficial immune modulation in a subset of at-risk individuals, although no overall effect was seen (113-115). Dosing and the choice of antigen could be improved (111). Indeed, c-peptide levels were maintained after therapy with the more immunogenic proinsulin peptide and an IL-10-driven antigen-specific response was noted (116). Other antigen-specific therapies were also found to be safe and conferred beneficial effects to at least a subgroup of patients (117-120). A new avenue was opened when antigen-specific therapies were combined with immunomodulatory therapies. For example, the combination of intralymphatic glutamic acid decarboxylase (GAD)-alum and vitamin D showed promising results with a decrease in HbA1c and maintained c-peptide levels in a small pilot study, but it lacked a control group (121). Several other trials are now being conducted with different drug additions to GAD-alum, such as etanercept and GABA (clinicaltrials.gov; NCT02002130; NCT02464033). Finally, the risk of inadvertent immune activation with antigen therapy should be acknowledged and this risk, together with efficacy, could be improved with adjuvant optimization by, for instance, optimizing cellular therapies that could carry the antigen.

Cellular immunotherapies

Cellular therapies have the promise of reinstating equilibrium in a more natural way than a specific targeted drug, as cells have a broad array of functions and feedback mechanisms. Indeed, cells secrete multiple factors instead of just modulating one factor by for instance blocking it with a monoclonal antibody. Often cells and their functions are plastic, which accounts for their strength as they adjust to their environment, but it comes with a caveat of the possibility of an ‘‘unstable’’ drug (122). In general, cellular therapies

can either consist of unaltered cells to repopulate a cell population that was found decreased in a disease or of cells that are altered in a way to make them more fit to combat the disease. The added advantage of using autologous cells is that there is no risk of rejection (123). Examples of the latter category are T regulatory cells (124, 125), tolerogenic dendritic cells and activated mesenchymal stromal cells.

Tolerogenic dendritic cells

Dendritic cells (DCs) are crucial to directing an adaptive immune response. Their antigen presenting capacity is mostly known to induce a pro-inflammatory immune response against non-self-antigens. In the thymus, however, DCs can also induce tolerance against self-antigens. Autoimmune disease in this respect seems to be due – at least in part – to DC mediated self-antigen presentation in an immune activating setting (126). As mentioned previously, dendritic cells of T1D patients indeed had an abnormal activation status, compared to healthy individuals (69, 71, 127). Thus, converting autologous DCs into tolerogenic cells (tolDCs) would be an attractive way to engage the immune system with a peptide therapy (**Figure 1B**). The first phase I clinical trial with autologous tolDCs made ex vivo was deemed safe, although this was without peptide added (128). TolDCs can be produced by multiple methods, including pharmacologically by for instance dexamethasone and VD3 treatment, or by increasing immunomodulatory molecules such as IL-10 or downregulating co-stimulatory molecules via gene therapy (129). VD3 is particularly poised to reinstate the balance in the immune system, as it is a known immune modulator and found to be deficient in T1D patients (130-132). Furthermore, VD3 is advantageous as it has been used as a dietary supplement for decades and safety was secured in T1D trials, which concluded that VD3 supplementation in early childhood may reduce the risk of developing T1D later in life (133, 134). VD3 has synergistic effects with dexamethasone, which is widely used in the clinic as an immunosuppressant and blocks DC maturation (135). An area of concern of pharmacologically induced tolDCs is their stability, however, as tolDCs could potentially convert to a pro-inflammatory phenotype and this should be addressed to safeguard its translation into the clinic. Furthermore, it should be validated that autologous tolDCs from T1D patients are similar to tolDCs from healthy individuals.

Mesenchymal stromal cells

Mesenchymal stromal cells (MSCs) are of interest as they are believed to be inherently immunomodulatory (**Figure 1B**) (136). Furthermore, the fact that MSCs are already used in the clinic could expedite its translation for T1D treatment (137). MSCs secrete immunosuppressive factors such as indoleamine 2, 3-dioxygenase (IDO) and express immune inhibitory factors such as PD-L1 (138, 139). Upon activation with pro-inflammatory cytokines the immunosuppressive properties of MSCs are thought to be

enhanced (140). There is a fear, however, that this manipulation (with pro-inflammatory cytokines) could result in inadvertent activation of the immune system, as was similarly feared for tolDC therapy (141). Besides this, MSC therapy is not antigen-specific. In conclusion, it is important to investigate the effect of pro-inflammatory cytokines on MSCs' immunosuppressive phenotype and examine the potential of MSCs to become antigen-specific.

2.3. Beta cell therapies

As argued before, immunotherapy may not suffice to cure T1D, as β cells appear actively involved in their own demise. The realm of β cell therapies has mainly consisted of efforts towards β cell replacement and to a lesser extent toward β cell recovery.

Beta cell replacement

The first attempt to replace β cells in T1D patients was successfully achieved by the advent of islet transplantation in the 1980's (**Figure 1B**) (142). Although this remains an important therapy for rare patients suffering from hypo-unawareness and uncontrolled blood glucose levels, the scarcity of islet donors and the immune suppression needed to prevent graft rejection halt its wide application in T1D (143). In addition, the viability and successful engraftment of islets are of concern and often times multiple islet infusions are needed to achieve insulin independence (144-147). In this sense, β cell recovery strategies could in addition be used to improve islet transplant viability and function. MSCs are a good example of this, as they improved β cell function in T1D patients by themselves and could be used in combination with islet transplantation as well (148, 149). Other strategies to replace β cells consist of producing β cells from other types of cells, such as stem cells, and are reviewed elsewhere (142).

Beta cell recovery

The field of β cell recovery therapies is still in its infancy. Although extrapolation from T2D therapies should be possible, currently there are no FDA-approved drugs for T1D therapy that specifically target the β cell. In fact, a systematic review of T1D clinical trials identified 2090 registered trials in 2018, of which 212 were investigational drugs and only 30% of these 212 trials focused mechanistically on the β cell (150). This suggests that there is a sea of opportunity for innovations regarding β cell recovery and survival. The glucagon-like peptide 1 (GLP-1) signaling pathway is by far most researched with 72% of clinical trials in β cell recovery dedicated to it (150). Most drugs targeting the GLP-1 pathway are analogues of GLP-1, such as liraglutide, and have been used in T2D management for more than a decade. In T1D, liraglutide has shown promising clinical results as well (**Figure 1B**) (151-154). Mechanistically, GLP-1 analogues could work by promoting β cell proliferation (155, 156) and glucose stimulated insulin secretion by the β cell (157).

3. Aims and outline of thesis

Drawing from the analysis of recent and new immune modulating and β cell therapies, my thesis aims to decipher promising treatment paradigms for T1D. Chapter two and three describe two studies in which T1D was successfully reversed. The first study involves a drastic reset of the immune system by autologous hematopoietic stem cell transplantation, whereas the second study is a case report of successful reversal of T1D in the setting of IVIG treatment. As these treatment strategies are associated with morbidity or only incidental success, respectively, other therapies that aim to reinstate a subtler immune balance are discussed in chapter four and five. Therein, the possibility of using tolerogenic dendritic cells or activated mesenchymal stromal cells as antigen-specific immunomodulation in T1D is discussed. Chapter six engages the islets of Langerhans as targets for therapy. In this chapter, MSCs show additional beneficial potency to improve the islet microenvironment. Chapter seven summarizes these different strategies and puts these in perspective, while their significance to future T1D therapies is discussed.

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