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From immune suppression to immune modulation in type 1 diabetes patients

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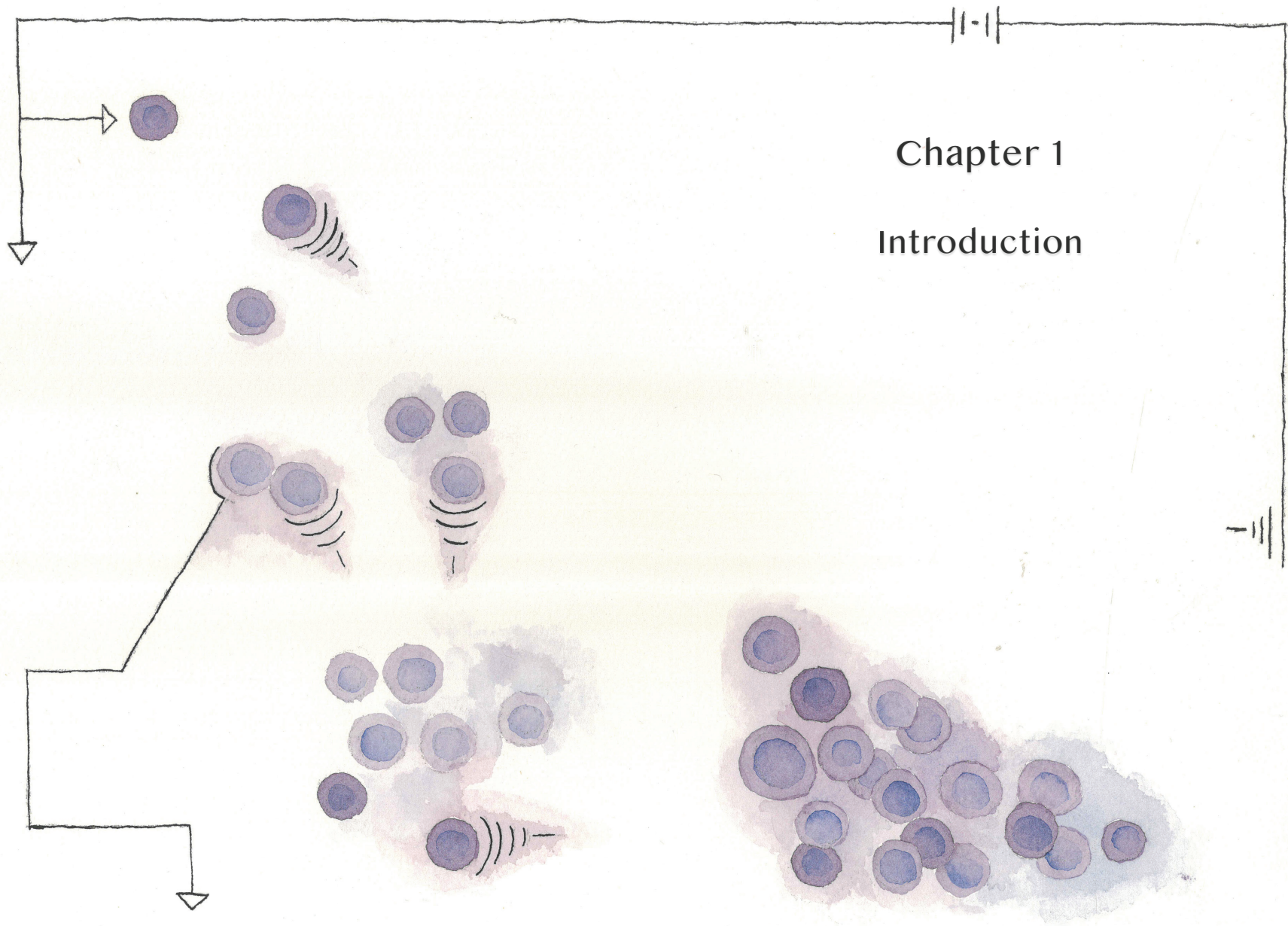
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Chapter 1

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 hemoglobin (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

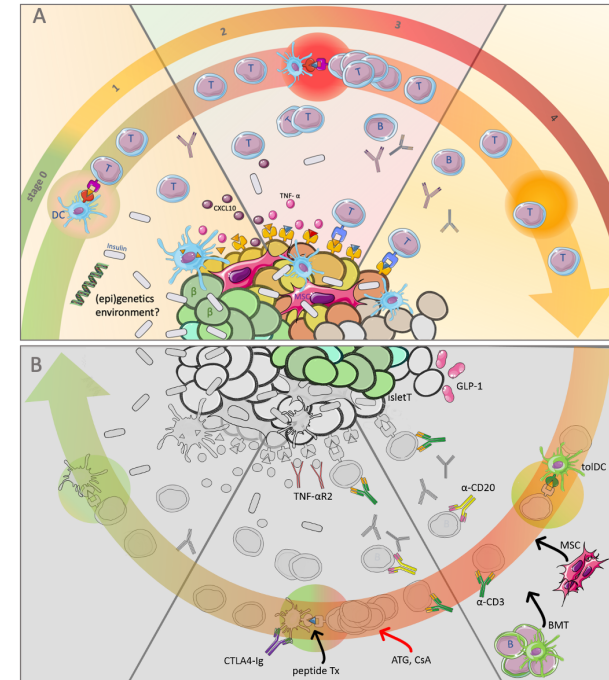


Figure 1: Natural History and Therapeutic Strategies in Type 1 Diabetes. (A) The natural history and stages of Type 1 Diabetes. It is yet unclear which environmental triggers cause the onset of islet autoreactivity in genetically susceptible T1D patients. This onset is characterized by beta cell-antigen 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 insulinitis, 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 glucagon-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- κ B 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 oteplizumab 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 (G-CSF) 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 G-CSF 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.

References

- Atkinson, M.A., G.S. Eisenbarth, and A.W. Michels, Type 1 diabetes. *The Lancet*, 2014. 383(9911): p. 69-82.
- Association, A.D. Diagnosis and classification of diabetes mellitus. *Diabetes care*, 2013. 36(Supplement 1): p. S67-S74.
- Insel, R.A., et al. Staging Presymptomatic Type 1 Diabetes: A Scientific Statement of JDRF, the Endocrine Society, and the American Diabetes Association. *Diabetes Care*, 2015. 38(10): p. 1964-1974.
- Thomas, N.J., et al. Frequency and phenotype of type 1 diabetes in the first six decades of life: a cross-sectional, genetically stratified survival analysis from UK Biobank. *The Lancet Diabetes & endocrinology*, 2018. 6(2): p. 122-129.
- Shields, B.M., et al. C-peptide decline in type 1 diabetes has two phases: an initial exponential fall and a subsequent stable phase. *Diabetes care*, 2018. 41(7): p. 1486-1492.
- Joensen, L.E., T.P. Almdal, and I. Willaing. Associations between patient characteristics, social relations, diabetes management, quality of life, glycaemic control and emotional burden in type 1 diabetes. *Primary care diabetes*, 2016. 10(1): p. 41-50.
- Berlin, K.S., E.M. Rabideau, and A.A. Hains. Empirically derived patterns of perceived stress among youth with type 1 diabetes and relationships to metabolic control. *Journal of pediatric psychology*, 2012. 37(9): p. 990-998.
- Strandberg, R.B., et al. Longitudinal relationship between diabetes-specific emotional distress and follow-up HbA1c in adults with Type 1 diabetes mellitus. *Diabetic Medicine*, 2015. 32(10): p. 1304-1310.
- Semenkovich, K., et al. Academic abilities and glycaemic control in children and young people with Type 1 diabetes mellitus. *Diabetic Medicine*, 2016. 33(5): p. 668-673.
- Lin, A., et al. Risk Factors for Decline in IQ in Youth With Type 1 Diabetes Over the 12 Years From Diagnosis/Illness Onset. *Diabetes Care*, 2015. 38(2): p. 236-242.
- Control, D. and C. Trial. Intensive diabetes treatment and cardiovascular outcomes in type 1 diabetes: the DCCT/EDIC study 30-year follow-up. *Diabetes care*, 2016. 39(5): p. 686-693.
- Control, D., et al. Effect of intensive diabetes therapy on the progression of diabetic retinopathy in patients with type 1 diabetes: 18 years of follow-up in the DCCT/EDIC. *Diabetes*, 2015. 64(2): p. 631-642.
- Mayer-Davis, E.J., et al. Incidence trends of type 1 and type 2 diabetes among youths, 2002–2012. *New England Journal of Medicine*, 2017. 376(15): p. 1419-1429.
- Diaz-Valencia, P.A., P. Bougnères, and A.-J. Valleron. Global epidemiology of type 1 diabetes in young adults and adults: a systematic review. *BMC public health*, 2015. 15(1): p. 255.
- Harjutsalo, V., L. Sjöberg, and J. Tuomilehto. Time trends in the incidence of type 1 diabetes in Finnish children: a cohort study. *The Lancet*, 2008. 371(9626): p. 1777-1782.
- Moltchanova, E.V., et al. Seasonal variation of diagnosis of Type 1 diabetes mellitus in children worldwide. *Diabetic Medicine*, 2009. 26(7): p. 673-678.
- Karvonen, M., et al. Incidence of childhood type 1 diabetes worldwide. *Diabetes Mondiale (DiaMond) Project Group. Diabetes Care*, 2000. 23(10): p. 1516-26.
- Pani, M.A., et al. Vitamin D receptor allele combinations influence genetic susceptibility to type 1 diabetes in Germans. *Diabetes*, 2000. 49(3): p. 504-507.
- Bailey, R., et al. Association of the vitamin D metabolism gene CYP27B1 with type 1 diabetes. *Diabetes*, 2007. 56(10): p. 2616-2621.
- Mohr, S.B., et al. The association between ultraviolet B irradiance, vitamin D status and incidence rates of type 1 diabetes in 51 regions worldwide. *Diabetologia*, 2008. 51(8): p. 1391-1398.
- Pociot, F. and Å. Lernmark. Genetic risk factors for type 1 diabetes. *The Lancet*, 2016. 387(10035): p. 2331-2339.
- Roche, E.F., et al. Clinical presentation of type 1 diabetes. *Pediatric diabetes*, 2005. 6(2): p. 75-78.
- Parkkola, A., et al. Extended Family History of Type 1 Diabetes and Phenotype and Genotype of Newly Diagnosed Children. *Diabetes Care*, 2013. 36(2): p. 348-354.
- Erich, H., et al. HLA DR-DQ Haplotypes and Genotypes and Type 1 Diabetes Risk. Analysis of the Type 1 Diabetes Genetics Consortium Families. 2008. 57(4): p. 1084-1092.
- Barrett, J.C., et al. Genome-wide association study and meta-analysis find that over 40 loci affect risk of type 1 diabetes. *Nature Genetics*, 2009. 41: p. 703.
- Redondo, M.J., et al. Concordance for Islet Autoimmunity among Monozygotic Twins. *New England Journal of Medicine*, 2008. 359(26): p. 2849-2850.
- Jerram, S.T., M.N. Dang, and R.D. Leslie. The role of epigenetics in type 1 diabetes. *Current diabetes reports*, 2017. 17(10): p. 89.
- Rewers, M. and J. Ludvigsson. Environmental risk factors for type 1 diabetes. *Lancet (London, England)*, 2016. 387(10035): p. 2340-2348.
- Paul, D.S., et al. Increased DNA methylation variability in type 1 diabetes across three immune effector cell types. *Nature Communications*, 2016. 7: p. 13555.

30. Miao, F., et al., Profiles of epigenetic histone post-translational modifications at type 1 diabetes susceptible genes. *Journal of Biological Chemistry*, 2012. 287(20): p. 16335-16345.
31. Elboudwarej, E., et al., Hypomethylation within gene promoter regions and type 1 diabetes in discordant monozygotic twins. *Journal of autoimmunity*, 2016. 68: p. 23-29.
32. Mohn, F. and D. Schübeler, Genetics and epigenetics: stability and plasticity during cellular differentiation. *Trends in Genetics*, 2009. 25(3): p. 129-136.
33. Ostreich, K.J. and A.S. Weinmann, Encoding stability versus flexibility: lessons learned from examining epigenetics in T helper cell differentiation, in *Epigenetic Regulation of Lymphocyte Development*. 2011, Springer: p. 145-164.
34. Willcox, A., et al., Analysis of islet inflammation in human type 1 diabetes. *Clinical & Experimental Immunology*, 2009. 155(2): p. 173-181.
35. Coppieters, K.T., et al., Demonstration of islet-autoreactive CD8 T cells in insulinitis lesions from recent onset and long-term type 1 diabetes patients. *The Journal of Experimental Medicine*, 2012. 209(1): p. 51-60.
36. Babon, J.A.B., et al., Analysis of self-antigen specificity of islet-infiltrating T cells from human donors with type 1 diabetes. *Nature Medicine*, 2016. 22: p. 1482.
37. Michels, A.W., et al., Islet-Derived CD4 T Cells Targeting Proinsulin in Human Autoimmune Diabetes. *Diabetes*, 2017. 66(3): p. 722-734.
38. Pinkse, G.G.M., et al., Autoreactive CD8 T cells associated with β cell destruction in type 1 diabetes. *Proceedings of the National Academy of Sciences of the United States of America*, 2005. 102(51): p. 18425-18430.
39. Allen, J.S., et al., 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*, 2009. 58(1): p. 138-145.
40. Hull, C.M., M. Peakman, and T.J.M. Tree, Regulatory T cell dysfunction in type 1 diabetes: what's broken and how can we fix it? *Diabetologia*, 2017. 60(10): p. 1839-1850.
41. Lindley, S., et al., Defective Suppressor Function in CD4⁺CD25⁺ T-Cells From Patients With Type 1 Diabetes. *Diabetes*, 2005. 54(1): p. 92-99.
42. Schneider, A., et al., The effector T cells of diabetic subjects are resistant to regulation via CD4+ FOXP3+ regulatory T cells. *The Journal of Immunology*, 2008. 181(10): p. 7350-7355.
43. Foulis, A.K. and J.A. Stewart, The pancreas in recent-onset Type 1 (insulin-dependent) diabetes mellitus: insulin content of islets, insulinitis and associated changes in the exocrine acinar tissue. *Diabetologia*, 1984. 26(6): p. 456-461.
44. Campbell-Thompson, M., et al., Insulinitis and β -Cell Mass in the Natural History of Type 1 Diabetes. *Diabetes*, 2016. 65(3): p. 719-731.
45. van Lummel, M., A. Zaldumbide, and B.O. Roep, Changing faces, unmasking the beta-cell: post-translational modification of antigens in type 1 diabetes. *Current Opinion in Endocrinology, Diabetes and Obesity*, 2013. 20(4): p. 299-306.
46. Dooley, J., et al., Genetic predisposition for beta cell fragility underlies type 1 and type 2 diabetes. *Nature Genetics*, 2016. 48: p. 519.
47. Bottazzo, G.F., Death of a Beta Cell: Homicide or Suicide? *Diabetic Medicine*, 1986. 3(2): p. 119-130.
48. Frigerio, S., et al., β cells are responsible for CXCR3-mediated T-cell infiltration in insulinitis. *Nature medicine*, 2002. 8(12): p. 1414.
49. Roep, B., et al., Islet inflammation and CXCL10 in recent-onset type 1 diabetes. *Clinical & Experimental Immunology*, 2010. 159(3): p. 338-343.
50. Richardson, S.J., et al., Islet cell hyperexpression of HLA class I antigens: a defining feature in type 1 diabetes. *Diabetologia*, 2016. 59(11): p. 2448-2458.
51. Roep, B.O., et al., A roadmap of the generation of neoantigens as targets of the immune system in type 1 diabetes. *Current opinion in immunology*, 2016. 43: p. 67-73.
52. Kracht, M.J., A. Zaldumbide, and B.O. Roep, Neoantigens and microenvironment in type 1 diabetes: lessons from antitumor immunity. *Trends in Endocrinology & Metabolism*, 2016. 27(6): p. 353-362.
53. Marré, M.L., E.A. James, and J.D. Piganelli, β cell ER stress and the implications for immunogenicity in type 1 diabetes. *Frontiers in cell and developmental biology*, 2015. 3: p. 67.
54. McLaughlin, R.J., et al., Human islets and dendritic cells generate post-translationally modified islet autoantigens. *Clinical & Experimental Immunology*, 2016. 185(2): p. 133-140.
55. Kracht, M.J.L., et al., Autoimmunity against a defective ribosomal insulin gene product in type 1 diabetes. *Nature Medicine*, 2017. 23(4): p. 501-507.
56. Robertson, R.P., et al., Glucose Toxicity in β -Cells: Type 2 Diabetes, Good Radicals Gone Bad, and the Glutathione Connection. *Diabetes*, 2003. 52(3): p. 581-587.
57. Brozzi, F., et al., Cytokines induce endoplasmic reticulum stress in human, rat and mouse beta cells via different mechanisms. *Diabetologia*, 2015. 58(10): p. 2307-2316.
58. Keenan, H.A., et al., Residual insulin production and pancreatic β -cell turnover after 50 years of diabetes: Joslin Medalist Study. *Diabetes*, 2010. 59(11): p. 2846-2853.
59. Hara, M., et al., Resting beta-cells—A functional reserve? *Diabetes & metabolism*, 2016. 42(3): p. 157-161.
60. Carloti, F., et al., Isolated human islets contain a distinct population of mesenchymal stem cells. *Islets*, 2010. 2(3): p. 164-173.
61. Bogdani, M., et al., Extracellular matrix components in the pathogenesis of type 1 diabetes. *Current diabetes reports*, 2014. 14(12): p. 552.
62. Rabinovitch, A., T. Russell, and D.H. Mintz, Factors from fibroblasts promote pancreatic islet β cell survival in tissue culture. *Diabetes*, 1979. 28(12): p. 1108-1113.
63. Ohnishi, S., et al., Effect of Hypoxia on Gene Expression of Bone Marrow-Derived Mesenchymal Stem Cells and Mononuclear Cells. *STEM CELLS*, 2007. 25(5): p. 1166-1177.
64. Anzalone, R., et al., Wharton's Jelly Mesenchymal Stem Cells as Candidates for Beta Cells Regeneration: Extending the Differentiative and Immunomodulatory Benefits of Adult Mesenchymal Stem Cells for the Treatment of Type 1 Diabetes. *Stem Cell Reviews and Reports*, 2011. 7(2): p. 342-363.
65. Alvarez-Perez, J.C., et al., Hepatocyte Growth Factor/c-Met Signaling Is Required for β -Cell Regeneration. *Diabetes*, 2014. 63(1): p. 216-223.
66. Ma, S., et al., Immunobiology of mesenchymal stem cells. *Cell Death & Differentiation*, 2014. 21(2): p. 216-225.
67. Price, J.D. and K.V. Tarbell, The Role of Dendritic Cell Subsets and Innate Immunity in the Pathogenesis of Type 1 Diabetes and Other Autoimmune Diseases. *Frontiers in Immunology*, 2015. 6(288).
68. Jansen, A., M. van Hagen, and H.A. Drexhage, Defective maturation and function of antigen-presenting cells in type 1 diabetes. *The Lancet*, 1995. 345(8948): p. 491-492.
69. Mollah, Z.U., et al., Abnormal NF- κ B function characterizes human type 1 diabetes dendritic cells and monocytes. *The Journal of Immunology*, 2008. 180(5): p. 3166-3175.
70. Irvine, K.M., et al., Peripheral blood monocyte gene expression profile clinically stratifies patients with recent-onset type 1 diabetes. *Diabetes*, 2012. 61(5): p. 1281-1290.
71. Nieminen, J.K., et al., Altered phenotype of peripheral blood dendritic cells in pediatric type 1 diabetes. *Diabetes care*, 2012. 35(11): p. 2303-2310.
72. Oras, A., et al., A study of 51 subtypes of peripheral blood immune cells in newly diagnosed young type 1 diabetes patients. *Clinical & Experimental Immunology*, 2019.
73. Chen, X., et al., Type 1 diabetes patients have significantly lower frequency of plasmacytoid dendritic cells in the peripheral blood. *Clinical immunology*, 2008. 129(3): p. 413-418.
74. Leete, P., et al., Differential insulinitis profiles determine the extent of β -cell destruction and the age at onset of type 1 diabetes. *Diabetes*, 2016. 65(5): p. 1362-1369.
75. Bloem, S.J. and B.O. Roep, The elusive role of B lymphocytes and islet autoantibodies in (human) type 1 diabetes. *Diabetologia*, 2017. 60(7): p. 1185-1189.
76. Martin, S., et al., Development of type 1 diabetes despite severe hereditary B-cell deficiency. *New England Journal of Medicine*, 2001. 345(14): p. 1036-1040.
77. Demeester, S., et al., Preexisting insulin autoantibodies predict efficacy of otezlizumab in preserving residual β -cell function in recent-onset type 1 diabetes. *Diabetes care*, 2015. 38(4): p. 644-651.
78. Triolo, T.M., et al., Identical and Nonidentical Twins: Risk and Factors Involved in Development of Islet Autoimmunity and Type 1 Diabetes. *Diabetes Care*, 2019. 42(2): p. 192-199.
79. Harrison, L., et al., Inverse relation between humoral and cellular immunity to glutamic acid decarboxylase in subjects at risk of insulin-dependent diabetes. *The Lancet*, 1993. 341(8857): p. 1365-1369.
80. Roep, B.O., et al., HLA-associated inverse correlation between T cell and antibody responsiveness to islet autoantigen in recent-onset insulin-dependent diabetes mellitus. *Eur J Immunol*, 1996. 26(6): p. 1285-9.
81. Weenink, S., et al., Autoantibodies and associated T-cell responses to determinants within the 831–860 region of the autoantigen IA-2 in Type 1 diabetes. *Journal of autoimmunity*, 2009. 33(2): p. 147-154.
82. Giorda, C.B., et al., Incidence and risk factors for severe and symptomatic hypoglycemia in type 1 diabetes. Results of the HYPOS-1 study. *Acta Diabetologica*, 2015. 52(5): p. 845-853.
83. Nathan, D.M. and D.E.R. Group, The diabetes control and complications trial/epidemiology of diabetes interventions and complications study at 30 years: overview. *Diabetes care*, 2014. 37(1): p. 9-16.
84. Miller, K.M., et al., Current State of Type 1 Diabetes Treatment in the U.S.: Updated Data From the T1D Exchange Clinic Registry. *Diabetes Care*, 2015. 38(6): p. 971-978.
85. 6. Glycemic Targets: Standards of Medical Care in Diabetes—2018. *Diabetes Care*, 2018. 41(Supplement 1): p. S55-S64.
86. Feutren, G., et al., Cyclosporin increases the rate and length of remissions in insulin-dependent diabetes of recent onset: results of a multicentre double-blind trial. *The Lancet*, 1986. 328(8499): p. 119-124.
87. Group, C.-E.R.C.T., Cyclosporin-induced remission of IDDM after early intervention: association of 1 yr of cyclosporin treatment with enhanced insulin secretion. *Diabetes*, 1988. 37(11): p. 1574-1582.
88. Myers, B.D., et al., Cyclosporine-associated chronic nephropathy. *New England Journal of Medicine*, 1984. 311(11): p. 699-705.

89. Feutren, G. and M.J. Mihatsch, Risk factors for cyclosporine-induced nephropathy in patients with autoimmune diseases. *New England Journal of Medicine*, 1992. 326(25): p. 1654-1660.
90. Drachenberg, C.B., et al., Islet Cell Damage Associated with Tacrolimus and Cyclosporine: Morphological Features in Pancreas allograft biopsies and clinical Correlation. *Transplantation*, 1999. 68(3): p. 396-402.
91. Keymeulen, B., et al., Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes. *New England Journal of Medicine*, 2005. 352(25): p. 2598-2608.
92. Sherry, N., et al., Teplizumab for treatment of type 1 diabetes (Protégé study): 1-year results from a randomised, placebo-controlled trial. *The Lancet*, 2011. 378(9790): p. 487-497.
93. Hagopian, W., et al., Teplizumab preserves C-peptide in recent-onset type 1 diabetes: two-year results from the randomised, placebo-controlled Protégé trial. *Diabetes*, 2013. 62(11): p. 3901-3908.
94. Herold, K.C., et al., An Anti-CD3 Antibody, Teplizumab, in Relatives at Risk for Type 1 Diabetes. *New England Journal of Medicine*, 0(0): p. null.
95. Orban, T., et al., Co-stimulation modulation with abatacept in patients with recent-onset type 1 diabetes: a randomised, double-blind, placebo-controlled trial. *The Lancet*, 2011. 378(9789): p. 412-419.
96. Pescovitz, M.D., et al., Rituximab, B-lymphocyte depletion, and preservation of beta-cell function. *New England Journal of Medicine*, 2009. 361(22): p. 2143-2152.
97. Pescovitz, M.D., et al., B-lymphocyte depletion with rituximab and β -cell function: two-year results. *Diabetes care*, 2014. 37(2): p. 453-459.
98. Rigby, M.R., et al., 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*, 2013. 1(4): p. 284-294.
99. Rigby, M.R., et al., Alefacept provides sustained clinical and immunological effects in new-onset type 1 diabetes patients. *J Clin Invest*, 2015. 125(8): p. 3285-96.
100. Mastrandrea, L., et al., Etanercept Treatment in Children With New-Onset Type 1 Diabetes. Pilot randomized, placebo-controlled, double-blind study, 2009. 32(7): p. 1244-1249.
101. Faustman, D.L., et al., Proof-of-concept, randomized, controlled clinical trial of Bacillus-Calmette-Guerin for treatment of long-term type 1 diabetes. *PLoS one*, 2012. 7(8): p. e41756-e41756.
102. Colagiuri, S., et al., Intravenous immunoglobulin therapy for autoimmune diabetes mellitus. *Clin Exp Rheumatol*, 1996. 14 Suppl 15: p. S93-7.
103. Moran, A., et al., Interleukin-1 antagonism in type 1 diabetes of recent onset: two multicentre, randomised, double-blind, placebo-controlled trials. *Lancet*, 2013. 381(9881): p. 1905-15.
104. Bayat Mokhtari, R., et al., Combination therapy in combating cancer. *Oncotarget*, 2017. 8(23): p. 38022-38043.
105. Gottlieb, P.A., et al., Failure to preserve β -cell function with mycophenolate mofetil and daclizumab combined therapy in patients with new-onset type 1 diabetes. *Diabetes care*, 2010. 33(4): p. 826-832.
106. Long, S.A., et al., Rapamycin/L-2 combination therapy in patients with type 1 diabetes augments Tregs yet transiently impairs β -cell function. *Diabetes*, 2012. 61(9): p. 2340-2348.
107. Haller, M.J., et al., Low-Dose Anti-Thymocyte Globulin (ATG) Preserves β -Cell Function and Improves HbA_{1c} in New-Onset Type 1 Diabetes. *Diabetes Care*, 2018. 41(9): p. 1917-1925.
108. Haller, M.J., et al., Low-dose Anti-Thymocyte Globulin Preserves C-Peptide and Reduces A1c in New Onset Type 1 Diabetes: Two Year Clinical Trial Data. *Diabetes*, 2019: p. db190057.
109. Couri, C.B., et al., C-peptide levels and insulin independence following autologous nonmyeloablative hematopoietic stem cell transplantation in newly diagnosed type 1 diabetes mellitus. *JAMA*, 2009. 301(15): p. 1573-1579.
110. Malmegrim, K.C.R., et al., Immunological Balance Is Associated with Clinical Outcome after Autologous Hematopoietic Stem Cell Transplantation in Type 1 Diabetes. *Frontiers in Immunology*, 2017. 8(167).
111. Roep, B.O., D.C. Wheeler, and M. Peakman, Antigen-based immune modulation therapy for type 1 diabetes: the era of precision medicine. *The Lancet Diabetes & Endocrinology*, 2018.
112. Smith, E.L. and M. Peakman, Peptide Immunotherapy for Type 1 Diabetes—Clinical Advances. *Frontiers in Immunology*, 2018. 9: p. 392.
113. Association, A.D., Effects of oral insulin in relatives of patients with type 1 diabetes: the Diabetes Prevention Trial—Type 1. *Diabetes care*, 2005. 28(5): p. 1068-1076.
114. Vehik, K., et al., Long-term outcome of individuals treated with oral insulin: Diabetes Prevention Trial—Type 1 (DPT-1) oral insulin trial. *Diabetes care*, 2011. 34(7): p. 1585-1590.
115. Krischer, J.P., et al., Effect of oral insulin on prevention of diabetes in relatives of patients with type 1 diabetes: a randomized clinical trial. *Jama*, 2017. 318(19): p. 1891-1902.
116. Ali, M.A., et al., Metabolic and immune effects of immunotherapy with proinsulin peptide in human new-onset type 1 diabetes. *Science translational medicine*, 2017. 9(402): p. eaaf7779.
117. Agardh, C.-D., et al., GAD65 vaccination: 5 years of follow-up in a randomised dose-escalating study in adult-onset autoimmune diabetes. *Diabetologia*, 2009. 52(7): p. 1363-1368.
118. Ludvigsson, J., et al., GAD65 antigen therapy in recently diagnosed type 1 diabetes mellitus. *New England Journal of Medicine*, 2012. 366(5): p. 433-442.
119. Roep, B.O., et al., Plasmid-encoded proinsulin preserves C-peptide while specifically reducing proinsulin-specific CD8⁺ T cells in type 1 diabetes. *Science translational medicine*, 2013. 5(191): p. 191ra82-191ra82.
120. Beam, C.A., et al., GAD vaccine reduces insulin loss in recently diagnosed type 1 diabetes: findings from a Bayesian meta-analysis. *Diabetologia*, 2017. 60(1): p. 43-49.
121. Ludvigsson, J., J. Wahlberg, and R. Casas, Intralymphatic Injection of Autoantigen in Type 1 Diabetes. *New England Journal of Medicine*, 2017. 376(7): p. 697-699.
122. Barcala Tabarrozzi, A.E., et al., Cell-based interventions to halt autoimmunity in type 1 diabetes mellitus. *Clinical & Experimental Immunology*, 2013. 171(2): p. 135-146.
123. Gage, F.H., Cell therapy. *NATURE-LONDON*, 1998: p. 18-24.
124. Marek-Trzonowska, N., et al., Therapy of type 1 diabetes with CD4⁺ CD25^{high} CD127[−] regulatory T cells prolongs survival of pancreatic islets—results of one year follow-up. *Clinical immunology*, 2014. 153(1): p. 23-30.
125. Bluestone, J.A., et al., Type 1 diabetes immunotherapy using polyclonal regulatory T cells. *Science Translational Medicine*, 2015. 7(315): p. 315ra189-315ra189.
126. Audiger, C., et al., The importance of dendritic cells in maintaining immune tolerance. *The Journal of Immunology*, 2017. 198(6): p. 2223-2231.
127. Creusot, R.J., J. Postigo-Fernandez, and N. Teteloshvili, Altered function of antigen-presenting cells in type 1 diabetes: a challenge for antigen-specific immunotherapy? *Diabetes*, 2018. 67(8): p. 1481-1494.
128. Giannoukakis, N., et al., Phase I (safety) study of autologous tolerogenic dendritic cells in type 1 diabetic patients. *Diabetes care*, 2011. 34(9): p. 2026-2032.
129. Obregon, C., et al., Update on dendritic cell-induced immunological and clinical tolerance. *Frontiers in immunology*, 2017. 8: p. 1514.
130. Pozzilli, P., et al., Low levels of 25-hydroxyvitamin D3 and 1, 25-dihydroxyvitamin D3 in patients with newly diagnosed type 1 diabetes. *Hormone and Metabolic Research*, 2005. 37(11): p. 680-683.
131. Takiishi, T., et al., Vitamin D and Diabetes. *Rheumatic Disease Clinics*, 2012. 38(1): p. 179-206.
132. Takiishi, T., et al., Effects of vitamin D on antigen-specific and non-antigen-specific immune modulation: relevance for type 1 diabetes. *Pediatric diabetes*, 2013. 14(2): p. 81-89.
133. Hyppönen, E., et al., Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. *The Lancet*, 2001. 358(9292): p. 1500-1503.
134. Zipitis, C.S. and A.K. Akobeng, Vitamin D supplementation in early childhood and risk of type 1 diabetes: a systematic review and meta-analysis. *Archives of disease in childhood*, 2008. 93(6): p. 512-517.
135. Ferreira, G.B., et al., Differential protein pathways in 1, 25-dihydroxyvitamin D3 and dexamethasone modulated tolerogenic human dendritic cells. *Journal of proteome research*, 2011. 11(2): p. 941-971.
136. Nauta, A.J. and W.E. Fibbe, Immunomodulatory properties of mesenchymal stromal cells. *Blood*, 2007. 110(10): p. 3499-3506.
137. Wang, L.-T., et al., Human mesenchymal stem cells (MSCs) for treatment towards immune- and inflammation-mediated diseases: review of current clinical trials. *Journal of biomedical science*, 2016. 23(1): p. 76.
138. Augello, A., et al., Bone marrow mesenchymal progenitor cells inhibit lymphocyte proliferation by activation of the programmed death 1 pathway. *European journal of immunology*, 2005. 35(5): p. 1482-1490.
139. Ryan, J., et al., Interferon- γ does not break, but promotes the immunosuppressive capacity of adult human mesenchymal stem cells. *Clinical & Experimental Immunology*, 2007. 149(2): p. 353-363.
140. Ryan, J.M., et al., Interferon- γ does not break, but promotes the immunosuppressive capacity of adult human mesenchymal stem cells. *Clinical & Experimental Immunology*, 2007. 149(2): p. 353-363.
141. Sivanathan, K.N., et al., Interferon-gamma modification of mesenchymal stem cells: implications of autologous and allogeneic mesenchymal stem cell therapy in allotransplantation. *Stem Cell Reviews and Reports*, 2014. 10(3): p. 351-375.
142. Castro-Gutierrez, R., A.W. Michels, and H.A. Russ, β Cell replacement: improving on the design. *Current Opinion in Endocrinology, Diabetes and Obesity*, 2018. 25(4): p. 251-257.
143. Foster, E.D., et al., Improved Health-Related Quality of Life in a Phase 3 Islet Transplantation Trial in Type 1 Diabetes Complicated by Severe Hypoglycemia. *Diabetes Care*, 2018. 41(5): p. 1001-1008.
144. Gaber, A.O., et al., Improved in vivo pancreatic islet function after prolonged in vitro islet culture. *Transplantation*, 2001. 72(11): p. 1730-1736.
145. Kin, T., et al., Risk factors for islet loss during culture prior to transplantation. *Transplant International*, 2008. 21(11): p. 1029-1035.
146. Kanak, M.A., et al., Inflammatory response in islet transplantation. *International journal of endocrinology*, 2014. 2014.
147. Tatum, J.A., M.O. Meneveau, and K.L. Brayman, Single-donor islet transplantation in type 1 diabetes: patient selection and special considerations. *Diabetes, metabolic syndrome and obesity : targets and therapy*, 2017. 10: p. 73-78.

148. Figliuzzi, M., et al., Mesenchymal stem cells help pancreatic islet transplantation to control type 1 diabetes. *World journal of stem cells*, 2014. 6(2): p. 163-172.
149. Carlsson, P.-O., et al., Preserved β -Cell Function in Type 1 Diabetes by Mesenchymal Stromal Cells. *Diabetes*, 2015. 64(2): p. 587-592.
150. Fenske, R.J. and M.E. Kimple, Targeting dysfunctional beta-cell signaling for the potential treatment of type 1 diabetes mellitus. *Experimental Biology and Medicine*, 2018. 243(6): p. 586-591.
151. Ahrén, B., et al., Efficacy and Safety of Liraglutide Added to Capped Insulin Treatment in Subjects With Type 1 Diabetes: The ADJUNCT TWO Randomized Trial. *Diabetes Care*, 2016. 39(10): p. 1693-1701.
152. Kuhadiya, N.D., et al., Addition of liraglutide to insulin in patients with type 1 diabetes: a randomized placebo-controlled clinical trial of 12 weeks. *Diabetes care*, 2016. 39(6): p. 1027-1035.
153. Mathieu, C., et al., Efficacy and Safety of Liraglutide Added to Insulin Treatment in Type 1 Diabetes: The ADJUNCT ONE Treat-To-Target Randomized Trial. *Diabetes Care*, 2016. 39(10): p. 1702-1710.
154. Wang, W., et al., Effects of Insulin Plus Glucagon-Like Peptide-1 Receptor Agonists (GLP-1RAs) in Treating Type 1 Diabetes Mellitus: A Systematic Review and Meta-Analysis. *Diabetes Therapy*, 2017. 8(4): p. 727-738.
155. Xu, G., et al., Exendin-4 stimulates both beta-cell replication and neogenesis, resulting in increased beta-cell mass and improved glucose tolerance in diabetic rats. *Diabetes*, 1999. 48(12): p. 2270-2276.
156. Tourrel, C., et al., Persistent improvement of type 2 diabetes in the Goto-Kakizaki rat model by expansion of the β -cell mass during the prediabetic period with glucagon-like peptide-1 or exendin-4. *Diabetes*, 2002. 51(5): p. 1443-1452.
157. Chon, S. and J.-F. Gautier, An update on the effect of incretin-based therapies on β -cell function and mass. *Diabetes & metabolism journal*, 2016. 40(2): p. 99-114.