Immunotherapy for Type 1 diabetes: past and future

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Practice Points

- The incidence of Type 1 diabetes is increasing, mainly owing to the influence of unknown environmental factors.

- Type 1 diabetes is considered to be an autoimmune disease due to the presence of islet autoantibodies and lymphocyte infiltration around β-cells.

- Several trials aiming to suppress and modify the immune response have been carried out.

- Immune intervention is usually effective in animal models but the results cannot be reproduced in human trials.

- Some immune intervention has shown limited effect in subgroups of patients, but effectiveness in the general patient population has not been demonstrated.

- New thinking and strategies are needed for the cure and prevention of Type 1 diabetes.

SUMMARY   Type 1 diabetes is regarded as an autoimmune disease and, in accordance with this, trials using immune suppression and general or specific immune modifications have been carried out. Most trials have been successful in animals, but it has not been possible to reproduce these results in humans. Some effects have, however, been observed in subgroups of patients, but no drug has shown a general effectiveness. The side effects have been acceptable. The failure has been suggested to be due to insufficient doses, late start or the need for a combination of drugs. It could also simply be that this is an inappropriate way to attack the disease. It is therefore time for a new approach in the battle against Type 1 diabetes. The autoimmune findings could be an expression of defense and an effort to restore damaged β cells. More effort has to be put into a critical evaluation of environmental factors to find the very early agents promoting Type 1 diabetes.

Diabetes: an autoimmune disease

Before the discovery of insulin in 1921 Type 1 diabetes was a lethal disease. The general incidence of Type 1 diabetes was falsely considered to be low due to the limited awareness of diabetes. Many patients died with the diagnosis of infection and other acute illnesses due to the difficulty of diagnosing diabetes. Treatment with insulin saved lives but, owing to insufficient metabolic control, patients developed severe complications,

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leading to suffering and a shorter life. Even though insulin treatment has improved both pharmacologically and also with the tremendous development of devices, insulin is still a substitution treatment and a cure for the disease cannot yet be offered to these patients.

In 1974, autoantibodies directed against islet cells in the pancreas were demonstrated by an immunofluorescence technique in the sera of patients with Type 1 diabetes [1]. Assays for islet cell antibodies were established, but the antigen behind the immune reaction was still a mystery. The antigen was defined as a protein of 64 kDalton [2,3] and later it was found that the previously known autoantigen present in patients with the neurological disease Stiff man syndrome, glutamic acid decarboxylase (GAD) with the molecular weight of 67 kD, was almost identical to the antigen for islet cell antibodies [4]. Patients with Stiff man syndrome also developed diabetes. GAD was an enzyme that, when present in the brain was called GAD67, with large sequences homologous and with the shorter GAD65 protein present in the β cells, and it seemed to be the antigen that had been searched for. The pathogenesis mechanism linked to the development of diabetes was, however, still unclear. With time the focus moved from the humoral immune activity, represented by antibodies, towards the cellular immune system, represented by killer T cells directly destructive to the β-cells [5]. In autopsy material from diseased patients with recent-onset diabetes, a cellular infiltration of lymphocytes starting in the periphery of the islets and developing to an insulitis was also shown as a proof of T-cell activity [6]. During the 1970s, the association between the HLA system on chromosome 6 and Type 1 diabetes was described [7]. This highly polymorphic region coded for immune responsiveness, and the HLA-DQ β region was of particular interest since certain sequences were associated with both disease susceptibility and resistance [8,9]. The protective HLA types seemed to dominate over the risk alleles [10]. Taken together, a pathogenic model was constructed where the HLA risk alleles were permissive for the disease to occur but one or several environmental trigger factors were necessary to start the immune process [11]. The question of whether there is one primary autoantigen, as for example insulin itself [12], or whether there are several remains unanswered. Recent animal studies have shown how autoimmunity can be induced, even independently of the HLA types,

by preventing the expression of organ-specific antigens in the thymus. If this negative selection with depletion of autoreactive T cells in the thymus fails, it opens up the possibility of autoimmune reactions [13]. The discovery of spontaneously diabetic animals such as the bio breed rat (BB rat) [14] and the non-obese-diabetes mouse (NOD-mouse) [15] started an extensive experimental research on autoimmune diabetes. Both the NOD mice and the BB rats have since then been extensively used and have served as models for human Type 1 diabetes [16]. The histological picture of insulitis could be reproduced in these animal models. With time, numerous different antigens were detected beside GAD and insulin, such as a tyrosine phosphatase (IA-2) and a zinc transporter protein (ZnT8), which could give rise to β-cell-specific autoantibodies that could be present long before clinical diagnosis. Despite the identification of these antigens no light was shed over the mechanism behind the development of the immune reaction. These β-cell-specific autoantibodies have been used as predictors for the disease [17], and later on the antigens corresponding to these antibodies have been used in vaccination trials [18]. It was revealed that, despite the acute symptoms at the onset of diabetes, the autoimmune activity had been present a long time before the diagnosis. With the expanding use of these markers in the clinic, it has been shown that Type 1 diabetes occurs not only in young individuals, but in all ages at an even frequency [19]. The phenotype of autoimmune diabetes in adult age is, however, more like Type 2 diabetes, with mild symptoms at diagnosis and a slower progress with eventual destruction and disappearance of the β cells. This special form is called latent autoimmune diabetes in adults (LADA). Since islet cell antibodies can be detected several years prior to diabetes onset, it indicates a long subclinical phase during which progressive β-cell destruction takes place, finally giving acute symptoms at onset.

Intervention trials with general immune suppression

On the basis of the knowledge we have about the activated immune system against β cells at the time of onset of diabetes, several trials have been performed in order to decrease the intensity of the immune system or to shift the direction to other targets. Plasmapheresis was carried out in Swedish children with newly onset diabetes to reduce the amount of islet cell

antibodies, since these were thought to be harmful [20]. The treatment resulted in a rebound effect with an increase of the antibody level after plasmapheresis treatment, and no positive effect on β-cell function could be demonstrated [21]. No correlation between antibody titer and degree of immune activation or rate of decline of C-peptide has been identified [22]. Several trials with general immunosuppressive treatment, such as azathioprine, corticosteroids [23] and cyclosporine [24] were performed throughout the following years [25].

Corticosteroids had the adverse effect of stimulating increased insulin resistance meaning that an increase in insulin demand, making β-cell function even more difficult to estimate [26]. The cyclosporine trial gave some positive effects in certain subgroups of patients but not for all patients [27]. However, owing to the adverse effects, with potential nephrotoxicity causing irreversible kidney damage, the trials were stopped and have not been continued. None of the other treatments gave any immediate or prolonged benefit.

Intervention trials with modification of the immune response

One of the disadvantages of general immunosuppression is the increased risk of infections due to the generally poorer defense against pathogenic agents and opportunistic infections. Further development in clinical trials has, therefore, tried to modify the character, strength and direction of the immune attack. The goal for these activities is to change the activated T cells into regulatory T cells (Tregs), which will increase the level of tolerance against specific antigens [28]. The amount of Tregs can be estimated by the expression of CD4$^+$ and CD25$^+$ markers [29]. However, these regulatory T cells can show plasticity with the presence of both FOXp3 and INF-γ [30]. In addition, it is desirable to change the secretion of cytokines from the inflammatory cells in direction from the more toxic IL-1 and TNF-α characterizing a Th1 reaction, to the more mild Th2 reaction characterized by IL-10 and IL-4. With increasing knowledge about how tolerance is induced and how the immune system can differ between self and nonself, this information can be applied in experimental trials.

One of the first modifications applied in immune treatments was to administer anti-thymocyte globulin, which had a positive response in diabetes by inducing immunomodulation, perhaps through a change in the expression of antigen in the thymus, leading to an increased tolerance [31,32]. This, however, gave an increased risk for thrombocytopenia, cytokine release and reactivation of latent viruses [33,34]. Another approach was to use nicotinamide, a B vitamin that was thought to inhibit the DNA repair enzyme poly-ADP-ribose polymerase and prevent β-cell NAD depletion. The trial was performed in prediabetic children as a multicenter trial in Europe, but the results were disappointing since no difference in the frequency of overt diabetes was found between treated and non-treated children [35]. Other vitamins, such as vitamin D, have been shown to modify cytokine expression, which could be of importance for Type 1 diabetes development [36,37]. Several studies have shown lower levels of vitamin D in all types of diabetic patients, but intervention studies failed to demonstrate any effect [38,39].

CD3 & IL-1 blockers

Anti-CD3 monoclonal antibodies (mAb) has been successfully used for years in the treatment of acute transplant rejection. However, the treatment is associated with severe symptoms of cytokine release, which is thought to be induced by the strong crosslinking between the T-cell receptor (TCR) and the anti-CD3 via an Fc receptor on the CD3 molecule. New preparations of CD3 without the Fc region have, therefore, been developed. These Fc receptor-nonbinding anti-CD3 mAbs, such as teplizumab and otelixizumab, have the same immunomodulatory effect but lack much of the adverse effects. This suggests that CD3 blockers. besides being simple blockers of the interaction, are also capable of changing the function of the TCR by phosphorylation, preferably of the CD3 epsilon chains. Anti-CD3 mAb therapy works in favor of suppressive Tregs and suppresses autoreactive T cells and thereby restores tolerance [40,41]. Anti-CD3 mAbs modulate the TCR (TCR–CD3 complex) and induce apoptosis of activated autoreactive T cells [42]. In addition, an induction of IL-10 secreting T cells and an increased number of CD8$^+$ T cells of a subset showing coexpression of CTLA4 and Foxp3 further contributed to an immunosuppressive effect [43].

Anti-CD3 mAbs given to mice with streptozotocin-induced autoimmune diabetes were shown to prevent the expected development of diabetes in these animals [44]. Antibodies against

CD3 T cells have since been tried in human clinical trials for patients with rheumatic diseases, for those with multiple sclerosis [45] and also for Type 1 diabetes, with promising initial results [46,47].

There are several positive reports from trials with the CD3 blockers [48,49], but only in selected patients and for a limited time. The side effects of these drugs are dominated by the patient's experience of symptoms caused by cytokine release manifested as flu-like symptoms, fever and arthralgia. In the trials, 10% of patients stopped treatment because of the cytokine-related symptoms. In order to diminish these symptoms, the Fc region in the mouse-derived mAb was modified to a human Fc region, which has reduced the antigenicity of the treatment [50]. Reactivation of the Epstein–Barr (EB) virus was another expected side effect, and patients with previous EB virus infection were therefore excluded from participation in the trials. Despite this, some patients experienced a transient reactivation of EB virus infection [33].

A trial with a mAb directed against CD20 (rituximab), which was thought to cause a selective depletion of B lymphocytes, demonstrated a better preservation of C-peptide 1 year after treatment in recent-onset Type 1 diabetic patients [51]. These results go against the hypothesis that Type 1 diabetes mellitus is associated only with T-lymphocyte autoimmunity and give support to the idea that this is also influenced by B lymphocytes.

Blockade of the cytokine IL-1β with the mAb, anakinra, has recently been performed in children with new-onset Type 1 diabetes. The drug was acceptably tolerated but no conclusive results regarding efficacy could be reported [52].

Intervention trials with specific immune suppression

Every exposure to a foreign antigen is a challenge for the immune system in the body to differ between self and nonself. Antigens are exposed and presented in the thymus and the T cells undergo a negative selection, meaning that T cells showing the tendency to react against autoantigens should be eliminated. However, this mechanism is not perfect and a few autoreactive T cells can escape thymic selection. Self-antigens are supposed to be present in a large quantity while nonself antigens are more rare. Tolerance could therefore be induced by a massive exposure of a certain antigen to the immune system.

The identification of different antigens present during the time around diagnosis and also later on in the disease course has lead to experiments with exposure to these antigens in both the preclinical and clinical phases. The administration of β-cell-specific antigens has been one strategy to induce selectively immunoregulatory T cells for a given self-antigen or peptide. One of the first antigen was insulin itself, and when administered to NOD mice it inhibited or delayed the expected onset of diabetes. Effectiveness has also been shown in a variety of other mouse models [53–55].

In the Diabetes Prevention Trial using oral insulin administered to high-risk individuals, subjects with high levels of insulin autoantibodies had a delay in the development of diabetes, whereas subjects with low levels of insulin autoantibodies showed no effect [56]. In humans, insulin has been given in intense doses and it is unclear if the eventual positive effect is due to good metabolic control or to insulin as such [57]. One study, using the B-chain insulin as a vaccination, showed an effect even after 2 years of follow-up [58]. Early insulin treatment to LADA patients, instead of conventional treatment with oral agents until insulin is necessary, has not shown any convincing positive effect [59]. Human trials with nasal and oral administration to nondiabetic individuals at high risk have hitherto been inconclusive [60]. In the Diabetes Prevention Trial using oral insulin administered to high-risk individuals, subjects with high levels of insulin autoantibodies had a delay in the development of diabetes, but subjects with low levels of insulin autoantibodies had a nonsignificant trend to get more diabetes in the insulin-treated group [56].

Vaccination is a further development of this concept, based on exposure of autoantigen in order to induce tolerance. Insulin and GAD are the main autoantigens present in β cells and are targets for a majority of autoreactive T cells in Type 1 diabetic patients. Recombinant human GAD is coupled to alum as an adjuvant and the formula is given as a repeated vaccination [61]. The expression of GAD is postulated to stimulate the formation of immunoregulatory CD4+ T cells and increase levels of IL-4 and IL-10, cytokines that suppress the general immune response [62]. Current clinical trials with GAD vaccination involve recent-onset diabetic children, patients with LADA, and high-risk marker-positive prediabetic children [63]. Recent results in a study in which newly diagnosed

patients with Type 1 diabetes were vaccinated did not show any benefit of vaccination [18]. Beside GAD, the antigens heat shock protein [64] and CTLA4 [65] have been considered to be appropriate for vaccination. CTLA4 is an immunoglobulin fusion protein (abatacept), with the ability to block interaction between CD80 and CD86 on antigen-presenting cells and CD28 on T cells. This interaction starts the activation of the T cells, and if it is inhibited the autoimmune process will be delayed. The results after 2 years demonstrate a slower progress of β cell reduction in the treated group [66]. Like many of the successful interventions carried out in the NOD mice, the positive effects of vaccinations were not possible to reproduce in humans [67].

Trials aiming to reduce aggressive cytokines and turn the reaction from a Th1 to Th2 response have been carried out. Levels of the anti-inflammatory cytokine IL-10 could be selectively increased, which would reduce the activity of macrophages and dendritic cells. This approach would result in effective immune suppression, and in NOD mice the treatment had the desired effects in both preclinical and clinical stages of Type 1 diabetes. However, one of the major concerns raised about this treatment is that it could be dangerous because the effective immune suppression allows pathogens to survive. It is therefore necessary to have strict control over the dose given and the ability to tightly regulate and interrupt the supply if needed [68]. A system for such a regulation can be constructed by the insertion of a regulatory gene, which is activated by feeding with tetracycline, in front of a vector for a gene encoding for IL-10. This approach has not yet been tested in humans.

Problems left to face

Rapidly increasing insight into the regulation of the immune system has given hope that a therapeutic intervention maybe found for the so-called autoimmune diseases. The TNF-α antagonists in rheumatoid arthritis and the immune modulating agents used in multiple sclerosis have been successful, but for Type 1 diabetes we are still waiting for the breakthrough. All types of immune interference have hitherto been more or less disappointing. There has been discussion on whether the doses given are too small, whether the treatment has to be repeated in order to last longer, or whether different types of treatment with complementary properties should be combined [69].

A matter of concern is that intervention is started late, when the immune activity has already reached a maximum at the time of clinical diagnosis. At the time of diagnosis, several epitopes are recruited in the autoimmune storm, and simultaneously the remaining β cells continue to activate the immune system, leading to a point of no return or at least a difficult situation to turn back upon. It is debatable whether the autoimmune attack starts with a single immune response that expands and involves more and more epitopes with time or whether multiple antigens are engaged from the beginning.

The time of intervention is therefore critical and it seems to be necessary to intervene early during the process. In the present situation, the best outcome of treatment seems to be a reduction of autoimmune activity, without any hope for a complete remission and cure for the disease. However, if autoimmunity against β cells is suppressed, it may facilitate regenerative mechanisms and the repair of β cells. Even a minor arrest of further β cell destruction is welcome, since a small but significant amount of remaining β cells will have a positive effect on the ability maintain a good metabolic control.

LADA has been proposed as a preferable human model for testing intervention therapy in, since these patients have slower progress of disease and often have a significant amount of β cells left to rescue at clinical onset [59]. These subjects are, however, hard to find, since they are mostly hidden in the large number of adult patients stricken by Type 2 diabetes, and routine testing of β-cell specific autoantibodies in these patients is not usually carried out. With time they require insulin but by then these patients are also in a late phase of the disease.

Complete regression of the process and rescue of the β cells requires very early intervention, probably in the preclinical stage. There are concerns about recruiting individuals in the preclinical phase, mainly because the predictive value of available markers is not specific enough. With a combination of genetic and serological markers in first-degree relatives to probands with Type 1 diabetes, the prediction for diabetes is fairly good, but still not 100% accurate. In the general population, the same markers would be really poor at predicting diabetes, which is a problem because most patients are recruited from families without diabetes. There is a substantial risk that subjects who would never develop diabetes could be exposed to immune intervention with

agents that are potentially harmful. The risk of the treatment, therefore has to be balanced against the safety and efficacy of the drugs.

Among the difficulties with intervention treatments in diabetes is the absence of hard end points in the evaluation of the effect. Clinical outcomes from different intervention programs analyze C-peptide, either after fasting or after stimulation with glucose or meals, as a measurement of remaining β-cell function, HbA1c as a measurement of metabolic control and the amount of insulin required as a measurement of β-cell failure. The most common is measuring C-peptide, which is secreted at an equivalent amount in relation to insulin. The disappearance rate of C-peptide from onset to follow-up could be described as percentage of the initial value, as the difference between levels in nmol/l or as a grade of inclination for a curve. It is well known that there is a strong correlation between initial C-peptide and C-peptide at follow-up – that is, the initial value of C-peptide is prognostic for future C-peptide levels. Subjects starting with high C-peptide will, therefore, have higher C-peptide at follow-up. This could be the explanation as to why several studies only show effect in patients with remaining C-peptide or in those who are in an early phase of the disease. In addition to this, the course of C-peptide is highly variable depending on whether remission occurs or not. During remission the β cells recover and regain part of the endogenous production of insulin. This is, however, unfortunately transient, and after 6–12 months the β-cell function starts to decline again, slowly but steadily. For an accurate monitoring of different intervention treatments it is necessary to find stable markers that could be followed to evaluate the β-cell function. An agreement needs to be achieved upon how C-peptide should be handled at least.

A final problem to face is the complexity of the immune system, and the difficulties of predicting outcome and transferring results from animal models into human situations. Tremendous work has been carried out with animal studies, especially in the NOD mouse, which is an animal model with several similarities to human Type 1 diabetes. The possibility of influencing the disease outcome in humans is not as easy as in the mouse, so the same intervention effect cannot be expected in humans. The immune system is also complex and unpredictable, and an autoreactive T-cell response can evoke inflammation in subjects with diabetes while being

harmless in healthy subjects [70]. Treatment could also have unexpected effects, since it has been observed that oral administration of auto-antigens can induce cytotoxic T cells in mouse models [71].

What is the real cause of diabetes?

Epidemiologic observations have demonstrated an influence of latitude on the incidence of childhood diabetes, meaning that there is an inverse correlation between the incidence of Type 1 diabetes in children and distance to the equator. Based on this it has been speculated that exposure to sunlight could decrease the incidence of diabetes, possibly through vitamin D, which has immune-modulating effects [39]. This hypothesis is supported by the observation that patients with Type 1 diabetes have lower levels of vitamin D compared with controls in the same country [37]. Countries with lower socioeconomic conditions have lower incidences of Type 1 diabetes, yielding a hypothesis that poor hygiene in terms of higher exposure to dust, soil and animals should make the immune system more tolerant to foreign antigens and lead to fewer cases of diabetes. Another current hypothesis is that Type 1 diabetes is induced after certain virus infections [72]. Rubella, parotitis, coxsacckie B and cytomegalovirus are among the most suspected ones [73].

The general increase in weight gain in the population affecting both newborns and older children is another area of concern, leading to a hypothesis about the uncontrolled demand of insulin during young age and an insufficiency to live up to these requirements, leading to stress and exhaustion of the β cells [74]. In addition to the almost unlimited access to food leading to obesity, it can also be concluded that dietary habits have changed during recent years in western societies and even more in developing countries. More processed food is served and the facilitation of transport has led to an exposure to foreign food in all developed populations. Owing to the long subclinical phase of diabetes, the initiation event is difficult to identify and the current hypothesis could neither be proved nor rejected.

Future perspective

The current treatment for Type 1 diabetes is insulin, which is a substitution therapy that works for the patient in the clinic but does not provide a cure. Even if intense efforts have been made with immune therapies against Type 1 diabetes, the results are disappointing. The

current opinion, however, is that intervention treatment, despite all of the negative results, has some effect in selected patients. Based on the available knowledge about Type 1 diabetes and owing to the good results obtained in animal models, immune intervention ought to work better. It is, therefore, time for a new approach in thinking about the prevention and cure of Type 1 diabetes. To find a curative treatment it may be necessary to use an earlier and more powerful intervention, and efforts to find and include subjects with a high risk of developing Type 1 diabetes in the future in trials, and to give higher doses, would perhaps improve the results. Similar to cancer treatment, a combined treatment using different modalities of action should be practiced to enable clinicians to hit from different fronts to stop the immune attack and keep adverse events as low as possible [75,76].

Even if immune therapy may be successful, it is still a treatment that is applied several steps after the initiating event has occurred. If an effective therapy is to be found, it will have to be one directed against the process that has initiated the primary insult. Even if genes are involved in the disease they only seem to play a role as a permissive factor in an interaction with environmental trigger events. Genes are stable and will not account for the great increase seen during recent decades.

The initiation factors are proposed to be found in the close environment and more efforts should be made for primary prevention and large-scale studies on lifestyle intervention. What has changed in our environment and lifestyle to such a degree that it can trigger an autoimmune process leading to the disappearance of important cells and organs in young people? These factors certainly have to be identified before we can find a cure and prevention for Type 1 diabetes. Factors related to lifestyle are, however, extremely hard to study owing to difficulties in protocol standardization and the requirement for long exposure time and tight compliance of the study population. Concerns regarding the different genetic susceptibility of the subjects must also be taken into account. Studies in animals can overcome these limitations, but results from animals are not always transferable to humans. Nevertheless, more efforts have to be put into a critical evaluation of diet, physical activity and other lifestyle factors to find the agents that are promoting Type 1 diabetes.
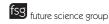
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