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Progress and challenges for treating Type 1 diabetes

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Abstract

It has been more than 30 years since the initial trials of Cyclosporin A to treat patients with new onset Type 1 diabetes (T1D). Since that time, there have been insights into genetic predisposition to the disease, the failures of immune tolerance, and mechanisms that cause the immune mediated β cell destruction. The genetic loci associated affect lymphocyte development and tolerance mechanisms. Discoveries related to the roles of specific immune responses gene such as the major histocompatibility complex, PTPN22, CTLA-4, IL-2RA, as well as the mechanisms of antigen presentation in the thymus have suggested ways in which autoreactivity may follow changes in the functions of these genes that are associated with risk. Antigens that are recognized by the immune system in patients with T1D have been identified. With this information, insights into the novel cellular mechanisms leading to the initiation and orchestration of β cell killing have been developed such as the presentation of unique antigens within the islets. Clinical trials have been performed, some of which have shown efficacy in improving β cell function but none have been able to permanently prevent loss of insulin secretion. The reasons for the lack of long term success are not clear but may include the heterogeneity of the immune response and in individual responses to immune therapies, recurrence of autoimmunity after the initial effects of the therapies, or even intrinsic mechanisms of β cell death that proceeds independently of immune attack after initiation of the disease. In this review, we cover developments that have led to new therapeutics and characteristics of patients who may show the most benefits from therapies. We also identify areas of incomplete understanding that might be addressed to develop more effective therapeutic strategies.

Keywords

Type 1 diabetes; autoimmunity; tolerance; immune therapy

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Conflicts of interest

The authors have no conflicts of interest to declare.

Introduction

Type 1 diabetes (T1D) accounts for 5-10% of all cases of diabetes and is estimated to have an annual incidence of 80,000 in children. The rates of Type 1 diabetes and other autoimmune diseases have been increasing over the past few decades in Western countries: and it is estimated to double every 20 yrs(1, 2). Although the reason(s) for the increasing rates have not been clearly identified, much interest has focused on the rising rates as a consequence of widespread use of anti-microbials and other practices of Western countries, i.e. the “hygiene hypothesis”.

The hallmark of T1D is immune mediated destruction of insulin producing β cells resulting in dependence on exogenous insulin for survival(3). The Diabetes Control and Complications Trial (DCCT), a landmark study, showed that tight control of blood glucose levels could reduce the rates of dreaded secondary microvascular complications such as retinopathy, nephropathy, and others(4). At the same time the study pointed out the limitations in achieving normal metabolic control because of hypoglycemia resulting from non-physiologic delivery of insulin and limitations of real time monitoring. Technologies for delivering insulin in a physiologic manner have been rapidly improving. Recombinant human insulins with kinetics closer to normal insulin secretion than earlier formulations have been developed and combinations of insulins that more closely mimic normal insulin secretion are widely used. Insulin pumps and continuous glucose monitoring that show glucose levels in real time are used in practice allowing patients to more closely track and correct glycemic excursions and recognize and avoid insulin induced hypoglycemia. Means of integrating these afferent and efferent limbs of management are in development(5).

However, despite the improved technologies, the levels of glycemic control that are generally achieved in practice currently, do not meet goals prescribed by the American Diabetes Association or other advisory panels based on results from the DCCT. For example, recent studies from the Type 1 Diabetes Exchange showed that, in the United States, children and young adults between the ages of 10 and 21 have average hemoglobin A1c levels that exceed 8.5% and even in older patients, average hemoglobin A1c levels exceed 7.0% despite the recommendations that levels < 7.5 and $< 7.0\%$ are maintained for children and adults(6-8) (Table 1). Likewise, in patients < 24 yrs in England and Denmark, average A1c levels are 8% and greater(9). The risk of hypoglycemia from the use of exogenous insulin remains a significant fear for patients and their families and limits their ability to achieve therapeutic goals, particularly in children. In addition, the chronic disease exerts an impact on the psychological function and quality of life for these patients.

Many studies have documented improvement in metabolic control in individuals who retain endogenous insulin production. Glycemic excursion is reduced and hemoglobin A1c levels are inversely related to endogenous insulin secretion(10). Rates of insulin-treatment related hypoglycemia are lower in patients who maintain some endogenous insulin production either from β cells in the pancreas(11). Therefore, the primary goal of interventions in T1D is to improve the prevent or at least dramatically delay the progression of β cell destruction and to restore the lost cells.

Alternative strategies for replacement of β cells have been championed. Islet transplantation, while not routinely rendering recipients insulin independent are able to reduce rates of severe hypoglycemia(12). The development of stem cell derived replacement β cells remains a key objective for a therapy that would potentially be a limitless source that can restore near normal metabolic control provided that issues of graft survival, immune rejection, and normal secretory function can be addressed(13).

Development of a model of the pathogenesis of T1D

Just over 3 decades ago, the first clinical studies of Cyclosporin A were reported to modify the natural course of β cell loss in patients with new onset Type 1 diabetes (T1D)(14). These trials followed earlier observational studies that identified autoantibodies that reacted with islet cells and in the serum of patients with T1D as well as prospective studies from a unique set of triplets that showed that the autoimmune disease was chronic and progressive(15). Rodent models, in which the cellular mechanisms that caused β cell destruction were developed that had their limitations: the BB rat, multi-low dose streptozotocin induced diabetes in mice, and viral induced diabetes(16-18). Since that time, studies of the cause and treatment of autoimmune diabetes have greatly enhanced insights into the disease mechanisms. The current “gold standard” rodent model, the NOD mouse, genetic analyses, defining mechanisms of immune tolerance, understanding the cellular molecular basis of immune responses, and insights into β cell biology have transformed the field although there are ongoing concerns about the application of studies in the NOD mouse to human clinical trials (19-21).

At the same time, clinical trials targeting pathways identified through these studies were performed. Newer therapeutics are more specific and safer than agents such as Cyclosporin A or azathioprine and prednisone that showed promise in affecting the natural history of the disease(22-24). However, the results from clinical trials have been disappointing since, similar to the findings with Cyclosporin A, they have caused transient but not permanent improvement in β cell loss. Extended follow up of patients treated with successful agents such as Cyclosporin A, anti-CD3 mAb, or CTLA4Ig have shown persistent differences in C-peptide responses for as long as 3 years but loss of the biologic differences after that time (25-27). The identification of autoantibodies against defined autoantigens including insulin, ICA512, GAD65, ZnT8 and ICA (islet cell autoantibodies) has sharpened the prediction of disease in relatives of patients with T1D. However, none of the agents tested for prevention have been successful, including Cyclosporin A that showed promise in patients with new onset disease (28).

Therefore while several immune modulators can affect disease in the short term, a careful evaluation of factors that lead to long term failures need to be addressed. In this issue, we consider factors that are likely to be involved including failures in immune tolerance, mechanisms of β cell failure, and therapies and clinical trial design that may be employed to improve long term outcomes.

Overview of disease mechanisms

1) Immune response and β cell genes are associated with disease risk: (Figure 1)

The factors which lead to the development of T1D are both genetic and acquired as the concordance rate of T1D is 30 – 50% in monozygotic twins compared to 10% in dizygotic pairs. However with time, up to 80% of initially discordant dizygotic twins may develop disease(29). Monogenic forms of diabetes have been associated with mutations in immune response genes. Patients with the autoimmune polyendocrine syndrome 1 (APS1 or APECED), caused by a mutation of *Aire* resulting in failure of negative selection in the thymus develop autoimmune diabetes(30). Robust autoimmunity, including T1D, develops in patients with mutations of *Foxp3*, needed for development and function of regulatory T cells(31, 32). These mutations are uncommon but illustrate the dependency of normal immunologic tolerance on these genes.

Genomewide association studies have revealed important risk loci linked to increased risk for T1D(33) Of the 25 loci most highly associated with disease, 19 involve candidate genes linked to immune responses and 3 are involved in β cell function or survival (www.t1dbase.org). The major histocompatibility complex is the locus most strongly associated with the disease and explains most risk. The specific HLA alleles conferring the greatest risk are DRB1*0301-DQA1*0501-DQB1*0201 (odds ratio [OR] 3.64) and the DRB1*0405-DQA1*0301-DQB1*0302, DRB1*0401-DQA1*0301-DQB1*0302, and DRB1*0402-DQA1*0301-DQB1*0302 haplotypes (ORs 11.37, 8.39, and 3.63), followed by the DRB1*0404-DQA1*0301-DQB1*0302 (OR 1.59) and the DRB1*0801-DQB1*0401-DQB1*0402 (OR 1.25) haplotypes. The most protective alleles are DRB1*1501-DQA1*0102-DQB1*0602 (OR 0.03), DRB1*1401-DQA1*0101-DQB1*0503 (OR 0.02), and DRB1*0701-DQA1*0201-DQB1*0303 (OR 0.02)(34). The Class I region also appears to impart risk in addition to and independently from HLA Class II alleles. The greatest association is with B*5701 and B*3906 but also A*2401, A*0201, B*1801 and C*0501 (35, 36). The MHC is responsible for presentation of antigens to immune cells and therefore, variation in these genes greatly affects the immune repertoire. Hence, a significant finding was that the Class II MHC alleles associated with T1D have a substitution for aspartic acid residue at position 57 of the β chain of HLA-DQ: aspartic acid at position 57 confers protection against T1D and 96% of the patients carry a polymorphism compared to 19.5% of healthy controls(37).

The locus encoding protein tyrosine phosphatase non-receptor 22 (PTPN22) which encodes lymphoid protein tyrosine phosphatase (LYP), is also to disease(38). This locus has also been associated with a number of other autoimmune disorders including rheumatoid arthritis, systemic lupus erythema, vitiligo, Graves' disease, and Crohn's disease, suggesting a common mechanism of autoimmunity (39-41). The disease-associated allele, a single nucleotide polymorphism (SNP) at nucleotide 1858 in codon 620 1858C>T (rs2476601), disrupts an interaction motif in the protein. PTPN22 inhibits early TCR signaling by dephosphorylating the activation loops of Lck, Fyn, ZAP-70 and ITAMs of the CD3 ζ -chain (41, 42). The polymorphism precludes the ability of CSK to bind and inhibit downstream signaling molecules critical to an appropriate level of TCR activation. The role of PTPN22

in B cell responses is less clear but the disease associated polymorphism is postulated to result in impaired B cell tolerance and the development of autoreactive B cells (43).

The gene locus encoding the negative costimulatory molecule, CTLA-4 is also associated with T1D (44, 45). CTLA-4 binds the same ligands as the costimulatory molecule CD28 but in activated T cells CTLA-4 imparts an inhibitory signal to cells. This function as a negative regulatory molecule identifies a likely mechanism for failure of inhibition of autoreactive T cells. Indeed, CTLA-4 knockout mice develop immune mediated diabetes (46). Moreover, CTLA-4 controls the development of both conventional and regulatory T cells (Tregs) through modulation of the TCR repertoire (47). CTLA-4 serves as a negative regulator of T cell activation by opposing the actions of CD28 through multiple mechanisms: CTLA-4 has stronger affinity for B7 than CD28, inducing inhibitory signaling in conventional T cells, increasing Treg engagement with APCs, and others (48-50).

The polymorphism in the alpha chain of the IL-2 receptor (IL-2RA) is thought to affect IL-2 signaling needed for normal regulatory mechanisms. IL-2 is required for generation of Tregs and their survival. Mice lacking IL-2 or its receptor CD25 succumb to lymphoproliferative disease due to absent thymic development of Tregs. The IL2RA haplotype identified by rs12722495 is associated with decreased IL-2 receptor signaling in both Tregs as well as conventional T cells and is associated with lower levels of FOXP3 expression and decreased suppressor function. Additional polymorphisms of the IL-2RA locus have been associated with an increased risk in T1D including rs706778 and rs3118470(51-53).

There are also loci associated with genes related to β cell killing and insulin expression. TNFAIP3 is involved in limiting NF κ B and TNF-mediated apoptosis(54). A locus associated with variable number tandem repeats (VNTR) in the insulin gene promoter is thought to affect levels of gene expression and structural variations both centrally and peripherally(55). Expression of the insulin gene is regulated by the VNTR region located upstream of the insulin translation initiation site. Three major classes of the VNTR minisatellites have been identified: class I, class II, and class III alleles. The class I and III alleles are most commonly found in Caucasians. The class I allele is associated with an increased risk for T1D whereas the class III allele is protective. The class III allele confers increased levels of protein expression whereas inheritance of the class I allele has an opposing effect. Protection provided by the class III allele is due to its increased levels of thymic expression of proinsulin mRNA that are 2-3 orders higher than the class I allele. Increased expression of the insulin gene at the thymus is thought to provide more effective negative selection of insulin reactive T cells(56).

2) Initiating events in T1D

The increasing rate of T1D, particularly in young children, has led investigators to search for environmental factors that interact with genetic factors in accelerating or modifying disease. A large observational study (TEDDY) seeks to identify environmental factors that may be involved in disease(57). Observations between colonies of NOD mice have added further evidence for a strong environmental influence on the disease. In these inbred mice, the rates of diabetes varies from ~80% to < 50% and the incidence is dramatically different in males and females. Krieger et al showed that a single bacterium, and the gut immune system

alterations associated with it, can either promote or protect from autoimmunity in predisposed mouse models, probably reflecting their variable dependence on different Th subsets (58). However, Markle et al described an interesting interaction between sex hormones and the microflora that may account for the sexual differences in NOD disease. They showed that early-life microbial exposures determine sex hormone levels and modify progression to diabetes. Colonization by commensal microbes lead to elevated serum testosterone resulting in greater protection of NOD males from T1D (59). In this issue, this group provides a review of these findings and insights into the mechanisms. Some early data suggests that reduced diversity of the microbiome is associated with progression to T1D in individuals at risk although it is not clear whether the changes that have been observed are the cause or effect of the changes in immune responses since the changes occurred between acquisition of autoantibodies and disease onset (60). Nonetheless, several studies from other fields indicate that regulatory and effector mechanisms can be modulated by commensals as well as oral antigens(61-64).

Innate immune responses are the initiators of adaptive T cell responses that can cause β cell destruction. However, experimental data suggests that there is a close interaction between the microflora and these initiating events. NOD mice that are deficient in MyD88, a signaling molecule for all TLRs except TLR3, do not develop diabetes when they are housed under specific pathogen free conditions. However, when the animals are made germ-free, they develop robust diabetes (65). These observations suggest a close relationship between the microflora and the dependence on innate signaling. Tai et al address innate immune signaling in this issue.

3) Failures of immune tolerance

A number of the genes associated with T1D, such as PTPN22, IL2RA, CTLA-4 and others are involved in the control of immune tolerance. Studies with samples from patients are consistent with failures of normal tolerance mechanisms in B and T cell lineages. Abnormalities in early B cell tolerance checkpoints in T1D result in the production of large numbers of autoreactive B cells that accumulate in the peripheral blood of patients. (Figure 2) (43, 66).

Moreover the frequency of CD69+ mature naïve B cells and the number of divisions of mature naïve B cells was increased in patients indicating that the B cells were activated and had undergone proliferation (67, 68). This finding was not explained by an increase in BAFF levels. The basis for these abnormalities is not established but blunted B and T cell receptor signaling has been associated with the *1858T PTPN22* variant found in patients with T1D and may account for failure to remove autoreactive B cells (43). Other susceptibility genes including BLK, LYN, PTPN2, and BANK1 may also affect BCR signaling and normal tolerance mechanisms. The basis for the failures of tolerance has direct relevance to the successes and failures of immune therapies. Chamberlain et al found that the frequency of autoreactive B cells was similar before and after patients with new onset T1D had been treated with rituximab (anti-CD20 mAb) that depletes B cells (66). Rituximab treatment transiently reduced the number of peripheral B cells. New emigrant B cells were increased after treatment, but mature naïve, IgM memory, and switched memory B cells were

remained reduced at 12 months, and after treatment. The frequency of polyreactive and Hep-2 reactive B cell clones was similar before and after treatment (n=4). Hence, these observations indicate that the failures of B cell tolerance are not an acquired phenomenon and recur after B cell recover in rituximab treated subjects.

However, other findings from peripheral B lymphocytes point to acquired factors that may accelerate disease progression. In healthy subjects, high-affinity insulin binding B cells are found exclusively in the anergic naïve B cell compartment whereas the high-affinity insulin binding B cells are absent from the anergic compartment in all prediabetic and new-onset T1D patients. Interestingly, they return to normal frequency in individuals with T1D for > 1 yr (69). Resistance of CD4+ T cells to suppression by Tregs has been suggested as a mechanism that can account for failure of normal tolerance mechanisms(70). This characteristic also appears to be acquired during the progression of disease. Thus, these prospective studies indicate that the ultimate failure of tolerance mechanisms may be acquired on top of an autoreactive repertoire that develops as a result of genetic factors.

In this issue, Buckner et al address mechanisms of failure of tolerance in T cells in T1D. They have identified resistance of effector T cells to the actions of Tregs in patients. Other investigators have postulated that the polymorphism of IL2RA may lead to failures of Tregs that may then fail to prevent the accumulation of autoreactive mature naïve B cells (51, 67). Prolonged signaling by IL-2 can increase Tregs in primates (71). However, precise dosing of IL-2 is critical. Worsening of β cell function in all participants in a pilot clinical trial of high dose IL-2 with rapamycin in patients with new onset T1D (72). Analysis of data from this trial highlighted the dose/response differences in immune cell subsets: an increase in NK cells in response to the high dose of IL-2 was thought to explain the deterioration in C-peptide responses. This issue has been recently addressed with clinical studies to identify a dose that would expand Tregs without significant expansion of NK cells (73-75). An alternative approach has also been developed by Bluestone et al and is described in this issue by Gitelman et al. They report the results of a pilot trial to expand and readminister autologous Tregs from patients with T1D(76). Interestingly, the failure of IL-2 signaling, manifest as decreased phosphorylation of STAT5 is improved during expansion of the Tregs in vitro. This finding suggests that more than just a manifestation of the genetic polymorphism of IL-2RA, the decreased signaling may be affected by in vivo by acquired factors. Studies are ongoing to evaluate the clinical outcomes with this therapy. It will also remain to be determined if boosting Treg numbers and function in T1D will be sufficient to restore the abnormal removal of autoreactive B cells in the periphery.

Preclinical models and human data support the hypothesis that effector cells that recognize β cell antigens drive the disease. Krishnamurthy et al in this issue describe features of antigen specific T cells from preclinical studies in this issue. *Ins1* and *Ins2* knockout NOD mice, that express a mutated proinsulin transgene, do not develop autoimmune diabetes. Human T cell lines and clones, reactive with islet antigens such as IGRP can cause destructive insulinitis when transferred into immune deficient NOD mice that express human MHC molecules(77, 78). However, more recent studies suggest a twist on the process of central tolerance in the T cell compartment, by showing that pathologic T cells may recognize neoantigens that are not presented during negative selection in the thymus. The islets contain CD103+ DC that can

present altered peptides (Ins 12-20). The 12-20 segment is not presented when an APC handles insulin but only when presenting denatured insulin or peptide fragments(79, 80). Ferris et al in this issue describe the features of the diabetogenic T cells and intraislet DC's in this issue. The insulin 9-23 peptide is a major target for pathogenic CD4+ T cells. However, the 9-23 reactive T cells can be divided into two types depending on whether their response are improved or inhibited by substituting a glycine for the B:21 glutamic acid at the p8 position of the peptide. Crawford et al reported that within the pancreas, unique processing of insulin generates truncated peptides that lack or contain the B:21 glutamic acid(81). In the thymus, the absence of this type of processing combined with the low affinity of B:9-23 binding to register 3 of IA^{g7} (the Class II allele of NOD) may explain the escape of insulin specific CD4+ T cells. Finally, DeLong et al identified hybrid insulin peptides that are formed by covalent cross-linking of proinsulin peptides to other peptides present in β cell secretory granules(82). These neoantigens were shown to be recognized by islet infiltrating T cells in NOD mice and by CD4+ T cell clones that infiltrate islets from patients who died with T1D.

4) β cells are more than targets

There is very little known about changes in β cells during pathogenesis of T1D. It has become clear from studies of β cells in settings of non-immunologic stressors that there may be changes affecting their differentiation and function(83). Changes in the patterns of insulin secretion have been identified prior to deterioration in glucose tolerance in individuals at risk for T1D, and these qualitative abnormalities persist even as total insulin secretory capacity declines as a result of immunologic destruction(84). In patients, functional readout of the immunologic process has been identified: β cell glucose sensitivity changed 1.45 years before diagnosis and predated loss of glucose tolerance that was identified 0.78 years before diagnosis in at-risk relatives who progress to overt disease(85). Processing of proinsulin is impaired reflected by a relative increase in the ratio of proinsulin:insulin that is found in other settings of abnormal β cell function such as in T2D and in patients with insulinoma(86, 87).

In addition, in NOD mice, epigenetic changes are induced in β cells by immune mediators (88). The consequences of these and other alterations may include impaired synthetic function but studies with other mediators of cellular stress raise the possibility that cellular transdifferentiation may account for β cell failure (89, 90). In FoxO1 knock-out mice, development of hyperglycemia is due to β cell differentiation (83), manifested by expression of Neurogenin3, Oct4, Nanog, and L-Myc. Functional insulin producing cells may develop in the gut following FoxO1 ablation. Neurog3+ enteroendocrine progenitors require active FoxO1 to prevent differentiation into insulin+ cells suggesting a strategy to restore insulin production by ablation of this transcription factor in gut cells (91). Wilcox et al review these and other findings pertaining to the effects of the immune response on β cell targets in this issue.

Can better trials deliver better results?

The progress resulting from preclinical and clinical studies has set the stage for significant clinical advances. These key achievements include 1) identification of antigens, 2) metabolic and clinical readouts of disease progression, 3) identification of relatives of patients who have normal metabolic function but whose serum contain autoantibodies that identify them at risk for development of disease, and 4) availability of drugs, some of which have been approved for other diseases with the potential to modify the progression of T1D.

The results of trials with single agents has suggested that combinations of agents will be needed to achieve and maintain a clinically useful treatment for patients with new onset disease and possibly for prevention. In this issue Ehlers describes strategies to improve efficiency in combination trials which include mechanistic studies to maximize information from the clinical studies. Future guidance on trial design may also depend on the answers to the following questions:

1) Is there recurrence of autoimmunity after treatment of new onset patients with agents that can reduce β cell loss in the earliest stages of disease?

Are the features of recurrent disease the same as the original pathogenesis or does it change? Does the pathologic immune response ever remit? Evidence for the latter is the finding of a high frequency of individuals with long standing T1D who retain β cell function. While the frequency is high when newer C-peptide assays have been used to detect residual insulin secretion, even with standard assays, the frequency of residual insulin production has been reported to be as high as 64.7% (92, 93).

2) Is there an opportunistic “window” for treatment and when does it occur?

Data from clinical trials has been conflicting. For example, anti-CD3 mAb, which was shown in preclinical studies to be most effective in treating an ongoing immune response, appeared to lose its robustness of efficacy when it was given to patients after the new onset period (94). However, the combination of ATG and GCSF appeared to show efficacy in patients with diabetes for 4mos-2 yrs (95). This may suggest that the best chance of successful therapy is in the prediabetes period i.e. prevention despite the failures of the agents that have been tested to date.

3) What are the features that distinguish responders and non-responders to treatments?

Subjects do not show identical responses to immunologics or any treatment. Identifying individuals who are most likely to respond to a treatment would minimize the risk:benefit ratio of experimental therapies and develop a more personalized approach to therapy. In clinical trials, immunologic, metabolic, and demographic features have identified those most likely to respond to treatment (Table 2). Apart from these known features, the microflora has also been identified to determine responses to biologics used to treat cancer including anti-CTLA-4 and anti-PD-L1 mAbs (96, 97). Modulation by unknown environmental factors may be an important determinant of differing responses to biologics among NOD colonies (21).

4) Can antigen specific therapies be used to treat a complex disease?

To date, antigen based therapies have not been successful. A number of explanations may account for the lack of success. First, despite the identification of a number of autoantigens, a primary antigen is not certain. The article by Ferris et al and Delong et al that describe robust responses to modified peptides of known diabetes antigens raise the possibility that these antigens would be optimal for clinical testing. The lack of precise knowledge of the initiating antigen may not preclude the use of antigen specific therapies. Through mechanisms described as “bystander suppression” and even the spread of regulatory phenotypes among antigen reactive cells that are activated in the presence of regulatory cytokines (“infectious tolerance”) responses to unknown antigens may be achieved(98). Second, the manner in which antigens have been delivered may not induce a protective response. In a recent trial of alumGAD65, high titers of anti-GAD65 antibodies were induced but the therapy did not alter the progression of disease(99, 100). Strategies that have been successful in allergic diseases, such as early oral administration of antigen have not been tested. Third the timing of therapies may be too late. By the time of diagnosis, several autoantigens are recognized and studies of β cell killing in NOD mice and at-risk relatives suggest that the time of maximal β cell killing begins before the onset of hyperglycemia(101, 102).

5) Is the loss of β cell function after immune therapy an intrinsic feature of injured β cells, the result of increasing demand on remaining cells, or the result of initial injury?

Finally, it is possible that the decline in β cell function over long term reflects the unfolding of damage that is responsible for initiating the disease or even failure of normal cellular repair mechanisms. Data from NOD mice and clinical experience indicates that the improved metabolic function that occurs following immune therapy of new onset diabetes is largely due to recovery of dysfunctional β cells rather than growth of new β cells(103). However, our analysis of β cell death in a successful trial of teplizumab showed that the immune therapy decreased β cell killing but it remained higher than levels seen in healthy control subjects (104). There is little mechanistic insight into the basis for long term β cell failure in T1D. Understanding this process would guide the use of therapies that may have a more sustained benefit.

Conclusions

In summary, the need for successful treatments and prevention of T1D persists. There have been many advances in understanding the immune and cellular biology of T1D. Translating these findings to clinical studies to achieve a clinically meaningful reversal or prevention of disease has been challenging but there have been some successes. The future development of therapies require understanding of the mechanisms responsible for disease combinations of agents that can address pathologic mechanisms involving tolerance induction, immune responses, β cell survival and regeneration, as well as optimal trial design.

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Highlights of “Progress and challenges for treating Type 1 diabetes”

- There has been considerable progress in understanding the genetics, molecular, and cellular immune mechanisms that lead to Type 1 diabetes through studies of rodent models and patients
- Some clinical trials have successfully modified the natural progression of the disease. However none have resulted in permanent remission
- Combinations of therapies, identification of individual differences in responses to therapy, careful timing of therapy may improve the long term outcomes of trials to treat new onset disease or in prevention

Figure 1. Genetic loci associated with Type 1 diabetes

The diagram illustrates where loci, identified through GWAS analyses may affect immune responses. The MHC is the loci with the highest risk of the disease. The INS-VNTR may affect expression of insulin by medullary thymic epithelial cells. Insulin reactive T cells may fail to be deleted during negative selection. The association with IL-2 RA may affect signaling in both Tregs and Teffector cells. A polymorphism of the PTPN22 affects signaling through the T cell and B cell (not shown) receptors which may affect signals needed for Teffector and Treg activation as well as purging the repertoire of autoreactive T and B cells during their selection. CTLA-4 is a negative costimulatory molecule. Failure of this negative costimulatory molecule may affect cell activation in the periphery as well as in the thymus. (see text for details)

Figure 2. Patients with T1D display elevated frequencies of autoreactive mature naïve B cells in peripheral blood

Recombinant antibodies cloned from mature naïve B cells from 12 healthy donors (HD) and 12 T1D patients were tested by ELISA for reactivity against HEp-2 human hepatocarcinoma cell line (A) and polyreactivity (B). Antibodies were considered polyreactive when they recognized three different antigens that included double-stranded DNA, insulin and lipopolysaccharide as previously reported (113, 114). (****p<0.0001)

Table 1

Patients meeting treatment goals set by the American Diabetes Association

Age range	ADA treatment goal(7, 8)	% meeting goal*
6-12	7.5	22
13-17	7.5	17
18-25	7	14
>25	7	30

Data from (6).

Table 2

Features of responders at baseline

Parameter	Responders	Reference
Time from diagnosis	Responses to teplizumab and cyclosporine A were greatest in patients who enrolled soon after diagnosis.	(105-107)
Age	Children with new and recent onset T1D show improved responses to rituximab, anti-CD3 mAb, abatacept	(94, 108)
Baseline C-peptide	Responders to cyclosporine A and otelexizumab had higher C-peptide	(107, 109)
Immune markers	The titers of insulin autoantibodies (IAA) were lower in rituximab responders. Cellular markers were significantly different in responses compared to non-responders treated with teplizumab. HLA-DR3 was associated with responses to abatacept.	(110-112)
Metabolic measures	Responders to teplizumab had lower HgbA1c and insulin needs at study entry	(111)