STEM CELLS

TRANSLATIONAL AND CLINICAL RESEARCH

Concise Review: Cell-Based Therapies and Other Non-Traditional Approaches for Type 1 Diabetes

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ABSTRACT

The evolution of Type 1 diabetes (T1D) therapy has been marked by consecutive shifts, from insulin replacement to immunosuppressive drugs and targeted biologics (following the understanding that T1D is an autoimmune disease), and to more disease-specific or patient-oriented approaches such as antigen-specific and cell-based therapies, with a goal to provide efficacy, safety, and long-term protection. At the same time, another important paradigm shift from treatment of new onset T1D patients to prevention in high-risk individuals has taken place, based on the hypothesis that therapeutic approaches deemed sufficiently safe may show better efficacy if applied early enough to maintain endogenous β cell function, a concept supported by many preclinical studies. This new strategy has been made possible by capitalizing on a variety of biomarkers that can more reliably estimate the risk and rate of progression of the disease. More advanced ("omic"-based) biomarkers that also shed light on the underlying contributors of disease for each individual will be helpful to guide the choice of the most appropriate therapies, or combinations thereof. In this review, we present current efforts to stratify patients according to biomarkers and current alternatives to conventional drug-based therapies for T1D, with a special emphasis on cell-based therapies, their status in the clinic and potential for treatment and/or prevention. STEM CELLS *2016;34:809–819*

SIGNIFICANCE STATEMENT

This article summarizes the significance of the paradigm shift in the thinking about the treatment of Type 1 diabetes. Current treatment strategies are now directed toward prevention of disease progression to maintain endogenous beta cell function. The use of immunomodulating strategies including antigen- and cell-based therapies as well as the need to identify new biomarkers that allow a measure of disease stage and time to onset of hyperglycemia for selection of appropriate patients to enroll in prevention trials are discussed.

INTRODUCTION

Type 1 diabetes (T1D), like its more common Type 2 counterpart, has been rising in prevalence and incidence primarily in Western countries [1, 2] (Fig. 1). Insulin replacement therapy has been the primary treatment of all forms of diabetes for almost 100 years, but inadequate control of its delivery has allowed a number of complications to markedly diminish the quality of life of affected individuals, and contributed to an increasingly intolerable financial burden. The realization that a subset of patients presents with an autoimmune form of insulin-dependent diabetes was made in the 1970s [3]. The initial model suggesting the potential pathogenesis of this disorder as a chronic autoimmune disease directed against β cells was proposed by George Eisenbarth in the mid-1980s [4]. This understanding led to subsequent attempts to develop more specific treatments for this autoimmune form of diabetes, initially with immunosuppressive therapies that had proven effective in other chronic autoimmune diseases, including cyclosporine A (CsA) or anti-thymocyte globulin and prednisone [5–7]. Despite initial suggestion of efficacy of CsA, no subsequent study has been able to confirm these initial results. In addition, the lack of lasting effects once CsA was withdrawn and the serious renal toxicity of the drug severely limited enthusiasm for this approach.

Subsequent natural history studies have made the approach to treatment more complex as these studies have demonstrated that underlying autoimmune responses are present for varying periods of time, usually years, in genetically predisposed individuals before the

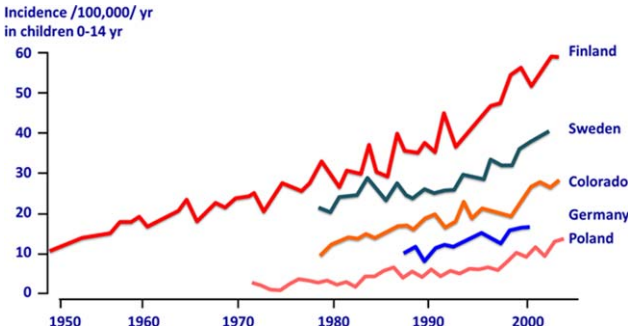

Figure 1. T1D incidence has doubled every 20 years. Data for Finland are from the Finnish National Public Health Institute; data for Sweden are from the Swedish Childhood Diabetes Registry; data for Colorado are from the Colorado IDDM Registry, the Barbara Davis Center for Childhood Diabetes, and SEARCH for Diabetes in Youth; data for Germany are a compilation of two reports; and data from Poland are from Diabetologia 2010;54:508-515. Reprinted with permission from the Ann NY Acad Sci 2008;1150:1-13, with additional modifications and permission from Marian Rewers and Jay Skyler.

appearance of overt hyperglycemia. Particular major histocompatibility class II haplotypes (HLA-DR3/4, HLA-DQ8) confer the greatest risk as genetic factors [8]. In addition to serving as a diagnosis tool for T1D in new onset diabetic patients, circulating autoantibodies against β cell proteins (specificity and quantity) in at-risk individuals, as well as abnormalities in the oral glucose tolerance test, can generally help predict the risk of, and time remaining, before the onset of hyperglycemia [9]. These predictive data have raised the possibility of attempting therapeutic intervention before the onset of hyperglycemia in high-risk individuals identified based on biomarkers like those mentioned above. Prevention of T1D may represent a viable alternative to an actual cure by permanently blocking the autoimmune response while there are sufficient β cells remaining, and may offer a more cost-effective approach in the short-term to deal with the alarming rise in the incidence of disease (Fig. 1). To make the matter even more complex, many patients may present a spontaneous but temporary remission after onset, known as the honeymoon period, possibly reflecting reduced stress on residual β cells after initial insulin treatment. This honeymoon period may perhaps represent a sweet window of opportunity (pun intended) to exploit for the use of intervention therapy.

In this review, we will discuss how our therapeutic arsenal to fend off this autoimmune disease has greatly diversified beyond traditional drugs and biologicals to include various forms of cell therapies, as well as other less conventional approaches. The field is witnessing a paradigm shift from immunosuppressive therapies applied after the onset of hyperglycemia (a time at which β cell function has generally been irreversibly lost) to prevention strategies attempting to shut off the autoimmune response and preserve β cell function in high-risk individuals. This of course entails the use of reliable biomarkers to identify the most appropriate at-risk subjects for such intervention trials, and perhaps also guide the type of therapy that should be employed, paving the way to more personalized therapies. Current studies using new techniques of transcriptomics and proteomics [10–13] are attempting to more precisely stratify those at risk by identifying novel biomarkers that may be superior to those currently used to define the stage or rate of progression of disease, and thus help select appropriate subjects to enter into prevention trials. Although the strategies described in this review have all shown remarkable efficacy in preclinical models, it should be noted that little or no clinical efficacy data is available for most of them, whether they are evaluated in the treatment of recent onset patients after safety has been demonstrated or in prevention studies following safe but ineffective use in recent onset patients.

A Shift from Treatment to Prevention Is Driven by Biomarkers Guiding When and How to Intervene

The progress in identifying the patients who are at "high-risk" and should be entered into prevention trials has been supported by an improved understanding of T1D disease processes that allows screening for at-risk individuals and stratification of the individual's risk and time of progression to the development of hyperglycemia. The first level of screening is comprised of family history (number of relatives with T1D and degree of relationship) and HLA haplotype (HLA-DR3/4 heterozygosy combined with HLA-DQ8 conferring the highest known risk) [9]. Although these risk factors are fixed from birth, new relatives may become diagnosed later and the relative risk re-evaluated. These parameters have served to enroll young subjects into studies on how environmental factors influence disease progression (e.g., primary prevention studies examining diet alterations in genetically at-risk babies with no evidence of autoimmunity, Table 1). These individuals can be closely and regularly monitored and undergo a second level of screening consisting of well-established biomarkers such as circulating autoantibodies to β cell antigens insulin, GAD65, IA-2, ZnT8, and IGRP [9, 14], which have served as good predictive tools [15–18] and enrollment criteria for prevention studies. In vitro immunoassays performed on peripheral blood cells including T cell responses to β cell antigens or identification of diabetogenic T cells by tetramer staining complete this assessment of the breadth (how many autoantigens targeted) and amplitude (antibody titers or frequency of tetramer-positive T cells) of the autoimmune response [14]. More recently, biomarkers based on epigenetic changes have been discovered, such as circulating demethylated insulin DNA [19, 20] and differences in methylation level at specific CpG sites in immune cells [21]. Increased levels of demethylated insulin DNA in the blood correlates with the extent of β cell damage [19], while the extent of insulitis may now be evaluated by refined imaging techniques [22].

These biomarkers of prediction are important to evaluate disease risk and rate of progression (indicating with a high degree of confidence if and approximately when a patient will progress to overt hyperglycemia unless the course of progression is altered by treatment), and, therefore, to determine when to treat. Individuals with a comparable risk level may experience a different rate of progression, according to their genetic makeup (other genes besides HLA) and their environment, which differentially affect mechanisms of immune tolerance and pathogenesis. As environmental factors change, the rate of progression may increase or decrease, placing the subject at greater risk or slowing down the development of the

Table 1. Main clinical trials focused on the prevention of T1D

Prevention trials	Drug	Type of study
Diet/supplement-based prevention		
NCT01055080 (FINDIA)	Baby diet alteration	Phase 1, primary prevention
NCT00570102 (MIP)	Baby diet alteration	Phase 2, primary prevention
NCT01115621 (BABYDIET)	Baby diet, delayed gluten	Phase 1, primary prevention
NCT00179777 (TRIGR)	Controlled diet in infants	Phase 2, primary prevention
NCT00333554 (NIDDK)	Omega-3-fatty acids	Phase 2, primary prevention
NCT00141986 (CDA)	Vitamin D	Phase 1, primary prevention
β cell antigen-based prevention		
NCT00004984 (DPT-1)	Parenteral or oral insulin	Phase 2, secondary prevention
NCT00419562 (NIDDK)	Oral insulin	Phase 3, secondary prevention
ISRCTN76104595 (Pre-POINT)	Oral insulin	Phase 1, primary prevention
NCT00654121 (BDR Trial)	Subcut. insulin (Actrapid HM)	Phase 2, secondary prevention
NCT00223613 (DIPP)	Intranasal insulin	Phase 3, secondary prevention
NCT00336674 (INIT-II)	Intranasal insulin	Phase 2, secondary prevention
NCT01122446 (DIAPREV-IT)	Diamyd (GAD-Alum)	Phase 2, secondary prevention
Combinations of the above		
NCT02387164 (DIAPREV-IT2)	Diamyd (GAD-Alum + Vit. D)	Phase 2, secondary prevention
Prevention using biological-based immunotherapy		
NCT01773707 (NIDDK, TN18)	Abatacept (CTLA4-Ig)	Phase 2, secondary prevention
NCT01030861 (NIDDK, TN10)	Teplizumab (anti-CD3)	Phase 2, secondary prevention
Cell-based prevention		
CoRD study (Sydney)	Umbilical cord blood	Phase 1, secondary prevention

Note: Clinical trials are color-shaded based on whether they are completed, ongoing, or planned.

disease (Fig. 2). As a result of multiple etiological factors acting in concert, the disease can progress from very fast in the case of fulminant T1D [23] to very slow in the case of latent autoimmune diabetes in adults (LADA) [24]. However, the most aggressive forms of disease, like fulminant T1D, might not benefit from prevention unless the trigger becomes well understood.

Because of the heterogeneity of etiological factors that may control the rate of progression, it is unlikely that patients stratified as having a similar risk will be equally responsive to a particular treatment (Table 2). Treating a high-risk patient with the wrong drug would cost precious time during which β cells will continue to be destroyed. In prevention studies, as opposed to new onset cases, more time will be needed before it can be determined whether the treatment is effective. Conversely, treating a low-risk patient, who may never advance to onset, even with the right prevention therapy, would involve unnecessary costs and risks. Thus, a third level of screening that is more sophisticated (using novel biomarkers featured in larger datasets) will be required to help determine how best to treat each patient by providing clues as to the underlying defects that need to be acted upon. A combination of genetic, transcriptomic, and proteomic tests performed on blood samples will likely be part of such screening in the future, and extensive research is being conducted to this end. Furthermore, advances in viromics have enabled the development of sensitive blood tests that can detect prior exposure to particular viruses, some of which have long been suspected to play a role as a trigger for the disease in some individuals [44]. If these tests help confirm a link between these pathogens (which are not uncommon, and therefore would not be sufficient to induce T1D), then the prospect of vaccinating genetically at-risk individuals becomes possible.

In recent years, it has become clear that combination therapies, selected to address multiple underlying defects, will become more prominent in our effort to tackle T1D heterogeneity in both prevention and treatment. Such combination therapies are expected to be effective in larger cohorts of patients with overlapping defects. Although many of the immunosuppressive strategies have not been effective when administered post-hyperglycemia, they might be appropriate to use at an earlier stage of disease where they may prove more efficacious. Besides efficacy and safety, the cost will

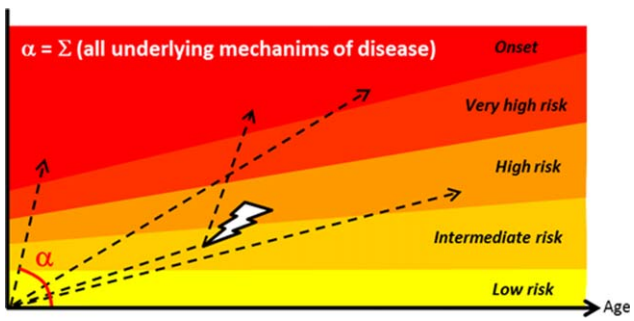

Figure 2. Rates of T1D disease progression. In T1D, earlier onset reflects a faster rate of progression through risk levels (represented by narrower height). The risk level can be evaluated according to family history and HLA haplotype, which are fixed at birth, as well as circulating anti-β cell autoantibodies and certain metabolic measurements, which are dynamic [9]. The rate of progression (α) takes into account all causes and factors that may contribute to pathogenesis, including (but not limited to) defective deletional tolerance, defective immune regulation, defective/delayed clearance of damaged β cells, viral infection (high tropism for β cells and/or molecular mimicry), and β cell intrinsic factors such as susceptibility to infection, apoptosis or dedifferentiation (Table 2). Progression through these stages may not be linear for all individuals, as precipitating events during life (depicted by a thunderbolt) may accelerate the rate, while other environmental changes may curb it. Furthermore, accumulation of genetic (prefixed) factors may cause an individual to start at an intermediate risk, but genetic analysis other than HLA haplotype is not yet routinely performed. Successful prevention of disease will require more advanced tools to evaluate the risk level, the rate of progression, and preferably, the nature of the deficiencies that contribute to the rate of progression (Table 2).

Table 2. Factors contributing to the rate of disease progression

Possible mechanisms (not mutually exclusive)	Possible causes or predisposing genes	Possible biomarkers	Possible therapeutic approach to prevent (and reverse) disease	References
Defective deletional tolerance: higher frequency of β cell-reactive T cells (and B cells)	HLA-DR, INS, PTPN22 polymorphism	HLA-DR, selected SNP analysis, MHC tetramer analysis/ELISPOT	Antigen-specific therapies	[8, 25–29]
Defective immune regulation: reduced number, responsiveness and/or function of regulatory T cells	IL2RA, IL2, CTLA4, IL10, PTPN22 polymorphism Vitamin D and/or fiber deficiency Foxp3 promoter methylation	Treg suppression, TSDR assay, STAT5 responsiveness	Expanding Tregs and/or boosting their function (***in vivo*** or ***ex vivo***); antigen-specific therapies	[8, 25, 30–36]
Antigen-presenting cells: hyperactivity under inflammatory conditions or defective tolerogenic properties	Genetic predispositions? Environmental factors?	Functional characterization of certain blood cells?	Blockade of specific costimulatory pathways or cytokines	[37, 38]
Response of β cells: apoptosis, stress ($\pm$ generation of neo-antigens), de-differentiation or trans-differentiation	IFIH1 polymorphism, inflammation, some unknown genetic determinants, inability to cope with excessive stress	Selected SNPs, circulating demethylated insulin DNA (also reflects β cell immune destruction); impaired glucose tolerance	Drugs increasing β cell replication, reducing β cell stress (imatinib?), or stabilizing β cell phenotype Anti-inflammatory drugs?	[20, 39–41]
Defective/delayed clearance of damaged β cells / β cell antigens (disease initiation)	Genetic predispositions?	None (too early to detect)?	None at the moment	[42, 43]
Interaction with microbes: molecular mimicry (cross-reactivity with β cell antigens), immune deviation or dysregulation	HLA-DR, IFIH1, TLR7/8 polymorphism; Infection (e.g., enterovirus); Dysbiosis (imbalanced microbiome)	Infection history (difficult) Microbiome profiling	Probiotics? Vaccines? Anti-inflammatory drugs?	[8, 35, 44–48]

Combinations of genetic and environmental factors contribute to the initiation and progression of T1D disease, leading to various deficiencies at the level of both immune cells and β cells. Gene-wide association studies have identified some 40 gene polymorphisms each contributing a small risk to the disease, some of which are listed here. The more deficiencies that exist in a particular individual, the faster the progression is expected to be. However, different patients are characterized by different combinations of such deficiencies, leading to substantial heterogeneity in how they progress and how rapidly. Biomarkers that assess not only the risk but also the underlying deficiencies will help inform the choice of prevention therapies to be applied to more homogeneous cohorts of patients for better efficacy. References are only provided as examples to illustrate the concepts.

need to be leveraged against the long-term benefits. Expensive therapies that induce durable tolerance and protection may, however, save money in the long run. It is unusual for new drugs to be approved for prevention of disease without prior testing in new-onset patients: the immunomodulatory drugs and cell-based therapies described below are no exception to this rule.

Overview of the Current Landscape of Non-Cell Therapies

Cell- or Pathway-Neutralizing Biologics

Regulatory authorities have historically prioritized treatment of new onset patients because these cases are more pressing, the risk tolerance greater, and the studies shorter, smaller, and less expensive. In an attempt to replace the use of globally immunosuppressive drugs, a number of promising biologics have been evaluated in new onset patients, such as anti-CD3 mAb [49–51], anti-CD20 mAb [52], CTLA4-Ig [53]. These drugs showed significant but limited (transient) efficacy in new-onset patients. These drugs are now being tested in high-risk normoglycemic patients where they might have a more pronounced and durable effect in sustaining euglycemia (Table 1). These drugs were deemed safe enough by the US Food and Drug Administration to be used prophylactically at a dose unlikely to cause serious adverse events. Current studies using such biologics to prevent disease in high-risk patients include two major TrialNet studies: TN10 (ClinicalTrial.gov NCT01030861) using Teplizumab (anti-CD3 mAb) and TN18 (ClinicalTrial.gov NCT01773707) using Abatacept (CTLA4-Ig). Many other biologics used to treat other autoimmune diseases are also being evaluated for T1D. However, these drugs remain relatively nonspecific and may still carry accrued risks of infections or malignancies in susceptible subjects.

Low Dose IL-2: A Safer and More Selective Approach?

Because of different sensitivities, it was found that regulatory T cells (Tregs), a subset of T cells that protects from autoimmunity, are selectively stimulated by low doses of IL-2, a T cell growth factor [54]. This new approach is particularly noteworthy because of its safety profile, based on several published studies on the use of low dose IL-2 to treat inflammatory diseases including chronic graft-versus-host disease (GvHD) [55–57] and hepatitis C virus-induced vasculitis [58, 59], and preliminary studies on its use as a potential treatment of T1D [60]. In addition, preclinical studies in non-obese diabetic (NOD) mice showed that low dose IL-2 administered after onset of hyperglycemia restored euglycemia in a majority of the treated mice [61]. In each instance, low dose IL-2 therapy was associated with a dose-dependent increase in the number of circulating Tregs and a marked diminution of inflammatory cytokine expression in the serum of the

Table 3. Main clinical trials using low-dose IL-2 or cell-based therapies in recent onset T1D patients

New onset trials	Drug	Type of study
Low-dose IL-2		
NCT01827735 (DILT1D)	Proleukin (IL-2)	Phase 1/2, onset $<$ 24 months
NCT02265809 (DILfrequency)	Aldesleukin (IL-2)	Phase 1/2, onset $<$ 60 months
NCT01353833 (DF-IL2)	Aldesleukin (IL-2)	Phase 1/2, onset $<$ 24 months
NCT01862120 (DFIL2-Child)	IL-2	Phase 2, recent onset
NCT02411253 (DIABIL-2)	rhIL-2	Phase 2, recent onset
Cell-based therapies		
ISRCTN06128462 (Gdansk)	Polyclonal Tregs	Phase 1, onset $<$ 2 months
NCT01210664 (UCSF)	Polyclonal Tregs	Phase 1, onset 3-24 months
NCT00445913 (Pittsburgh)	Autologous DCs	Phase 1, long-term T1D (5y+)
NCT02354911 (Pittsburgh)	Autologous DCs	Phase 2, new onset $<$ 100d
NCT01068951 (Uppsala)	MSCs	Phase 1, new onset
NCT00690066 (Mesoblast)	Prochymal (MSCs)	Phase 2, onset 2-20 wks
NCT02057211 (Uppsala)	MSCs	Phase 2, new onset $<$ 3 weeks
NCT01322789 (Sao Paulo)	MSCs	Phase 1/2, new onset $<$ 6 weeks
NCT00305344 (Florida)	Umbilical cord blood (UCB)	Phase 1/2, post-onset
NCT00989547 (Munich)	Umbilical cord blood (UCB)	Phase 1, post-onset
NCT01350219 (Tianhe)	UCB-derived stem cells	Phase 2, post-onset
NCT01996228 (Tianhe)	UCB-derived stem cells	Phase 1/2, post-onset
NCT00315133 (Sao Paulo)	Autologous HSCs	Phase 1/2, onset $<$ 12 weeks
NCT01285934 (Northwestern)	Autologous HSCs	Phase 1/2, onset $<$ 5 months

Note: Clinical trials are color-shaded based on whether they are completed, ongoing, or planned. Stem cells used for the generation of new β cells are not covered here. DCs: dendritic cells; HSCs: hematopoietic stem cells; MSCs: mesenchymal stem/stromal cells; Tregs: regulatory T cells.

treated mice or patients in the initial short-term safety trial [60]. An additional dose finding study to determine the optimal dose of IL-2 required to increase the number and response of Tregs has been completed in T1D patients (ClinicalTrial.gov NCT01827735). Subsequently, an efficacy trial has begun in patients with recent onset T1D (ClinicalTrial.gov NCT01862120). Once these studies (in Table 3) are completed and the safety profile confirmed, a move to recruit high-risk patients in low dose IL-2 studies will be expected.

However, the potential success of low dose IL-2 therapy in T1D patients rests on two assumptions: (i) Tregs are functionally defective and (ii) IL-2 production is impaired. Studies on whether $CD4^+$ $CD25^+$ Tregs are defective in T1D have yielded conflicting results (decreased frequency [62], decreased function [63], or normal frequency and function [64]), which may reflect inadequate identification of Tregs by available markers; recruitment of patients different in age and disease progression and differences in experimental conditions. Follow-up studies using more specific markers ($FOXP3^+$ $CD127^{low}$ and demethylation of regulatory elements of the *FOXP3* gene) showed that both the frequency and function of Tregs are normal in the blood of T1D patients, even though a transient decrease of suppressor activity may occur early after diagnosis [65], and in a subset of T1D patients [30]. Studies from the Battaglia lab showed that reduced suppressive function of Tregs may be restricted to the pancreatic lymph nodes in patients with long lasting T1D [31]. A defect in IL-2 production by total peripheral blood mononuclear cells of patients with new onset T1D was reported several years ago [66] but never confirmed as a key immunological feature of T1D patients. A recent study showed that the T1D-susceptibility IL2RA haplotype identified by rs12722495 is associated with decreased signaling via the IL-2 pathway in both memory T cells and Tregs and that this is linked to diminished Treg function [32]. However, this phenotype is limited to carriers of this single nucleotide polymorphism (SNP) and not to all individuals. Thus, it is likely that this treatment may benefit some patients more than others, again based on their underlying defects that contribute to disease.

A Wide Array of Approaches to Reestablish Antigen-Specific Tolerance

The overall objective of this strategy is to deliver β cell antigens in particular ways such that their presentation in vivo results in elimination or inactivation of antigen-specific diabetogenic T cells, or induction of antigen-specific immunoregulatory populations, to confer durable protection from autoimmunity without compromising the general immunosurveillance for infectious agents and malignant cells. The traditional method has been to administer protein antigens via tolerogenic routes (mainly oral or intranasal insulin and GAD65/Alum), but this approach has not produced significant clinical benefit in recent onset patients [67]. Because of lack of adverse side effects, these therapies are now being tested in secondary prevention trials (i.e., in patients with ongoing autoimmunity evidenced by circulating autoantibodies) (Table 1). It is worth pointing out that oral insulin has also been tested in a primary prevention trial (in young subject with no evidence of autoimmunity, Pre-POINT trial, Table 1) and data suggest that insulin-specific Tregs were induced at the highest dose [68]. Antigens coupled with apoptotic cells have been known for several decades to be very tolerogenic and showed efficacy in preclinical models of T1D [69]. This strategy has now been tested in patients with multiple sclerosis and was well tolerated [70]. Massive apoptosis resulting from depletion of B cells and $CD8^+$ T cells (using a short course of biologics) is accompanied by release of TGF-β, which combined with exogenous antigens such as GAD65 peptides, supports the generation of protective Tregs, because $CD4^+$ T cells are left untouched and available for conversion [71]. This promising approach validated in mouse models of T1D and multiple sclerosis remains to be tested for safety in humans.

A less conventional alternative to protein antigen delivery lets the body produce specific antigens in cells or sites

amenable for tolerance induction following gene transfer [72]. Plasmid DNA encoding autoantigens such as insulin or its $InsB_{9\text{-}23}$ immunodominant peptide prevented disease in NOD mice [73–75] and was given to recent-onset T1D patients in a phase 1 trial [76]. Data from this trial demonstrated both safety and diminution of insulin-reactive $CD8^+$ T cells, thus tolerogenic DNA vaccines merit consideration for prevention trials. Delivery of autoantigens by viral vectors used for gene therapy has also been explored [77, 78]. One legitimate concern when using viral components is the inadvertent activation of antigen-specific effector T cells that could exacerbate β cell autoimmunity, especially if expression with ubiquitous promoters is allowed in professional antigen-presenting cells (APCs) than can mature and become immunogenic [79]. The insertion of a microRNA-142 target sequence to abrogate transgene expression in professional APCs and other hematopoietic lineage cells, together with the use of a liver-specific promoter resulted in a lentiviral vector, which specifically targets the antigens to hepatocytes [79, 80]. Treatment with such a vector encoding the $InsB_{9\text{-}23}$ peptide-induced some antigen-specific effector T cells but also antigen-specific $CD4^+$ $FoxP3^+$ Tregs, which halted islet immune cell infiltration, and protected mice from T1D [79]. When combined with a single suboptimal dose of anti-CD3 mAb, it was effective in reversing hyperglycemia after onset in a Treg-dependent manner [79]. The use of nonintegrating forms of lentiviral vectors will offer an additional level of safety when implementing such an approach clinically [81].

So far, antigen-specific therapies for T1D have been proved efficient in mouse models and to be among the safest in patients, but evidence for clinical efficacy is lacking. One possible reason may have to do with the choice of β cell antigens used, which is limited in two aspects: (1) only a single antigen (mostly insulin or GAD65) is used despite the evidence of epitope spreading reflected by different types of autoantibodies, and (2) only native antigens are used while it has become increasingly clear that many diabetogenic T cells respond to post-translationally modified or processed neo antigens. Accomplishing long-term antigen-specific tolerance, whether it is with Tregs or other regulatory cells will require these issues to be addressed.

Cell-Based Therapies: Current and Future Applications

Cell-based therapies are individualized approaches that currently involve the transfer of autologous cells that have immunoregulatory properties and can provide a counterbalance for effector T cells that mediate β cell destruction. While certain drugs aim at expanding and potentiating Tregs *in vivo* (see low dose IL-2 above), cell-based therapies generally involve Tregs or cells that have the ability to induce or potentiate such immunoregulatory populations *in vivo*. We will also discuss the use of different types of stem cells as part of cell-based therapies to block autoimmune responses, but we will not cover the generation of new β cells from stem cells for transplantation, which is reviewed elsewhere [82].

Regulatory T Cells (Tregs)

Several preclinical animal studies have established that the adoptive transfer of Tregs can prevent various autoimmune diseases, T1D included. However, only a few studies showed that Treg cell transfer is efficacious in reverting active disease and, when it occurred, the transferred cells needed to be antigen-specific [83–85]. Based on this evidence, Tregs are now being used in phase 1/2 studies in patients with autoimmune diseases [86]. Increasing doses of autologous *ex vivo*–expanded polyclonal $CD4^+CD25^+$ Tregs have been used safely in newly diagnosed T1D patients [87]. These studies were instrumental in demonstrating the safety and feasibility of such a complex approach of personalized medicine, but efficacy has still to be demonstrated in larger trials.

Based on the data in animal models, to be of any therapeutic use, Tregs have to be transferred prior to overt hyperglycemia or have to be antigen-specific. It has been, up to now, difficult to obtain sufficient antigen-specific $CD4^+$ $FoxP3^+$ Tregs, but this might be more feasible by inducing Tregs de novo in vitro. Such an approach has been used in the context of allogeneic hematopoietic stem cell transplantation to prevent GvHD where host-specific Tr1 cells were generated in vitro from donor peripheral blood and transferred to transplanted hosts [88]. The induction of self-specific Tr1 cells in NOD mice *in vivo* is feasible and they protect from diabetes development [89]. However, the generation of human diabetes-related antigen-specific Tregs in vitro has yet to be achieved. Studies on adoptive T cell therapy have demonstrated the possibility of engineering T cells using lentivirus, either by expressing a relevant (β cell antigen-specific) T cell receptor into polyclonal Tregs [90] or by overexpressing FoxP3 in T cells [91], potentially in antigen-specific T cells that have been enriched and *ex vivo* expanded.

Regulatory B Cells (Bregs)

Recently, the IL-10–producing regulatory Bregs have attracted attention as being altered in autoimmune diseases and thus represent another potential tool for cell therapy [92]. As with Tregs, their numbers and function might be compromised in T1D patients [93], indicating the possibility of using Breg therapy, alone or in concert with Tregs. However, the importance of antigen-specificity in this case is not clear, and considering that we are still at the beginning of cell therapy with Tregs, using Bregs is even more futuristic.

Dendritic Cells

As the most specialized of APCs, dendritic cells (DCs) have long been a candidate of choice for their ability to engage T cells through presentation of β cell antigens, and under a tolerogenic phenotype, to achieve deletion or inactivation of diabetogenic T cells, converting them into Tregs or restimulating preexisting Tregs. DC infusions have shown remarkable efficacy in numerous preclinical studies, even in the absence of exogenously provided antigens [94]. The first clinical trial using autologous DCs in recent onset T1D patients demonstrated both safety and the potential to induce Bregs [95]. In this phase 1 study, the first of its kind for the treatment of autoimmune diseases, the monocyte-derived DCs were locked in a nonimmunogenic state by silencing important costimulatory molecules (CD40, CD80, and CD86), but were not provided exogenous antigen. Autoantigen expression and maturation stage are two crucial considerations, because immunogenic DCs expressing β cell antigens could boost autoimmune T cell

Table 4. Phenotype of MSCs used in T1D studies

Source	CD11c	CD14	CD29	CD31	CD34	CD44	CD45	CD73	CD90	CD105	Reference
Mouse bone marrow	−	−	+	−		+	−	+		+	[104]
Mouse bone marrow			+			+	−	+	−	+	[108]
Mouse adipose tissue	−				−	+	−	+	+	+	[105]
Human bone marrow		−		−	−		−	+	+	+	[109]
Human bone marrow		−	+		−	+	−	+	+	+	[*]
Human adipose tissue							−	+	+		[110]

Note: Additional characterization may include ability to terminally differentiate (e.g., adipocytes) or to suppress T cell responses. [*] Prochymal MSCs (NCT00690066).

responses against β cells. The antigen-specific therapies previously described rely on the acquisition and presentation of relevant antigens by tolerogenic APCs that are not that well characterized but possibly comprising different subsets of DCs and other types of APCs. In the case of the recent DC trial, the mechanism of action is not completely understood as no exogenous antigen was provided, and whether these DCs could pick up and present relevant autoantigens *in vivo* remains unclear. Furthermore, there is evidence that DCs expressing costimulatory molecules but not inflammatory cytokines (termed semimature DCs) may induce tolerance as well [96].

Another phase 1 trial employing tolerogenic DCs pulsed with citrullinated peptides for the treatment of rheumatoid arthritis has demonstrated safety as well as immunological responses reflective of regulation [97], suggesting that provision of autoantigens may not lead to exacerbated responses as long as the DCs are maintained tolerogenic, which in this case was achieved by pretreatment with an NF-κB inhibitor. An alternative or complementary approach to silencing the expression of costimulatory genes is the overexpression of tolerogenic products for which the list is long and includes immunoregulatory cytokines, inhibitory ligands, and metabolism-altering enzymes [98]. A safe and clinically viable way to overexpress genes and achieve a significant therapeutic outcome is by mRNA electroporation, which has been used widely for autologous DC therapy in cell therapy of cancer [99, 100]. This method of modification allows for coexpression of multiple products of interest in the same cell and at the same time, including relevant antigens for added specificity if desired [101]. Although transient, expression of genes in DCs by mRNA is sufficient to induce long-lasting responses resulting in prevention of T1D in mice [102]. A follow-up trial of the first safety study of autologous DCs in T1D patients is set to begin in the near future.

Mesenchymal Stem/Stromal Cells

Mesenchymal stem/stromal cells (MSCs) are endowed with regenerative and immunosuppressive properties that have fueled their popularity in cell therapy, yet controversies remain regarding their name and definition [103]. Although they can generally suppress immune responses on their own in a nonspecific manner, they have also been shown to induce or expand Tregs, including in preclinical T1D studies [104–106]. Because MSCs are nonprofessional APCs, it is unclear if and how they specifically interact with Tregs and diabetogenic T cells, and their effect may be indirect through inflammation relief [107]. Unlike DCs, MSCs are nonimmunogenic, and can provide protection even in an allogeneic host, which makes them attractive for the clinic [108]. They have been proven to be well-tolerated in T1D patients whether they were isolated by bone marrow aspiration [109] or from adipose tissue [110], and associated with improvement of disease parameters such as C-peptide preservation. Careful characterization of the phenotype and properties of MSCs used in cell therapy is crucial to demonstrate consistency between studies and draw meaningful conclusions, regardless of the source of the cells, isolation, and culture conditions (Table 4). Two follow-up clinical trials are currently recruiting in Sweden and Brazil to demonstrate long-term efficacy (Table 3). All trials so far are being conducted in recently diagnosed T1D patients using MSCs that are either autologous or from first-degree relatives. Although no serious side effects have been reported so far, there remains a concern that slow growing tumors may appear in the long term in some patients receiving autologous cells, according to some preclinical studies [108].

Umbilical Cord Blood

Although this approach is limited to a few individuals who have banked samples, umbilical cord blood (UCB) is a great source of abundant MSCs and Tregs, which might work in synergy when infused into patients. As many important self-reactive Tregs appear to be released early in life [111], these Tregs may also include more antigen-specific Tregs of relevance. Autologous UCB infusion was found to be safe but did not have any significant therapeutic effect despite increased numbers of Tregs [112, 113] and even with oral docosahexaenoic acid and vitamin D supplementation [114]. Another phase 1 trial, also in new onset pediatric subjects, is well under way in Germany (Table 3). As previously mentioned, it is possible that therapies involving Tregs would be more effective when applied prior to disease onset. In that model, the Cord Reinfusion in Diabetes (CoRD) study, enrolling high-risk children with banked UCB, was recently initiated in Australia and represents the first cell-based therapy used for secondary prevention of diabetes [115]. A distinct process is being tested in China, whereby lymphocytes are obtained from the blood by leukapheresis, "reeducated" *ex vivo* in contact with UCB-derived stem cells, and then reinfused into the patient. These studies suggest that this treatment improved preservation of β cell function without notable adverse effects, but caution must be exercised in the interpretation of these studies, which were improperly controlled [116].

Hematopoietic Stem Cells

Perhaps the most drastic of all cell therapies consists of a major immunological reset with the transient ablation of

circulating T cells and their replacement with hematopoietic stem cells (HSCs). Multiple completed studies have involved autologous HSCs mobilized from peripheral blood and administered after a nonmyeloablative regimen consisting of cyclophosphamide and anti-thymocyte globulin [117–119]. This treatment performed in new-onset T1D patients demonstrated a remarkable ability to normalize glycemia in a majority of the subjects. Independence from insulin lasted between several months and several years, up to 3-4 years (as reported in the last meeting of the Immunology of Diabetes Society), and a larger trial is now enrolling patients (ClinicalTrial.gov NCT01285934). However, this therapy is fraught with considerable side effects associated with the nonmyeloablative regimen [117–119], which make this approach unattractive in its current form to many prospective patients and precludes its use for prevention. Furthermore, the contribution of HSCs in maintaining normoglycemia is unclear as the immunosuppressive effect of the regimen may account for part if not all of the therapeutic benefit. Finally, new T cells (and other immune cells) generated from autologous HSCs would still carry any inherent genetic defects that may play a role in disease etiology. Transplantation of bone marrow-derived allogeneic HSCs with induction of mixed chimerism has also been tested in a minority of patients with autoimmunity, including T1D [120]. The use of HSCs in combination with islet transplant to induce chimerism and immunological tolerance has been tested in a recent trial at the University of Miami based on campath-1H and infusion of donor $CD34^+$ HSCs (ClinicalTrials.gov NCT00315614), but did not show any significant benefit. Although allogeneic HSCs from a compatible and healthy donor may help correct some genetic abnormalities, this must be preceded by high doses of chemotherapy and radiation to ablate the patient's bone marrow and is followed by prolonged immunosuppression to prevent GvHD. The consequent transplant-related morbidity and mortality limit this approach to patients with concomitant hematological malignancies [121]. It should be noted that preclinical data with purified allogeneic $CD34^+$ $CD90^+$ HSCs showed complete reversion of T1D in the absence of GvHD [122]. In addition, novel biologicals are under investigation for use as safer and less toxic drugs to myeloablate the patient's bone marrow [123]. Thus, as safer and more effective HSC transplantation protocols become available, allogeneic HSCs might also be indicated in T1D. A more detailed review of the different applications of stem cells (including MSCs and UCB) to treat T1D can be found elsewhere [124].

In parallel to efforts in generating insulin-producing cells from stem cells (embryonic stem cells or induced pluripotent stem cells) [82], there is an expanded interest in growing tissues specialized in tolerance induction, such as thymic tissue [125, 126]. Although much can be learned from these studies, the clinical implementation of such advances is elusive, including where and how to implant the new tissue.

Conclusion

A variety of original therapeutic strategies for treating or preventing T1D have emerged in the past decade, with the latest approaches clearly dominated by cell-based therapies. As the least expensive and most conventional therapies have failed to deliver efficient and durable protection from diabetogenic immune responses, testing of more expensive, and individualized therapies has become justified as long as preclinical studies indicate a strong prospect of durable efficacy achieved with a minimal number of treatments. Strategies that are more antigen-specific and less immunosuppressive tend to have the best safety profile, and their poor efficacy in new onset patients should not discourage evaluation in prevention trials in high-risk patients in which they might perform surprisingly well. The enrollment of subjects for prevention studies should be guided by more refined biomarkers, which may help the diabetes community to better understand the underlying defects behind the autoimmune response in each patient, and better tailor the treatment type, dose, and timing. When appropriate, two or more of these therapies may be combined in order to address multiple defects and benefit a larger number of patients. A clear advantage of cell-based therapies is that they can perform multiple tasks. For example, one can envision tolerogenic APCs engineered to express selected β cell antigens (to specifically engage diabetogenic T cells), additional immunoregulatory ligands or cytokines (to potentiate T cell deletion or Treg induction), homing molecules (for targeting to inflamed islets or their draining lymph nodes), anti-inflammatory cytokines (to quench inflammation), and even growth factors to promote β cell replication.

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Author Contributions

All authors wrote and edited the manuscript

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