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Advances in immunotherapy of type I diabetes

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ABSTRACT

Type 1 diabetes mellitus (T1DM) is an autoimmune disease affecting 3 million individuals in the U.S. The pathogenesis of T1DM is driven by immune-mediated destruction of pancreatic β cells, the source of glucose regulator insulin. While T1DM can be successfully managed with insulin replacement therapy, approaches that can modify the underlying immuno-pathology of β cell destruction has been long sought after. Immunotherapy can attenuate T cell responses against β cell antigens. Given the detailed cellular and molecular definitions of T1DM immune responses, rational immunomodulation can be and have been developed in mouse models, and in some instances, tested in humans. The possibility of identifying individuals who are predisposed to T1DM through genotyping lend to the possibility of preventive vaccines. While much has been accomplished in delineating the mechanisms of immunotherapies, some of which are being tested in humans, long-term preservation of β cells and insulin independency has not been achieved. In this regard, the drug delivery field has much to offer in maximizing the benefits of immune modulators by optimizing spatiotemporal presentation of antigens and co-stimulatory signals. In this review, we attempt to capture the current state of T1DM immunotherapy by highlighting representative studies.

Contents

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1. Introduction

T1DM is an autoimmune disease caused by the immune-mediated progressive destruction of β cells in the pancreatic islets. The etiology of T1DM is multifaceted and has not been fully delineated, but class II HLA alleles and environmental triggers have been implicated [1–3]. The nature of the autoimmunity encompasses both innate and adaptive mechanisms, leading to propagation of autoreactive, antigen-specific T and B cells. T1DM immunotherapy intends to attenuate destructive autoimmune responses, thus halting the progress of autoimmune diabetes. Clinical application of biomarkers that are directly related to T1DM immunopathogenic process includes 1) hyperglycemia measurement, and 2) levels of C-peptide which is a specific indicator for T1DM onset and progression [4]. Indeed, a hallmark of T1DM is the emergence of β cell-destructive autoantibodies against endogenous antigens, which include proinsulin (biosynthetic precursor of insulin), proinsulin C-A junction (connection of C-peptide and A chain of proinsulin), glutamic acid decarboxylase 65 (GAD65, tolerogenic vaccine for T1DM prevention), islet antigen 2 (IA-2), and zinc transporter 8 (ZnT8) [5–9].

1.1. Pathogenesis and immunological mechanisms involved in T1DM

Insidious autoimmune responses against β cells precede the clinical onset of T1DM, at which point 80–90% of the β cell mass are lost. By monitoring autoimmune responses in infants at risk for T1DM shortly after birth, Heninger et al. found that in children who eventually develop autoimmunity, autoantigen-specific $CD4^+$ T cells preceded detectable autoantibodies [2,10]. The pathogenesis of T1DM is characterized by infiltration of islet antigen-specific T cells and proinflammatory antigen presenting cells and concomitant loss of $Foxp3^+$ regulatory T cells (T_{reg}) [11]. The ongoing immune destruction would eventually destroy all β cells within a few years, leading to almost complete loss of ability to produce insulin in the patients. The resulting chronic hyperglycemia induces renal failure and cardiovascular diseases, among others [12,13]. The lack of curative therapies means that T1DM patients must be treated aggressively with insulin replacement therapy. While life-long insulin therapy can alleviate symptoms and delay organ damage, it does not reverse the T and B-cell reactions toward β cells. Although T1DM patients can expect a reasonable quality of life with rigorous insulin regimens, experimental therapeutics have been explored to restore glucose homeostasis.

Allotransplantation of insulin-producing β cells or surrogate insulin-producing β cell mimics have been studied for reversal of T1DM in humans. While progress has been made, these approaches are limited by recognition of the transplanted cells by allospecific and islet antigen-specific T cells and antibodies in recipients. Immunotherapies, on the other hand, broadly speaking, are aimed to modify the underlying trajectory of the disease by deleting the reactive T cells and/or inducing T cell tolerance. A variety of T1DM immunotherapy approaches [14–16] have been in development aimed to prevent or delay T1DM onset in predisposed individuals or preserve insulin production in T1DM patients (Fig. 1).

1.2. Overview of the current status of T1DM immunotherapy

Experimental T1DM immunotherapies can be categorized nonautoantigen-specific and autoantigen-specific interventions. Non-antigen specific approaches include immunosuppressive drugs, T- cell or B-cell depletion, and induction of tolerogenic dendritic cells (DCs) and polyclonal T_{reg}s. Antigen-specific treatments include protein or peptide-based vaccines, adoptive transfer of antigen-specific T_{reg}s, and clonal deletion of effector T cells. Given the complexity of T1DM pathogenesis, combination immunotherapy is favored, due to the perceived notion that simultaneous or synergistic modulation is necessary to correct the autoimmunity [17,18]. Immunotherapies can be combined with standard-of-care insulin replacement therapy, or transplantation of β cells and islets. Advances made in pharmacoengineering and drug delivery technologies have accelerated the testing of immunotherapeutics in T1DM models. Complex functionalities can be engineered into the drug or antigen formulation, allowing fine-tuning of the dosing, timing, and routes of administration [18].

2. Nonautoantigen-specific therapies

Immunotherapy of T1DM in the absence of targeting specific antigens is built upon the premise that enhancing (often global) immune regulatory mechanisms can ameliorate the destructive autoreactive immune responses, including those against β cells. Early attempts in modifying T1DM pathogenesis had employed nonspecific immunosuppressants as global anti-inflammatory agents [19]. While inhibition of β cell destruction was observed in some cases [20], the efficacy was often transient, with any benefits outweighed by severe immunosuppression and toxicities in long-term treatment.

T1DM is associated with class II MHC alleles, with HLA-DQ8 implicated in humans [21] and $I—Ag^7$ in mice [22]. The conserved charged Asp at position 57 in the β chain is substituted with a neutral amino acid in these diabetogenic alleles. Because Asp57 is located in the cleft of the peptide-binding groove, the repertoire of endogenous peptides presented is altered in the diabetogenic alleles because of changes in binding selectivity. Consequently, central tolerance is breached, leading to the escape of autoreactive T cells [23]. An attempt to restore the negative selection was tested in non-obese diabetic (NOD) mice, which express the allele $I—Ag^7$. Mouse bone marrow cells were genetically engineered to express a resistance MHC molecule that has a normally strong binding affinity to autoantigens, and the *ex vivo* engineered cells were then reintroduced into NOD mice [24]. The corrected MHC restored T cell tolerance in the mice. This early study may be re-visited using the recently discovered genome editing technologies in Clustered Regularly Interspaced Short Palindromic Repeats-associated protein (CRISPR-cas) systems in advancing the approach by targeted correction of the mutation in the MHC-II locus.

2.1. T_{reg} cell therapy

The most promising immunotherapy for autoimmune immune diseases tested in humans is autologous T_{reg} cell therapy [25]. Animals studies have shown that $CD4^+Foxp3^+$ T_{reg}s can induce tolerance by inhibiting the functions of Th1-type T cells and DCs. These cells form the main mechanism of peripheral tolerance [26]. Since T_{reg}s are impaired in T1DM, it was hypothesized that the immune balance could be restored, thereby protecting β cells from destruction. In a recent phase I clinical trial, Bluestone and co-workers demonstrated the principle and safety of this approach [25]. In this study polyclonal T_{reg} cells (polyT_{reg}) ($CD4^+CD25^+CD127^+Foxp3^+$) were sorted from T1DM patients and expanded *ex vivo* during which the polyclonal T cell receptors were retained and the functional activity of these T cells was preserved. The expanded autologous polyT_{reg} cells were then transferred back into the patients from whom the cells were isolated. It was found that a significant fraction of the polyT_{reg} cells infused survived for months (Fig. 2). Monitoring the C-peptide levels demonstrated the efficacious immune regulation of polyT_{reg}s against β cell-targeted autoimmune responses. Despite the large number of polyT_{reg}s (0.05 to 26×10^8 cells) infused, no serious side effects were observed. The drawbacks, however, are that *ex vivo*-expanded autologous T_{reg}s can exhibit functional instability and that the manufacturing process is costly and laborious. In addition, there are indications that antigen-specific T_{reg}s may confer superior islet protection [27]. Nevertheless, autologous polyT_{reg} cells remain as a promising therapy for T1DM.

A cell-free alternative is to induce T_{reg} expansion *in vivo* using IL-2. High-dose IL-2 therapy has been used for many years to treat renal carcinoma and melanoma by stimulating cytotoxic T cells [28]. At low dose, IL-2 stabilizes and expand T_{reg} cells [29]. Convincing data from animal

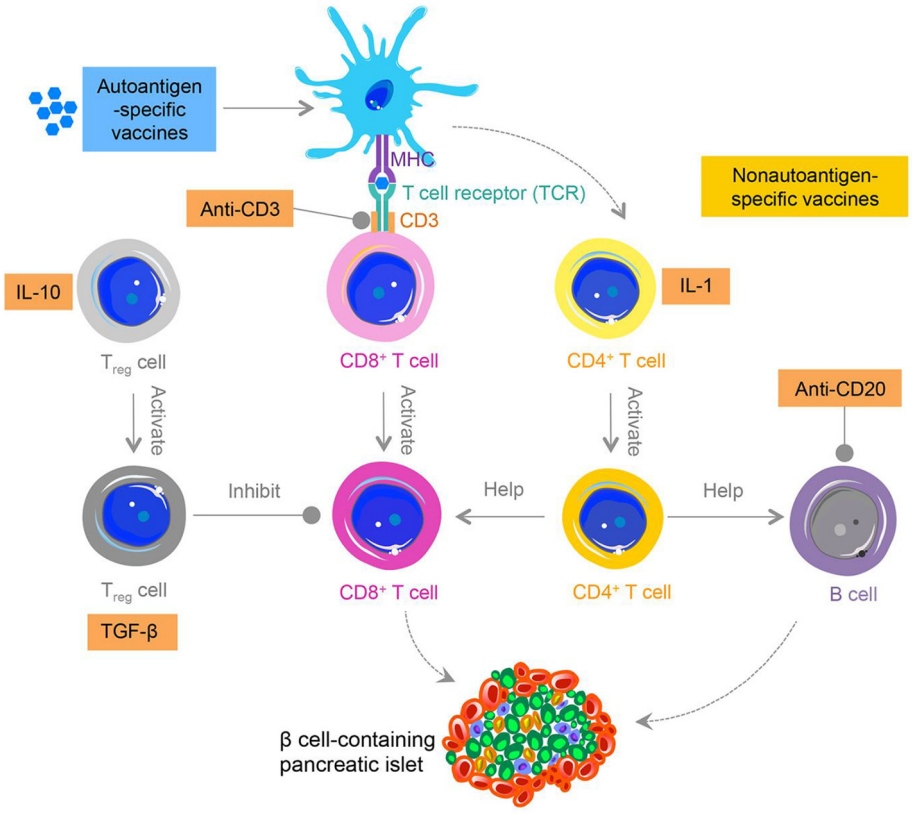

Fig. 1. Roadmap for immune mechanisms and cell-based interventions involved in T1DM. Cross-presentation of β cell specific autoantigens between MHC and TCR enables activation and proliferation of β cell-reactive T cells. Once activated, these autoreactive T cells are responsible for the greatest β cell damage and destruction by inducing β cell apoptosis. The absence of interaction between MHC and TCR on DCs and $CD8^+$ T cells using anti-CD3 will trigger function silencing or apoptosis of $CD8^+$ T cells. T_{reg} cells are also involved in modulating T1DM pathogenesis, and increased numbers of T_{reg}s intensify the inhibition of autoreactive T cells against pancreatic islet β cells. Autoantigen-specific therapies aim to restore tolerance between T_{reg} cells and DCs in order for the remission of T1DM. DC-based interventions help induce T_{reg} expansion by producing proinflammatory cytokines such as IL-1 and TNFα. B cells function as APCs and are required for T1DM development. Anti-CD20 treatment will lead to autoreactive B cell depletion. MHC, major histocompatibility complex; TCR, T cell receptor; DC, dendritic cell; T1DM, type 1 diabetes mellitus.

experiments and the long experience of the agent in humans led to the testing of low-dose IL-2 in four patients with T1DM. Low dose IL-2 induced $CD4^+Foxp3^+$ and $CD8^+Foxp3^+$ T_{reg} numbers with effector T cell responses against β cell antigens were suppressed [30]. Nevertheless, translating IL-2 therapy to humans is not straight forward; a phase I trial in T1DM patients with a combined regimen of IL-2 rapamycin showed an increase in T_{reg}s but also transient β cell dysfunction as evidenced in worsening of metabolic data temporarily [31]. A study of 70 T1DM patients showed that low T_{reg} frequency is correlated to dysfunctional IL-2 receptor signaling due to PTPN2 gene polymorphism. This discovery led to the notion PTPN2 genotyping might serve to identify individuals who are more likely to benefit from IL-2 treatment [32].

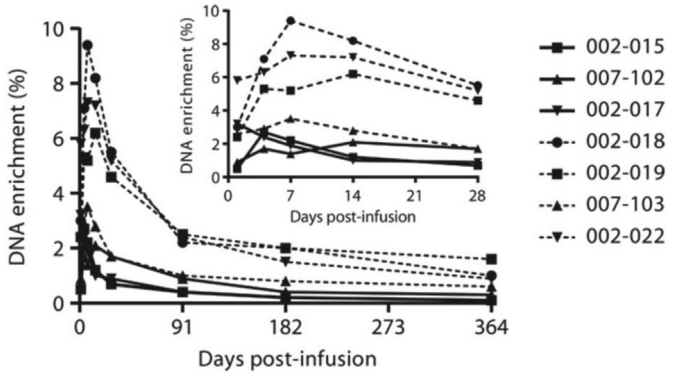

Fig. 2. PolyT_{reg} cells sorted from T1DM patients live up to years as indicated by the amount of DNA enrichment from transferred cells [25]. During *ex vivo* expansion, ^2H was labeled in replicating DNA of polyT_{reg}s. Three subjects (002–015, 007–102, and 002–017) were treated with 3.2×10^8 T_{reg}s, and four subjects (002–018, 002–019, 007–103, and 002–022) were treated with 26×10^8 cells that were about 60% enriched for the ^2H label. The ^2H isotopic enrichment in the genomic DNA of polyT_{reg} cells sorted from peripheral blood was analyzed by gas chromatography–mass spectrometry.

2.2. Depletion of autoreactive T cells

Depletion of T cells has also been studied in T1DM in order to remove the autoreactive population by which β cells are destroyed. As early as 1994, Chatenoud et al. demonstrated in NOD mice that anti-CD3 antibodies could be a promising strategy for T1DM treatment [33]. The anti-CD3 treatment was shown to induce T cell anergy and apoptosis by modulating T cell receptors (TCR) and blocking IL-2 signaling pathway [34]. Unexpectedly, higher numbers of T_{reg}s were detected in subjected treated with low dose anti-CD3, which might be attributed to the cells being less susceptible to antibody-induced apoptosis. A clinical trial in 2002 reported encouraging results in patients who, after receiving anti-CD3 antibody treatment, showed reduced insulin dependency and preserved plasma C-peptide levels for up to 2 years [35–37]. The sustained C-peptide level was correlated to an expanded regulatory T cell population, as evidenced in T-cell-mediated immunosuppression in the NOD mice [38–40]. The adverse events of anti-CD3 treatment were predominantly "Flu-like" symptoms, such as arthralgia, fever, and headaches. In rare instances, viremia and gastrointestinal symptoms were reported, but the patients generally recovered over the course of the therapy. Taken together, these studies could ultimately prove that anti-CD3 antibody treatment is beneficial for potential T1DM therapy [34,41].

2.3. B-cell-targeted therapy

Although T cells have been long considered as the central player in T1DM, recent reports of therapeutic benefits after B cell intervention have been controversial. B cells and plasma cells are involved in β cell destruction *via* production of autoantibodies. Selective depletion of B cells impaired the production of autoantibodies against β cell autoantigens and prevented diabetes in NOD mice, thereby establishing the indispensable role of B cells in T1DM pathogenesis [42]. In a phase II clinical trial, a sustainable C-peptide level preservation and decreased insulin dependency were observed in T1DM patients treated with anti-CD20 antibodies [43]. However, B cell depletion does not appear to completely prevent C-peptide decline or reduce insulin requirements. Thus, it remains to be determined the extent to which B cell depletion (*e.g.* anti-CD20) could be effective in halting T1DM disease progression [44].

2.4. Proinflammatory cytokine-based therapy

Proinflammatory cytokines are implicated in the development of T1DM [45,46]. Apart from recruiting immune cells to inflammatory reaction regions, cytokines can also interfere with cellular transcription and translation process through specific inflammatory signals. As has been confirmed in T1DM by transcriptome analysis, cytokine inhibition therapy induced profound gene expression changes in islet β cells [47]. These and other evidence in animal studies provided the basis for testing cytokine blockade in clinical trials [48]. To date, therapeutic blockade of proinflammatory cytokines have shown to be clinically beneficial in several autoimmune diseases [46]. IL-1α and 1β, key immune mediators secreted by monocytes, have been known to have direct toxic effects on β cells [49]. Anti-IL-1 treatment was initially used in rheumatoid arthritis and cryopyrin diseases found to be well tolerated in patients [50]. Several studies offer mechanistic insights of IL-1 in T1DM progression, such as promoting the differentiation and proliferation of helper T cells, and improving naïve and memory T cell expansion [51]. Consistent with findings in mouse models of T1DM, data from a clinical trial of 15 recent-onset T1DM patients suggested that IL-1 antagonist conferred protection of β cells with reduced insulin dependency [52]. Mechanistically, the effects of anakinra are attributed to proinflammatory signals inhibition by counteracting the IL-1 mediated β cell apoptosis [50]. TNF acts as a potential mediator of autoimmune diseases. Anti-TNF therapy has also been broadly used for the effective treatment of chronic proinflammatory autoimmune diseases such as rheumatoid arthritis and Crohn's disease. Clinical cases of T1DM suggested that patients treated with recombinant TNF receptor-IgG fusion protein (etanercept) had preservation of C-peptides and subtle reduction of glycated hemoglobin. Nevertheless, in NOD mice, the effects of TNF or TNF antagonism are conflicting, for which the responses and outcomes varied depending on the dose and timing at which treatment was given [53]. While the factors that delineate the timing of the effects of TNF are not clear, consequently, translation of these findings to the clinical setting is challenging. In general, cytokine pathways are intrinsically redundant and malleable. Cytokine inhibition emerges as a critical approach to mediate the flexible receptors. But because of the malleable properties of cytokine pathways, it seems that combination therapy instead of cytokine inhibition alone is of great importance to achieve durable therapeutic efficacy.

3. Autoantigen-specific T1DM immunotherapy

B cell specific T cell responses can be induced in ectopic sites to expand antigen-specific T_{reg}s, which would traffic to and accumulate in islets to abrogate pancreatic islet β cell destruction [54,55]. Although the underlying mechanism remains to be fully understood, the dysfunction and imbalance of T_{eff} and T_{reg} cells are crucial factors contributing to the development of autoimmunity [56]. Based on a study of postmortem pancreases, both $CD8^+$ and $CD4^+$ T cells, as well as $CD68^+$ macrophages and $CD20^+$ B cells, were observed in infiltrated islets in patients with emerging T1DM. Besides, the authors claimed that β cell death corresponded to numbers of $CD8^+$ T cells and B cells [57]. Therefore, different approaches have been employed to fight against β cell autoimmunity by targeting autoreactive T cells and hampering the pathogenesis of autoimmune activity [54,58].

Priming of $CD8^+$ T cells depends on cross presentation of epitopes by DCs through MHC-I [59]. Cross presentation between DCs and $CD8^+$ T cells requires the interaction of $CD4^+$ T cells. $CD4^+$ T cells help the activity of $CD8^+$ T cells by mediating Fas or perforin based cytolytic killing or releasing cytokines of crucial importance for promoting immune activity against islet β cells [60,61]. It is also presumed that $CD4^+$ T help cell depletion can decrease the incidence of diabetes [62]. High level of IFN-γ is produced by islet antigen-specific $CD4^+$ T cells, whereas $CD4^+$ T cells recognizing the same antigen secrete IL-10, emphasizing that IFN-g is of pivotal importance for $CD4^+$ T cells in mediating diabetes progression [10,63]. T_{reg} cells act as primary peripheral mediators of immunological tolerance [64]. The absence of T_{reg} is correlated with pathogenesis of autoimmune diabetes. T_{reg} abrogates T_{eff} proliferation and Th1 polarization in part through secretion of IL-10 and TGF-β [65]. Expression of transcription factor Foxp3 is thought to be indispensable in T_{reg} function. Depletion of Foxp3 results in severe systemic autoimmune syndromes, indicating the importance of T_{reg} in maintaining self-tolerance [66,67]. When transfecting Foxp3 promotor mediated diphtheria toxin receptor (DTR) into NOD mice, the deletion of T_{reg} compartment leads to approximately 100% penetrance of diabetes within days, directly support the role T_{reg} impairment in the pathogeneses of T1DM [68]. Other populations of T cells such as classical invariant natural killer T (iNKT) cells also function as controllers in regulating T1DM onset, as demonstrated in NOD mice that diabetes progression could be halted by activation and expansion of iNKT cells. The mechanism of iNKT in T1DM progression has been revealed to be associated with Th2 cell responses induced by islet autoantigens in NOD mice, while little direct evidence has acknowledged the association between iNKT frequencies and T1DM onset and progression in T1DM patients [69,70].

Compared with nonautoantigen-specific immunomodulation, autoantigen-specific immunotherapy of T1DM is expected to selectively modulate T1DM-related autoimmunity while preserving the global immune homeostasis intact [71–74]. This can be achieved by inhibiting the deleterious β-cell specific T cell responses and restoring tolerance toward T1DM-associated autoantigens.

3.1. Autoantigen-specific T cell modulation

Modulation of autoantigen-specific T cells has been pursued in concert with technological advances in nanomedicines and biomaterials, which will be further discussed in the following section. In one example, Santamaria and colleagues developed nanoparticles coated with autoantigen-related MHC-II/peptide complex molecules (pMHCII) (Fig. 3A) [74]. Systemic delivery of these nanoparticles remarkably resolved established autoimmune phenomena. Specifically, the systemically administered pMHCII-coated nanoparticles elicited or expanded the repertoires of autoantigen-specific regulatory $CD4^+$ T cell type I (T_R1)-like cells and promoted the differentiation of autoreactive T cells into T_R1 cells (Fig. 3B). T_R1 cells ($Foxp3^-CD4^+CD25^-$) represent an alternative regulatory T cell subset that is different from conventional T_{reg} cells ($Foxp3^+CD4^+CD25^+$) that have been recently investigated for disease treatment. Such pMHCII-coated nanomedicines also induce and expand regulatory B cells, which further suppressed T1DM development by activating $CD4^+$ T cells (Fig. 3C). These autoantigen-specific APC-mimicking nanomedicines may represent an attractive alternative to cellular therapies using autologous DCs. Compared with *ex vivo* expansion of T_{reg}s for adoptive cell transfer therapy, systemic administration of these nanomedicines for *in vivo* immunomodulation of T_{reg}s bypasses the need for effective strategies of *ex vivo* antigen-specific

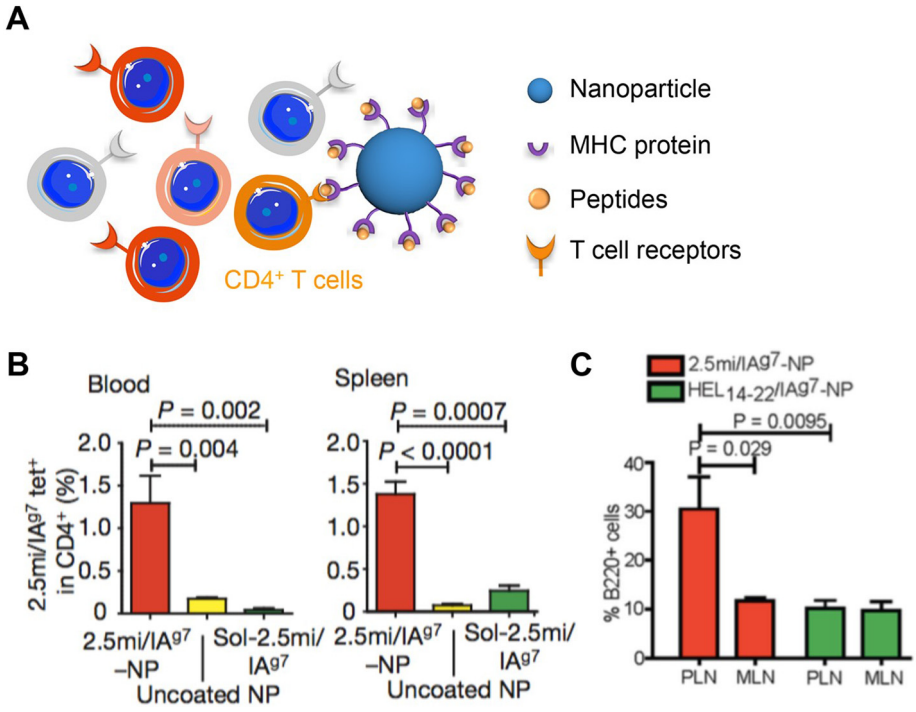

Fig. 3. (A) Nanoparticles coated with T1DM-relevant pMHC-II (pMHC-NPs) triggered T1DM-specific $CD4^+$ T cells. (B) Systemic delivery of pMHC-NPs promotes the expansion of $CD4^+$ T cells in blood and spleens. (C) pMHC-NPs increased percentages of $B220^+$ cells in NOD mice [74].

T_{reg}s. These nanoparticles may hold the potential to treat a wide range of autoimmune diseases by autoantigen-specific T cell modulation.

3.2. Autoantigen-specific B cell modulation

Non-specific inhibition of B cells has shown limited efficacy and raised safety concerns over increased risk of infectious diseases. Inhibition of autoantigen-specific B cells has been shown to be more promising [71], for example, by depleting insulin-reactive B cells. It was shown in T1DM-prone mice that insulin-reactive B cells escaped immune regulation and were able to respond to autoantigens (insulin in this case) by upregulating costimulatory molecules in presentation epitopes to T cells. Henry and colleagues infused in NOD mice monoclonal antibodies targeting insulin-specific BCR and observed disease protective effects and elimination of insulin-reactive B cells. Nonetheless, challenges against this approach may stem from the currently incomplete understanding of autoantigen profiles involved. In theory, among polyclonal β cell-destructive B cell subsets, one needs to deplete all or as many of such as possible to eliminate the broad spectrum of autoreactive B cells in order to minimize residual β cell-destructive antibodies.

3.3. Autoantigen-targeted vaccines

An accepted paradigm is that β cell autoantigens presented in non-inflammatory contexts could serve to regulate autoreactive T cells and generate β cell protection [75]. The concept led to the development of vaccination strategies to induce T cell tolerance against autoantigens. To this end, T cell epitopes of insulin and glutamic acid decarboxylase (GAD) have been mapped in detailed [76–79]. The C19-A2 proinsulin peptide, spanning the C-A junction and restricted by T1DM-associated HLA-DRB1*0401, has been demonstrated to modulate autoreactive CD4 T cells in patients expressing the same class II allele [80]. In recently diagnosed individuals (within 100 days), administration of the peptide exhibited higher C-peptide plasma concentrations without systemic or local hypersensitivity [81]. The biomarker levels were associated with the expansion of IL-10 and Foxp3 expressing T_{reg} cells. Another dominant T1DM autoantigen is GAD. Aluminum salts of GAD65 (a 65-kD isoform of GAD), GAD65-Alum, was studied in mice to attenuate GAD-specific Th1 T_{eff} cells [7,82]. Short-term normoglycemia was observed in more than 70% overtly diabetic mice with a treatment, while repeated administration of GAD65-alum restored long-term normoglycemia in approximately 80% NOD mice. An expansion of IL-10+ $CD4^+$ T cells was observed, indicating the induction of the regulatory compartment [83]. Despite the success of these vaccines in mouse models, the disparities in the autoantigens between mice and humans, and the polymorphisms of the β cell autoantigens, may complicate translation of such strategies to the clinic.

4. Pharmacoengineering of biomaterials for T1DM immunomodulation

To date, no intervention has conferred durable reversal of autoimmunity in T1DM patients [84]. Materials engineering have been used to realize the potential of molecular approaches in diabetes therapy based on our previous studies [85,86]. Nano- and micro- scale polymeric particles and scaffolds have been developed for the prevention and intervention of autoimmune disorders [87]. Carriers of antigens and immune modulators have been generated endowed with diverse characteristics in terms of physical size, surface charge, as well as surface functional groups. These properties can be tuned to tailor the delivery of the bioactive molecules for targeting multiple signaling pathways. Supramolecular nanostructures can be loaded with small molecule drugs, antigenic peptides, and cytokines [88]. In many applications, the carriers are administered locally, designed to accumulate in draining lymph nodes targeting T cells and APCs trafficking to a particular organ system.

Particulate systems have been used to generate tolerance toward the polyclonal T cell responses in T1DM [89]. Shea and coworkers have used β cell antigens loaded poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles to restore tolerance in NOD mice through activation of β-cell-specific $Foxp3^+$ T_{reg}s in PD-1 and CTLA-4 dependent manner [90]. Microparticles, formulated with or with islet antigens, loaded with antisense oligonucleotides targeting costimulatory molecules CD80, CD86 and CD40 were shown to increase antigen-specific $Foxp3^+$ T_{reg}s in NOD mice

[91]. Keselowsky and co-workers have developed dual-sized (1–1.5 μm and 30 μm) microparticles for biphasic delivery of vitamin D3, insulin B peptide, TGF-β1 or GM-CSF [92]. T1DM in NOD mice was prevented when the formulations were administered before 5 weeks of age. Microneedle has been used to present β-cell antigens through intradermal administration [93]. The localized delivery the antigen-coated microneedle resulted in measurable antigen presentation in draining lymph nodes as long as 10 days later. Repeated administration of the microneedle led to a reduced proliferation of antigen-specific T cells in the pancreatic lymph nodes.

Optimal subunit vaccines often require the co-delivery of immune adjuvants and subunit antigens. Nanostructures with porous cores and large surface area serve as excellent platforms for engineering adjuvants and antigens into a single carrier, which is conducive for steering DCs toward a tolerogenic phenotype. The nanocarriers can be optimized for tissue penetration through the extracellular matrix and cellular uptake through endocytosis [94,95]. In one study, Quintana and colleagues developed a gold nanoparticle formulation that efficiently co-delivered a tolerogenic adjuvant and proinsulin for *in vivo* modulation of autoantigen-specific T_{reg}s in a T1DM mouse model [96]. Specifically, the gold nanoparticle-based nanovaccine co-delivered a tolerogenic adjuvant of 2-(1′*H*-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE) and a β cell autoantigen proinsulin. The resulting nanovaccine, termed $NP_{ITE+Ins}$ induced tolerogenic DCs *in vivo* and promoted the generation of T_{reg} (Fig. 4A). These results demonstrated the potential of using nanoparticulate platforms in T1DM and perhaps autoimmune diseases in general. As another example, biomaterial scaffold was engineered to be loaded with antigens or cytokine adjuvants for T1DM immunotherapy. Specifically, Thelin et al. developed a scaffold fabricated with antigen proteins mixed with poly(lactide-*co*-glycolide) (PLG) microspheres (Fig. 4B) [97]. The obtained scaffold induced the recruitment and proliferation of antigen-specific T cells without promoting diabetes progression. Interestingly, they further encapsulated β cell lysate into scaffolds, resulting in the local enrichment of autoimmune T cells (β-cell-reactive T cells). NOD mice would suffer diabetes when transplanted with the derived T cells, providing a novel approach to reveal the pathogenicity of antigen-specific T cells in T1DM (Fig. 4C). In a similar approach, Keselowsky and colleagues utilized peptide hydrogel for local delivery of CpG and GM-CSF as well as PLGA microparticles encapsulated with denatured insulin. With the treatment of hydrogel, diabetes onset of NOD mice was delayed to 19 weeks, demonstrating the possibility of the degradable hydrogel to prevent T1DM [98]. These strategies may lead to novel avenues of translational T1DM research.

Molecular engineering has also been employed to develop tools for T1DM immunotherapy. One platform is antibody-autoantigen conjugates, designed aimed to enhance antigen internalization, processing, and cross-presentation in APCs [99,100]. DEC-205, a surface receptor expressed selectively on DCs, has been exploited to direct antigens and modulators to the adaptive immune system [100]. Anti-DEC-205 antigen conjugated with autoantigens have been shown to induce T cell tolerance by selectively targeting a population of DCs [101,102]. In mice, low dose antigen delivered in conjugation with DEC-205 promoted conversion of $CD4^+$ T cells into T_{reg} cells and enhanced antigen-specific T_{reg} functions. Thus, specific targeting of surface receptors by antibody-antigen conjugates presents a viable strategy for promoting immune tolerance [103]. Hess et al. developed saporin toxin and MHC-I tetramer conjugates to selectively deplete islet-specific glucose-6-phosphatase catalytic subunit relate protein (IGRP) specific T cells in NOD mice model. As demonstrated, T1DM development was successfully delayed after administrated of this specific conjugate [104].

As mentioned above in the example of pMHC-NPs, nanoparticles can act as artificial APCs by conveying antigen signals to immune cells in a form of peptide-MHC complexes [74]. Treatment of NOD mice with peptide-MHC I-nanoparticles was able to trigger the expansion of autoregulatory $CD8^+$ T cells, thus remarkably restoring normoglycemia and immune tolerance in diabetic mice. Immune tolerance ensued attributed to both prevention of autoantigen-loaded APCs apoptosis and inhibition of autoantigen presentation in second lymphoid organs. Santamaria et al. developed nanoparticle coated with peptide-MHC to reverse autoimmunity by expanding memory-like autoregulatory T cells in diabetic mice (Fig. 5) [105]. Disease onset was delayed after administration of low avidity pMHC-NP by expanding memory-like autoregulatory T cells, which in turn suppress presentation of the autoantigens by DCs. A limitation of some of these strategies is diabetes can return once the therapy is withdrawn. Thus, repeated

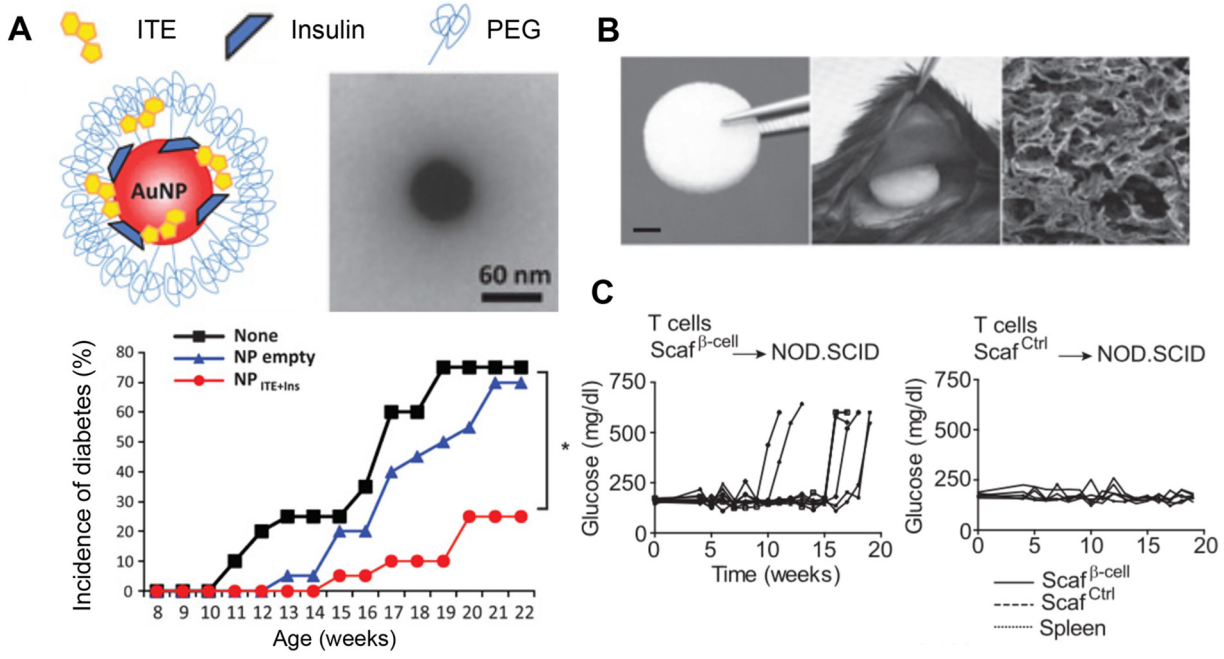

Fig. 4. (A) Gold nanoparticles guided co-delivery of proinsulin and ITE, which effectively suppressed autoimmunity in a T1DM model [96]. (B) A biomedical scaffold composed of antigen and PLG microspheres for recruitment of antigen-specific T cells. (C) T cells isolated from scaffold loaded with β cell lysate induced higher blood glucose levels in NOD mice [97].

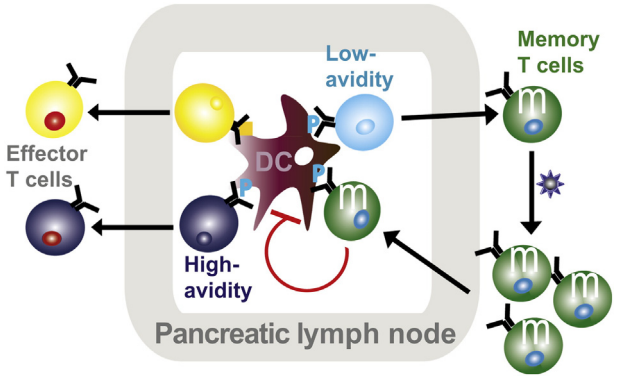

Fig. 5. Treatment with pMHC-NPs expanded autoregulatory T cells. Low-avidity autoreactive T cells become memory-like T cells which in reverse suppress autoantigen-loaded APCs [105].

administration may be necessary. Interestingly, another study by Shen, et al. reported a biodegradable PLGA microparticle (killer MP) on which both ovalbumin (OVA) antigen-MHC complexes and anti-Fas monoclonal antibody were attached. The obtained killer MPs exhibited a longer retention time of 48 h *in vivo* after intravenous administration. Moreover, antigen-specific autoreactive T cells were effectively depleted for up to 4 days with two injections of killer MPs in transgenic OT-1 mice, which further appeared to prolong the allo-skin graft survival for approximately 40 days in bm1 mice [106].

5. Conclusions and outlook

While the full spectrum of underlying immunological mechanisms remains to be elucidated, significant progress has already been made through either nonautoantigen-specific immune modulation or T1DM autoantigen-specific immunotherapy. Nevertheless, there is thus far no T1DM immunotherapy that can replace standard insulin replacement therapy. Novel therapies should aim at the benchmark of β cell preservation and normglycemia with reduced or no insulin dependency. This is most likely to achieve in patients with newly onset T1DM, and in individuals with genetic predispositions for the disease. The polyclonal adaptive immune responses in T1DM have complicated the development of rational immunotherapies. A combinatorial drug delivery approach may be a solution [17]. Much insight can be gleaned from the recent breakthroughs in cancer immunotherapy, in particular, the role of immune check points PD-1/PD-L1 and CTLA-4. As such, one might contemplate the feasibility of engineering into β-cell agonists of the checkpoint molecules. Another path going forward is to mimic the personalized, neoantigen-guided cancer immunotherapy, by isolating all autoreactive T cells or B cells in a given patient, map the autoantigen epitopes, and develop multi-epitope tolerogenic individualized vaccines. In addition, biomaterials-based approaches can engineer into the drug formulation physical properties conducive for optimal spatiotemporal engagement of T cells and DCs operating in the pancreatic lymphatics. In conclusion, despite the long history, T1DM immunotherapy is entering into a new chapter with wide open possibilities.

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Conflict of interest

The authors declare no conflict of interest.

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