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REVIEW ARTICLE

Advanced Delivery Strategies for Immunotherapy in Type I Diabetes Mellitus

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Abstract

Type 1 diabetes mellitus (T1DM) has been defined as an autoimmune disease characterised by immune-mediated destruction of the pancreatic β cells, leading to absolute insulin deficiency and hyperglycaemia. Current research has increasingly focused on immunotherapy based on immunosuppression and regulation to rescue T-cell-mediated β-cell destruction. Although T1DM immunotherapeutic drugs are constantly under clinical and preclinical development, several key challenges remain, including low response rates and difficulty in maintaining therapeutic effects. Advanced drug delivery strategies can effectively harness immunotherapies and improve their potency while reducing their adverse effects. In this review, we briefly introduce the mechanisms of T1DM immunotherapy and focus on the current research status of the integration of the delivery techniques in T1DM immunotherapy. Furthermore, we critically analyse the challenges and future directions of T1DM immunotherapy.

Key Points

Although insulin maintains normal blood glucose levels in type 1 diabetes mellitus (T1DM) patients, it cannot prevent or reverse the destruction of the islet β cells.

T1DM occurs directly due to an imbalance between the $CD4^+$ effector T cells (Teffs) and regulatory T cells (Tregs). Teffs can promote β-cell death and the production of immunoglobulins, which are markers of the autoimmune process. Immunotherapy can preserve the islet β cells by preventing T cells from recognising and attacking the antigenic epitopes of pancreatic β cells or promoting immune self-tolerance and homeostasis of Tregs.

Development of localised targeted delivery strategies is crucial in overcoming challenges such as off-target effects and systemic toxicity of T1DM immunotherapy.

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1 Introduction

Type 1 diabetes mellitus (T1DM) has been defined as a potentially multifactorial autoimmune disorder characterised by T-cell-mediated destruction of the pancreatic β cells, resulting in absolute deficiency of insulin synthesis and secretion [1]. According to the International Diabetes Federation Diabetes Atlas, the global prevalence of DM among people aged 20–79 years was estimated as 10.5% (536.6 million people) in 2021, and T1DM accounts for approximately 10% of this proportion (approximately 30 million individuals worldwide). In one observational study of paediatric and adult patients with T1DM in the United States, diabetes-related costs totalled more than $800 per month [2, 3]. T1DM is initially characterised by the appearance of islet autoantibodies (AAbs), followed by the clinical manifestation of dysglycaemia due to partial destruction of the β cells, and ultimately leading to fatal complications due to absolute insulin deficiency such as hyperglycaemic hyperosmolar state (HHS) and diabetic ketoacidosis (DKA) [4]. HHS causes extreme dehydration and coma [5], whereas DKA causes death mainly through cerebral injury or cerebral oedema [6] in T1DM patients. Abundant evidence indicates that T1DM is a dangerous chronic disease that leads to disability and death. Moreover, it has become a rapidly growing global problem with huge social, health, and economic consequences, and effective control is the need of the hour.

Since the initial model of T1DM pathogenesis proposed by George Eisenbarth in the 1980s, we have understood the chronic process of the development of T1DM through decades of research [7]. Currently, the synergistic effects of genetic and environmental factors are believed to be the aetiology underlying T1DM. According to the database of genome-wide association studies, the human leukocyte antigen (HLA) class II haplotypes HLA-DR and HLA-DQ (HLA-DR3-DQ2 or HLA-DR4-DQ8 haplotypes) within the major histocompatibility complex (MHC) region contribute to approximately 50% of the genetic susceptibility by affecting T-cell recognition and tolerance to foreign and autologous molecules [3, 8]. Recent studies have shown that rare and low frequency variants, including TYK2, IFIH1, RBM17, PTPN22, STK39, and LRP1B, may be potential detection and therapeutic targets in T1DM [9]. In the presence of genetic susceptibility, environmental factors such as enteroviruses, intestinal microbiota, infant's diet, and vitamin D can induce or aggravate the occurrence of T1DM [3, 10].

Owing to the lack of radical therapy, the first-line empirical treatment for T1DM patients is still insulin replacement therapy [11]. Although insulin has a significant therapeutic effect in relieving symptoms of both T1DM and type 2 DM (T2DM) and delaying organ damage, it does not hinder the T-cell-mediated progressive destruction of β cells [7]. Moreover, a rare fraction of patients with diabetes may present with euglycaemic DKA (EDKA), which is characterised by increased anion gap metabolic acidosis, ketosis, or ketonuria in euglycaemia (serum glucose level < 250 mg/dL). EDKA is commonly observed in patients with a history of hypoglycaemia due to starvation, chronic liver disease, pregnancy, infection, and alcohol abuse [12, 13]. Moreover, recent evidence demonstrates that sodium-glucose cotransporter 2 (SGLT-2) inhibitors (e.g. dapagliflozin) may lead to a significant increase in the incidence of EDKA [14]. SGLT-2 inhibitors competitively block the reabsorption of 30–50% of filtered glucose from the original urine in the proximal convoluted tubule, which stimulates increased secretion of glucagon and enhances lipolysis and ketogenesis [12]. Therefore, the existing treatments only enable T1DM patients to achieve normal blood glucose levels; however, they cannot reverse the destruction of β cells, and euglycaemia could even lead to misdiagnosis or delayed diagnosis by physicians. Thus, T1DM patients miss the optimum treatment window, which results in disease progression. Therefore, researchers have attempted to develop a novel approach in immunotherapy to rescue the functional loss of β cells by alleviating the autoimmune attack of T and B cells on the β cells [15].

According to the targeted intervention on immune cells, the current T1DM immunotherapies can be divided into T-cell-targeted, $CD4^+$ regulatory T cell (Treg)-targeted, and dendritic cell (DC)-targeted immunotherapies. Owing to the complexity of the pathogenesis of T1DM, combination immunotherapies can exert simultaneous or synergistic modulation to correct the autoimmune process in these patients [7, 16]. Whether used alone or in combination, the success of immunotherapy depends on its interaction with the epitopes. Currently, various cytokines and antibodies face the challenge of transportation to the target sites. Another limitation is that continuous immunosuppressive effects cannot be induced, leading to an off-target effect [17]. Hence, one of the core purposes of developing novel delivery strategies for immunotherapeutic agents is to achieve a targeted and controlled release [7]. Advanced delivery strategies for T1DM immunotherapy, including nanoparticles (NPs), liposomes, plasmids, engineered microorganisms, and microneedles (MNs), have been developed for localised on-demand delivery of drugs, cell factors, and antibodies to minimise toxicity in vivo. In this review, we summarise the fundamental immune pathogenesis of T1DM and several major types of T1DM immunotherapy, and focus on the advanced delivery strategies to overcome these challenges. Furthermore, we discuss the current limitations of T1DM immunotherapies and their corresponding prospects for improving the efficacy and safety in T1DM patients.

2 Immune Pathogenesis

T1DM is a polygenic disease in which susceptibility genes or genetic variations cause disease risk, primarily involving the HLA region on chromosome 6 and several β-cell-specific genes [18, 19]. The pathogenesis of T1DM is characterised by the infiltration of islet antigen-specific T cells and proinflammatory antigen-presenting cells (APCs), as well as the concomitant loss of forkhead box protein 3 ($Foxp3^+$) Tregs [18, 20]. The development of T1DM can be divided into three stages. In the first stage, the occult autoimmune response to β cells precedes the clinical onset of T1DM. APCs such as DCs and macrophages present autoantigens that initiate the activation of β-cell-specific T cells, mainly $CD4^+$ and $CD8^+$ T cells. Interleukin (IL)-12 is produced to induce the generation of $CD4^+$ and $CD8^+$ effector T cells (Teffs), Th1, and Tc1, marked by the expression of the transcription factor T-bet and cytokines such as interferon (IFN)-γ and tumour necrosis factor (TNF)-α [21, 22]. The autoreactive Teffs then migrate to the pancreatic islets and induce β-cell destruction through perforin and granzyme, the Fas/FasL ligand pathway, and the TNFα-dependent pathway [23-25]. The β-cell autoimmunity is triggered and accompanied by the appearance of β-cell-targeted AAbs against endogenous antigens [18, 26]. An histological examination of the islets showed a decrease in

the β-cell mass and residual insulin-containing islets and an increase in $CD8^+$, $CD4^+$ and $CD20^+$ T-cell infiltration in T1DM patients aged < 7 years [27]. The AAbs commonly observed in T1DM patients include proinsulin (biosynthetic precursor of insulin), proinsulin C-A connection (C-peptide and proinsulin A-chain connection), glutamic acid decarboxylase (GAD) 65, tyrosine phosphatase IA-2 and IA-2β, zinc transporter 8 (ZnT8), and insulin. These epitopes can induce the activation of $CD4^+$ and $CD8^+$ T cells and destruction of β cells [3, 26]. Peripheral Tregs ($CD4^+$, $CD25^+$, and $Foxp3^+$ T cells) showed a significant reduction in a paediatric cohort with T1DM [28]. As the disease progresses to the second stage, individuals suffer from different degrees of β-cell loss, which can be detected by measuring the serum C-peptide levels, a byproduct of insulin synthesis [29]. In the first and second stages, the main strategy of T1DM immunotherapy is to preserve the remaining β-cell mass as well as inhibit β-cell autoimmunity [30]. The administration of immunosuppressive drugs in children with new-onset T1DM can partially delay the developmental process [31]. The residual β cells can still secrete insulin compensatively and maintain euglycaemia [7] (Fig. 1).

However, when the disease progresses to the third stage, β-cell mass reduces by 70–90%. The residual β cells are unable to synthesise and secrete enough insulin, thus clinical manifestations appear gradually. Therefore, the main goal of immunotherapy at this stage is to preserve

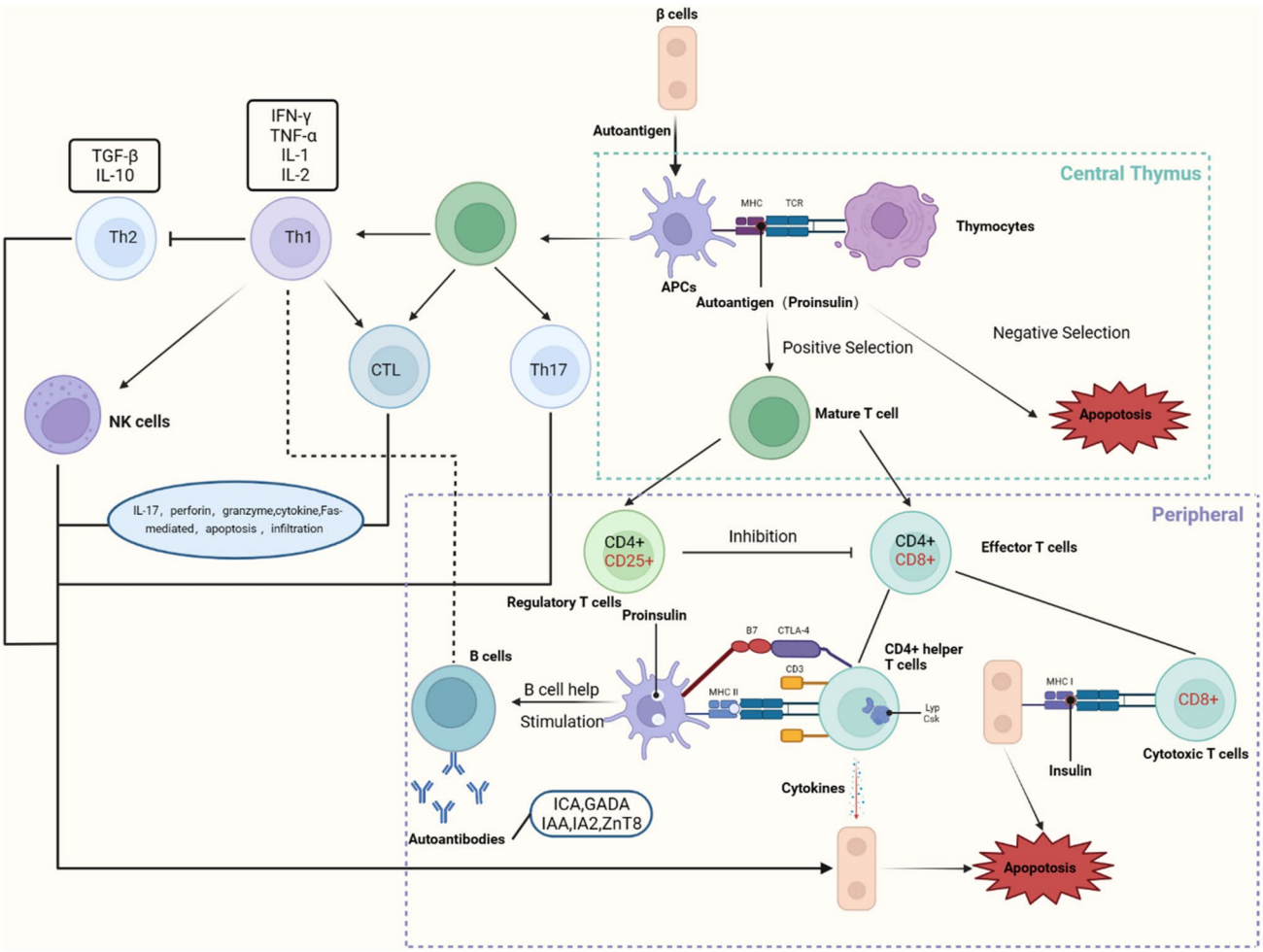

Fig. 1 Immune pathogenesis of T1DM. During thymocyte maturation, positive and negative selection takes place in the thymus. This process involves interaction between the proteins of the MHC on the APCs, proinsulin and the TCR on thymocyte. In addition, autoantigens from pancreatic islet β-cells are presented by APCs, thereby activating T cells, including Th cells type 1 and type 17 and CTLs. Abbreviation: *T1DM* type 1 diabetes mellitus, *MHC* major histocompatibility complex, *APCs* antigen-presenting cells, *TCR* T-cell receptor, *Th* T-helper, *CTLs* cytotoxic T lymphocytes, *GADA* glutamic-acid-decarboxylase antibody, *IA2* islet tyrosine phosphatase 2 antibody, *IAA* insulin autoantibody, *ICA* islet cell antibody, *TGF* transforming growth factor, *IL* interleukin, *IFN* interferon, *TNF* tumour necrosis factor, *APCs* antigen-presenting cells, *CTLA-4* cytotoxic T lymphocyte antigen 4

the number and function of the remaining β cells [32]. Although $CD4^+$ and $CD8^+$ T cells have been identified as specific epitopes on β cells, it is difficult to target the autoimmune destruction caused by them [26]. Researchers have found that there are significant heterogeneities between insulitis and β-cell destruction in T1DM patients. For instance, although insulitis can be found in insulin-containing islets in newly diagnosed T1DM patients, it is far less in insulin-deficient islets [33]. Notably, in one T1DM patient, different islet lobules probably exhibited different degrees of immunocyte infiltration and destruction [34]. Additionally, high expression of HLA-I [35] and endoplasmic reticulum stress markers (e.g. NLRP3 inflammasome) has been detected in insulin-containing lobules [36]. Available evidence indicates that understanding how to increase the response rates to targeted immunotherapy in T1DM patients is the key to improving the efficacy and reducing off-target effects (Fig. 2).

3 Targeted Immunotherapy

The purpose of immunotherapy is to prevent, delay, or even reverse the development of T1DM by inhibiting the reactive T cells and/or inducing T cells to tolerate the antigenic epitope on β cells [19]. T1DM immunotherapy can be classified into non-autoantigen-specific and autoantigen-specific interventions [7]. Most immunotherapeutic drugs and cytokines target T cells, Tregs, B cells, and DCs to induce T-cell tolerance and regulate the autoimmune process.

In the absence of targeted antigen, the immunotherapy for T1DM is based on the enhancement of local or systemic immunomodulatory mechanisms, thus improving the destructive autoimmune response and immune response targeting the β cells. In early studies, a series of broad-spectrum immunosuppressive schemes was used; however, it did not achieve the desired results [37-39]. Although β-cell destruction was inhibited and islet function was partially restored

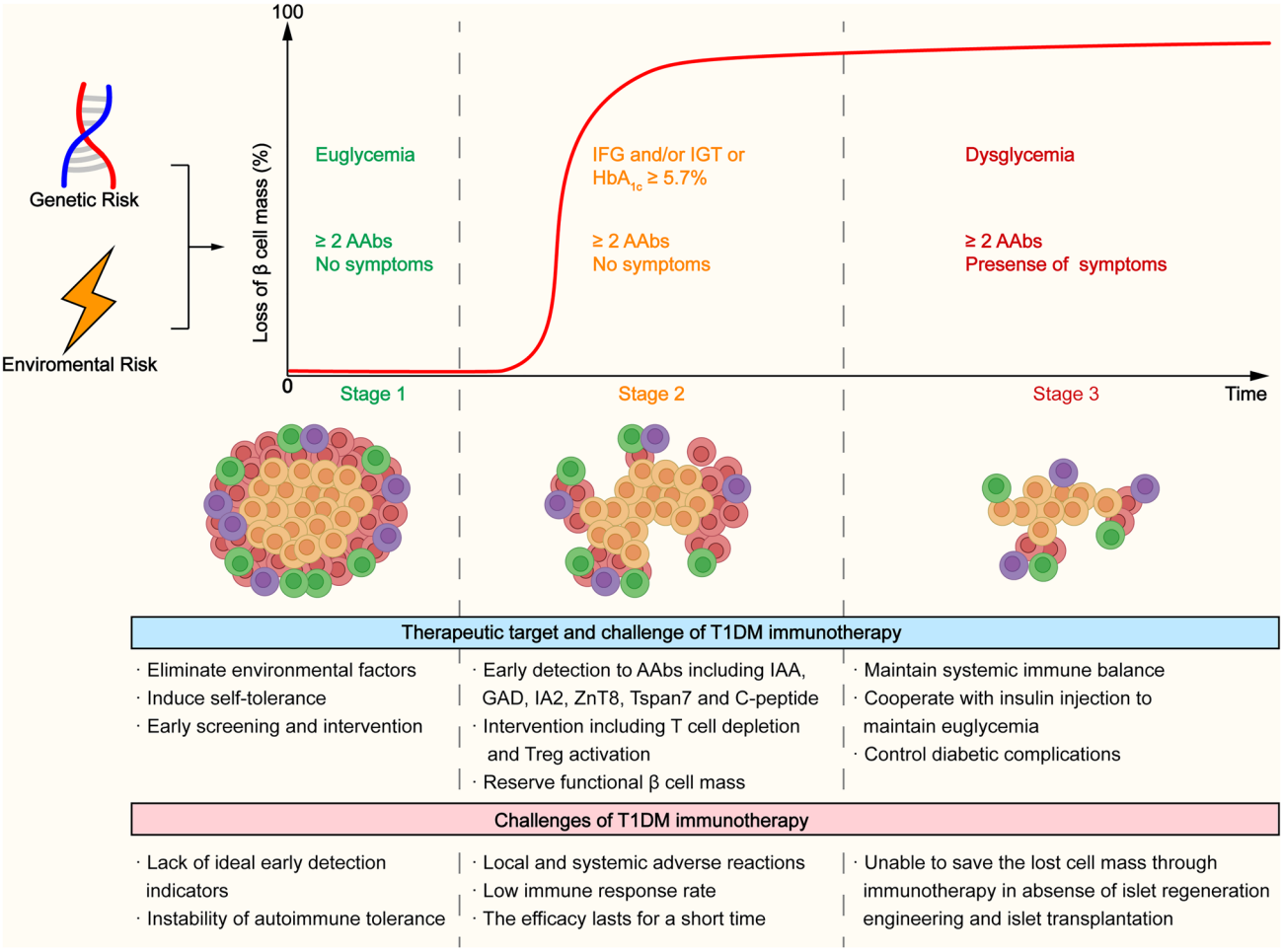

Fig. 2 T1DM stages. Abbreviation: *T1DM* type 1 diabetes mellitus, *IFG* impaired fasting glucose, *IGT* impaired glucose tolerance, *AAbs* autoantibodies, *Treg* regulatory T cell

in some cases, the curative effect was often temporary, and the occurrence of long-term immunosuppression and severe systemic toxicity far exceeded its benefits [40, 41]. Hence, researchers have turned to developing targeted immunotherapeutic drugs and innovative delivery strategies to increase the response rates and reduce systemic immunosuppression and toxicity [42-45]. Therefore, the development of T1DM innovative immunotherapy depends on understanding the molecular basis of the T-cell response to the islets in T1DM patients [16, 46]. Several immunotherapeutic products have been used in preclinical studies or clinical trials (Table 1, Fig. 3).

3.1 T-Cell-Targeted Immunotherapy

The key step in initiating the T-cell response is to activate antigen-specific T cells through T-cell receptor (TCR)/CD3 and co-stimulatory signals, which are transmitted by the antigen-MHC and other molecules expressed on the surface of the APCs [47]. TCR/CD3 signalling controls and participates in many processes such as thymus T-cell development, Tregs production, and immature T-cell activation by activating various intracellular pathways [48]. Theoretically, the downregulation of TCR/CD3 signal intensity probably affects the autoimmunity in T1DM. The relationship between autoreactive $CD8^+$ T cells and the progress of T1DM makes these phenotypes potential biomarkers of disease trajectories and responses to immunotherapeutic intervention [49]. In a cross-sectional study of patients with T1DM, the increasingly depleted islet-specific $CD8^+$ T cells were consistent with the slowdown of β-cell loss [20].

The use of monoclonal antibodies (mAbs) is one of the promising strategies for inducing an autoimmune drive against

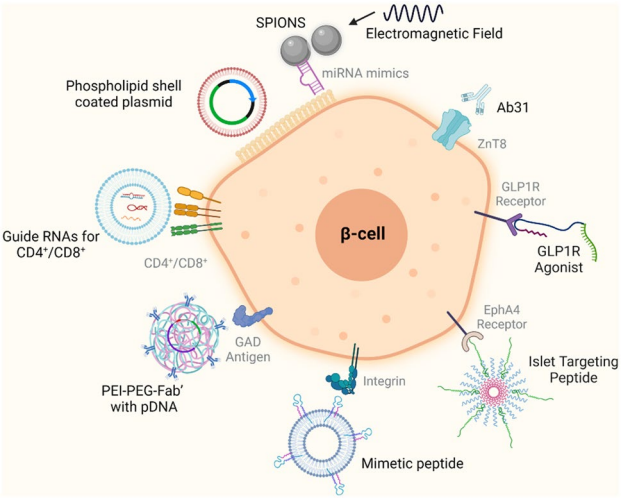

Fig. 3 β-cell immune target sites and advanced delivery strategies. Abbreviation: *Ab* antibody, *SPIONS* superparamagnetic iron oxide nanoparticles

specific immunocyte populations. Several mAbs have been proven clinically safe, and they have shown varying degrees of efficacy in regulating autoimmune disorders, including T1DM [50-52]. To date, one of the most common advanced immunotherapeutic drugs for T1DM is the CD3 mAb teplizumab, an Fc receptor-nonbinding anti-CD3 mAb [44, 45, 53]. Teplizumab binds to the TCR complex on the surface of T cells and undergoes continuous phosphorylation to generate TCR signals [54]. TCR signalling elicits diverse cellular responses such as regulating cell metabolism, inducing cell division, and driving effector functions, including cytolytic activity and secretion of signal molecules, such as IL, IFNγ, TNFα,

Table 1 Target areas for immunotherapy development and current status for T1DM management or prevention

Target pathways	Epitopes	Immunotherapeutic agents	Available products	References and ClinicalTrials.gov identifiers
Teff exhaustion	Anti-CD3	Teplizumab, alefacept, otelixizumab	Amevive®	[53, 64], NCT00965458
Inhibition of T-cell activation	CTLA-4/CD28	Abatacept	Orencia®	[108], NCT03929601
Induction of Treg	Foxp3, IL-2, 21E-22E, CD80/CD86-CD28	Polyclonal Tregs, insulin-specific vaccination, rhIL-2, belatacept	Proeukin®, Nulojix®	[95], [179, 180], NCT02772679 NCT01862120
B-cell antagonists	CD20	Rituximab	Rituxan®	[181]
DC antagonists	JAK1	Ruxolitinib (JAK inhibitor)	Jakafi®	[182–184]
Induction of tolDCs	GM-CSF, IL-4, IL-10	Antisense oligonucleotides, proinsulin peptide		[185, 186], NCT02354911
Cytokine antagonists	IL-6, CXCL-8 (IL-8)	Tocilizumab, allosteric inhibitor	Actemra®, Ladarixin®	NCT02293837 NCT04628481

T1DM type 1 diabetes mellitus, *Teff* effector T cell, *Treg* regulatory T cell, *DC* dendritic cell, *tolDCs* tolerogenic dendritic cells, *CTLA-4* cytotoxic T lymphocyte antigen 4, *Foxp3* forkhead box protein 3, *IL* interleukin, *JAK* Janus kinase, *GM-CSF* granulocyte-macrophage colony-stimulating factor, *CXCL* chemokine (C-X-C) ligand, *rhIL-2* recombinant human interleukin-2

and transforming growth factor (TGF)-β [55]. A clinical trial demonstrated that the proportion of diabetes-free individuals in the teplizumab group was 57% compared with 28% in the placebo group. Correspondingly, antidrug antibodies were observed in the peripheral blood of 20–55% of teplizumab-treated participants after the first course [56]. Another randomised controlled trial of non-diabetes relatives at high risk for T1DM demonstrated that glucose tolerance decreased, whereas area under the curve (AUC) of C-peptide (1.94 vs. 1.72 pmol/mL of the control group) and insulin secretory rates increased after teplizumab treatment. Moreover, the teplizumab-treated group demonstrated a reduced secretion of IFNγ and TNFα [57]. Generally, teplizumab can modulate pathologic T-cell signals and delay the development of T1DM; however, investigation of the clinical safety and target accuracy in further preclinical studies is warranted.

Additionally, IL-7 promotes autoimmune diabetes by maintaining memory T cells (T_{EM}) in a functional state. Studies have shown that IL-7Rα blocking approaches can be one of the established treatments for T-cell-dependent autoimmune diseases [58]. IL-7Rα blockade alters the balance of Tregs and T_{EM} cells by promoting exogenous cellular regulation and further increasing the threshold of Teffs activation [59]. The durable efficacy and multipronged tolerogenic mechanisms of anti-IL-7Rα therapy indicate a prospective disease-modifying approach for T1DM [58]. Similarly, other immunotherapeutic drugs targeting Teffs that have been tested in clinical trials include antithymocyte globulin polyclonal antibody (wide-range, nonspecific immunosuppressants) [60, 61], abatacept (inhibits B-cell activation and other DCs by acting on T follicular helper cells) [62] and alefacept (inhibits the co-stimulatory receptor CD2) [63, 64].

In contrast to non-autoantigen-specific immune regulation, T1DM autoantigen-specific immunotherapy provides a more accurate targeted approach for selectively regulating T1DM-related autoimmunity while maintaining systemic immune homeostasis. The β-cell autoantigens presented in a non-inflammatory microenvironment can be used to regulate the autoreactive T cells for rescuing β cells, leading to the development of autoantigen-specific vaccination strategies [65, 66]. T-cell epitopes against icariside, GAD, and insulin are considered suitable candidates [67-69]. Both non-obese diabetic (NOD) mice and T1DM patients treated with GAD65 conjugated to aluminium hydroxide (GAD-alum) showed increased secretion of Th2/Tc2-related chemokines that suppressed the self-reactive Th1/Tc1 effector cells [70]. In a phase II clinical trial, adult patients with latent autoimmune DM received a subcutaneous injection of recombinant human GAD-alum. During the 30-month follow-up period, the treated group showed higher fasting and stimulated C-peptide concentrations as well as lower glycated haemoglobin levels than those in the placebo group ($r = -0.40$; $p = 0.006$) [71]. Furthermore, antigen-specific peptide immunotherapy regulated the pathogenic T-cell response, providing the potential to maintain immune homeostasis and prevent further β-cell destruction [67]. Interestingly, the incidence of T1DM has been accidentally found to be associated with various enterovirus infections, such as group B coxsackievirus (CVB). CVB infection in NOD mice demonstrated not only an obvious relationship with the pathogenesis but also a possible reduction in the incidence of T1DM [72]. Researchers conducted a study to investigate whether rotavirus vaccination could reduce the incidence of T1DM in children aged 8 months–11 years, however no significant results were observed [73]. Nevertheless, we believe that the inactivated virus is a promising candidate for antigen-specific vaccination in T1DM immunotherapy. Available evidence indicates that some viruses, such as rotavirus and severe acute respiratory syndrome coronavirus 2, are closely related to the pathogenesis of T1DM; however, the underlying mechanisms need to be investigated [74].

Immune checkpoints, including anti-cytotoxic T lymphocyte antigen 4 (CTLA-4) and anti-programmed cell death (PD)-1/PD ligand 1 (PD-L1) play indispensable roles in regulating T-cell activation and maintaining immune haemostasis [75, 76]. Antibodies against CTLA-4 and PD-1/PD-L1 are associated with an increased risk of immune checkpoint inhibitor-T1DM, indicating that the CTLA-4 and PD-1/PD-L1 pathways are related to T1DM development [77]. Nasr et al. [78] revealed that haematopoietic stem and progenitor cells (HSPCs) are deficient in PD-L1 using transcriptomic profiling and subsequent genome-wide profiling in NOD mice, indicating that the immunoregulatory molecule PD-L1 inhibits the activation of T cells. Pharmacologically regulated or genetically engineered HSPCs overexpressing PD-L1 were found in the pancreas of hyperglycaemic NOD mice in vivo and induced the inhibition of the autoimmune response, which was also confirmed in human HSPCs from T1DM patients in vitro. These findings demonstrate that targeted and specific immune checkpoint defects may contribute to a novel immunotherapeutic strategy for T1DM. Thus, the selection of immune targets and accuracy of immune drug delivery technology are particularly important.

3.2 Regulatory T-Cell-Targeted Immunotherapy

Although small, Tregs are a critical cell subgroup. They specifically express the transcription factor Foxp3 in the nucleus and CD25 and CTLA-4 on the cell surface actively participating in immune self-tolerance and homeostasis. Tregs deficiency or dysfunction has been observed in multiple autoimmune diseases, including T1DM, indicating that autoreactive T cells cannot be cleared from the thymus [79]. TCRαβ pairs from islet Tregs can be captured through single-cell TCR sequencing, and specific islet-derived antigens including insulin B:9–23 and proinsulin have been detected. Moreover,

islet Tregs from prediabetes NOD mice effectively prevented T1DM in Tregs-deficient NOD CD28$^{-/-}$ recipients [80]. Therefore, strategies for upregulating or replacing Tregs in T1DM may reverse autoimmunity and protect the remaining β cells [81].

Various Tregs-mediated inhibition mechanisms have been proposed, including cell–cell contact and humoral factor-mediated mechanisms. Currently, many molecules are involved in Tregs-mediated inhibition mechanisms such as cell surface molecules (CTLA-4, CD25, CD39, CD73, and GITR), cytokines (IL-2, IL-10, IL-35, and TGFβ), and intracellular molecules (granzyme B, cyclic adenosine monophosphate, and indoleamine-2,3-dioxygenase) [79, 82]. In the process of Tregs-mediated inhibition, Foxp3 controls the expression of key molecules that are vital for Tregs to perform their function, especially that of inhibition [83, 84]. Recently, CD80 and CD86 were identified as activation markers of Tregs by investigating the dynamics of endogenous B7 protein acquisition and dependence on IL-2 signalling in purified CD4+ T cells incubated in vitro for 2 weeks with or without IL-2 through protein-RNA single-cell analysis. In the absence of TCR stimulation, incubation with IL-2 significantly induced the upregulation of CD80 and CD86 expression, whereas Foxp3^{+} memory Tregs expressed significantly higher levels of IL-2RA than that of memory Teffs [85].

Strategies to increase the number of Tregs and/or enhance Tregs function have become potential treatments for T1DM, as shown in several clinical trials (NCT01210664, NCT02772679). In a phase I clinical trial, participants received a single infusion of different concentrations of amplified clone Tregs (poly Tregs), and the researchers found that Tregs increased the STAT5 phosphorylation in response to IL-2, Tregs inhibitory activity, and long-term survival in vivo (> 1 year) [86].

Tregs are known to have defects in T1DM patients; therefore, recent studies have focused on restoring self-tolerance through the amplification and enhancement of Tregs both in vivo and in vitro [87]. *IL-2RA* is one of the genes intrinsically regulated by Foxp3 with IL-2 as its ligand, which inhibits immunopathology by preferentially expanding Tregs [88]. In NOD mice, daily administration of low-dose IL-2 significantly prevented the development of T1DM [89]. However, IL-2 is often limited by off-target effects caused by the expansion of pathogenic cells [88], whereas frequent high-dose IL-2 administration probably accelerates the development of T1DM by enhancing the immune response of Teffs and natural killer (NK) cells [90]. Furthermore, some organic compounds, such as D-mannose [91] and ethyl pyruvate [92], can delay T1DM development in NOD mice by stimulating and activating Tregs. However, these agents rarely achieve the expected therapeutic effects owing to their lack of target specificity. Accurate dose control and delivery technologies are urgently needed to improve the therapeutic effects of Tregs and to reduce their systemic adverse effects.

To improve target specificity, chimeric antigen receptors (CAR) and gene editing technology (e.g. CRISPR-Cas9) have been used to modulate Tregs [93, 94]. Antigen receptors can be knocked out at precise genome locations using CRISPR-Cas9 genome editing, and multiple genes that regulate Tregs function can be edited simultaneously [94]. CAR-modified Tregs (CAR-Tregs) can be efficiently designed and engineered with antigen specificity in a non-MHC-restricted manner. CAR-Tregs show less dependence on IL-2 during their growth. After maturity, CAR-Tregs maintain stable phenotypes and functions, preferentially migrate to target sites, and exert more potent and specific immunosuppression compared with the polyclonal Tregs. However, it is unclear whether CAR-Tregs cause a cytokine storm and neuronal cytotoxicity similar to that caused by anti-tumour CAR-T cells. Moreover, self- or alloantigens have not yet been fully characterised in the construction of antigen-specific CAR-Tregs. Finally, CAR-Tregs depletion may also limit their efficacy in immunosuppression [95]. Thus, although we have some achievements in tumour treatment with CAR-T cells, we are not fully prepared to take advantage of CAR-Tregs. Related research needs to be improved using animal models and preclinical trials.

3.3 B-Cell-Targeted Immunotherapy

Although T1DM is considered an autoimmune disease mediated by T cells, B cells are also involved. AAbs that identify islet antigens usually appear in the circulating peripheral blood in the early stages of T1DM, before the onset of clinical manifestations [96]. Several altered B-cell-intrinsic signals, including the B-cell receptor, toll-like receptors, co-stimulatory molecules (including CD40, CD80, and CD86), and cytokine receptors, are involved in promoting the pathogenesis of autoimmunity [97]. B cells play a pathogenic role through multiple mechanisms of producing AAbs, presenting antigens, and secreting proinflammatory cytokines. Nearly 70% of B cells and plasma cells participate in the destruction of β cells by producing AAbs; hence, they should be silenced to prevent autoimmunity [98]. Rituximab, a chimeric human anti-CD20 mAb, can reverse the abnormal functional activity of monocytes and generate proinflammatory cytokines through B-cell depletion in the early stages of T1DM [99]. Intervention with rituximab reduced the incidence of T1DM in NOD mice, promoted insulin secretion, controlled blood glucose levels, and alleviated insulitis [100]. In a phase II clinical trial (NCT00279305), early rituximab treatment delayed the decrease in C-peptide levels by 8.2 months, resulting in overall better retention of β-cell function when considering all time points. However, during the 30-month follow-up period, the reduction

rate of the C-peptide AUC was similar to that in the control group, indicating that rituximab did not fundamentally change the underlying pathophysiology of T1DM [101]. Therefore, from a therapeutic point of view, rituximab may be recommended for innate immune regulation and inflammatory conditions in the prevention and adjuvant treatment of T1DM [99].

3.4 Dendritic Cell-Targeted Immunotherapy

DCs are the most important subgroup of professional APCs that initiate and regulate the function of the adaptive immune system and are characterised by high levels of expression of MHC molecules and integrin CD11c [102, 103]. A recent study demonstrated that DCs with CD103 integrin could be detected in the islets of NOD mice [104]. DCs not only induce the activation of Teffs, NK cells, and helper T cells to control infection and cancer development but also inhibit inappropriate self-immune responses. DCs deficiency and dysfunction have been observed in the immune pathogenesis of T1DM. For instance, NOD mice have been shown to express diabetes genetic susceptibility regions and insulin-dependent diabetes loci, which make epitopes on the DCs ideal targets for immunotherapeutic interventions [105].

A DCs scan can recognise a variety of antigens, including microorganisms released by dead cells, extracellular fluid, and apoptotic cells [106]. These antigens can be processed and presented on class MHC I and II molecules to naive T cells in the form of polypeptides. Most DCs reside in the body in an immature state, and these immature DCs (iDCs) are usually regarded as tolerant DCs (TDCs). Under these circumstances, iDCs lack many features and processes that lead to strong T-cell responses, such as increased MHC presentation, expression of co-stimulatory molecules (CD80 and CD86), and production of inflammatory cytokines, such as IL-12, IL-23, and TNFα, which are effective in detecting and isolating antigens. Designing an effective treatment method to induce DCs should fulfil two main objectives: (1) passing the 'autoantigen' of the disease to the TDCs population for processing and presentation; and (2) regulating or covering the expression profile of these DCs and presenting signals that inhibit T-cell activation to T cells, thereby inducing tolerance. DC receptor/co-stimulatory molecules such as CD80/CD86, also identified as B7-1 and B7-2, produce the necessary signals to initiate the induction and differentiation of immature T cells and may inhibit immune tolerance, similar to that in T1DM [107]. CD80/CD86 can bind to CD28 on the surface of T cells for self-regulation and intercellular binding or to CTLA-4 produced by T cells to reduce immunosuppression and cell dissociation. The first immunotherapeutic product targeting CD80/CD86 co-stimulatory binding to CD28 was the fusion protein CTLA4-immunoglobulin (Ig) called abatacept. Abatacept is a kind of co-stimulatory modulator that inhibits CD28-mediated T-cell co-stimulation by binding to CD80/86 on APCs, thus inhibiting T-cell activation to protect the β cells from attack [108, 109]. In a clinical trial, the CTLA4/Ig treatment group exhibited a smaller percentage of change in the C-peptide AUC from that at baseline and a lower overall C-peptide rate within 24 months when compared with the placebo group [62]. Interestingly, the decrease in central memory (CM) $CD4^+$ T cells induced by CTLA4/Ig can also be used as a surrogate indicator of decreased C-peptide levels in patients newly diagnosed with T1DM [110]. Abatacept treatment drove peripheral contraction of the CM $CD4^+$ T cells and expansion of naive ($CD45R0^-$ $CD62L^+$) CD4 T cells, with a significantly slow rate of C-peptide decline. These findings suggest that quantification of CM $CD4^+$ T cells may provide a surrogate immune marker for C-peptide decline following T1DM diagnosis, and that co-stimulation blockade may exert beneficial therapeutic effects by modulating this subset [111]. Moreover, CTLA4-Ig was approved by the US FDA in 2005 for the treatment of rheumatoid arthritis, and in 2009 for the treatment of juvenile idiopathic arthritis, demonstrating the efficacy and potential for the clinical application of CTLA4/Ig treatment [110]. However, CTLA4/Ig does not maintain long-lasting immune tolerance in recipients, probably because of the increase in activated B cells, reprogrammed co-stimulatory ligand gene expression, and reduced inhibition of anti-insulin antibodies [112]. This seems to be a short-term effect and may reduce as the autoimmunity in T1DM patients fades over time [62].

3.5 Combination Immunotherapies

Ideally, a combination of two or more immunotherapeutic drugs can exert synergistic tolerance induction effects to achieve better therapeutic effects or reduce adverse effects. He et al. [113] observed the protective effects of combined rapamycin/γ-aminobutyric acid (GABA) therapy in NOD mice via two different mechanisms. Rapamycin (sirolimus) is a macrolide immunosuppressant that inhibits the mechanistic target of rapamycin (mTOR) protein kinase, blocks the activation of T and B lymphocytes, and induces the expansion of Tregs by inhibiting the response of DCs to IL-2 [114]. Simultaneously, GABA exerts synergistic protective and regenerative effects in both mice and humans by promoting the proliferation of the pancreatic α cells and regulating peripheral blood glucose levels significantly [115]. Under the combined treatment of rapamycin and GABA, insulitis was significantly alleviated and the structure and function of the islets were partially restored in the NOD mice [113]. Similar synergistic effects were observed in a phase I clinical trial involving low doses of IL-2 and rapamycin. Rapamycin/IL-2 combination therapy transiently increased $Foxp3^+$ natural Tregs, eosinophils, and NK cells, with a direct correlation

with soluble IL-2RA levels. The absolute white blood cell count, frequency of lymphocyte and monocyte populations, and CD4:CD8 ratio with combination therapy showed no significant changes in the serum cytokine analysis and flow cytometry, indicating that rapamycin/IL-2 combination therapy did not alter CD4 or CD8 Teffs differentiation [116]. In another study, NOD mice were administered a combined medication loaded with polyethylene glycol (PEG)-mediated anti-CD28Fab antibody fragment (PV1-PEG) and rapamycin. Compared with monotherapy with either agent, the combination of anti-CD28/rapamycin treatment exhibited a complementary style in remarkably inhibiting the activation and infiltration of T cells in the islets and T1DM development [117].

Immunotherapy is also frequently combined with standard insulin replacement therapy, islet transplantation, stem cell therapy, or other immunomodulators to produce a synergistic effect owing to the complexity of T1DM. The combination of immunotherapy and insulin replacement therapy enables the remaining β cells to retain and secrete part of the endogenous insulin while a sufficient supplement of exogenous insulin is obtained. This synergism is conducive for maintaining euglycaemia and homeostasis in T1DM patients [118]. Moreover, oral administration of insulin with a CD6 inhibitor may induce immune tolerance, although the therapeutic effect is not sustainable [119].

Currently, transplantation of vascularised islet tissues or stem cells is an emerging T1DM therapy to promote islet β-cell regeneration [120, 121]. However, the biggest challenge for these stem cell-derived β cells or β-cell transplantation is transplant rejection, indicating that combination immunotherapy is necessary for tackling self-immune-mediated attack by the host [122]. Ideally, immunosuppressive therapy should minimise lymphatic depletion and enhance Tregs function by inducing islet antigen-specific Tregs or diminishing the production of the targeting cytokines needed for Teffs activation and expansion [123]. To date, mTOR inhibitors (e.g. sirolimus and everolimus) are most frequently used for immunosuppression to prevent immune rejection against islet transplantations and β-cell regeneration engineering in clinical settings. In a previous study, a diabetic-humanised NOD/severe combined immunodeficiency IL2R null (NSG) mouse model received renal subcapsular human islet allografts with a transfusion of 1×10^7 human spleen mononuclear cells to test the therapeutic regimen of low-dose recombinant human IL-2/rapamycin. Monotherapy with either IL-2 or rapamycin for 3 weeks did not prolong survival, whereas the combination therapy of IL-2/rapamycin significantly prolonged human islet allograft survival for up to 62 days, indicating the importance of synergism. The proportion of $hCD45^+$ cells in the peripheral blood of NSG mice treated with IL-2/rapamycin significantly declined with reduced IFNγ production and perforin-1 expression, indicating that IL-2/rapamycin may have the potential to inhibit transplant rejection of human islet allograft by expanding Tregs in vivo and suppressing Teffs function [124]. However, available evidence indicates that long-term administration may mediate the loss of islet cell function and vitality by inhibiting mTOR complex 2 [123, 125]. A recent preclinical study showed that Tregs with mixed donor and recipient haematopoietic chimerism might be an effective method for inducing islet transplantation tolerance. According to the Edmonton protocol, some researchers combined myeloablative and/or non-myeloablative haematopoietic stem cell transplantation with Tregs to induce continuously mixed chimerism and allograft tolerance in islet transplantation [126]. This chimera avoids the use of mTOR inhibitors and is expected to be a substitute for immunosuppressants in islet transplantation.

4 Delivery Strategies for Immunotherapy

Advances in medical engineering and drug-delivery strategies have accelerated immunotherapy testing in T1DM models. Drug or antigen formulations can be designed in complex functional forms, allowing fine-tuning of the dose, timing, and route of administration [42]. Nanoscale particles or scaffolds have been commonly used in combination immunotherapy with islet transplantation [127, 128]. Nanopreparations enhance the characteristics of immunity medicine through modification, possess specificity for targeted delivery sites, and reduce off-target effects and adverse effects due to systemic immunosuppression [129]. For instance, the overexpression of phospholipids (PS) on the cell membrane can lead to the progression of diabetic microvascular complications, such as diabetic kidney disease and diabetic retinopathy. Liposome coating helps PS to be efficiently taken up by the DCs with an improved immune response rate to induce immune tolerance while reducing systemic adverse effects caused by off-target effects [130]. Some scaffold materials have been reported to help recruit Tregs to induce T-cell tolerance in islet grafts. In a previous study, primary Tregs and transplanted islets loaded in polylactic acid-glycolide copolymer (PLGA) microporous scaffolds were co-located in the abdominal fat of NOD mice. The survival rate of the grafts in the NOD mice was prolonged and blood glucose returned to a normal state, indicating that the transplanted Tregs may be replaced by receptor-derived Tregs through the mechanisms of infection tolerance and systemic tolerance toward islet antigens [127]. Moreover, advanced delivery strategies and biomaterials can provide convenient, efficient, and minimally invasive drug administration to improve the comfort and compliance in T1DM patients [131]. MNs are considered a promising drug delivery system facilitating the transmission

of autoantigens through the epithelial barrier in a minimally invasive approach. Compared with conventional intravascular or subcutaneous injections, MNs can directly penetrate the epithelial layer of NOD mice and significantly stimulate the proliferation of antigen-specific $CD8^+$ T cells in skin-draining lymph nodes by local injection of proinsulin *in vivo* [131]. Herein, we describe a series of advanced drug delivery strategies to improve the safety and efficacy of T1DM immunotherapy and discuss the expected clinical effects (Table 2).

4.1 Nanoparticles

NPs are structures sized from 1 to 100 nm with a solid core surrounded by suitable chemicals that affect the size, polarity, and electric charge of the NPs [132]. Nanoscale polymeric particles and scaffolds have been designed for multiple immune tolerance induction strategies for the targeted inhibition of T-cell activity [133]. Supramolecular nanostructures can be loaded with or combined with small-molecule drugs, antigenic peptides, and cytokines, thereby altering and optimising the approach for presenting autoantigens [128]. Nanomaterials are commonly classified into organic materials such as liposomes, polymers, including PLGA, polylactide, poly(β-amino esters), and PEG, or inorganic materials such as Au and porous Si [134]. PLGA is the most commonly used polymer in multiple drug delivery applications, mainly because it has been approved by the FDA. The byproducts generated after PLGA degradation can downregulate the MHC II molecules. This potentially useful immunosuppressive effect is in contrast to that of other polymer-based NPs that have an adjuvant effect, highlighting the importance of the properties of the polymeric carrier used to promote tolerance. In a previous study, syngeneic apoptotic cellular carriers and synthetic NPs were covalently cross-linked to diabetogenic peptides or proteins using ethylene carbodiimide (ECDI) to induce antigen-specific T-cell tolerance [133]. Autoantigenic peptides chemically cross-linked to splenic leukocytes (Ag-ECDI-SP) can directly engage in TCR signalling in the absence of co-stimulatory molecules, probably because of the indirect mechanisms involving host APCs. The Ag-ECDI-SP NPs readily induce apoptosis and are sequentially phagocytosed by macrophages and DCs, thus inducing immunosuppression of the host to the autoantigens [135]. Combined with the B-cell depletion strategy with anti-CD20 rituximab and a short course of rapamycin, Ag-ECDI-SP seemed to induce indefinite exoantigen-specific tolerance in diabetic C57Bl/6 mice; however, there is a concern whether the normal immunity of experimental animals is also impaired [133]. Another carboxylated 500 nm biodegradable PLGA NP (either surface-coupled with or encapsulating the cognate diabetogenic peptides) was used as a surrogate antigen carrier for the induction of tolerance in diabetogenic BDC2.5 $CD4^+$ and NY8.3 $CD8^+$ T cells. Reduced infiltration of Teffs and cytokine production were observed within the pancreas of p31-PLG tolerized mice, concomitant with selective retention in the spleen via the CTLA-4 and PD-1 pathways [136]. The strategy of encapsulating Ag into PLG NPs has been improved to become more effective and safer for targeting and tolerising both $CD4^+$ and/or $CD8^+$ diabetogenic T cells [136, 137]. Notably, the encapsulation of NPs not only delivered antigens to induce tolerance in MHC II-restricted T cells but also delivered them to APCs for cross-presentation to regulate $CD8^+$ T-cell responses, as observed in PLG (NRPA7)-treated recipients of $CD8^+$ NY8.3 T cells [138] (Table 3).

Except with the Teffs-targeted immunosuppression strategy, the transplanting islet-loaded microporous PLG scaffold focused more on recruiting Tregs to induce tolerance to pancreatic β-cells. In combination with immunotherapy and islet transplantation, the PLG scaffold provided a platform to co-localise Tregs with the islets for extrarenal and extrahepatic islet graft protection. The PLG scaffold induced recipient-derived Tregs to replace the transplanted Tregs, indicating the successful establishment of systemic tolerance to islet antigens [127].

NPs are usually designed to be captured by APCs via phagocytosis or micropinocytosis and accumulate in the

Table 2 Characteristics of delivery strategies for T1DM immunotherapies

Delivery strategy	Classes of delivery	Advantages	Limitations
Nanoparticle	mRNA/siRNA	Target-specific organs or cells	Immune heterogeneity
Plasmid	Compounds	Biodegradability and biocompatibility	Potential systemic toxicity and adverse effects
Engineered bacteria and BLP	mAb	Localised delivery	
Liposome	Insulin	Protect cargo from degradation	Insufficient bioavailability
Microneedle	Antigen	Sustained and on-demand release	Lack of research on long-term safety
	Cytokine	Possibility to provide stealthiness	Difficult to commercialise
	Antigenic peptide and protein	Interaction with ECM	Low drug loading
	Enzyme and kinase	US FDA approval	

T1DM type 1 diabetes mellitus, *BLP* bacterium-like particle, *mAb* monoclonal antibody, *ECM* extracellular matrix, *mRNA* messenger RNA, *siRNA* small-interfering RNA

Table 3 Nanoformulation for T1DM immunotherapies

Material	Cargo	Size	Targeted cell	Target site	Outcome	References
PLGA microsphere	Antisense oligonucleotide	1–1.8 μm	DC	CD40, CD80 and CD86	Antigen-specific $Foxp3^+$ Tregs increased in the lymph nodes draining the site of administration	[187]
PLGA microparticle	VD3 and insulin B9–23 (1 μm) GM-CSF and TGF-β1 (30 μm)	~ 1 μm (phagocytosable); ~ 30μm (nonphagocytosable)	DC	CD86 and MHC II	Boosted pancreatic lymph node and splenic Tregs, upregulated PD-1 on $CD4^+$/$CD8^+$ T cells; reversed hyperglycaemia for ~100 days	[143]
PEG-PLGA	Autoimmune diabetes-relevant peptide (2.5 mi); pCas9, gRNA	~138 nm	DC	CD40, CD80 and CD86	Tolerogenic DCs triggered autoantigen-specific Tregs generation and expansion by presenting the 2.5 mi peptide to $CD4^+$ T cells in the absence of costimulatory signals	[188]
mPEG-b-PCC-g-DC-g-TEPA	Sunitinib, siRNA against Alox15 (siAlox15)	97.63 ± 0.20 nm	PBMC	Akt, PDGFR	Inhibited PBMC proliferation and activation by downregulating the phosphorylation of Akt, PDGFR at protein and mRNA levels.	[189]
PLGA micelles	Dex, CTLA4-Ig	1–100 μm	Macrophage, DC	CD28	Converted $CD4^+$ T cells into IL-10-secreting Tregs, thereby inhibiting the proliferation of Teffs and supporting long-term survival of islet engraftment and allograft	[190]
AuNP	ITE and proinsulin	60 nm	DC, Treg	CD40, CD80/CD86, MHC II	The coadministration of β-cell antigen and tolerogenic AhR ligand ITE suppressed the spontaneous development of T1DM in NOD mice	[191]
AuNP	PIC19-A3 peptide	> 5 nm	DC, LC	HLA-DR4 (DRB1*0401)	LCs and DCs significantly internalised the AuNP-peptide complex and reduced the capacity of these cells to activate naive T cells	[162]
SPIONS	miR-216a mimics/ASO	~ 30 nm	β cell	PTEN, Ki67	miR-216a mimics expressed higher insulin-producing functionality compared with controls and miR-216a ASO	[192]

Table 3 (continued)

Material	Cargo	Size	Targeted cell	Target site	Outcome	References
PS-liposome	Insulin A and B peptides	1 μm	DC	PS receptors (CD36, MERTK); immunoregulatory molecules (PPARG, TNFAIP3, TNFSF14)	Peripheral DC subsets were altered and the expression of tolerogenic markers upregulated in the first stage of T1DM paediatric patients	[185]

PLGA poly (lactic-co-glycolic acid), *PEG* polyethylene glycol, *mPEG-b-PCC-g-DC-g-TEPA* poly(ethylene glycol)-block-poly(2-methyl-2-carboxyl-propylenecarbonate-graft-dodecanol-graft-tetraethylenepentamine, *AuNP* gold nanoparticle, *SPIONS* superparamagnetic iron oxide nanoparticles, *PS* phosphatidylserine, *VD3* vitamin D3, *GM-CSF* granulocyte-macrophage colony-stimulating factor, *TGF-β1*, transforming growth factor-β1, *Dex* dexamethasone, *ITE* 2-(1'H-indole-3'-carbonyl)-thiazole-4-carboxylic acid methylester, *DC* dendric cell, *PBMC* peripheral blood mononuclear cell, *Treg* regulatory T cell, *LC* Langhans cell, *ASO* antisense oligonucleotides, *Teff* effector T cell, *pCas9* CRISPR-Cas9 plasmid, *gRNA* guide RNA, *siRNA* small-interfering RNA, *CTLA-4* cytotoxic T lymphocyte antigen 4, *Ig* immunoglobulin, *MHC* major histocompatibility complex, *PDGFR* platelet-derived growth factor receptor, *Foxp3* forkhead box protein 3, *PD-1* programmed cell death-1, *mRNA* messenger RNA, *IL* interleukin, *NOD* non-obese diabetic, *T1DM* type 1 diabetes mellitus, *HLA* human leukocyte antigen, *PPARG* peroxisome proliferator-activated receptor gamma, *TNFAIP3* tumour necrosis factor-α-induced protein 3, *TNFSF14* tumour necrosis factor superfamily member 14

draining lymph nodes targeting T-cells and APC trafficking [128]. Immune tolerance induced by DC-targeted nanomedicines is an outstanding immunotherapeutic strategy for treating T1DM [134]. Compared with conventional immunosuppressive therapies, tolerogenic immunotherapy of DCs reduces the activation of T and B cells by inhibiting the presentation of autoantigens, thus indirectly protecting the β cells from being attacked [134]. iDCs not only present antigens and induce tolerance to autoimmunity but also take up a wide range of exogenous materials, including NPs, via multiple internalisation mechanisms, resulting in alteration of the transport kinetics of the antigens [134, 139]. Several NP factors, including size, shape, surface structure, and surface charge, have been reported to alter immune targets and engineer DCs [140, 141]. Kim et al. [142] prepared an NP system containing ovalbumin (OVA) + dexamethasone (Dex) by encapsulating Dex and model antigen OVA with PLGA NPs. Among the available size of polymeric NPs (ranging from 300 nm to 2 μm) that can be efficiently taken up by DCs via phagocytosis, the researchers determined that the average particle size of 864.8 nm was the optimal size. These findings provide a prospective DC regulatory strategy for adjusting the sizes of NPs. Moreover, synergistic and superposition effects appear to exist in this adjustment strategy. A dual-sized microparticle (MP) system established by phagocytosable (approximately 1 μm) and non-phagocytosable (approximately 30 μm) MPs exhibited the outstanding property of simultaneously delivering tolerance-promoting factors both intracellularly and extracellularly to DCs. In recent-onset NOD mice, the PLGA-MP system enhanced Tregs in the pancreatic lymph nodes and spleen, with upregulated PD-1 on $CD4^+$ and $CD8^+$ T cells, resulting in the induction of T-cell tolerance and preservation of hyperglycaemia for up to 100 days [143].

4.2 Plasmid

A DNA vaccine has been developed to regulate the immune responses during immunotherapy for cancer and autoimmune diseases [144]. Plasmids are circular DNA molecules extracted from bacteria that can replicate independently from the genomic DNA of bacterial chromosomes, making them ideal vectors for constructing DNA vaccines [145]. Plasmid DNA (pDNA) represents a critical starting point for many genetic engineering pursuits, including the development of recombinant proteins, viral vectors, and advanced immunotherapeutics [144]. Known diabetic autoantigen-specific epitopes, such as preproinsulin2, GAD65, and ZnT8 as well as epitopes expressing intracellular apoptosis-inducing signalling molecules, such as BAX, have been inserted in pDNA [146-148]. Engineered plasma was designed to induce TDC migration to the draining lymph nodes, which subsequently present antigens to regulate

autoantigen-specific Tregs, thus inducing Tregs expansion and suppressing the corresponding Teffs [147]. Moreover, plasmids can be simultaneously inserted into multiple antigen epitopes to ensure the success of mediating immune tolerance in T1DM. A multiple-epitope vaccination was conducted to co-deliver pDNA of multiple epitopes/mimotopes from four β-cell antigens (proinsulin, GAD65, ChgA, and islet-specific glucose-6-phosphatase-related protein) and then efficiently presented to $CD4^+$ and $CD8^+$ T cells. $CD25^+$ TCR-transgenic T cells and $Foxp3^+$ cells were found to increase the draining lymph nodes in NOD mice, and the induced Tregs exerted partial immunosuppression in the late stage of the disease [149].

4.3 Engineered Bacteria with Derived Materials

Current studies have demonstrated that *Lactococcus lactis*, a safe engineering strain, has been developed as a novel vector for the delivery of immunotherapeutic drugs. *L. lactis* is commonly prepared for oral administration, which encapsulates and protects antigens from undergoing enzymatic hydrolysis or hydrolysis [150]. In previous studies, NOD mice were orally administered with heat shock protein65-6IA2P2-loaded *L. lactis*. The recombinant *L. lactis* successfully delivered antigens to the intestinal mucosa and maintained euglycaemia for approximately 32 weeks [150]. Recently, an innovative strategy for early intervention in T1DM targeting neutrophil extracellular traps (NETs) with staphylococcal nuclease (SNase) has been developed. NOD mice were administered recombinant *L. lactis* encapsulating and expressing SNase, which effectively diminished the NETs and alleviated intestinal inflammation, as proven by the increased IL-4 levels and well-controlled levels of infection markers or inflammatory indicators such as C-reactive protein and TNFα in the early stages [151].

To avoid additional inflammatory responses mediated by the endogenous antigens of engineered bacteria, inanimate lactic acid bacteria have been proposed as antigen delivery platforms (i.e. bacterium-like particles [BLPs]) [152]. These mechanisms are similar to those of engineered bacteria, which are used as protective vectors or immune stimulants in T1DM prevention owing to their immunostimulatory properties. Furthermore, BLPs exhibit improved stability and safety [153]. These findings suggest that engineered bacteria with derived materials have shown remarkable potential for preparing convenient, safe, and low-cost oral administrations that mediate antigen-specific immune tolerance in T1DM.

4.4 Liposome

Liposomes (lipoplexes), characterised by a fluid lipid bilayer membrane and nanoscale size, are widely used in drug delivery systems because of their ease of fabrication, low toxicity, and outstanding biodegradability. With optimal physical and chemical properties (e.g. size, charge, and membrane fluidity) and appropriate components (properties and proportion of components), liposomes provide an on-demand adjunctive strategy [154]. Liposomes have been shown to enhance the in vivo delivery of insulin and particularly increase its oral bioavailability. Arginine-insulin complex (AINS)-loaded liposome was incorporated into cysteine-modified alginate hydrogel to form AINS-Lip-Gel. The intestinal permeability of AINS and AINS-Lip-Gel is approximately 200% and 600% in vitro, respectively, which is significantly higher than that of free insulin; moreover, the in vivo hypoglycaemic effect was an excitonic effect [155]. Targeted delivery can be achieved using functionalised liposomes, such as for specifically targeted delivery of mAbs to epitopes [3]. Pellegrino et al. [156] targeted downregulation of the *PTPN22* mutant gene by using a liposome vector carrying the small-interfering RNA (siRNA) duplex, leading to specific target mRNA downregulation and increased release of IL-2. Moreover, the combination of liposomes and siRNA did not affect the conformational stability of siRNA or celiac disease spectrum, indicating that liposomes can maintain the stability and activity of the cargos.

Additionally, studies have shown that liposomes themselves have an interventional effect on the progression of T1DM. PS connect the receptors on the macrophages and T cells, promote the polarisation of macrophages to the M2 phase, and secrete IL-10 and TGFβ, thereby mediating the immune tolerance of β cells [157]. Liposomes are efficiently phagocytosed by DCs, and suppress latent Teffs in the peripheral blood mononuclear cells of T1DM patients [158]. They have been shown to encapsulate various components, including insulin, and mediate immune tolerance in experimental NOD mice. However, only insulin-containing liposomes significantly reduced the incidence of T1DM, whereas liposomes containing C-peptide, GAD65, and IA2 peptides did not achieve the desired effect [159]. These findings suggest that insulin-containing PS liposomes have a unique role in the immune response.

4.5 Microneedles

In previous studies, the main components delivered through the MNs were mainly insulin and T2DM therapeutic drugs. Recently, researchers have focused on the use of MNs in immunotherapeutic drug delivery owing to the abundance of immunoreactive APCs in the skin. MNs are considered a suitable drug delivery system and an available replacement for subcutaneous injections to deliver autoantigens in a minimally invasive manner [160]. The available components include autoantigenic peptides, active pharmaceutical ingredients, hydrophobic peptides, and NPs [131, 161, 162]. The components remain physically stable upon extrusion through

the MN chamber and diffuse rapidly and widely throughout the dermis; they are presented by the APCs, including Langerhans cells (LCs) and dermal DCs, to induce antigen-specific immunotherapy (ASI) [162]. ASI aims to induce regulatory immune response and autoimmune tolerance to reduce or prevent autoimmune-mediated β-cell destruction [67]. Abundant immunoreactive LCs and DC make the skin an attractive target for T1DM immunotherapy. MNs have been proposed as a suitable drug delivery device to promote intradermal delivery of autoantigens in a minimally invasive and painless manner. Researchers constructed a stainless steel MN array comprising 30 projections of 500 μm to deliver a therapeutically relevant dose of proinsulin into the skin. The MN array punctured the skin of the NOD mice, and 86% of the therapeutic payload was reproducibly delivered into the local tissue after a single insertion for 150 s [131]. Interestingly, an innovatively coated MN system addressed the problem of preserving drug activity. Yang L et al. [160] proposed an optimised triple-component (proinsulin–diluent–surfactant) coating formulation that facilitated the quantitative, uniform, and reproducible coating of the immunotherapeutic drugs in the MNs. Advanced coating provides an innovative preparation strategy for a stable, convenient, and targeted transdermal drug delivery device. These results indicated that MNs are an efficient, comfortable, and safe autoantigen delivery device for ASI induction. However, the issues of time and money for mass production as well as the difficulty of storage and transportation need to be addressed [131, 163].

4.6 Exosome

Exosomes play a role not only in immune stimulation but also in immune tolerance, and they are becoming an alternative tool for T1DM immunotherapy to induce and re-establish self-tolerance [164, 165]. Current studies demonstrated that stem cell-derived exosomes have innate therapeutic potential and may protect pancreatic β cells from autoimmune attack, thereby ameliorating T1DM progression [166]. Exosomes isolated from menstrual blood-derived mesenchymal stromal cells (MSCs) enhanced β-cell regeneration and insulin secretion through the pancreatic and duodenal homeobox 1 pathways in a T1DM mice model [167]. A recent study showed that streptozotocin (STZ)-induced diabetic mice treated with MSC-derived exosomes showed reduced blood glucose levels and elevated plasma insulin levels, indicating the activation of insulin-producing β cells [168]. Histopathological examination also demonstrated an increase in the size and number of islet β cells and a decrease in islet fibrosis and inflammation. Notably, some researchers have pointed out that exosomes can be used as an alternative to stem cell therapies, because they are safer, faster, easier to inject, more effective, and have a longer storage time [169, 170].

MSC-derived exosomes also have immunomodulatory effects [171, 172]. *In vitro* studies showed that bone marrow MSC (BMSC)-derived exosomes inhibit the maturation of DCs, reduce the secretion of the proinflammatory cytokine IL-12, and promote the production of the anti-inflammatory cytokine TGFβ, thus contributing to the regulation of DC-induced immune responses [173]. *In vivo* experiments revealed that exosomes derived from adipose-derived MSCs exerted protective effects in STZ-induced T1DM mice by increasing Tregs and their products without altering the proliferation index of lymphocytes [174].

Some animal experiments have shown that exosomes ameliorate diabetic complications. Brain endothelium-derived exosomes ameliorate cognitive dysfunction in STZ-induced diabetic rats by improving the cerebral vascular dysfunction and enhancing neurogenesis [175, 176]. Another study showed that exosomes derived from adipose stem cells inhibited the activation of MPC5 and mTOR signalling by promoting the expression of miR-486, enhancing autophagic flux, and diminishing podocyte damage, thus improving the symptoms of diabetic nephropathy [177]. Furthermore, an exosome isolated from human umbilical cord mesenchymal stem cells upregulated the expression of vascular endothelial growth factor and TGFβ-1, promoting granulation tissue regeneration and angiogenesis, thereby facilitating skin wound healing [178].

5 Discussions and Prospects

Although there is an overall understanding of the pathogenesis of T1DM, immunotherapy drugs based on existing epitopes do not meet our needs for long-term control or reversal of the T1DM development process. We need to develop novel therapeutic targets with their corresponding targeted immunotherapeutic drugs. However, T1DM immunotherapy inhibits the Teffs activation and pancreatic β-cell induction while causing deficiency or dysfunction in the host systemic immune system. Immunity dysfunction is likely to cause irreversible damage to the host when coping with infectious diseases or cancer, especially in growing children. Therefore, the development of localised targeted delivery strategies is the key to overcome challenges such as off-target effects and systemic toxicity.

Furthermore, we confirmed that immunotherapy should be used in combination with other therapies, including insulin replacement therapy or islet transplantation, to achieve optimal outcomes. In the third-stage patients, the remainder pancreatic β cells cannot produce enough insulin to maintain normal blood sugar levels even when T1DM is completely reversed. Therefore, other therapies that supplement

endogenous or exogenous insulin are necessary in T1DM immunotherapy. Currently, combination immunotherapy is usually associated with insulin replacement therapy; however, precisely controlling the amount of exogenous insulin remains a concern. The combination of immunotherapy with β-cell regeneration engineering may be the optimal treatment regimen. Additionally, advanced drug delivery systems have demonstrated their potential in immunotherapy against various diseases, such as tumours, as they improve the immune response rate and reduce systemic off-target effects. However, there is a long way to go before they can be used in preclinical studies and clinical trials. In summary, immunotherapy is a promising treatment for T1DM because it fundamentally targets the pathogenic mechanism of T1DM. The development of novel antigen-specific targets and targeted delivery strategies is the future direction for T1DM immunotherapy.

Declarations

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