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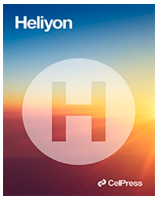

Review article

Biological function of type 1 regulatory cells and their role in type 1 diabetes

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ABSTRACT

The collapse of immune homeostasis induces type 1 diabetes (T1D). In T1D, uncontrolled immune attacks against islet β cells reduce insulin secretion, resulting in hyperglycaemia and various complications. Type 1 regulatory (Tr1) cell therapy is a promising approach for the treatment of T1D. Tr1 cells are a subset of regulatory T (Treg) cells that are characterised by high interleukin-10 secretion and forkhead box protein P3 non-expression. Tr1 cells are reduced and have impaired function in patients with T1D. Immunotherapy is used to treat various diseases, and Treg cells have been applied to treat T1D in animal models and clinical trials. However, the safety and efficacy of Tr1 cells in treating diabetes and other diseases remain unclear. In this review, we aim to investigate the identification and biological function of Tr1 cells and related studies on immune diseases; additionally, we discuss the feasibility, limitations, and possible solutions of Tr1 cell therapy in T1D. This review shows that T1D is caused by an immune imbalance where defective Tr1 cells fail to control effector T cells, leading to the destruction of islet β cells. However, Tr1 cell therapy is safe and effective for other immune diseases, suggesting its potential for treating T1D.

1. Introduction

Type 1 diabetes (T1D) is a chronic autoimmune disease characterised by insulin deficiency, which is induced by the loss and dysfunction of pancreatic β cells, resulting in hyperglycaemia, ketoacidosis, and vascular complications [1,2]. Over 8.4 million patients were diagnosed with T1D worldwide in 2021, and the number is expected to increase to approximately 13.5–17.4 million in 2040 [3]. T1D can occur at any age; however, it peaks in adolescents aged 10–14 years [4]. Multiple factors are involved in the pathogenesis of T1D, including genetic, environmental, and immunological factors, of which immunological factors play a crucial role. The high proinflammatory/cytolytic activity of monocytes is associated with T1D susceptibility and progression [5]. Dendritic cells, macrophages, natural killer cells, and neutrophils participate in the earliest stage of T1D, and $CD4^+$ T cells and $CD8^+$ T cells accelerate the disease process. Regulatory T (Treg) cells are crucial in preventing disease progression [6]. Patients with T1D have a more active immune profile with a higher percentage and number of neutrophils, monocytes, B cells and activated $CD4^+CD25^+$ T cells in the blood than healthy individuals, whereas the number of Treg cells is reduced [7]. Although insulin replacement therapy helps maintain blood

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glucose stability and revert normal metabolism [8], it cannot inhibit immune attacks against pancreatic β cells. Immune modulation, which includes augmenting regulatory function and targeting destructive immune responses, is a promising strategy for T1D treatment.

Type 1 regulatory T (Tr1) cells, an important subset of regulatory $CD4^+$ T cells that have been tested to treat multiple autoimmune diseases, are ideal cells for immunotherapy. Unlike conventional forkhead box protein P3 ($FOXP3^+$) Treg cells, Tr1 cells do not constitutively express FOXP3 and originate in the periphery upon antigen exposure in a tolerogenic situation, secrete high interleukin (IL)-10 levels [9], and play a crucial role in maintaining immune homeostasis and inducing long-term immune tolerance. Tr1 cells can secrete a high level of IL-10 after allogeneic bone marrow transplantation, and adoptive transfer treatment mitigates the severity of graft-versus-host disease (GVHD) [10]. Moreover, patients with various autoimmune diseases exhibit reduced Tr1 cell numbers and functionality. In inflammatory bowel disease (IBD), fewer Tr1 cells infiltrate into the involved lesions of patients and produce lower IL-10 levels than controls. Furthermore, the number of Tr1 cells is negatively correlated with the severity of disease [11,12]. The diminution of Tr1 cell numbers and the suppression of their function have also been identified in patients with autoimmune thyroid disease, and the decreased cell number is associated with disease severity [13]. Declines in Tr1 cell frequency and function also participate in multiple sclerosis (MS) progression [14]. A reduction in Tr1 cell numbers was related to the spontaneous emergence of experimental autoimmune encephalomyelitis (EAE) in humanised double transgenic mice expressing human leukocyte antigen (HLA)-DR15 and a specific T cell receptor (TCR) from a patient with MS [15]. The suppressive capacity of Tr1 cells is also defective in patients with MS. Impaired CD46 glycosylation contributes to the abnormal induction of Tr1 cells in MS [16]. Patients with psoriasis also have lower proportions of Tr1 cells than healthy controls, and a reduction in Tr1 cells is correlated with the psoriasis area and severity index [17]. In addition, Tr1 cells are also dysfunctional in allergies, wherein peanut-specific Tr1 cells from patients with allergies secrete high IL-4 and IL-5 levels, further promoting allergy progression [18]. IL-10 deficiency accelerated the development of

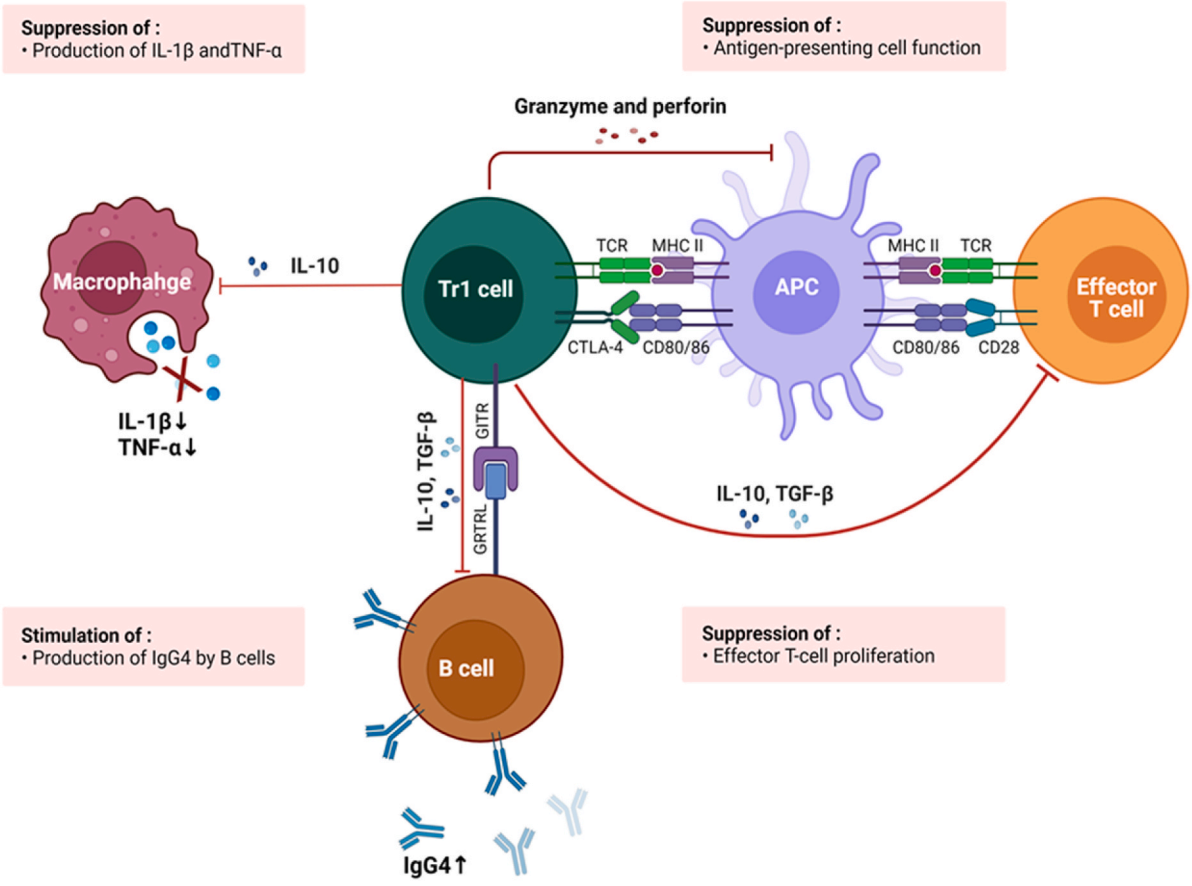

Fig. 1. Suppressive mechanism of type 1 regulatory cells. Type 1 regulatory (Tr1) cells exert their immunosuppressive function through several mechanisms. The first immunosuppressive mechanism involves cytokines such as tumour growth factor (TGF)-β, interleukin (IL)-Tr1, and direct killing of effector or antigen-presenting cells (APCs) by perforin, granzyme B, or cell-to-cell interaction. The second immunosuppressive mechanism involves APCs and CTLA-4. This mechanism includes decreased CD80/86 expression by APCs when binding to CTLA-4 expressed by activated Tr1 cells, leading to impaired APC maturation and reduced T cell proliferation. The third immunosuppressive mechanism involves Tr1 promoting the production of immunoglobulin (Ig) G4 by B cells via IL-10, TGF-β, and glucocorticoid-induced tumour necrosis factor (TNF) receptor-related protein (GITR)/GITR-L interaction. Lastly, Tr1 cells can suppress IL-1β production by macrophages by secreting IL-10.

T1D in a mouse model [19]. Patients with T1D also display fewer Tr1 cells than healthy controls [20]. Tr1 cells were developed through IL-10 lentiviral transduction into $CD4^+$ T cells, and these engineered Tr1 cells inhibited myeloid leukaemia progression and prevented GVHD [21]. Therefore, Tr1 cells are theoretically feasible as a treatment for T1D; however, the safety and efficacy of Tr1 cells in disease treatment must be established through further assessment. Therefore, this review summarises relevant research into the role of Tr1 cells in T1D to provide insights into novel immunotherapy for T1D.

1.1. Identification and biological function of Tr1 cells

Tr1 cell clones were first successfully generated *in vitro* and designated as Tr1 cells by Groux et al., in 1997 [22]. Tr1 cells were induced from murine and human $CD4^+$ T cells in the presence of IL-10 and were characterised by high IL-10 level production [20]. Tr1 cells can also secrete moderate levels of transforming growth factor (TGF)-β, interferon (IFN)-γ, granzyme B, and IL-22, but cannot secrete IL-2, IL-4, or IL-17, or only in low amounts [22–24]. Tr1 cells exert anti-inflammatory and regulatory functions by secreting these cytokines. Initially, IL-10-secreting $CD4^+$ T cells were used to identify Tr1 cells, and IL-10-reporter mice were established to obtain purified Tr1 cells [25]. The co-expression of CD49b and lymphocyte-activation gene 3 (LAG3) enriched Tr1 cells in murine and human $CD4^+$ T cells, which made it easier to isolate Tr1 cells [26]. However, the specificity of this strategy is controversial because other cells (including some $FOXP3^+$ Treg cells) also co-express CD49b and LAG3 [27]. Moreover, no differences were observed in CD49b and LAG3 expression between $CD4^+IL\text{-}10^+$ and $CD4^+IL\text{-}10^-$ T cells, and the expression levels of these two markers changed dynamically over time [28]. Therefore, purifying Tr1 cells limited further research. In 2019, Cook et al. isolated viable high-purity Tr1 cells from human memory $CD4^+$ T cells using an IL-10 capture assay and enrichment kit; Tr1 cells obtained through this method were the most consistent with the nature of Tr1 cells, both in terms of phenotype and function [24]. Mouse Tr1 cells had also been isolated in the same manner [29]. B-lymphocyte-induced maturation protein 1 and cMAF are crucial transcription factors for IL-10 production in Tr1 cells [30,31]. Eomes expression in $CD4^+$ T cells drives a Tr1-like program, including secreting IL-10 [32]. Thus, Tr1 cells will continue to be characterised, and improved extraction methods will be developed in the future.

Tr1 cells are relatively infrequent in the peripheral blood of healthy humans, accounting for approximately 1 % of memory $CD4^+$ T cells [24,33]. However, Tr1 cells play a vital role in maintaining immune balance owing to their extensive and potent immunosuppressive effects (Fig. 1). Tr1 cells primarily exert their physiological functions through two mechanisms: paracrine and cell-to-cell contact. Tr1 cells inhibit the proliferation and proinflammatory abilities of $CD4^+$ and $CD8^+$ T cells by secreting IL-10 and TGF-β [22,23,34]. Their inhibitory effect on T cells is comparable to that of $FOXP3^+$ Treg cells [24]. The impacts of Tr1 cells on B cells are intricate; Tr1 cells promote immunoglobulin (Ig) G4 production by B cells to eliminate parasitic infection via IL-10, TGF-β, and glucocorticoid-induced tumour necrosis factor (TNF) receptor-related protein (GITR)/GITR-L interaction [35]. Furthermore, Tr1 cells also suppress IgG production to participate in B cell tolerance via unknown soluble factors [36]. In particular, Tr1 cells can kill myeloid antigen-presenting cells in a granzyme B- and perforin-dependent manner through cell-to-cell contact [37]. In addition, Tr1 cells can suppress IL-1β production in macrophages by secreting IL-10, whereas $FOXP3^+$ Treg cells cannot [24,38]. Tr1 cells also reduce TNF-α production in myeloid cells [24]. IL-10 facilitates the function of $FOXP3^+$ Treg cells to suppress Th17 responses by activating STAT3 [39]. However, whether Tr1 cells induce $FOXP3^+$ Treg cells via IL-10 is unclear. Surprisingly, in addition to their immunosuppressive ability, Tr1 cells can also exert a protective role in the intestinal mucosal barrier by releasing IL-22 [24].

1.2. Alteration of Tr1 cells in T1D

T1D is classified into three subtypes according to the different manners of onset and progression: acute-onset, slowly progressive, and fulminant [43,44]. However, abnormal immune response persists in all patients with T1D. The gene expression of IL-10 was higher in infiltrated islets than in healthy controls [46]. T cells from patients with T1D released IFN-γ when stimulated by specific islet peptides, which was distinct from healthy controls that released IL-10 [40]. The T cell response to different islet antigens varies considerably among the different T1D subtypes. The response of $CD4^+$ T cells was detected in patients with T1D to four islet antigens *in vitro*, and peripheral blood mononuclear cells (PBMCs) from patients with fulminant T1D showed a lower response of Tr1 cells to all islet antigens and PBMCs from patients with acute-onset T1D had more T helper (Th) 1 cells than healthy controls. These patients also exhibited a stronger Th17 cell response to some islet antigens, and PBMCs from patients with slowly progressive T1D presented a stronger Th2 response to some islet antigens than the controls [45]. Furthermore, Tr1 cells are less frequently observed in patients with T1D and their first-degree relatives (FDRs) than in healthy controls, and the IL-10 secretion level of Tr1 cells in patients with T1D is lower than in FDRs [41]. The islet-specific glucose 6 phosphatase catalytic subunit-related protein-specific Tr1 cell number decreased in adult-onset T1D, and higher frequencies of Tr1 cells at onset positively correlated with better future blood glucose control [20]. In contrast, the number of $FOXP3^+$ Treg cells is negatively correlated with T1D prognosis [42]; the number of Tr1 cells can be a prognostic index. In particular, the local immune microenvironment of T1D is complicated. However, extensive investigations focusing on the local immune environment of infiltrated tissues are restricted because of the limited access to the pancreas from living patients with T1D; therefore, the infiltration of different immune cells around islets remains unclear. Nonetheless, various platforms and organisations, such as the Network for Pancreatic Organ Donors with Diabetes and T1D Exchange, now provide biospecimens, pathology information, and associated data to address these issues.

1.3. Tr1 cell therapy in T1D

An imbalance in immune homeostasis triggers T1D. If the abnormal immune status is not restored, the inflammatory reaction will

be aggravated, and pathology will progress. Therefore, strengthening the immunosuppression of Treg cells and weakening the response of effector T cells are crucial processes for restoring the immune homeostasis of T1D [47]. Although immense efforts have been made in T1D treatment, no practical approach currently exists to cure T1D [48]. However, Tr1 cell therapy has been used to treat various immune diseases and has achieved satisfactory effects. Therefore, Tr1 cell therapy could be used to treat T1D in the future.

1.3.1. Applications of Tr1 cells in immune diseases

Recent animal models and clinical trials have proven that the direct infusion of Tr1 cells, or the induction of Tr1 cells through other methods, can alleviate the symptoms of various immune diseases (Table 1). In an acute GVHD murine model, the lack of Tr1 cells exacerbated disease progression, with all mice dying within 40 days after bone marrow transplantation; however, the adoptive transfer of Tr1 cells remarkably prolonged survival [10]. Bacchetta et al. conducted a small trial that included 12 patients with high-risk haematopoietic malignancies, as Tr1 cells could be induced from T cells in the presence of IL-10. These patients received combined transplantation of T cell-depleted haploidentical–haematopoietic stem cells and IL-10-pretreated T cells from donors. Four patients achieved successful immune reconstitution and complete disease remission after a 7.2-year follow-up [49]. A similar trial, which included paediatric patients with blood disorders, is currently underway (NCT03198234), and preliminary published data confirmed that donor-derived Tr1 cells survived for approximately one year after adoptive transfer [50]. In addition to GVHD, Tr1 cells have also been used to treat autoimmune diseases. Tr1 cell injection inhibited Th17 cell response and blocked the progression of intestinal inflammation through the IL-10 signalling pathway in a $CD4^+CD45RB^{hi}$ cell transfer-induced IBD murine model [26,51]. Furthermore, the application of autologous ovalbumin (OVA)-specific Tr1 cells was reported in 20 patients with refractory Crohn's disease. OVA-specific Tr1 cells were induced and expanded in the presence of OVA, and patients ingested OVA-rich foods after being injected with Tr1 cells. The Crohn's disease activity index was reduced in a dose-dependent manner, with no severe adverse reactions occurring related to Tr1 cells. Therefore, Tr1 cells were well-tolerated and effective in this small-scale IBD clinical trial. This study also represented the first study of Tr1 cell therapy for human autoimmune diseases [52]. In addition, injecting Tr1 cells into OVA-sensitised mice decreased the number of eosinophils and neutrophils and suppressed airway hyper-responsiveness in allergic asthma [29,53]. In MS, administering anti-inflammatory mediators, such as C-X-C motif chemokine (CXCL) 11 and CXCL12, in an EAE murine model induced the differentiation of Tr1 cells from effector T cells, recruited Tr1 cells to the lesion site, and mitigated symptoms [54,55]. Moreover, the expansion of Tr1 and $FOXP3^+$ Treg cells is promoted by regulatory B cell therapy in the EAE model and associated with disease relief [56]. Furthermore, regulatory B cells induced naive T cells into Tr1 cells through IL-10 secretion and cell-to-cell contact *in vitro*, even in patients with rheumatoid arthritis [57]. In an animal model of rheumatoid arthritis, exogenous Tr1 cells decreased the incidence and attenuated the severity of collagen-induced arthritis [58], while the infusion of Tr1 cells combined with mesenchymal stem cells (MSCs) enhanced the treatment effect [59]. Notably, intra-lymphatic MSC infusion increased the number of Tr1 cells and $FOXP3^+$ Treg cells and reduced symptom scores in a collagen-induced arthritis model. This result indicates that MSC treatment can induce Tr1 cells, suggesting that the therapeutic mechanism and its effects may differ depending on the administration route [60]. These studies demonstrate that the recovery of Tr1 cell function, either by direct or indirect approaches, is greatly beneficial for reconstructing the immune state and achieving remission in immune diseases.

Table 1
Type 1 regulatory cells can alleviate the symptoms of various immune diseases.

Disease	Species	Tr1 definition	Role on disease	Authors
Multiple sclerosis	Murine	IL-10-producing $CD4^+CD25^+$ Treg cells	Suppressed ongoing EAE	Meiron et al., 2008 [54]
	Murine	IL-10 hi Tregs	Suppressed autoimmune encephalomyelitis	Zohar et al., 2014 [55]
	Murine	$CD49b^+CD223^+$ regulatory Treg cells	Associated with the relief of EAE	Pennati et al., 2016 [56]
	Murine	$LAG3^+CD49b^+CD25^+CD69^+$IL-10-producing T cells	Reduced Tr1 cells related to the spontaneous emergence of EAE	Leno-Duran et al., 2021 [15]
Allergic asthma	Murine	IL-10-producing $CD4^+$ T cells	Suppressed allergic airway inflammation	Matsuda et al., 2017 [33]
	Murine	$IFN\gamma^-IL4^-IL17A^-Foxp3^-$IL-10-producing $CD4^+$ T cells	Suppressed allergic asthma in mice	Matsuda et al., 2019 [29]
Inflammatory bowel disease	Murine	$CD4^+Foxp3^-$IL-10-producing (Tr1) cells	Blocked the progression of intestinal inflammation through the IL-10 signalling pathway	Huber et al., 2011 [51]
	Human	IL-10-producing type 1 Tregs	Reduced the Crohn's disease activity index in a dose-dependent manner	Desreumaux et al., 2012 [52]
Rheumatoid arthritis	Murine	NA	Decreased the incidence and attenuated the severity of collagen-induced arthritis	Vicente et al., 2016 [58]
	Murine	IL-10-producing type 1 Tregs	Enhanced immunoregulation of mesenchymal stem cells in collagen-induced arthritis	Lim et al., 2016 [59]
GVHD	Human	$IL-10^+IL4^-IL17^-CD49b^+LAG3^+$	Tr1 cells can be an adjuvant treatment for malignancies and immune-mediated disorders	Bacchetta et al., 2014 [49]
	Murine	$IL-10^+Foxp3^-CD4^+$ T cells	Tr1 cells were crucial for preventing GVHD	Zhang et al., 2017 [10]

EAE, experimental autoimmune encephalomyelitis; IL, interleukin; GVHD, graft-versus-host disease; Tr1, type 1 regulatory; Treg, regulatory T cell.

1.3.2. Applications of Tr1 cells in T1D

Compromised immune homeostasis dominates the erroneous attack of immune cells on islet β cells, resulting in insufficient insulin release and T1D onset. Defective Treg cells, which various factors can cause, cannot restrict the over-activation of autoreactive T cells, thus leading to immune imbalance [61]. Regaining and enhancing the immunosuppressive ability of Treg cells is an encouraging approach to restoring islet β-cell tolerance in T1D treatment. Soluble receptors for advanced glycation end products prevented diabetes in mice by expanding FOXP3$^+$ Treg cells [62]. Therefore, Treg cell therapy could cure T1D, and studies have preliminarily confirmed its feasibility and safety [63] (Fig. 2). Infusing FOXP3$^+$ Treg cells in a T1D rat model reduced proinflammatory cytokine secretion, such as that of IFN-γ and IL-17, increased the IL-10, TGF-β, IL-2, and IL-4 levels, and improved islet function [64]. The infusion of autologous polyclonal FOXP3$^+$ Treg cells was feasible and well-tolerated in 14 patients with recent-onset T1D [65]. Marek-Trzonkowska et al. followed up with 12 children with T1D who received expanded FOXP3$^+$ Treg cell injections for one year; C-peptide levels were upregulated in most patients. Furthermore, their insulin demand decreased, highlighting the effectiveness of FOXP3$^+$ Treg cells in treating T1D [66]. A single dose of autologous FOXP3$^+$ Treg cells in children with new-onset T1D improved C-peptide preservation [67]. A trial involving the administration of FOXP3$^+$ Treg cells and IL-2 in T1D is currently ongoing (NCT02772679). Compared to FOXP3$^+$ Treg cells, the adoptive transfer of Tr1 and FOXP3$^+$ Treg cells prevented diabetes in non-obese diabetic (NOD) mice; however, Tr1 cells are essential in mitigating new-onset T1D [68]. In a monkey model of allogeneic islet transplantation, Tr1 cells, but not FOXP3$^+$ Treg cells, induced long-term immune tolerance [69]. Intestinal-specific Tr1 cells can migrate to the periphery via several chemokine receptors, suppress Th1 cell responses in the pancreas, and prevent T1D onset [70]. System delivery of nanoparticles coated with T1D-relevant peptide-major histocompatibility complex class II molecules can convert T-follicular helper-like cells into Tr1-like cells [71]. Jide et al. identified an immunogenic preproinsulin determinant ($PPI_{L4\text{-}20}$) that increased the frequency of Tr1 cells [72]. However, studies into Tr1 cell therapy in T1D are still in their infancy compared to those into FOXP3$^+$ Treg cells, and clinical trials are relatively rare. No clinical trials have reported the safety and efficacy of Tr1 cells in treating T1D. Therefore, four crucial issues must be addressed to accelerate research: 1) Almost all studies related to Tr1 cells in T1D are based on blood samples, and few studies have investigated the infiltration and function of Tr1 cells in the insulitis microenvironment; therefore, the current understanding of the tissue distribution and heterogeneity of Tr1 cells in T1D is limited and insufficient. 2) Tr1 cells are extremely rare, which makes it challenging to realise their large-scale expansion *in vitro* for subsequent administration. 3) Because of the low proliferation of Tr1 cells, considering the contamination risk from the excessive proliferation of effector T cells is essential [22]. Although the inherent phenotype and immunosuppressive function of expanded Tr1 cells have been elucidated [24], more evidence is needed. 4) The appropriate time, route, dosage, and single or combined administration should be investigated as these are related to adverse reactions.

Although various of these issues are challenging to resolve, the application of Tr1 cells in T1D is not impossible. First, the infiltration of Tr1 cells and other immune cells in patients' pancreatic samples should be explored. Nevertheless, fresh pancreatic samples from patients with T1D are scarce and evaluating the association between T1D and the dysfunction of islet Tr1 cells remains challenging. Recently, imaging mass cytometry has been applied to T1D to demonstrate its potential pathogenesis [73,74]. No difference was observed in the frequency of islet β cells between patients with recent-onset T1D and the control group; however, islet β cells expressed lower levels of specific markers in patients with T1D, and cytotoxic T and Th cells infiltrated into islets earlier, before islet β

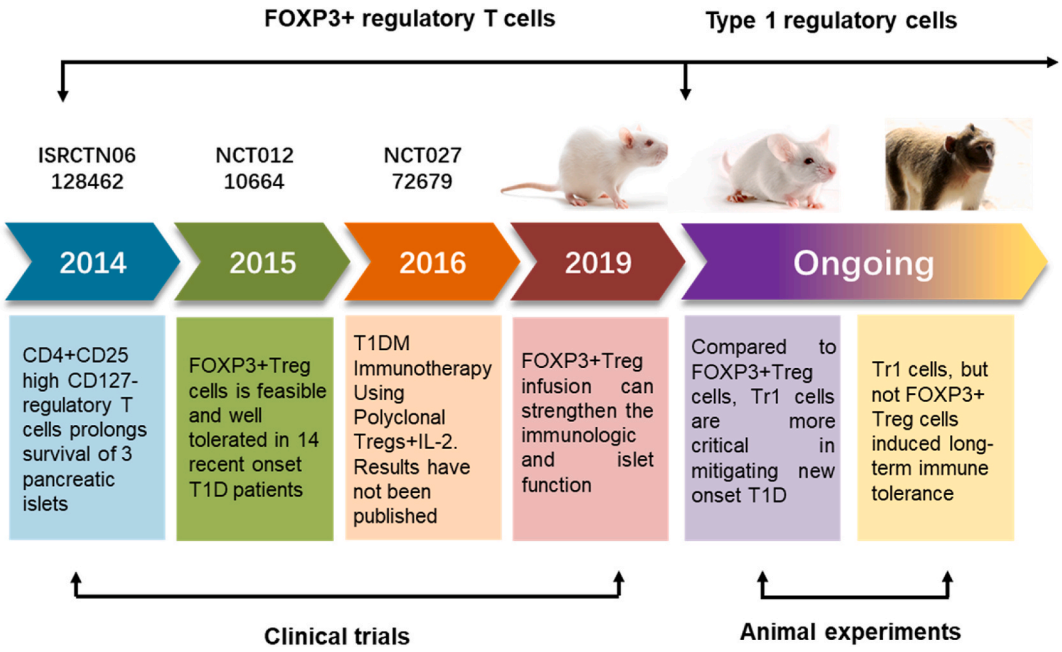

Fig. 2. Recent shifts in regulatory T cells in type 1 diabetes research. With more profound insight into regulatory T cells in type 1 diabetes (T1D), Tr1 cells are considered more vital than FOXP3$^+$ regulatory T (Treg) cells in preventing diabetes and inducing long-term immune tolerance.

cell destruction. New technology could help to realise the localisation and tracking of Tr1 cells in T1D islets. As Tr1 cells are limited in number, more cells for in-depth clinical trials can be obtained by expanding *in vitro* studies. However, no uniform culture conditions are specified for Tr1 cell culture and expansion. In mice, the addition of IL-10 or IL-27 to the culture media can induce Tr1 cells from naive $CD4^+$ T cells; however, IL-10 is not indispensable in this process [23,75]. In humans, IL-10 is necessary for Tr1 cell differentiation [9], and IL-10 receptor signalling is essential to maintain the function of mature Tr1 cells [33]. Antigen-specific Tr1 cells can be induced in mice and humans in the presence of antigens such as OVA [29,49,53]. IL-2 promotes the proliferative ability of Tr1 cells in appropriate stimulation environments, including anti-CD3/CD28 antibodies and mixed lymphocyte reactions, which can accelerate Tr1 cell expansion *in vitro* [22,49]. Therefore, a relatively precise gating strategy can minimise the risk of mixing other cells. Cook et al. sorted viable cells using the $CD4^+CD45RA^-IL\text{-}10^+$ gating strategy from human peripheral blood through an IL-10 capture assay; the resulting cells possessed the classic phenotype and function of Tr1 cells. After 14 days of expansion stimulated with anti-CD3/CD28 beads and IL-2, Tr1 cells still secreted high IL-10 levels and inhibited T cell proliferation and IL-1β secretion by macrophages [24]. Notably, the proportion of IL-10-positive cells decreased to approximately 40 % after expansion *in vitro,* and the contamination of effector T cells cannot be excluded. Therefore, the secondary purification of expanded Tr1 cells may help to improve therapeutic effects and reduce side effects if applied in clinical trials (Fig. 3).

In addition, the administration of Tr1 cells may be more effective during the onset stage than in the late stage, as most cells are destroyed during the late stage [76]. The timely infusion of Tr1 cells in patients with recent-onset T1D who have sufficient β cells can block the immune attack, thereby avoiding the continuous reduction of β cells. The optimal dose of Tr1 cells varies depending on the disease. A dose of 3×10^5 IL-10-pretreated $CD3^+$ T cells/kg showed an apparent efficiency in the prevention of GVHD in haematopoietic stem transplantation [49]. A dosage of 10^6 Tr1 cells achieved the best effect, compared with dosages of 10^7, 10^8, and 10^9 Tr1 cells in the treatment of refractory Crohn's disease [52]. However, no reliable dose for reference has been specified, and the safety and efficacy of Tr1 cells in T1D should be verified at multiple doses. Islet antigen-specific $FOXP3^+$ T cells are enriched in the lesions of NOD mice, especially insulin-specific $FOXP3^+$ T cells, to protect islets from damage [77]. Notably, the effect of antigen-specific $FOXP3^+$ T cell transfer is superior to that of polyclonal $FOXP3^+$ T cells in NOD mice [78]. Thus, antigen-specific Tr1 cells can work better in the treatment of T1D.

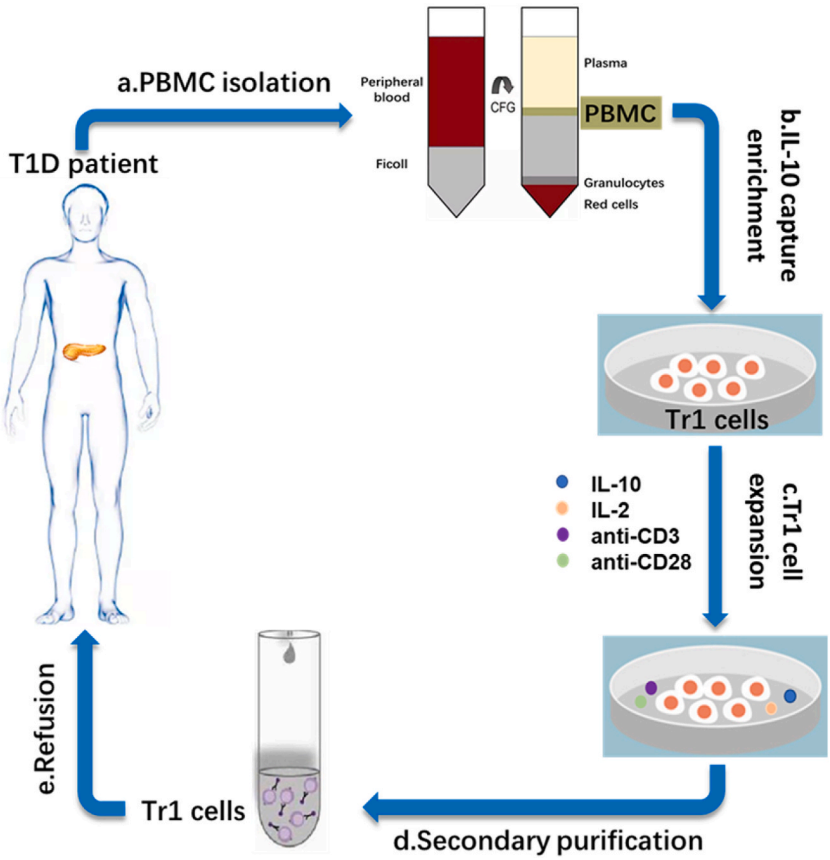

Fig. 3. Idea map of Tr1 cell therapy in T1D. T1D is an incurable autoimmune disease induced by the collapse of immune homeostasis. Peripheral blood mononuclear cells (PBMCs) were collected from patients with T1D (a). The $CD4^+CD45RA\text{-}IL10^+$ gating strategy was used to sort Tr1 cells using an IL-10 capture assay (b). Two weeks of expansion of Tr1 stimulated by anti-CD3/CD28 beads and IL-2 and IL-10 (c). Secondary purification of Tr1 cells may improve the therapeutic effects and reduce side effects (d). Refusion of Tr1 into patients with T1D (e).

Genetic engineering may be of great value in enhancing the migratory ability of Tr1 cells to islets. Chimeric antigen receptor (CAR) technology has been successfully applied as an innovative remedy in tumour-targeted therapy and has also been adopted to generate engineered antigen-specific FOXP3^{+} Treg cells (CAR-Treg) to treat autoimmune diseases in animal models [79,80]. Engineered Tr1 cells can be constructed with islet antigen receptor expression using CAR technology. These engineered Tr1 cells will gather in inflammatory islets to suppress the immune response once infused into patients with T1D. Notably, maintaining the immunosuppressive function of CAR-Tr1 cells is essential after genetic modification.

2. Conclusion

T1D is caused by immune imbalance, in which defective Tr1 cells lose control of effector T cells and islet β cells are destroyed. The exogenous infusion of Tr1 cells might reverse this status. Various studies have confirmed the safety and effectiveness of Tr1 cell therapy in the treatment of other immune diseases, which provides a reference for its application to T1D treatment. However, precise gating, appropriate expanded conditions, and improvement in specificity are needed for the further development of Tr1 cell therapy.

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Ethical considerations

Not applicable as this is a review article.

Data availability statement

The authors declare that no data associated with this work has been deposited into a publicly available repository since no data was used for the research.

CRediT authorship contribution statement

Lingli Qi: Formal analysis. **Zhichao Wang:** Investigation, Methodology. **Xinxing Huang:** Project administration. **Xiuzhu Gao:** Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Glossary

Table of abbreviations

Abbreviation	Definition
CAR	Chimeric antigen receptor
CXCL	C-X-C motif chemokine
EAE	Experimental autoimmune encephalomyelitis
FDR	First-degree relatives
FOXP3	Forkhead box protein P3
GITR	Glucocorticoid-induced tumour necrosis factor receptor-related protein
GVHD	Graft-versus-host disease
HLA	Human leukocyte antigen
IBD	Inflammatory bowel disease
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
LAG3	Lymphocyte-activation gene 3
MS	Multiple sclerosis
MSC	Mesenchymal stem cell
NOD	Non-obese diabetic
OVA	Ovalbumin
PBMC	Peripheral blood mononuclear cell
T1D	Type 1 diabetes
TCR	T cell receptor

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TGF	Transforming growth factor
Th	T helper
TNF	Tumour necrosis factor
Tr1	Type 1 regulatory
Treg cells	Regulatory T cells

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