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Review Article

Recent Advances and Evidence in Regulatory T Cell (Tregs) Based Therapy for Type 1 Diabetes

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Abstract

Type 1 diabetes (T1D) arises from autoimmune destruction of pancreatic β-cells in genetically susceptible individuals, leading to insulin deficiency. A key pathogenic factor is the breakdown of immune tolerance, compounded by dysfunction in regulatory T cells (Tregs). In T1D, Tregs exhibit impaired function, manifested by Foxp3 expression instability, aberrant proinflammatory cytokine production (e.g., IFN-γ), reduced suppressive capacity, and plasticity, contributing to disease progression. Targeting Treg stability, survival, and function is therefore a promising therapeutic strategy to restore immune tolerance. While polyclonal autologous Treg adoptive transfer has proven safe in clinical trials for recent-onset T1D, efficacy has been limited by key barriers: lack of antigen specificity for pancreatic targets, poor long-term persistence, difficulties in generating sufficient cell numbers, and inherent Treg defects in patients. Advanced genetic engineering offers solutions to overcome these hurdles. Strategies include enhancing FoxP3 expression for stability and expansion, engineering T cell receptors (TCRs) or chimeric antigen receptors (CARs) for antigen-specific targeting, and modifying cytokine signalling to improve survival and function. Although these engineered Treg approaches hold significant preclinical promise across immune disorders, T1D presents distinct challenges and opportunities for clinical translation. This review examines the role of Tregs in T1D pathogenesis, the rationale and limitations of current Treg-based therapies, recent advances in engineered Treg strategies specifically for T1D, and key challenges for integrating these novel cell therapies into clinical practice.

Keywords: Type 1 Diabetes; Regulatory T Cells; FoxP3 Expression; Proinflammatory Cytokine Production, Chimeric Antigen Receptors

Abbreviations

APC: Antigen-Presenting Cell; CAR: Chimeric Antigen Receptor; CGM: Continuous Glucose Monitoring; CIA: Collagen-Induced Arthritis; CTLA-4: Cytotoxic T Lymphocyte Antigen 4; DMARDs: Disease-Modifying Anti-Rheumatic Drugs; FDA: Food and Drug Administration; FOXP3: Forkhead Box P3; GN: Glomerulonephritis; JIA: Juvenile Idiopathic Arthritis; MG: Myasthenia Gravis; NOD: Nonobese Diabetic; OVA: Ovalbumin; PKB: Protein Kinase B; RA: Rheumatoid Arthritis; TCR: T Cell Receptor; TGF-β: Transforming Growth Factor Beta; Tregs: Regulatory T Cells; T1D: Type 1 Diabetes.

Introduction

Type 1 diabetes (T1D) is an autoimmune disease characterized by the T cell-mediated destruction of pancreatic insulin-secreting

beta cells, leading to insulin deficiency, hyperglycemia, and a lifelong dependence on exogenous insulin therapy [1,2]. Globally, the incidence of T1D is rising at an average annual rate of 3-4%, with approximately 8.4 million individuals affected in 2021. This burden is expected to increase rapidly, particularly in resource-limited settings [3,4]. Despite significant advances in insulin delivery systems and continuous glucose monitoring (CGM), optimal glycemic control remains elusive for many patients, contributing to both acute complications (hyperglycemia, diabetic ketoacidosis, hypoglycemia) and chronic micro- and macrovascular complications (cardiovascular disease, neuropathy, nephropathy, retinopathy) [5-7]. Furthermore, individuals with T1D exhibit an increased susceptibility to other autoimmune conditions (e.g., autoimmune thyroid disease, celiac disease, Addison's disease, vitiligo), particularly those diagnosed at an older age, underscoring the systemic nature of immune dysregulation [8-10].

Current management focuses on insulin replacement but fails to address the underlying autoimmune pathogenesis [11-13]. Consequently, there is a critical need for disease-modifying therapies that target the root cause: immune-mediated beta cell destruction. Suppressing this autoimmune response without causing broad immunosuppression is a major therapeutic goal. Regulatory T cells (Tregs), defined by expression of the transcription factor FOXP3 and the high-affinity IL-2 receptor CD25 [1,6], represent a highly promising avenue. Tregs are essential for maintaining peripheral immune tolerance and preventing autoimmunity, as dramatically evidenced by the development of T1D in over 60% of patients with IPEX syndrome caused by FOXP3 mutations [6-8]. Critically, Tregs can suppress immune responses in an antigen-specific manner through diverse mechanisms, including immunosuppressive cytokine production (e.g., IL-10, TGF-β), modulation of antigen-presenting cell (APC) function, IL-2 consumption, and direct cytolysis of effector T cells [1] presented in figure 1. This specificity offers the potential for targeted immunomodulation without compromising overall immune defense.

However, Tregs in T1D patients are often numerically or functionally deficient, contributing to disease progression [16,17]. Studies in animal models and humans support the presence of Treg dysfunction [19]. Restoring functional Treg populations holds significant therapeutic potential to suppress autoreactive T cells, modulate aberrant immune responses, and potentially halt or reverse beta cell destruction [17]. Intriguingly, histological analyses of pancreata from T1D patients (e.g., via nPOD) reveal a variegated pattern of islet destruction, with some individuals retaining insulin-positive beta cells even in long-standing disease. This suggests autoimmune destruction may be a localized event within the pancreas, potentially influenced by the dynamic interface between effector T cells and Tregs at specific sites [9, 10]. This heterogeneity implies an opportunity for therapeutic intervention to restore local immune regulation and preserve residual or transplanted beta cell mass.

Consequently, Treg cell-based therapies are at the forefront of translational research for T1D [13,14,17,19]. Approaches range from the expansion and reinfusion of unmodified polyclonal Tregs to advanced genetic engineering strategies designed to enhance Treg specificity (e.g., for islet antigens), stability, function, and survival [1,17]. Furthermore, combination therapies targeting both immune dysfunction and metabolic defects may prove more effective than monotherapies in preserving beta cell function across disease stages [18]. Despite this exciting potential, key challenges remain before widespread clinical implementation, including defining optimal cell purity, ensuring long-term stability *in vivo*, establishing functional efficacy, determining the necessity for tissue or antigen specificity, and clarifying whether Treg function must be assessed directly within the pancreatic microenvironment or if peripheral blood suffices [19].

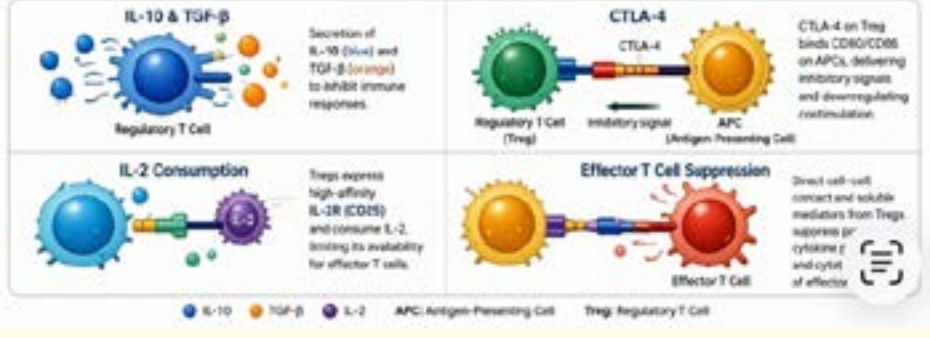

Figure 1: Mechanisms of regulatory T-cell mediated immune suppression including IL-10, TGF-β, CTLA-4 signaling and IL-2 consumption.

This narrative review explores the role of regulatory T cells (Tregs) in the pathogenesis of type 1 diabetes (T1D) and evaluates their therapeutic potential as disease-modifying agents. We first discuss the involvement of Tregs in T1D development and early attempts to employ unmodified Tregs in treatment. Subsequently, we examine emerging genetic engineering strategies designed to enhance Treg phenotype, antigen specificity, and functionality, thereby improving the efficacy of adoptive Treg therapies for T1D.

Overview of regulatory T cells (Tregs)

Regulatory T cells, commonly known as Tregs, are a type of white blood cell essential for maintaining balance in the immune system. They help regulate immune responses by preventing the body from overreacting to foreign substances called antigens. Antigens are typically unwanted intruders that trigger an immune reaction, prompting the body to produce antibodies—specialized proteins that target and neutralize these threats. In the absence of Tregs, the immune system may become overactive, potentially attacking the body's own healthy cells and tissues. This misdirected response can result in autoimmune diseases. In such situations, a suppressed immune reaction is often more beneficial. Tregs play a critical role in determining when the immune system should activate and when it should remain inactive [1].

Tregs represent a distinct subpopulation of T cells equipped with specialized regulatory mechanisms that suppress key elements of both the adaptive and innate immune systems. In humans, Tregs are identified by the expression of the Forkhead box P3 (FoxP3) protein, along with differential expression levels of the CD45RA surface marker. Functionally and phenotypically, Tregs can be divided into three main subsets: 1. $CD45RA^{+}FoxP3$-low resting Tregs, 2. $CD45RA^{-}FoxP3$-high activated Tregs, both of which possess immunosuppressive functions, and 3. $CD45RA^{-}FoxP3$-low non-suppressive subset that primarily secretes cytokines [2].

Tregs play a vital role in maintaining immune homeostasis and promoting self-tolerance. Given their capacity to regulate immune responses, especially through FoxP3 and $CD4^{+}$ expression, numerous studies have highlighted their therapeutic potential in managing autoimmune diseases and severe allergic conditions [3-6].

Efficient Treg function depends not only on FoxP3 expression but also on several key Treg-associated genes, such as CD25 and cytotoxic T lymphocyte antigen 4 (CTLA-4). Moreover, antigen-specific Tregs are particularly efficient in modulating immune response intensity, offering a promising avenue for the treatment of autoimmune disorders [7]

Regulatory T cells in autoimmune diseases

Autoimmune diseases arise from a breakdown in the body's tolerance to its own components, leading to local tissue inflammation. This loss of tolerance can occur when immune-suppressive mechanisms are compromised—either due to functional impairments in regulatory cells or because tissue injury triggers the presentation of multiple autoantibodies that overwhelm anti-inflammatory defenses. Such situations can develop during certain inflammatory or infectious conditions, where tissue damage activates the adaptive immune system and ultimately disrupts self-tolerance. Regulatory T cells (Tregs) play a crucial role in preventing further injury under these circumstances. Once tolerance is lost, ongoing tissue damage can drive the progression and manifestation of disease. The following sections will illustrate this process in various immune-mediated disorders.

Tregs undergo expansion at sites of local inflammation. This is observed in both rheumatoid arthritis (RA) and juvenile idiopathic arthritis (JIA), where their numbers are elevated within affected joints, but remain comparable to healthy controls in peripheral blood. In JIA, Tregs display a restricted T cell receptor repertoire, reduced FOXP3 stability, and diminished CD25 expression, along with altered cytokine and chemokine secretion and decreased responsiveness to IL-2, indicating impaired Treg function [8,9]. However, several studies report that Tregs from JIA synovial fluid and peripheral blood are fully demethylated and exhibit robust suppressive activity outside the joint *in vitro* [9–11]. These findings suggest that the inflammatory microenvironment likely drives the functional impairment of JIA Tregs—an effect described as "resistance to suppression," which relates to altered effector T cell function. This resistance depends on activation of protein kinase B (PKB)/c-Akt and can be reversed by TNFα antagonist therapy. Furthermore, addition of synovial fluid to *in vitro* cultures not only enhances FOXP3 expression in Tregs, but also induces changes in effector T cells that render them resistant to Treg-mediated suppression ex vivo [10-14].

Tregs possess the ability to regulate immune activity, curb inflammation, and prevent tissue damage by modulating the actions of various cell types. These include classical $CD4^+$ helper T cells, B cells involved in antibody production and affinity maturation, $CD8^+$ cytotoxic T lymphocytes responsible for granule-mediated killing, and antigen-presenting cells affecting their function and maturation status [15,16]. In total, more than 15 distinct functions have been ascribed to Tregs, encompassing mechanisms such as the secretion of suppressive cytokines.

Role of Tregs in T1D pathogenesis

Recent evidence indicates that the inability of regulatory T cells (Tregs) to effectively suppress autoreactive T cells can contribute to the development of autoimmune diseases such as type 1 diabetes (T1D) [17,18]. The nonobese diabetic (NOD) mouse remains one of the most well-characterized and widely used spontaneous models for studying autoimmune diabetes. In this model, female mice typically develop overt T1D between 12 and 16 weeks of age, following an extended asymptomatic phase. This prediabetic phase is marked by progressive insulitis beginning around three weeks of age. Tregs have been shown to play a protective role in preventing both spontaneous diabetes in NOD mice and diabetes induced by diabetogenic T cells. First, disease onset in NOD mice is associated with a gradual decline in Treg suppressive function [19,20]. Moreover, studies have shown that conventional T cells in both diabetic humans and NOD mice become increasingly resistant to Treg-mediated regulation. Second, co-transfer of Tregs—either from young, prediabetic mice or as islet-specific induced Tregs (iTregs)—prevents disease induction by diabetogenic T cells in immunodeficient NOD.scid mice [21,22]. Third, depletion of Tregs in young NOD mice using anti-CD25 monoclonal antibodies accelerates diabetes onset and increases disease incidence in both sexes. Treg-mediated protection can be either antigen-specific or nonspecific; however, islet antigen-specific Tregs, such as those derived from the BDC.2.5 transgenic mouse, demonstrate superior suppressive efficiency, requiring fewer cells to prevent disease compared to polyclonal Tregs [23].

Mechanistic insights from the NOD model indicate that Tregs exert their function in T1D through key immunoregulatory molecules such as cytotoxic T lymphocyte antigen-4 (CTLA-4) and transforming growth factor beta (TGF-β). Disruption of CTLA-4 or TGF-β signaling leads to increased disease incidence and earlier onset of T1D. Notably, treatment with a combination of anti-CTLA-4 and anti-TGF-β antibodies—unlike anti-TNFα—results in a poly-autoimmune syndrome featuring colitis, sialitis, and gastritis. Furthermore, CD28-deficient NOD mice, which lack functional Tregs, exhibit accelerated T1D onset when treated with anti-CTLA-4 and anti-TGF-β [24], though it remains unclear whether TGF-β from other cellular sources also contributes to disease modulation [18].

Therapeutic strategies targeting Tregs for the treatment of T1D are under active investigation. Two primary approaches have shown promise: *in vivo* induction of Tregs and adoptive transfer of *in vitro*-expanded Tregs. In NOD mice, administration of non-mitogenic anti-CD3 antibodies induces $FoxP3^+$ Tregs in a TGF-β-dependent manner, effectively suppressing disease progression [25]. This strategy has translated into human clinical trials, where treatment with hOKT3γ1(Ala-Ala)—a humanized Fc-mutant anti-CD3 monoclonal antibody—successfully halted disease progression for over one year [26,27]. Additionally, rapamycin therapy, known to promote the expansion of Tr1 and $FoxP3^+$ Tregs, has been effective in preventing diabetes in murine models [28]. Finally, adoptive transfer of ex vivo-expanded, antigen-specific Tregs has demonstrated the ability to suppress ongoing disease, offering a promising therapeutic avenue for T1D management [29].

Treg-based therapeutic approaches

Regulatory T cell (Treg)–based therapies hold considerable potential for the treatment of type 1 diabetes (T1D). Multiple strategies have been explored in both preclinical models and clinical trials, with the goal of leveraging the immunosuppressive functions of Tregs to protect pancreatic beta cells from autoimmune destruction presented in Figure 2. Below, we outline several key Treg-focused therapeutic approaches for T1D [30].

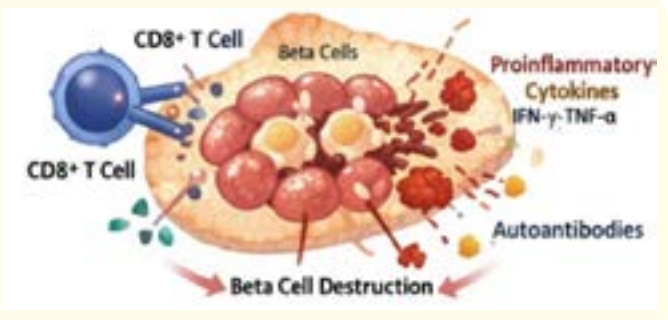

Figure 2: Immune-mediated destruction of pancreatic β-cells in type 1 diabetes by autoreactive T cells and inflammatory cytokines.

Recently, adoptive cell therapy utilizing genetically engineered regulatory T cells (Tregs) has emerged as a promising approach to mitigate transplant rejection and autoimmune diseases. However, a major limitation of this technique lies in the absence of standardized protocols for the isolation, expansion, and genetic modification of murine Tregs. Notably, protocols involving the purification, expansion, and retroviral transduction of mouse Tregs with a chimeric antigen receptor (CAR)-encoding vector have demonstrated significant advancements in the generation of engineered Tregs presented in Figure 3. It has been shown that selecting Tregs based on GFP expression results in high-purity populations. While rapamycin inhibits the proliferation of Tregs during expansion, it does not compromise their suppressive function. This methodology facilitates the *in vivo* study of transduced Tregs in animal models and provides a platform for investigating genetically modified Treg-based therapies for various inflammatory conditions. Furthermore, in the future, genetically engineered Tregs may overcome current limitations in Treg expansion and purification while also enhancing their immunosuppressive efficacy.

Figure 3: CAR-engineered regulatory T cells designed for antigen-specific immune suppression in autoimmune diseases.

Therapeutic strategies that leverage Treg-mediated immunosuppression have been explored in multiple contexts, including organ transplantation and autoimmune diseases. Moreover, Treg-based interventions are being investigated for their potential to treat other pathological conditions such as cardiovascular disease, obesity, and systemic inflammatory syndromes. The therapeutic potential of Tregs in managing a variety of autoimmune diseases is discussed in the following section.

Inflammatory bowel disease /Crohn's disease

In vitro–generated IL-10–producing, ovalbumin (OVA)-specific regulatory T cells (Tr1) have been shown to prevent colitis in SCID mice induced by $CD4^+$ CD45RB^hi^ T cells. This finding demonstrates that IL-10–secreting $CD4^+$ Tregs possess immunosuppressive properties and can actively suppress pathological immune responses *in vivo* [31]. Additionally, experimental transfer of $CD4^+CD25^+$ Tregs into immunodeficient mice exhibiting clinical signs of colitis led to significant disease amelioration. Recovery was observed within two weeks following Treg infusion at a dose of 10^6 cells per mouse, in contrast to control groups receiving $CD4^-CD25^-$ T cells, which did not show improvement [32].

According to published studies, the first clinical trial involving a single infusion of freshly isolated peripheral blood mononuclear cell (PBMC)-derived Tregs (10^6–10^9 cells) in a patient with active Crohn's disease was safely conducted in 2012, without any adverse effects. Interestingly, clinical improvement was primarily observed in patients who received the lowest Treg cell doses, suggesting that minimal immune suppression may be more effective in this context [33]. As previously described, Crohn's disease involves chronic intestinal inflammation. Notably, inflamed mucosa in inflammatory bowel disease (IBD) cases shows a relatively low presence of Tregs compared to other inflammatory conditions, such as diverticulitis, which may explain the insufficient immune regulation observed in IBD [34,35]. IL-10 knockout mice have been widely used as a suitable model for colitis, highlighting the essential role of IL-10 in immune regulation. Infusion of IL-10–producing Tregs in these models has been shown to reverse disease pathology, underscoring IL-10's critical function in Treg-mediated immunosuppression [36].

A humanized mouse model—developed by transplanting fetal small bowel tissue and inducing colitis via *Escherichia coli* infection—has also been utilized to study Treg function. Following transfusion of 10^6 Tregs along with recombinant IL-10 (rIL-10), these cells preferentially migrated to the inflamed lamina propria, where they suppressed conventional T cells and alleviated inflammation [37]. Moreover, surgical specimens from IBD patients have revealed defective $CD8^+$ Tregs in the lamina propria, in contrast to healthy individuals who possess functionally active $CD8^+$ regulatory T cells at this site. It is proposed that intestinal

epithelial cells play a key role in the induction of these regulatory T cells by processing and presenting antigens. These cells are crucial for maintaining immune homeostasis and controlling inflammation in the intestinal mucosa [38].

Rheumatoid arthritis (RA)

Rheumatoid arthritis (RA) is a systemic, heterogeneous autoimmune disease clinically characterized by symmetrical polyarthritis. It is a chronic inflammatory joint disorder that can lead to cartilage and bone destruction, ultimately resulting in disability conditions in many patients [39]. The pathogenesis of RA is multifactorial, with regulatory T cell (Treg) dysfunction proposed as a key mechanism contributing to the breakdown of self-tolerance and disease progression. In patients unresponsive to conventional disease-modifying anti-rheumatic drugs (DMARDs), Treg-based therapies have been explored with varying degrees of success [40].

Clinical studies have reported an increased number of Tregs in the peripheral blood of some RA patients [41]. In contrast, other studies have found either no significant change or a reduced Treg population in patients exhibiting active disease, raising concerns regarding the interpretation of Treg involvement [42]. This variability may stem from differences in defining Tregs, commonly based on FoxP3 and CD25 expression—markers that can be influenced by inflammation and T cell activation. Importantly, Tregs from RA patients exhibit impaired cytokine profiles, including reduced IL-2 and IFN-γ production, compared to those from healthy individuals, reinforcing the role of FoxP3 as a key Treg marker [43]. Recovery in RA patients treated with anti-TNF-α antibodies has been associated with the emergence of novel Treg subsets expressing FoxP3 but lacking CD62L expression [44]. However, the direct role of TNF-α in modulating Treg function remains a subject of ongoing investigation [45]. These observations suggest that using more stringent criteria to identify Tregs may reveal a reduced Treg count in peripheral blood and an elevated count in synovial fluid. Nevertheless, the functional competence of synovial fluid–resident Tregs remains to be fully elucidated [46].

Adoptive transfer of Tregs has shown promise in suppressing disease progression, supporting their therapeutic potential in autoimmune conditions. In murine models of collagen-induced arthritis (CIA), adoptive transfer of Tregs effectively inhibited disease development [47]. Notably, Treg therapy in these models not only suppressed T and B cell responses but also reduced osteoclast-mediated bone destruction, thereby protecting joint injury [48]. Overall, adoptive Treg cell therapy serves to restore immune regulation by supplementing the activity of endogenous Tregs that may be functionally compromised in the inflammatory synovial environment [49].

Psoriasis

Regulatory T cells (Tregs) have also emerged as key players in the pathogenesis of psoriasis—a systemic inflammatory disorder influenced by genetic predisposition and environmental triggers. The disease is primarily driven by Type 1 and Th17 cytokine-producing cells, which are normally kept in check by Tregs in healthy individuals. Tregs are essential for maintaining immune homeostasis by suppressing inflammatory immune responses, thereby preventing autoimmune conditions such as psoriasis and other skin-related autoimmune disorders [50]. In psoriasis, it is postulated that Treg-mediated immune suppression is impaired, resulting in an altered Th17/Treg ratio. Although Treg dysfunction has been associated with disease progression, the precise mechanisms regulating their impairment in psoriasis remain unclear. Recent studies underscore the importance of Tregs in the management and potential treatment of psoriasis. While the exact role of Treg quantity is still being investigated, reduced Treg function and an imbalanced Th17/Treg ratio have been closely linked to the development and worsening of the disease [51].

Other autoimmune diseases

Recently, Huang., *et al*. highlighted the critical roles of regulatory T cells (Tregs) in the initiation, treatment, and recovery phases of uveitis—a recurrent autoimmune disease characterized by relapsing-remitting ocular inflammation that often requires corticosteroids and systemic immunosuppressive therapy to prevent severe vision loss. Both Th17 (pro-inflammatory) cells and Tregs are involved in the pathogenesis of uveitis, and modulating Treg function has been proposed as a promising therapeutic strategy. The protective role of Tregs has also been demonstrated in Myasthenia Gravis (MG), a T cell–dependent, B cell–mediated autoimmune disorder caused by the production of antibodies targeting nicotinic acetylcholine receptors and other components of the neuromuscular junction. In rodent models, adoptive transfer of Tregs has been shown to suppress the autoimmune response, supporting their therapeutic potential in MG. Notably, imbalances

in the ratio of T-helper (Th) cells to Tregs have been associated with poor prognosis in autoimmune diseases such as MG. Similarly, Herrnstadt., *et al*. proposed a protective role for Tregs in renal injury, particularly in glomerulonephritis (GN), where pathogenic Th1 and Th17 effector cells contribute to tissue damage. Tregs have been shown to counteract these pathogenic immune responses by suppressing components of the innate immune system in inflamed renal tissue. These findings provide a basis for exploring the immunosuppressive functions of Tregs in kidney inflammation and injury. Several clinical trials targeting autoimmune diseases have employed both polyclonal Tregs and antigen-specific Tregs engineered using T cell receptor (TCR) or chimeric antigen receptor (CAR) constructs, which significantly enhance their therapeutic efficacy. Moreover, the use of antigen-specific Tregs has been proposed for the treatment of autoimmune skin disorders, aiming to replenish the Treg population and suppress hyperactive immune responses.

In summary, current therapeutic strategies for managing autoimmune diseases primarily focus on restoring immune tolerance. Treatments are increasingly aimed at promoting the *in vivo* induction and expansion of Tregs. Pharmacological agents such as the mTOR inhibitor rapamycin, cytokines like IL-10 and low-dose IL-2, TNF receptor 2 agonists, and Flt3 ligand are widely used to modulate Treg activity and maintain immune balance. Moving forward, further research is needed to better understand Treg persistence and the factors necessary for their survival in peripheral circulation, which will be essential for optimizing their clinical application in immunotherapy.

Challenges in Treg therapy

The clinical application of regulatory T cells (Tregs) must adhere to stringent guidelines established by the U.S. Food and Drug Administration (FDA). Cell therapy products are required to meet rigorous standards for sterility, identity, purity, and potency before being administered to patients. Among these, demonstrating cell identity and purity is particularly challenging. The transcription factor FoxP3 is widely recognized as a definitive marker for Treg identity; however, ex vivo expansion processes rarely yield a population consisting entirely of $FoxP3^+$ cells. Therefore, the proportion of contaminating non-Treg cells must remain within acceptable qualitative and quantitative limits to ensure product safety and efficacy. Contaminating cells, especially alloreactive populations, can provoke immune responses in recipients, and thus must be minimized to the lowest possible levels. Currently, researchers are working to develop disease-specific models to evaluate the potency of enriched and expanded Treg products, as a single formulation may not be universally applicable across all autoimmune or inflammatory conditions. Additional limitations in the clinical use of Tregs include concerns regarding their phenotypic and functional stability in peripheral blood, which may affect their therapeutic efficacy. Moreover, for widespread clinical application, cryopreservation of Tregs is essential—but it presents significant challenges. Cryopreservation can compromise Treg viability, reduce functional durability, alter cytokine secretion patterns, and affect the expression of critical surface molecules necessary for Treg function and homing. Therefore, optimizing culture techniques, refining cryopreservation protocols, and establishing reliable storage conditions are crucial steps toward the successful and consistent use of Treg-based therapies in clinical settings.

Future Directions

Recent technological advancements have significantly enhanced our ability to identify and classify regulatory T cells (Tregs) and understand their immunosuppressive mechanisms. These developments have improved our comprehension of the dynamic relationship between Treg plasticity—required for effective immune suppression—and Treg destabilization, which can contribute to the onset of autoimmune diseases. The observed plasticity and heterogeneity of both murine and human-derived Treg populations have provided new insights into Treg biology. However, due to inconsistent findings across various studies, the precise contribution of Tregs in different autoimmune disease models remains uncertain.

Tregs are characterized by the expression of lineage-defining transcription factors, notably FoxP3, which serves as a master regulator of their development and function. Despite this, isolating pure Treg populations remains a challenge due to the absence of a single definitive surface marker, especially in humans. Human Tregs often express low, intermediate, or high levels of CD25, making it difficult to establish clear boundaries for isolation and purification. The use of human Tregs as therapeutic agents is an emerging field in clinical research. Preliminary clinical findings suggest that Tregs may be used as adjuncts to conventional therapies to

reduce disease recurrence and improve long-term quality of life in individuals with autoimmune conditions. Nevertheless, the interpretation of Treg-related data in patients with inflammatory and autoimmune diseases remains complicated by the lack of highly specific and reliable Treg markers. These markers are crucial, not only for identifying true Tregs but also for understanding their immunosuppressive capabilities. Therefore, identifying definitive surface and functional markers is essential for designing more targeted and effective therapeutic strategies. A comprehensive understanding of Treg-mediated immune suppression, coupled with detailed analysis of immune responses in patients with Treg dysfunction, is necessary to develop more efficient treatment regimens. Moreover, targeted investigations into the role of Tregs under specific inflammatory conditions are vital for advancing personalized therapeutic strategies tailored to distinct clinical manifestations. Advanced technologies such as single-cell RNA sequencing are proving invaluable for characterizing Treg subsets and elucidating their T cell receptor (TCR) repertoires. These tools allow for the identification of antigen-specific Tregs and provide insight into their functional diversity in both healthy and diseased states. This progress opens exciting possibilities, including the use of TCR-transduced Tregs for therapeutic applications.

In addition, the development of genetically engineered Tregs and CAR-Tregs shows promise in preclinical models of autoimmune disease. These approaches may offer therapeutic benefit, especially in contexts where extracellular target recognition is critical. In conclusion, the identification and characterization of immunosuppressive pathways and associated markers will be key to developing reliable and effective Treg-based therapies. Continued research in this area holds the potential to revolutionize treatment strategies for autoimmune diseases and advance the field of precision immunotherapy.

Conflict of Interest

The authors declare that there is no conflict of interest.

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