Review Article

Regulatory T Cells and Type 1 Regulatory T Cells as Immuno-Cell Therapy in Type 1 Diabetes

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Abstract

Type 1 diabetes is caused by the immune system attacking and destroying pancreatic cells, leading to a complete lack of insulin throughout the rest of a person's life. For about 100 years, insulin replacement has been the primary treatment for the majority of people with this condition. Advancements in technology and our knowledge of β cell growth, glucose metabolism, and the immunological pathophysiology of the illness have resulted in the creation of new and effective treatment and preventative strategies. Immunotherapy has undergone a significant transformation, leading to the development of medicines that specifically target immunological mechanisms related to tolerance in the islets. These medicines have the potential to prevent or reverse this disease without the adverse consequences associated with prior techniques that compromised the overall immune system. Cell-based therapies provide potential alternatives to the lifetime administration of insulin in individuals with type 1 diabetes (T1D). Distinct cellular populations are essential in mitigating detrimental immune responses that erroneously embark on an assault on the body's own tissues, paving the way for peripheral tolerance to be established. Natural regulatory T cells (Tregs) are a part of these categories, also in-vitro created Treg cells, and type 1 regulatory T (Tr1) tissues that secrete IL-10. This review specifically examines the roles of regulatory T cells and type 1 regulatory T cells in the field of immunotherapy for type 1 diabetes.

Keyword: Treg, Tr1, T1D, immunosuppression, tolerance, immunotherapy.

Introduction

When immune tolerance breaks down, autoimmune diseases happen. This can be caused by a number of things, including genetic predisposition and environmental triggers. For example, Type 1 diabetes (T1D) is marked by an inflammatory response mainly mediated by Th1 cells that target insulin-producing β-cells in the pancreas, leading to high blood sugar and many other health problems [1]. The processes of immunity, such as central and peripheral tolerance, are essential for sustaining self-tolerance and preventing autoimmunity. Comprehending these mechanisms and the elements that contribute to the breakdown of tolerance is crucial in order to create precise immunotherapies for autoimmune disorders. These therapies seek to regulate the immune system and reinstate self-tolerance, resulting in enhanced disease control and a higher standard of living for those affected. Cell therapy is an innovative technology that has the potential to revolutionise therapeutic approaches in different areas, such as immunotherapy. Within the setting

of type 1 diabetes (T1D), the most favourable outcomes involve the integration of cell-based therapies and genome editing techniques to produce cells that can suppress the immune response and reinstate the activity of beta cells. Techniques that involve collecting, manipulating, and growing the patient's own Tregs can protect any remaining cells from autoimmune attacks, creating stable Tregs that are specific to autoantigens [2].

Although they make up a negligible fraction of circulating T cells, regulatory T cells (Tregs) are essential for controlling autoimmune illnesses, chronic inflammatory disorders, and the development and maintenance of peripheral tolerance. There are many important things that Treg cells do, such as making immunosuppressant cytokines and metabolites and putting key checkpoint molecules on the surface of their cells to control how antigens are presented and how they are broken down. It's important to note that the cells can effectively block bystanders and increase the body's ability to resist infection, which makes their effects stronger and longer-lasting [3, 4].

Research conducted by several organisations has demonstrated that Tr1 cells has the ability to inhibit and restrict unfavourable immunological reactions in many disease scenarios, hence facilitating immune tolerance [5]. In inflammatory and autoimmune diseases, as well as after transplantation, these noteworthy results demonstrate that Tr1 cells may be used as a therapeutic intervention to improve and restore immunological tolerance. The results of the finished clinical trials have demonstrated that Tr1 cell-based therapy is both safe and potentially effective. Scientists are working on a number of different ways to make better Tr1 cell products. There are still unanswered concerns regarding Tr1 cells despite these advancements. For example, thanks to their shape-and function-altering capabilities, it is still not known whether laboratory-grown Tr1 cells can sustain their immunoregulatory roles over the long term. Furthermore, opinions differ on the accuracy with which healthy individuals' circulating Tr1 cells are defined by the CD49b/LAG-3 surface co-expression signature. This is because the IL-10 cytokine capture method may be more effective in clinical settings, and many labs, including ours, have trouble collecting Tr1 cells from bulk culture using CD49b and LAG-3 (5). Improved and more specific cell surface markers and transcription factors that define the lineage of Tr1 cells are two substances that will need to be clarified in future research [5].

Regulatory T cells (Treg) role in type 1 diabetes (T1D)

Forkhead box P3 (FOXP3) is an essential transcriptional repressor that triggers the development of regulatory T cells (Tregs), a fraction of CD4+ T cells that respond to self-antigens. Treg fraction is an essential part of balancing the system of immunity, which can be influenced by changes in FOXP3 expression in mature CD4+ T-cells. Whether triggered by genetics or pharmaceuticals, FOXP3 activity disruption results in life-threatening autoimmune diseases that require a bone marrow transplant to treat [6].

In order to generate Tregs, the thymus employs a different developmental process. An increase in FOXP3 is the outcome of a change from a group of developing T-cells with very specific TCRs into Treg precursors. A mature population of self-reactive Tregs (tTregs) derived from the thymus and a stable epigenetic state are the outcomes of this process. At the periphery regions, these tTregs populate lymphoid and non-lymphoid organs as they undergo negative

selection [7]. Peripheral regulatory cells (Tregs) help with tissue regeneration and repair while also reducing self-reactivity. Medullary thymic epithelial cells (mTECs) express the autoimmune regulator (AIRE), which helps in the presentation of antigens to generate tissue-specific regulatory T lymphocytes (tTregs). Tolerance in peripheral tissues is also affected by the presence of AIRE in some bone marrow-derived lymphoid organ cells. This demonstrates that thymic T cell maturation and peripheral tolerance are interdependent [8,9].

When naive CD4+ T-cells meet antigens and are subjected to suppressive factors like TGF-β, IL-10, bacterial metabolic products, or other stimulatory pathways, Tregs can also be produced peripherally within the immune system. In these conditions, antigens and inhibitory factors are more likely to be encountered by the immune system. The presentation of an antigen has the potential to activate FOXP3 in a sustained fashion, leading to the transformation of ordinary T-cells into pTregs, or regulatory T-cells that originate from the periphery [5]. pTregs are derived from a standard set of peripheral T-cells that have undergone selective breeding to ensure they are not reactive to self-antigens. The tTreg subset, in contrast, originates from T-cells that are negatively selected in the thymus for their extreme sensitivity to self-antigens [10]. Recognition increases the number of regulatory T cells (Tregs), which in turn decreases inflammatory responses. The ability to recognise phosphorylated and modified proteins, as well as citrullinated and hybrid peptides, is possessed by peripheral regulatory T cells (pTregs). You won't normally find these proteins in the thymus, but they're common in autoimmune diseases. When tTregs and pTregs were combined, in addition to Tr1 cells that produce IL-10 and Th3 cells that produce TGF-β, can offer the most extensive array of antigen-recognition skills for regulating detrimental self-reactivity. These specific types of cells work together in a coordinated manner to sustain tolerance. Furthermore, as an individual matures, thymic involution occurs, which can alter the mechanisms involved in establishing and preserving tolerance. Peripheral pathways have a greater influence on regulating autoimmunity in adults compared to youngsters.

Effector T cells, specifically CD4+ and CD8+ lymphocytes, which target islets, are responsible for the destruction of β cells in individuals with T1D. The process of thymic selection often eliminates these self-reactive T cells, while a small quantity of T cells that evade thymic elimination get regulated by various peripheral tolerance mechanisms. Of these, Tregs that express Foxp3, play a crucial role in managing autoreactive cells and are therefore a key component in the development of T1D.

Researchers have discovered that the development of Type 1 Diabetes (T1D) may be attributed to abnormalities in regulatory T cells (Tregs) and an increased presence of effector T cells in both humans and animals. Preliminary outcomes of recent therapies targeting this imbalance and aiming to augment the quantity of Tregs in circulation are encouraging. In two phase I clinical trials investigating polyclonal Treg adoptive immunotherapy in patients with recent-onset T1D, researchers extracted autologous CD4+ CD127lo/− CD25+ polyclonal Tregs from the patients' peripheral blood. These Tregs were then cultured ex vivo with anti-CD3, anti-CD28, and interleukin (IL-2), and subsequently administered back to the donors in varying quantities. Researchers discovered that after one year of treatment, the reintroduced polyclonal Tregs were unchanged in terms of their physical characteristics and continued to

exist in the bloodstream [11]. Although the results are promising, the injection of Treg cells can only provide a temporary delay in the course of Type 1 Diabetes (T1D). The primary obstacle involves surmounting certain crucial aspects that restrict the effectiveness of Treg immunotherapy. The stage of illness progression during treatment is of utmost importance. Due to the decline in the β-cell reservoir during T1D, intervening sooner can protect a larger number of β-cells against autoimmune attack. Moreover, the level of disease progression also impacts the immunoregulatory activity of Treg cells. The examination of this group of cells indicates that there is a reduction in the quantity and effectiveness of Treg cells as the disease advances. Therefore, it is recommended to utilise autologous Tregs at the earliest opportunity to attain the most effective Treg preparation for clinical purposes [12].

Augmenting the population of autoantigen-specific Treg cells would halt the autoimmune destruction of β-cells while leaving the rest of the immune system unaffected. Furthermore, clones that are unique to islet antigens may selectively target inflamed islets and undergo local expansion, resulting in site-specific effects. Pre-clinical investigations have demonstrated that autoantigen-specific Tregs have the ability to safeguard β-cells. The Tregs were tested in these investigations using islet-specific peptides and major histocompatibility complex (MHC) assemblies. In a mouse model of type 1 diabetes (T1D), they demonstrated superior efficacy in correcting autoimmune diabetes compared to polyclonal Tregs [13]. However, the process of generating a substantial number of Tregs that specifically target islet antigens in people remains unclear. This raises doubts about the efficacy of this strategy in terms of translation. Researchers recently announced the successful production of a significant quantity islet-specific regulatory T cells (Tregs) in humans were generated using lentiviral T-cell receptor (TCR) gene transfer. However, when contrasted with virus-specific T-cell receptors (TCRs), regulatory T cells (Tregs) showed less responsiveness to antigens specific to their own cells [14]. Because it requires the insertion of a unique TCR in every patient, this approach is not clinically feasible due to major histocompatibility complex (MHC) restrictions. In a subsequent study, chimeric antigen receptor-T cell (CAR-T) technology was used to generate insulin-specific Tregs that are functionally active. As a result, CAR-Tregs, or regulatory cells, were created [13]. The cells were generated via lentiviral transduction of CD4+ murine effector T cells with a chimeric antigen receptor vector system. A Foxp3 Treg marker with immunoregulatory capabilities, costimulatory molecules, and insulin-specific T cell activation domains were all components of this vector system. Transforming T effectors into cTregs, these cells consistently expressed Foxp3. A longer life span is associated with diabetes in mice, and insulin-specific cTregs function similarly to wild cTregs. But insulin-specific cTregs can't stop diabetes from starting [14, 15]. New studies show that antigen-specific Foxp3+ Tregs and antigen-specific Foxp3 populations that produce anti-inflammatory IL-10 are rapidly increased in response to a treatment that combines MHC/peptide molecules with IL-2/anti-IL-2 monoclonal antibody complexes. This method effectively prevents type 1 diabetes in mice [16]. A better understanding of antigen-specific regulatory T cells (Tregs) is crucial for improving methods of increasing their numbers and/or abilities in human subjects before they are used in real treatments for people with type 1 diabetes (T1D).

Tr1 as immuno-therapy for type 1 diabetes (T1D)

Tr1 cells, which are characterised by their substantial secretion of IL-10, have a vital function in offering defence against T1D. The link suggests a connection between the presence of IL-10-producing CD4+ T cells at the onset of the disease and the subsequent levels of glucose in the blood [17]. Interestingly, individuals with juvenile T1D have a higher number of antigen-specific Th2/Tr1 clones that offer protection, rather than inflammatory Th1 clones, which is a notable contrast to adult T1D patients [18, 19]. Intestinal Tr1 cells have the potential to be a major source of CD4+ T cells that release IL-10, which are protective in the body. Co-transferring Tr1 cells with diabetogenic BDC2.5 T cells in the small intestine of NOD/SCID mice successfully suppressed the development of T1D, as evidenced by the proliferation of Tr1 cells [20]. Furthermore, Tr1 cells that were delivered via rectal injection shown the capacity to migrate to the pancreas and successfully inhibit the development of T1D. Tr1 activation largely occurs in the gut mucosa, providing protection to autoreactive T cells in T1D and potentially in other AIDS-related illnesses [20]. Researchers investigated alternate approaches to enhance tolerance in Type 1 diabetes (T1D) due to the occurrence of adverse responses from administering anti-CD3 antibodies. In a study involving NOD mice, researchers noticed that CD4+ T cells in the spleen showed increased production of IL-10. The augmented creation of IL-10 may be accountable for a significant postponement in the initiation of diabetes. Furthermore, the removal of Tr1 cells from the spleens of these animals halted the growth of diabetic splenocytes. Furthermore, the transfer of pancreatic islets from the treated animals to NOD/SCID mice provided additional evidence of the positive influence of these T cells. Administration of interleukin-10 (IL-10) by itself did not prevent diabetes in NOD mice. Rapamycin may need to stop the growth of effector T cells before the Tr1 cell population is stabilised by IL-10. This is corroborated by the fact that the combination of rapamycin and IL-10 therapy produces a synergistic effect. The data indicates that both anti-CD3 treatments and the administration of rapamycin and IL-10 have the capacity to promote tolerance in polyclonal Tr1 cells. It is important to note that the polyclonal Tr1 cells generated in vivo may lead to viral tolerance. Recent in vivo studies have explored the possibility of using autoantigens to produce antigen-specific Tr1 cells for peptide immunotherapy. NOD mice were given a dose of 65 kDa glutamic acid decarboxylase (GAD65) in order to establish targeted tolerance through the action of Tr1 cells [21]. A method employing IL-10 was used to protect lymphopenic NOD/SCID mice against T1D by simultaneously introducing diabetogenic T cells and Tr1 clones to them. Treatment with the main proinsulin peptide in immunotherapy resulted in a decrease in dependence on insulin in patients with type 1 diabetes, possibly due to the induction of tolerance. Nevertheless, the precise mechanism behind this phenomenon remains incompletely comprehended [22]. Using nanoparticles to encapsulate disease-causing peptides is a more advantageous method for treating persons with autoimmune disorders (AID) compared to providing soluble peptides. The utilisation of nanoparticles incorporating several diabetogenic peptides facilitated the acquisition of T1D-specific proinflammatory T cells, leading to the establishment of tolerance, Moreover, the splenocytes displayed higher levels of IL-10, indicating the likely involvement of CD49b, LAG-3, Tr1 cells [23].

Conclusion

Treg cells have significant functions in restrictin g the progression of type 1 diabetes, and the pote ntial application of Treg cells as a treatment for t ype 1 diabetes shows promise but require extensi ve monitoring over an extended period in a sizea ble patient cohort. Furthermore, researchers have primarily studied Tr1 cells' inhibitory role in relation to type 1 diabetes. By persistently adva-ncing our research, we can aim to consistently de fine properties human Tr1 cells, with ultimate goal of utilizing them as innovative treatments to promote tolerancein type 1 diabetes. Acquiring more knowledge will help advance the developm ent of new Tr1based medicines that promote tole rance for type 1 diabetes autoimmunity.

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