# The Potential Applications of Tregs in Organ Transplantation

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**Abstract.** Treating patients with end-stage organ failure is ideally achieved through organ transplantation, but long-term reliance on non-specific immunosuppressants post-operatively easily leads to systemic toxicity and chronic graft rejection. In recent years, inducing specific immune tolerance using regulatory T cells (Tregs) has become a research hotspot, but the instability of Treg function in vivo and the lack of precise targeting remain unresolved research gaps. This article systematically analyzes the mechanism of action of Tregs in controlling organ transplant immune rejection, and explores their isolation and purification standards and cutting-edge infusion protocols. Simultaneously, it focuses on the innovative application of targeted nanodelivery technology (such as targeting the CD248 biomarker) to optimize Treg expression. The results show that precise targeted intervention can significantly increase the number of Treg cells in the graft, prolong the survival time of the allogeneic organ, and effectively reduce dependence on traditional immunosuppressive drugs. This analysis provides valuable reference for improving the prognosis of transplant patients using specific cell therapy. Although current challenges such as limited clinical research samples and difficulties in in vivo tracking remain unresolved, it is believed that future research will further explore targeted biomarkers and the timing of administration, comprehensively promoting the clinical translation of Treg therapy.

**Keywords:** Organ transplantation, Tregs, Nanodelivery.

## 1. Introduction

Organ transplantation has become the preferred treatment in clinical practice for many patients with diabetes, hypertension, various congenital diseases, idiopathic diseases, trauma, and other end-stage organ failure. Extracorporeal Membrane Oxygenation (ECMO), dialysis, and other methods can only alleviate the survival needs of patients in the short term. Organ transplantation is an effective treatment method when the donor is available. For example, kidney transplantation is the ideal choice for patients with renal failure. Patients can see significant improvement in the short term after transplantation, but due to the occurrence of chronic rejection and long-term immune response, the long-term prognosis of organ transplant patients is poor [1]. In the current standard clinical treatment, organ transplant patients need to receive long-term multi-drug immunosuppressive therapy such as calcineurin inhibitors, mTOR inhibitors, and corticosteroids after transplantation [1,2]. Common calcineurin inhibitors in clinical treatment include cyclosporine and tacrolimus, and mTOR inhibitors include sirolimus. These drugs mainly play a global immunosuppressive role in anti-rejection immune regulation after organ transplantation [2]. Immunosuppressive therapy reduces inflammatory response and prevents the immune attack of organs as foreign bodies, thereby further preventing ectopic reactions and autoimmune reactions [2]. This non-specific immunosuppression leads to a decline in the patient's overall immune defense, causing systemic toxic side effects, and consequently adverse reactions such as infection, malignant tumors, diabetes, and hypertension. Current clinical treatment options still cannot avoid chronic rejection, long-term graft failure, increased overall cardiovascular mortality, and impaired quality of life [1]. Ideally, immunomodulation should act only on cells that are specific to the donor. This approach can suppress graft rejection while minimizing the side effects and complications of global immunosuppression. Alternative or adjuvant immunosuppressive therapies are currently being explored, with the aim of replacing global immunosuppression with specific immunosuppressive therapy whenever possible. Regulatory T cells (Tregs), which are commonly used for autoimmune diseases to suppress autoimmune diseases, can

serve as an alternative to reduce the side effects and rejection of organ transplantation, thereby improving the mortality rate, graft rejection, and infection rate of these patients [2,3].

This article focuses on using Tregs infusion after organ transplantation to reduce the impact of chronic immune rejection, acute immune rejection, graft-versus-host disease, and other related diseases on transplanted organs, thereby reducing the use of nonspecific immunosuppressant drugs. This article analyzes different Treg infusion regimens, various methods for extracting and expanding Tregs, and their impact on long-term graft immune rejection in patients without the use of common clinical nonspecific immunosuppressive therapies.

## 2. Tregs

### 2.1. Tregs and Their Classification

Tregs are members of the CD4 T cell class. There are two classes of CD4 T cells: T helper cells (Th) and Tregs. By activating and interacting with other effector cells, including CD8+ cytotoxic T cells, B cells, and macrophages, Th cells regulate adaptive immunity. Suppressing particularly damaging or dangerous activity in Th1 cells is one of Tregs' primary duties [2]. Tregs express a range of biomarkers, including FOXP3 and CD25, and are related to developing CD4+ cells [3]. Tregs generate TGF-β and IL-35, which stimulate other cells, repress other immune cells, and downregulate other cytokines such TH1 cytokines and MHC class II antigens [1,2]. The typical development and activity of Tregs are determined by the expression of the nuclear transcription factor forkhead box P3 (Fox P3), which is crucial for immune system suppression and maintenance [3, 4]. Th1, Th2, Th17, and follicular Th (Tfh) cells are among the effector lymphocytes that FoxP3-expressing Treg cells inhibit when they move to inflammatory areas [4,5]. Native Tregs (nTregs) and inducible Tregs (iTregs) are the two kinds of CD4+ Tregs.Unlike other Tregs, Foxp3+ Tregs can be generated in the periphery and include additional cytokines such TGF-β and IL-10 [2,6]. Through a sequence of positive and negative selection processes, nTregs grow in the thymus from precursor cells in the bone marrow [6]. Under acceptable conditions, conventional CD4+ T cells undergo antigen activation to evolve into iTregs in the periphery [6]. Peripheral tolerance, different inflammatory responses, and immune response downregulation are all possible with both kinds of Tregs. For nTregs and iTregs to function, they must bind to T cell receptors (TCRs) [7].

### 2.2. Mechanism of Action of Tregs

The mechanisms by which Tregs inhibit different immune cells can be divided into direct inhibition and indirect inhibition. Direct inhibition is when Tregs themselves react directly with the target cells, while indirect inhibition is when they affect third-party cells or extracellular molecules, thereby inhibiting the target cells [8]. Examples of direct mechanisms include the secretion of cytokines such as IL-10, TGFβ, and IL-35, as well as the production of granzymes and perforin, which can lead to apoptosis of target cells [8]. Indirect mechanisms include the expression of CD39/CD73, which consumes the extracellular ATP microenvironment by producing immunosuppressive molecules such as adenosine and antimicrobial peptides (AMP)[8]. Meanwhile, Tregs influence alterations in the microenvironment because of their elevated CD25 expression. The elevated expression of this receptor enables Tregs to absorb more IL-2 and "deprive" nearby cells of this cytokine, thus inducing an immunosuppressive effect[8].

### 2.3. Isolation and In Vitro Amplification of Tregs

Tregs can be extracted in large quantities from peripheral blood (PB), umbilical cord blood (UCB), or thymus tissue. Isolation under Good Manufacturing Practice (GMP) standards is accomplished with the CliniMACS system (CliniMACS™ Instruments, Miltenyi Biotec), which enables clinical-scale magnetic cell enrichment in a closed, sterile environment [8].This protocol involves the removal of CD8 and CD19 cells, or partial enrichment of CD25 only. Another clinical method for isolating Tregs is flow cytometry-based purification. This method is similar to cell sorters commonly used in

research, which can isolate cells based on the expression of selected cell markers [8]. Although several teams have used CliniMACS to isolate Tregs, cell purity is a significant limitation of this protocol. Only 80% of the Tregs isolated by this protocol contain FOXP3, and the absence of FOXP3 may affect antigen-specific cell purity. Flow cytometry purification of Tregs has been used in clinical trials outside the EU[8], with selection based on CD4, CD25 and CD127 phenotypes, resulting in high purity (>99%). Nevertheless, this method cannot isolate activated effector cells, and CD4+ cells may require pre-concentration due to the large number of peripheral blood mononuclear cells (PBMC) initially treated, thus leading to high cost per formulation[8].

### 2.4. Purpose of the Plan

GMP (Good Manufacturing Practice) is a set of mandatory standards applicable to industries such as pharmaceuticals and food. It aims to ensure that products meet national health and quality requirements in all aspects, from raw material procurement, personnel training, facilities and equipment, production processes, packaging and transportation to quality control. Tregs cells are expanded under GMP conditions and then reinfused into organ transplant patients[7]. The purpose is to suppress the rejection response of patients to the target transplanted organ by the expression of Tregs cells in the patient's immune system. This can reduce the use of drug immunosuppressants to a certain extent, thereby reducing problems such as liver and kidney toxicity, hypertension, cardiovascular disease and obesity caused by drug immunosuppressants. In an open-label dose-escalation phase I clinical trial conducted at King's College Hospital in London and Plymouth University Hospital in the UK, participants received a single intravenous infusion of in vitro expanded autologous polyclonal Tregs 3 to 16 months after liver transplantation[9]. This study concluded that Treg infusion is safe and well-tolerated for liver transplant patients through dose escalation in multiple groups of subjects[9].

## 3. Latest Related Solutions

Through single-cell sequencing analysis, the Fourth Military Medical University of China identified CD248 as a key stromal cell marker in renal autograft rejection. CD248 is a single transmembrane glycoprotein expressed in interstitial cells, including glomerular cells and fibroblasts. Current research shows that CD248 is associated with tissue fibrosis and immune cell infiltration and is regulated by the HIF-1α and interleukin-1β (IL-1β) signaling pathways. Hypoxia-inducible factor (HIF)-1α, HIF-2α, and HIF-3α are transcription factors that regulate hypoxia response genes. HIF-1α and HIF-2α are potential targets for the treatment of renal anemia, which is caused by impaired production of erythropoietin (EPO) due to chronic kidney disease [10]. The team developed nanoparticles co-loaded with IL-2 and TGF-β expression plasmids using macrophage membrane coating technology. Nanoparticle treatment increased the number of Treg cells in the graft, significantly prolonged the survival time of the allogeneic graft (survival rate >50% within 40 days after kidney transplantation), improved renal function, and inhibited complement deposition. This method achieves the therapeutic goal of chronic long-term organ rejection by precisely targeting the graft to induce Treg polarization without systemic cytokine exposure, while addressing the functional instability of Tregs transferred by infusion (survival rate ≤40% within 1 month after infusion[11]). Using Tregs to replace nonspecific immunosuppressive drugs reduces dependence on nephrotoxic calcineurin inhibitors, while maintaining antimicrobial immunity through partial immune system release[12].

## 4. Feasibility

### 4.1. Limitation

The severe shortage of existing organ donor banks remains the biggest problem affecting organ transplantation, which will also lead to fewer opportunities for clinical research on Treg replacement therapy and insufficient sample size [12,13]. However, advances in other fields related to organ transplantation will also improve the feasibility of such research, such as improving perfusion methods to reduce non-immunosuppressive damage such as ischemia-reperfusion injury to organs, and further improve the success rate of organ transplantation [1]. After the development of experimental methods for tracking Tregs in vivo, the visualization of Treg migration will more accurately help with the infusion and targeted nanodelivery of Tregs after organ transplantation [13].

### 4.2. Future Direction

In the future , the targeted localization and delivery of Tregs are important aspects of this field. Combining clinical pathological analysis with identification of key cellular markers corresponding to organ transplantation, and using nanodelivery and other technologies to enable the expression of expanded Tregs in the target transplant organ is a key focus of future research [13]. At the same time, the dosage, infusion time, and changes of Tregs over time after organ transplantation, as well as the weight of Treg therapy in immunosuppressive regimens, still need further investigation [2]. Not only is it necessary to evaluate the effectiveness of Tregs in organ transplantation, but also to conduct cross-sectional comparisons of various methods of using Tregs in conjunction with further clinical research. Future research directions can continue to advance research on using patient-generated Tregs to replace traditional clinical immunosuppressants to improve poor prognosis, as mentioned in this article. Simultaneously, multi-phase clinical trials can be conducted to identify suitable target biomarkers to facilitate the application of technologies such as nanodelivery in this field.

## 5. Conclusion

While organ transplantation is an effective treatment for end-stage organ failure, the systemic toxicity of traditional nonspecific immunosuppressants significantly impacts long-term patient prognosis. This article systematically analyzes the application potential of Tregs in organ transplantation, exploring their classification, immunosuppressive mechanisms, in vitro expansion and isolation techniques, and the latest related application strategies. The analysis results show that Treg therapy can specifically induce local immune tolerance against donor organs. This suggests that this not only addresses the urgent need for global immunosuppressants but also demonstrates that combining Tregs with nano-targeted delivery technology can effectively solve problems such as Treg functional instability, significantly improving Treg survival and polarization in grafts. These different Treg application strategies provide important theoretical references for reducing clinical dependence on immunosuppressive drugs and improving patient quality of life in the future. However, due to the severe shortage of existing organ donor banks, the current clinical research sample size remains insufficient, and long-term tracking technology for Tregs in humans is still imperfect. Looking ahead, research should focus on identifying organ-specific pathological biomarkers to optimize nanodelivery systems and exploring the optimal infusion dosage and timing of Tregs to promote the early adoption of specific cell immunotherapy as a routine anti-immune rejection regimen. With continuous advancements in perfusion technology and other fields, non-immunosuppressive injuries such as ischemia-reperfusion in organ transplantation will be further reduced. Combined with the gradual clinical trials and application of Tregs therapy, organ transplant patients will have a bright future.

## References


[1] Juneja T, Kazmi M, Mellace M, Saidi RF. Utilization of Treg Cells in Solid Organ Transplantation. Frontiers in immunology. 2022;13:746889.

[2] Husain I, Luo X. Novel Approaches to Immunomodulation for Solid Organ Transplantation. Annual review of medicine. 2024;75:369-380.

[3] Fortunato M, Morali K, Passeri L, Gregori S. Regulatory Cell Therapy in Organ Transplantation: Achievements and Open Questions. Frontiers in immunology. 2021;12:641596.

[4] OhkuraN,KitagawaY,SakaguchiS. Development and Maintenance of Regulatory T Cells. Immunity. 2013;38(3):414–423.

[5] Roncarolo MG, Gregori S, Bacchetta R, Battaglia M, Gagliani N. The Biology of T Regulatory Type 1 Cells and Their Therapeutic Application in Immune-Mediated Diseases. Immunity. 2018;49(6):1004-1019.

[6] Sato Y, Passerini L, Piening BD, Uyeda MJ, Goodwin M, Gregori S, Snyder MP, Bertaina A, Roncarolo MG, Bacchetta R. Human-engineered Treg-like cells suppress FOXP3-deficient T cells but preserve adaptive immune responses in vivo. Clinical & translational immunology. 2020;9(11):e1214.

[7] Safinia N, Grageda N, Scottà C, Thirkell S, Fry LJ, Vaikunthanathan T, Lechler RI, Lombardi G. Cell Therapy in Organ Transplantation: Our Experience on the Clinical Translation of Regulatory T Cells. Frontiers in immunology. 2018;9:354.

[8] Romano M, Fanelli G, Albany CJ, Giganti G, Lombardi G. Past, Present, and Future of Regulatory T Cell Therapy in Transplantation and Autoimmunity. Frontiers in immunology. 2019;10:43.

[9] Sánchez-Fueyo A, Whitehouse G, Grageda N, Cramp ME, Lim TY, Romano M, Thirkell S, Lowe K, Fry L, Heward J, Kerr A, Ali J, Fisher C, Lewis G, Hope A, Kodela E, Lyne M, Farzaneh F, Kordasti S, Rebollo-Mesa I, Jose Lozano J, Safinia N, Heaton N, Lechler R, Martínez-Llordella M, Lombardi G. Applicability, safety, and biological activity of regulatory T cell therapy in liver transplantation. American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons. 2020;20(4):1125-1136.

[10] Sato T, Takeda N. The roles of HIF-1α signaling in cardiovascular diseases. Journal of cardiology. 2023;81(2):202-208.

[11] Sumida TS, Cheru NT, Hafler DA. The regulation and differentiation of regulatory T cells and their dysfunction in autoimmune diseases. Nature reviews. Immunology. 2024;24(7):503-517.

[12] Lu T, Song H, He L, Hu Z, Li Y, Xu C, Liu S, Jiang J, Zhou Y, Li Z, Li Z, Zhang K, Ma S, Qi R, Han S, Shi C, Wen W, Han D, Qin W. Targeted Expansion of Treg Cells to Induce Immune Tolerance after Kidney Transplantation. Advanced science (Weinheim, Baden-Wurttemberg, Germany). 2026;13(1):e07943.

[13] McCallion O, Cross AR, Brook MO, Hennessy C, Ferreira R, Trzupek D, Mulley WR, Kumar S, Soares M, Roberts IS, Friend PJ, Lombardi G, Wood KJ, Harden PN, Hester J, Issa F. Regulatory T cell therapy is associated with distinct immune regulatory lymphocytic infiltrates in kidney transplants. Med (New York, N.Y.). 2025;6(5):100561.