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Review

Cell based Therapy in Transplantation

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Abstract

One of the major hurdles still facing the field of transplantation is the management of immunosuppression and the morbidity that results from treatment. Due to toxicity and complications from a maintenance immunosuppression therapies, a necessary improvement in post-transplant immunosuppressive therapies must be the development of a low-side effect therapy. Cell-based therapies as an emerging candidate offer a novel approach to generating graft tolerance, and when utilized within a combination therapeutic strategy, they may allow for targeted allograft protection with higher safety. In this review, the results and advances of these cell-based approaches including regulatory T cells, IL-10 producing Tr1 cells, tolerogenic dendritic cells and mesenchymal stem cells in animal studies and clinical trials will be discussed and compared.

Keywords

Organ transplant; cell-based therapy

1. Introduction

Solid organ transplantation (SOT) and hematopoietic stem cell transplantation (HSCT) have become widely utilized procedures in today's clinical environment, and this is due to a variety of factors. First, in recent years significant progress has been made in the investigation of the mechanisms of allograft rejection [1]. Second, the development and application of numerous immunosuppression agents such as Tacrolimus have dramatically improved graft survival rates in organ transplantation [2]. Although the development of modern immunosuppression agents has resulted in dramatic improvement of short-term graft survival, however, the long-term survival rates remain a challenge [3]. Furthermore, toxicities of conventional immunosuppression, including susceptibility to a wide variety of pathogens, nephrotoxicity, diabetes mellitus, anemia, and many others [4-6] have now become major roadblocks in today's clinical transplantation setting.

As a result of the challenges associated with pharmacologic immunosuppression, research groups across the world have begun focusing on generating allograft tolerance as a mechanism to promote long-term graft survival. One of the most exciting movements within this research field, that of cell-based therapies, offers a novel approach to generating graft tolerance, and when utilized within a combination therapeutic strategy, these cell-based strategies may allow for targeted allograft protection without compromising the host's overall protective immunity. Within this framework of cell-based therapies, protocols that utilize regulatory T cells (CD4+ Treg, CD8+ Treg), IL-10 producing Tr1 cells [7], regulatory macrophages (Mreg) [8, 9], tolerogenic dendritic cells (tol-DCs) [10, 11], and Mesenchymal stem cells (MSCs) have emerged as the most promising approaches. In this review, the results and advances of cell-based immunosuppressive approaches in animal studies and clinical trials will be discussed and compared.

2. $CD4^+$ Regulatory T Cells

Regulatory T cells (Tregs) are an important element in the development of transplant tolerance, and they are commonly utilized in cell-based immunosuppressive protocols. The rationale behind using Tregs is that they will shift the host's inflammatory environment from a Th1 effector response to regulatory response, thereby lowering the risk of rejection and reducing the required conventional immunosuppression. There is a wide spectrum of heterogeneity among the cells grouped as "Tregs," and each of the subsets carries out a unique tolerance-promoting function [12]. However, the most important Treg subset is the class of conventional Treg cells that are $CD4^+CD25^{hi}Foxp3^+CD127^{low}$, within which Foxp3 is a transcription factor that plays a critical role in Treg development and function [13-16]. There are multiple mechanisms by which these $CD4^+CD25^{hi}Foxp3^+CD127^{low}$ Tregs exert their inhibitory effects and limit T cell activation, including cytotoxicity, secretion of IL-10 [17, 18], disruption of IL-2 signaling [19], disruption of antigen presenting cell (APC) signalling [20, 21], and induction of T cell anergy [22]. While many properties and characteristics of human Tregs remain to be studied, critical advances have been made in identifying specific types and subtypes of Tregs based on their markers, in expanding Tregs in *ex vivo* cultures, and in elucidating the pathways by which Tregs promote immunosuppression. As a

result of these advances, Tregs are becoming easier to culture and study, and research centers around the world are exploring new therapeutic applications of Tregs.

2.1 Animal Models

Within the realm of transplantation there is evidence of a useful array of Treg functions, and recent animal studies show that Tregs may interact with Calcineurin inhibitors (CNI) to control memory T cells and promote long-term graft survival [23]. It is well-established that regulatory T cells are critical for inducing and maintaining tolerance in the transplant setting, and previous animal transplantation studies have indicated that Treg-based therapy can control allograft rejection and potentially induce tolerance through a pathway involving anti-CD4 antibodies [24]. In one such study, the recipient and donor-derived Tregs proved to be effective in inhibiting alloreactivity both *in vitro* and *in vivo*; although interestingly the effect of donor-derived Tregs could be countered by exogenous IL-12 [25]. Furthermore, within the field of bone marrow transplantation, regulatory T cells are proving to play an important role in containing graft-versus-host disease [26]. In particular, ex vivo expanded CD4+CD25+ regulatory T cells have the ability to inhibit graft-versus-host disease while at the same time preserving graft-versus-tumor activity [27, 28]. Within the fields of solid organ transplantation, *in vitro* alloantigen stimulated regulatory T cells have been shown to induce long-term tolerance in both skin and cardiac transplants [29].

Treg utilization may also benefit from recent advances in T cell research such as the genetic engineering of T cells to overexpress transgenic TCRs, which is a research model that is currently being tested in settings such as cancer immunotherapy [30], autoimmune diseases [31] and allograft rejection [32, 33]. By developing chimeric antigen receptors (CARs) on Tregs, these cells could target designated allo-antigens and induce highly specific tolerance toward a small group of antigens. In one recent study [32] CAR technology was used to modify Tregs, and the results indicated that CAR-Tregs exhibit more potent suppressive activity both *in vitro* and in human skin xenografts when compared to polyclonal Tregs. Additionally, in a separate study utilizing a murine islet transplant model, mAb-directed CARs were bound to Tregs and administered to the mice that were recipients of the islet cell transplants. The administration of these Tregs led to donor specific tolerance and extended both islet cell graft survival and secondary donor skin graft survival [33].

2.2 Clinical Application

Human Tregs have been well-characterized over the past two decades, and the important roles they have in human transplantation outcomes are becoming evident. In 2009, a study by Trzonkowski et al [34] demonstrated that *ex vivo* expanded Tregs are effective at preventing chronic GvHD in humans. In particular, they found that Treg infusion was associated with a significant decrease in pro-inflammatory cytokines, and they also found that if Treg administration was delayed, the prevention of GVHD was less successful. Later, in 2010 Brunstein et al [35] managed to prevent allogeneic acute GvHD in bone marrow transplant recipients by infusing *ex vivo* expanded Tregs along with a standard immunosuppressive therapy into patients. They also found that cryopreservation may negatively impact the overall functioning of Tregs. In 2011, Di Ianni et al [36] proved that the infusion of freshly isolated donor Tregs into bone marrow transplant recipients can make immune reconstitution with $CD4^+$ and CD8+ T cell administration a feasible and safe option in the clinical setting. Finally, a study by Marek-Trzonkowska et al [37]

showed that Treg infusion is an effective therapeutic option to prolong the length of remission of type 1 diabetes in children.

More recently, a phase 1 Treg Adoptive Cellular Transfer (TRACT) trial tested the use of Tregs in kidney transplantation through the use of polyclonal expanded Tregs from recipients' cryopreserved leukopheresis products (NCT02145325) [38]. A total of nine patients were enrolled in the TRACT study, and all of them received Tacrolimus and mycophenolate immediately after transplantation, followed by conversion to Sirolimus monotherapy prior to administration of Tregs. Compared to pre-transplant Treg levels, in all patients a 5-20 fold increase in Tregs was achieved at one year follow up, indicating that the Treg infusions resulted in a stably elevated Treg presence within the recipients. Additionally, the study demonstrated the long-term safety of Treg therapy in humans. The pilot TASKp trial (NCT02088931) also explored the potential of *ex vivo* expanded polyclonal Tregs in kidney transplantation. The TASKp trial therapy included an infusion of 320×10^6 Tregs into each patient, and the resulting 100-fold expansion (or greater) of infused Tregs over the course of one week resulted in the infused Treg population comprising 7.5% of the patients' total peripheral Tregs. Furthermore, the results of a 1-year follow-up indicated decreased graft inflammation and increased uCRM scores compared to pre-Treg infusion levels. Finally, another pilot study recently applied regulatory T cell-based therapy in the setting of living donor liver transplantation. *Ex vivo*-produced Tregs were infused into recipients, and 7 out of 10 patients were able to completely withdraw from immunosuppression [39].

Through all of these clinical trials, Treg infusion has been shown to be a safe and effective method of modulating the immune system while simultaneously minimizing the use of immunosuppressive drugs. Furthermore, these studies show that the efficacy of Treg therapy is dependent on proper dosage, time of administration, and quality of the Tregs themselves. Future utilization of Treg infusion therapy will rely on the development of novel Treg expansion protocols as well as further discoveries into the mechanisms by which Tregs suppress immune functions.

3. $CD8^+$ Regulatory T Cells

While it has become well-established that CD4+ regulatory T cells are critical for immune regulation, researchers have just recently discovered that some sub-populations of $CD8^+$ T cells may also act as potent regulators of immune tolerance through the promotion of suppressive activity. In humans, $CD8^+CD28^-$ Tregs have been found in the settings of chronic allograft rejection [40-42] and autoimmune disease [43], and along with other $CD8^+$ subtypes including $CD8^+CD103^+$ [44] and $CD8^+CD122^+$ [45] cells, they have been shown to inhibit T cell activation and proliferation. Due to their recent discovery, the underlying inhibitory mechanisms of these $CD8^+$ Tregs have not yet been fully illustrated. However, perforin-mediated cytotoxicity, cell contact-dependent inhibition, the production of inhibitory cytokines, and the upregulation of inhibitory markers on APCs have all been linked to their suppressive activity [46, 47]. Moreover, there is evidence that $CD8^+Foxp3^+$ Tregs may indirectly inhibit proliferation of effector $CD4^+$ and $CD8^+$ T cells and the production of pro-inflammatory cytokines by stimulating an increase in CTLA-4 expression on dendritic cells [48].

3.1 Animal Models

Numerous pre-clinical research models have demonstrated the potential benefits of $CD8^+CD28^-$ Tregs in the transplant setting. In *ex vivo* models, the $CD8^+CD28^-$ Tregs have been shown to anergize xenoreactive $CD4^+$ T cells [49]. These $CD8^+CD28^-$ Tregs have also been demonstrated to have a beneficial effect on the long-term survival of rat liver transplants [50]. Additionally, adoptive transfer of $CD8^+CD122^+$ Tregs has been shown to extend graft survival in murine allogeneic islet transplantation models [51]. Finally, the infusion of human $CD8^+$ regulatory T cells in a murine model proved to be effective in inhibiting GvHD while persevering the protective immunity of the mice [52].

In summary, while $CD8^+$ Tregs have only recently been discovered and there have not been any clinical trials involving their administration into human patients, they are considered a novel and promising cell population with many potential future applications in the prevention allograft rejection.

4. Tolerogenic Dendritic Cells

Dendritic cells (DCs) have become well-known as the most potent of the antigen presenting cells. As critical orchestrators of the immune response, DCs have multiple roles in the upregulation and downregulation of the immune response as well as the induction of tolerance [53]. DCs comprise a very heterogeneous group of cells, and they can be sorted into conventional and plasmacytoid DCs based on their phenotypes and functions. Additionally, DCs can be sub-divided into immature and mature DCs based on their development stages. Of particular interest is a subpopulation of DCs known as regulatory or tolerogenic DCs (tol-DCs). These cells arise from immature DCs and function as suppressors of the immune response [54]. Tol-DCs play a vital role in maintaining both central and peripheral tolerance, and they promote tolerance through T cell inhibition and apoptotic depletion, induction of T cell anergy, activation of Tregs, and the production of immunosuppressive cytokines such as IL-10, TGF-β and IDO [55, 56]. Regarding central tolerance within the thymus, these tol-DCs enforce negative selection of self-reactive and antigen-specific reactive thymocytes. Furthermore, they also promote central tolerance by inducing regulatory T cell generation [57-59].

4.1 Animal Models

Many experiments have been conducted using tolerogenic DCs as a treatment both prior to and after the establishment of autoimmune disease symptoms, and the results suggest that tolerogenic DCs have a strong inhibitory effect on the development of autoimmune diseases [60]. In rodent heart and islet cell transplant studies it has been shown that tol-DCs are capable of decreasing effector T cell frequency and inducing splenic T cell unresponsiveness to allo-antigens [61, 62]. Furthermore, adoptive transfer of tol-DCs into transplant recipients has been shown to promote the development of donor-specific tolerance [63]. In mice, a series of studies showed that when pre-transplantation adoptive transfer of tol-DCs was utilized in addition to conventional immunosuppression, the median survival of the cardiac grafts exceeded 100 days [63-66].

In non-human primate transplant models, the Thomson group showed that infusion of tol-DCs along with CTLA4-Ig administration reduced allogenic T cell responses and led to an increase in

immune-modulating IL-10 producing T cells [67]. In another non-human primate transplant study, it was shown that tol-DC administration one week prior to transplantation increased graft survival by over 70 days [68]. Most recently, tol-DCs have also been shown to modulate the immune response by decreasing IL-17 production and promoting renal allograft survival in primate models [69].

4.2 Clinical Application

Tol-DCs are already being utilized in clinical trials to test for therapeutic benefits in inflammatory and autoimmune diseases such as Type 1 Diabetes [70], Rheumatoid Arthritis [71, 72] and Crohn's Disease [73]. Within the transplant setting, tol-DCs, and indeed, APCs in general are beginning to enter the clinical realm. Most recently, researchers have been studying the immunosuppressive benefits of another APC, which is a donor splenic macrophage referred to as a "transplant acceptance inducing cell" (TAIC) [74, 75]. In Hutchinson JA's study on TAICs and their immunosuppressive effects after renal transplantation, his team found that 10 of 12 patients were able to gradually withdraw from the conventional immunosuppression after 8 weeks due to the addition of TAICs to the treatment protocol [74]. Another study investigating the efficacy of regulatory macrophages (Mregs) in the renal transplant setting involved the administration of Mregs one week prior to transplantation. The patients then received multi-drug immunosuppression for 6 months before being tapered down to low-dose Tacrolimus monotherapy. Flow cytometry-based *in vitro* suppression assays indicated profound suppression of both CD4+ and CD8+ T cells in the early post-op period, and both patients in the study had stable graft function at 3 year follow-up visits [9]. As evidenced by these numerous successful clinical and pre-clinical trials, tol-DCs and other regulatory APCs have proven to be safe for use in humans and are demonstrating promise as potent additions to post-transplantation immunosuppression protocols. Given the promising results of these initial studies, then, several new phase I/II clinical trials are now underway to further investigate the use of tol-DCs and other APCs as additions to the immunosuppressive protocol in a variety of transplant settings. (NCT02088931, NCT02091232, NCT02129881, NCT02188719, NCT02244801)

5. Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) have long been considered an exciting cell population with the potential to enhance immunosuppressive protocols while minimizing side effects. In the field of transplantation, MSCs can be exogenously cultured and infused in order to influence immune microenvironments within the host in a paracrine fashion [76], and the dual roles of MSCs in regeneration and immune regulation make them uniquely attractive compared to previously mentioned cell lines such as T cells and DCs. What is more, allogeneic MSCs do not trigger classic rejection episodes by the host's immune system, and their immunomodulatory effects have shown promise in reducing cell-mediated cytotoxic effects on transplanted organs [77].

Mesenchymal stem cells possess a wide variety of immunoregulatory functions and have been shown to suppress the proliferation and activity of immune cells both *in vivo* and *in vitro* [1, 78, 79]. MSCs can suppress Th1 and Th17 cell proliferation via a contact-dependent method, and they can induce the expansion of immunomodulatory Tr1 IL-10+ T cells and Th3 TGF-β+ T cells. One study showed that MSCs can influence the development and maturation of APCs and decrease the

ability of these APCs to activate a T cell response [80]. In addition to inhibiting lymphocyte activation and cytokine production, there are also multiple studies indicating that MSCs can stimulate the maturation of regulatory T cells and regulatory B cells [81].

Although therapies based on MSC administration are generating exciting results, it is important to note that there are controversies surrounding the classification and characterization of "MSCs." Cells classified as MSCs can originate from bone marrow, skin, pancreatic tissue, the lungs, the kidneys, and many other sources. Furthermore, the term "MSC" represents such a diverse class of cells that, unlike many other stem cell types, there's no defining epitope unique to all MSCs [82]. As such, isolating MSCs requires an extensive enrichment process involving multiple markers, and the final enriched product may be a heterogeneous mixture of MSC subpopulations [83, 84] .

5.1 Animal Models

The preclinical pilot study on the immunosuppressive potential of MSCs was conducted on a baboon model by Bartholomew et al [85]. The infusion of allogeneic MSCs led to extended skin graft survival, and the MSCs did not elicit a proliferative response from the host immune system. Since this initial trial, numerous studies have been conducted to explore the mechanisms by which MSCs influence and enhance graft survival in various transplantation models [86-88].

5.2 Clinical Application

The first clinical trial regarding the infusion of MSCs in a transplantation setting was reported in 2011, in which two renal transplant patients received autologous MSCs from living-related donors [89]. Through the generation of an increase in the $CD4^+$ Treg population and a simultaneous decrease in the $CD8^+$ memory T cell population, cell-based therapies that utilized the MSCs were proven to be safe and effective in the clinical transplant setting. Currently, five completed clinical trials [90-94] have investigated the effects of MSC infusion in transplant patients, and all have shown MSCs to be effective immunomodulators. Furthermore, there are eight ongoing clinical trials involving MSCs in transplantation, and these trials will provide further information regarding the efficacy of MSCs in the clinical setting. (NCT02490020, NCT01690247, NCT02563366, NCT02409940, NCT01429038, NCT02561767, NCT02492308, NCT02957552)

Although MSCs are relatively new within the field of cell-based transplant treatment and their therapeutic benefits have not yet been fully elucidated, the research that has been done has shown MSCs to be a safe and promising therapeutic option of the future.

6. Conclusion

Cell based therapies within the field of transplantation are relatively new and must be rigorously standardized, optimized, and generalized before becoming a crucial addition to immunosuppressive protocols. However, with minimal side effects and clear benefits in an era of personalized medicine, cell-based therapies are ideally suited to become a new class of immunomodulatory treatments and to revolutionize our approach to developing transplant immunosuppression regimens.

Author Contributions

Y.G., M.G., and B.O. prepared the manuscript.

Competing Interests

The authors have declared that no competing interests exist.

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