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The trend for transplant medicine development: induction of immune tolerance or regulation of immune response?

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Abstract

One of the greatest medical advances of the last century has been the introduction of organ transplantation. However, despite the considerable potential of transplantation as often the only therapy for severe diseases, the toxicity of immunosuppressive drugs supporting the transplant remains a serious problem for its further development. Modification of immune response in order to form tolerance to the transplanted organ can play an important role on the way to minimize immunosuppression. Successful cases of withdrawal of immunosuppressive drugs for medical reasons in kidney and liver transplantation recorded in the literature, as well as the results obtained in the process of modeling such a situation in

the experiment, prove that achieving tolerance in organ transplantation is fundamentally possible.

The aim of this review is to investigate the ways of immunologic suppression and fundamental mechanisms of immunologic tolerance in the field of transplantation and to review the latest clinical achievements in this respect.

The review describes various approaches to the induction of central tolerance in solid organ transplantation implemented in the framework of the original clinical protocols. Special attention is given to a new direction in transplantation medicine - cell technologies providing tolerogenic effect by means of peripheral mechanisms activation, in particular due to activation of suppressor function of regulatory T cells.

We draw the attention to the advantages and disadvantages of these two trends. Which of them is preferable? In which direction will scientific thought be developed for realization of the long-term goal of transplantologists: to avoid allograft rejection without affecting the physiological homeostasis of the body? Possible answers to these questions are discussed in the text of this review.

Keywords: solid organ transplantation, immune tolerance, graft rejection, cell chimerism, immunosuppression, regulatory cells, graft versus host disease

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APCs, antigen-presenting cells

HIV, human immunodeficiency virus

HSCs, hematopoietic stem cells

DCs, dendritic cells

BM, bone marrow

GVHD, graft-versus-host disease/reactions

IL, interleukin

Tregs, regulatory T cells/lymphocytes

Introduction

The immune response to transplantation is determined by complex interactions between the components of the innate and adoptive immune responses, leading to the activation of cell- and antibody-mediated rejection mechanisms [1]. The graft rejection reaction occurs as a result of direct and indirect recognition of donor antigens by T cells and their activation via one of the signaling pathways [2]. The antigen-specific signaling pathway involves the interaction of the T-cell receptor presented in the context of molecules of the major histocompatibility complex. The second pathway is implemented through the interaction of costimulatory CD28/B7 molecules, whose activity is regulated by inhibitory signaling molecules, in particular CTLA-4, and PD-1 [3].

It is known that the main factors limiting the success of organ transplantation are the host immune response to an allograft and the adverse effects of long-term immunosuppressive therapy required to suppress this immune response. The standard immunosuppressive therapy based on tacrolimus and mycophenolates form the basis of long-term maintenance immunosuppression. It is generally accepted that these drugs are effective in preventing acute episodes of rejection, and therefore provide quite satisfactory immediate results, but do not prevent chronic allogeneic graft dysfunction [4].

Immunosuppression based on tacrolimus (calcineurin inhibitor) significantly improves the survival of liver transplant recipients. However, calcineurin inhibitors have a narrow therapeutic window and significant pharmacokinetic variability between recipients [5]. Plasma concentrations that are too low can lead to organ rejection, while too high concentrations can cause nephrotoxicity or neurotoxicity. Cancer is also major adverse complication of solid organ transplantation [6-8]. The risk of developing cancer is 2-4 times higher in patients after transplantation and is largely due to immunosuppression [9, 10]. The spectrum of cancer resembles the one observed in human immunodeficiency virus (HIV) infection [11]. The risk is especially high for virus-associated malignancies, including non-Hodgkin's lymphoma and Hodgkin's lymphoma (Epstein-Barr virus), Kaposi's sarcoma (human herpesvirus type 8), genital cancer (human papillomavirus), and liver cancer (hepatitis C and B viruses). Renal cell carcinoma occurs among patients who have undergone kidney transplantation, with an almost 6-fold excess risk compared with patients without transplantation [12]. The incidence of some other malignant neoplasms, such as lung, skin, and thyroid cancer, is also increased in transplant recipients.

After transplantation of solid organs while taking calcineurin inhibitors, the complication rates due to the clinical toxicity of immunosuppressive drugs increases. There are cardiovascular diseases, metabolic syndrome, bone loss, progression of opportunistic and community-acquired infections, and chronic kidney disease among them [13].

To reduce these effects, clinicians often empirically try to minimize doses of calcineurin inhibitors through trial and error, or switch to alternative drugs [14]. In addition, general immunosuppressive therapy may become ineffective over time as the patient's physiology changes,

poorly differentiated immune responses occur, or the pathological mechanisms of the disease change under constant therapeutic pressure. The use of these lifelong therapies and their ongoing monitoring is costly and has a significant impact on patients' quality of life.

All these circumstances cause an increased need to develop more effective and safer methods of treatment aimed at inducing immune tolerance to donor tissue by reprogramming the recipient's immune system, aimed at improving graft survival and eliminating the adverse effects of chronic drug therapy. To achieve this goal, it is necessary to understand the complex mechanisms of interaction between the antigenic structure of the graft and the immune system of the recipient, taking into account the effect of non-specific immunosuppressive, biological, chemo- and hormonal drugs used to prevent or stop rejection processes.

Transplantation tolerance

Since the first successful human kidney transplant by Dr. Joseph Murray in 1954 between identical twins, transplantologists had sought to move away from aggressive broad-spectrum immunosuppressive regimens to tolerogenic strategies that promote a long-term graft survival without side effects. Reports of successful kidney and liver transplants in which immunosuppressive drugs have been discontinued for medical reasons, together with the results of experimental transplantation models, prove that it is fundamentally possible to achieve tolerance in organ transplantation. However, the translation of the process of reformatting immune responses in clinical settings is a complex task associated with the superposition of many interacting factors amid the general variability of the course of the disease. If the body's tolerance to its native tissues (autotolerance) is formed as a result of embryonic development, the operational tolerance has a number of specific features.

The study of these features is demonstrated in the experimental work of R.E. Billingham and P.B. Medawar, which was published in 1951 under the title: "The technique of free skin grafting in mammals", where research was focused on the induction of "actively acquired tolerance" by exposing animals to donor antigens in the perinatal period [15]. This research laid the foundation for what would become the field of transplantation immunology. The basis for this approach was the observation of the effect of erythrocyte chimerism in most dizygotic twins of cattle, in the presence of a common placenta [16], which persisted in the postnatal period. Subsequently, on this basis, it was assumed that the presentation of an alloantigen during intrauterine and early neonatal life somehow leads to acquired tolerance. The authors showed that the acquired tolerance during organ and tissue transplantation is predetermined by the so-called mixed chimerism. Mixed chimerism is a form of the hybrid immune system in which donor pluripotent hematopoietic stem cells (HSCs) coexist with recipient stem cells, giving rise to hematopoietic lines in the recipient.

According to the classic definition formulated by R.E. Billingham and P.B. Medawar, with transplantation tolerance, the productive activation of an antigen-specific clone of lymphocytes does not begin, and the immune system steadily perceives an alloantigen as its native one and does not respond to it [17]. In cases where the productive activation of an alloreactive clone begins, is realized, and then suppressed, there is a mechanism for inducing the immune suppression, in other words, that for immunoregulation. The mechanisms of suppression imply the clone deletion by apoptosis, followed by the maintenance of an anergic state based on the cells possessing these properties. In this regard, immunological tolerance, by definition, has a significant difference from immunological suppression, in which an already established immune

response is suppressed. These two processes (tolerance and suppression) are formed and implemented at different stages of lymphopoiesis and lymphocyte immunogenesis, therefore, at least, they are not identical.

However, the current, not entirely correct interpretation of the term "transplant tolerance" overlooks the signs of the graft immune response in the long term without the use of immunosuppressive drugs while maintaining the immune system competence, regardless of which way this is implemented.

Induced mixed chimerism

From the point of view of the classical understanding, the induction of hematopoietic chimerism should be considered as the main mechanism for achieving transplantation tolerance [18]. The chimerism-based tolerance established by co-transplantation of hematopoietic stem cells with a kidney from the same donor has emerged from extensive preclinical studies as a promising approach for clinical application [19]. The induction of mixed chimerism and refusal of immunosuppression with achieving a stable graft function in cases of sequential bone marrow transplantation using myeloablation followed by kidney transplantation for myeloma-induced renal failure in patients from Massachusetts General Hospital [20-21] and Stanford [22] opened the way for clinical trials in patients with end-stage renal disease without malignancy [23]. However, the risks of toxicity from ablative conditioning that the authors encountered, acceptable for HSC transplantation in hemoblastoses, turned out to be unacceptable for establishing donor tolerance in the context of solid organ transplantation.

This circumstance was the reason to draw the attention of researchers to previous studies, which were based on the principle of non-myeloablative and low-intensity treatment methods [24, 25]. An

important role in this was played by the experimental work carried out by S.T. Ildstad et al. (1984) [26]. The authors compared the tolerance of mixed/syngeneic bone marrow (BM) chimeras and complete allogeneic BM chimeras. Mixed allogeneic mice were injected with T cells from syngeneic (native) BM and allogeneic (donor) BM. As a result, mixed chimeras showed significantly higher tolerance and immunocompetence compared to full allogeneic mice, both in studies in vitro on lymphocytes, and in studies in vivo on recipient's skin. Donor skin flaps adhered, and no graft-versus-host reactions (GVHD) were noted in the recipients. The fact that recipients with less than 1% donor chimerism were tolerant gave grounds to believe that the complete replacement of the recipient hematopoietic system with the donor one is not a prerequisite for the induction of tolerance, and the achievement of immunological tolerance did not depend on the intensity degree of donor chimerism.

Thus, the presence of chimerism cannot act as an independent biomarker of tolerance. A number of studies have reported a dissociation between tolerance and chimerism [27, 28]. It is believed that this dissociation is caused by the lack of the acceptance of donor T cells [29]. Donor T cell production in mouse chimeras is directly correlated with the tolerance of donor skin graft, but chimeras without donor T cell production reject donor skin grafts despite the persistence of hematopoietic chimerism [30]. The role of donor T cells in the induction and maintenance of tolerance was actually proven in a clinical study aimed at inducing tolerance to renal allografts through chimerism [31].

The mechanism underlying the lack of donor T cell production in grafted chimeras remains unclear, but clear is the fact that it is possible to confer tolerance through non-myeloablative conditioning without providing complete chimerism by focusing on T cell chimerism, as is customary in the hematology community for HSC transplantation [32–

34]. This can significantly reduce the risk of complications from ablative conditioning. In this regard, it has been suggested that syngeneic components of BM allow hosts to overcome the restrictions of immune cellular interactions that are observed in completely ablated allogeneic animals, while allogeneic elements contribute to the formation of host tolerance to the donor graft. This important discovery has been the basis of recent studies to develop low-intensity conditioning to establish chimerism and induce tolerance in kidney transplantation.

The clinical feasibility of this approach in kidney transplantation was described by Y. Fudaba et al. (2006), when 6 patients with multiple myeloma and renal insufficiency underwent bone marrow transplantation followed by kidney transplantation from HLA-identical sibling donors, after non-myeloablative conditioning, including cyclophosphamide, antithymocyte globulin, and thymus exposure to irradiation. Mixed chimerism was initially achieved by all, but subsequently was lost by 4. However, despite the loss of chimerism, 3 of 4 patients were in sustained complete remission for a long period (1.3 to 7 years) without immunosuppression [35].

To date, there are several centers that have an experience in combined kidney and donor bone marrow transplantation for the induction of transient donor chimerism and tolerance to renal allograft: Stanford University (Stanford Institute for Immunity, Transplantation, and Infection) [36], Massachusetts General Hospital [37] and Northwestern University Chicago (Comprehensive Transplant Center, Northwestern Memorial Hospital, Chicago, IL) [38]. Each of these centers uses its own unique conditioning regimens to induce acceptance of donor hematopoietic cells and uses different post-conditioning protocols with their own advantages and disadvantages. Recently, this trend has been developed in other medical centers as well [39].

Approximately 70 patients have been registered to date.

Nevertheless, despite encouraging results, the existing conditioning regimens are not optimal, extremely costly, and logistically complex, and have many side effects. Thus, one of the main complications is a GVHD occurrence. Active T-cell depletion of the allogeneic graft can reduce the incidence of GVHD, but has its drawbacks, including a delayed immune recovery and impaired donor cell inactivation [40]. However, the largest obstacle to making this approach more accessible is that currently it is feasible only with living donors [41]. All this casts doubt on the expediency of using the chimerism-induced tolerance in a broader sense [42]. Therefore, a longer follow-up and well-designed multicenter studies are required to ensure the efficacy and safety of the procedures.

Induction of immunosuppression (the immune response regulation)

The paradigm of modern immunology states that a key factor in maintaining immune homeostasis is a dynamic balance on a competitive basis between immunogenic and tolerogenic mechanisms of activation. In solid organ recipients, the balance of these mechanisms is shifted towards graft-damaging effectors, i.e., towards allograft destruction [43]. The development of methods to control and manage the balance of effector and regulatory responses to suppress or abolish alloreactivity, rather than searching for and activating mechanisms unique to a tolerant state, is currently a priority for transplantologists. In this regard, in order to achieve the transplantation tolerance, it is necessary either to deplete alloreactive T cells or selectively inhibit their activity without compromising protective immune functions or causing nonspecific toxicity. The induction of immunological tolerance can also be achieved by increasing the absolute number or increasing the activity of the

suppressor function of regulatory T lymphocytes (Tregs) with the phenotype $CD45^{+}RA^{+}$ $CD4^{+}$ $CD25^{high}CD127^{low/neg}$, which ultimately leads to the depletion of alloreactive T cells by triggering apoptosis processes in them [44-46].

Notably, most tolerogenic strategies that have been undertaken experimentally or in the clinic include depleting factors [47]. Lymph depletion in the form of "induction therapy" is an effective strategy to reduce the rate of alloreactive progenitors during organ transplantation to prevent acute allograft rejection [48]. Deletion approaches have also proven to be therapeutically effective in transplant recipients, although they are accompanied by toxic side effects during the conditioning process [49]. Since the fate of transplanted organs, as noted, is determined by the balance between effector and regulatory activities, another method for stimulating tolerance is to enhance the suppressor functions of regulatory cells by transferring them to the recipient after transplantation [50].

In 1995, S. Sakaguchi et al. demonstrated that a small cell population (5%-10% of peripheral CD4 lymphocytes, called Tregs), which are naturally formed in the thymus, were responsible for the T-cell-mediated mechanism of peripheral tolerance, and play a key role in both the prevention of organ-specific autoimmune diseases and in the induction of transplantation tolerance [51]. Subsequently, this was confirmed by other authors [52, 53]. There are two main types of Tregs: natural Tregs (nTregs), which develop in the thymus and migrate to the periphery, and induced Tregs (iTregs), which arise in the periphery by converting CD4+ T cells after immune stimulation [54]. iTreg cells have T-cell suppression properties similar to those of nTregs [55]. All Tregs express a wide repertoire of α/β T-cell receptors with specificity for both native and alien antigens. A unique cellular marker that distinguishes nTregs

from iTregs has not yet been found. Functionally active Tregs are characterized by constitutive pronounced expression of the α-chain of the IL-2 (CD25) receptor and low or negative expression of the α-chain of the IL-7 receptor (CD127) [56]. In addition, these cells express the transcription factor FoxP3. Its suppressor effect is realized through the repression of the IL-2 gene and other genes necessary for the activation of effector cells [57]. This contributes to the achievement of tolerance during transplantation [58].

The dominant function of Tregs is to control all aspects of the immune response. The mechanisms of immunoregulation by Tregs can be divided into those that target effector T cells (the secretion of inhibitory cytokines, disruption of metabolic processes, and induction of apoptosis) and those that target antigen-presenting cells (APCs) (the reduction of co-stimulation or the reduction of antigen presentation) [59, 60]. Tregs have been found to express additional markers such as cytotoxic T-lymphocytic antigen-4 (CTLA-4) and human leukocyte antigen - DR (HLA-DR). CTLA-4 expression on T lymphocytes occurs only after their activation, but on Treg cells, it is expressed on constitutively, preventing unwanted immune activation by reducing the expression of co-stimulatory molecules CD80 and CD86 on APCs through CTLA-4-mediated trogocytosis [61], as well as by the uptake of IL-2 and other common γ-chain cytokines [62, 63]. Inhibition of APCs activity prevents the proliferation of a clone of effector T cells [64, 65]. The expression of HLA-DR on Treg cells increases the suppressor potential of the total pool of Tregs [66].

During an active immune response, Treg cells proliferate, migrate, and accumulate at the inflammation site, especially in the later phase of the response, in order to restore normal immune homeostasis using a wide range of effector mechanisms, including the production and secretion of

the immunosuppressive cytokines IL-10, IL-35, TGF-β, etc. [67]. In this regard, during inflammation and graft rejection, the number of Treg cells often increases [68, 69]. However, in transplant settings, this increase is usually insufficient and too late to prevent damage to the graft. A decrease in the Tregs population is associated with the severity of acute rejection processes [70].

In addition, immunological tolerance caused by Treg cells has the effect of so-called "infectious" tolerance through their expression of IL-35, which has the ability not only to directly suppress the response of effector T cells, but also is able to enhance and spread suppressive functions by transformation of the total T-cell population into IL-35 producers called "iTr35 cells" [71, 72]. This effect opens up promising opportunities for induction and maintenance of a stable tolerogenic effect during transplantation of solid organs.

On the other hand, APCs also have the ability to induce, maintain, or increase the amount of Tregs, which in turn causes the generation of new tolerogenic APCs [73]. Upon encountering Tregs, all major APC subpopulations, i.e., dendritic cells (DCs), B cells, and monocytes/macrophages, respond with a decrease in antigen-presenting function with a simultaneous increase in the expression of inhibitory molecules and the secretion of immunosuppressive cytokines. DCs and macrophages are capable of both stimulating and suppressing T-cell mediated responses depending on the state of their activation [74, 75]. Immature DCs and macrophages present their own and harmless antigens during inflammatory processes. The antigen presentation without costimulation inactivates effector T cells. Thus, the antigen presentation by non-activated myeloid APCs contributes to the maintenance of stable both autotolerance and tolerance to alloantigen [76, 77]. In addition,

separate populations of Tregs and tolerogenic APCs act synergistically to maintain the immunological balance [78].

To achieve and maintain peripheral tolerance in the post-transplant period, cloning of Treg cells in vitro after their isolation is required. Experimental data show that Tregs cannot prevent a rejection as a standalone therapy. The use of Tregs as a long-term graft survival requires a short-term supplemental immunosuppression to create a therapeutic window. To induce tolerance in combination with a 90% deletion of endogenous T cells requires 150×10^6 to 1×10^9 alloantigen-reactive Tregs [79]. A prospective controlled trial is currently under way to look into the prospect of combination cell therapy using recipient Treg cells and donor bone marrow together with IL6 blockade as a potential strategy to induce transient chimerism and proto-tolerant immunomodulation in kidney transplantation [80]. The study is expected to provide valuable data on the potential of this approach, which could eventually become a new immunomodulatory therapy in kidney transplantation, with the ultimate goal of improving long-term outcomes.

When conducting clinical trials to study the efficacy of polyclonal nTregs as adjunctive therapy in living donor kidney transplantation in three study groups, it was found that immune cell therapy with minimizing an immunosuppressive load resulted in fewer episodes of acute rejection [81-82]. Moreover, the data obtained indicate that the adoptive transfer of Tregs does not interfere with protective immunity against infections and does not lead to global immunosuppression. In liver transplantation, the use of polyclonal Tregs isolated both from patients with end-stage liver disease awaiting transplantation and from stable liver transplant recipients during maintenance immunosuppression contributed to the prevention of the donor organ rejection in the absence of adverse reactions and complications [83].

Further studies of the heterogeneity of the Treg cell population revealed different subpopulations with different functions in the control of the immune response and the induction of peripheral tolerance [84]. It was found that in human blood there are both inhibitory and activating Treg cells, which are indistinguishable from each other by using classical markers: CD25 and Foxp3. Mechanisms leading to the dysfunction of human Treg cells and specific immunophenotypic markers have not yet been determined. The study of these mechanisms, and the physiological properties of individual subpopulations of Treg cells as a control of the immune response and induction of peripheral tolerance have recently been given a great importance [85].

Many researchers consider the technology of developing specific chimeric antigen receptors on nTreg cells (CAR-Tregs) to be attractive and promising for achieving stable tolerance with minimal doses of immunosuppression or even completely cancelling them, which will allow these cells to more successfully migrate into the target organ for realization of the inhibitory potential [86]. In recent years, a hopeful and promising scientific trend to achieve a tolerogenic result in transplantation medicine has been represented by a new medical technology based on the adoptive cellular immunotherapy that is extracorporeal photopheresis. It has been found to provide a direct stimulation of Treg cells by increasing the expression of the transcription factor FoxP3, reducing the expression of the coactivation receptor on unprimed T cells (CD28) and of its ligands (CD 80 and CD86) on antigen -presenting dendritic cells, as well as the profile changes in CD4 T lymphocytes towards the increase of Th 2 subpopulation producing anti-inflammatory cytokines: IL10, TFG-β, CTLA-4, etc., which together provide a tolerogenic potential. This method has already been widely used in solid organ transplantation as a prevention of rejection, as well as

for the control of acute and chronic rejection in transplantation of the heart, lungs, liver, and kidneys [87–90].

Calcineurin inhibitors, paradoxically, may play a decisive role in the induction of the tolerogenic effect. The action of these drugs at the recommended therapeutic dose is ultimately realized by blocking the production of the main T-cell growth factor IL2. However, this achieves the effect of canceling the proliferation of a clone of both alloreactive effector T cells, and also others, including the Treg cells. At present, it is known that the main, non-excessive function of this cytokine is the activation and proliferation of Treg cells in order to regulate and maintain peripheral tolerance of T cells. This selectivity is based on the extremely high affinity of IL-2 to the α-chain of this receptor on Treg cells, higher than on effector cells. In this regard, it is quite logical to assume that in order to achieve a stable tolerogenic effect during transplantation, it is necessary to minimize the dose of calcineurin inhibitors to that sufficient to maintain the production of IL-2, which will be able to activate only the suppressor population of Treg cells and is not able to activate the clone of effector alloreactive T lymphocytes. Given this circumstance, it is quite justified for some researchers to develop cell technologies aimed at minimizing rather than completely abandoning immunosuppressive drugs in order to induce a tolerogenic effect during transplantation [91].

Conclusion

It is known that the main factors that limit the success of organ transplantation are the host immune response to an allograft and the adverse effects of the long-term immunosuppressive therapy required to suppress this immune response. It is quite reasonable to believe that the regulation of such a pronounced and multifaceted immune response to a donor graft requires a similarly powerful and versatile impact by

reprogramming the recipient's immune system, without compromising its overall immune competence, in order to improve graft survival in the absence of adverse effects from the therapy.

Implementation donor-specific tolerance has been esteemed as the “Holy Grail” in organ transplantation. This goal has been actively sought to achieve for more than 6 decades. Despite promising experimental advances, the clinical application often remains unacceptable. The development of bone marrow transplantation methods together with kidney transplantation in clinical practice has given encouraging results, which may, in the near future, may radically change the role of immunosuppression in transplant recipients of other organs, as well. Hematopoietic stem cell transplantation has been widely used as a therapeutic option for the treatment of hemoblastoses. The ultimate result of allogeneic bone marrow transplantation is the establishment of a classic variant of immunological tolerance based on mixed chimerism. However, the standard bone marrow transplant procedure involves the use of aggressive myeloablative conditioning, which is absolutely unacceptable in the context of solid organ transplantation, where recipients have a severe physiological disorder as a result of end-stage organ failure. The success of " bone marrow mini-transplantation" using non-myeloablative conditioning in patients with hematological malignancies has opened up a promising new way for achieving immunological tolerance in solid organ transplantation based on the achievement of donor chimerism. However, according to the available data, much work remains to be done in this direction in understanding the mechanisms of tolerance and the adaptation of conditioning protocols for severe patients in the terminal stage of organ failure. Long-term follow-up is required to establish the stability of the achieved tolerance and exclude the occurrence of graft-versus-host reactions.

As an alternative to the induction of donor chimerism, the cell therapy represents a promising new approach aimed at activating the immune system's own suppressive capabilities to suppress its effector function without the side effects associated with pharmacological immunosuppression. In this regard, since the fate of transplanted organs is determined in part by the balance between the effector and regulatory activities, one of the approaches to stimulate tolerance is to enhance regulatory functions by transferring or activating the recipient's Tregs after surgery in combination with the attenuation or deletion of alloreactive effector cells. Treg cells have the desired specificity, versatility, and adoptability. Many studies have shown their therapeutic efficacy in transplantation. However, they do not have sufficient efficacy as a monotherapy in transplantation; and the factors that determine the efficacy of Tregs therapy in transplantation include the balance of effector and regulatory cells, their specificity (monoclonality), and additional immunosuppression.

In conclusion, it should be noted that new discoveries in the field of cell biology and transplantation immunology have led to many new therapeutic protocols. Meantime, one should take into account that clinical trials of new cell technologies should both meet a high level of safety, and also be oriented towards standardization of the procedure itself, taking into account the clinical and immunogenetic characteristics of the recipient, in order to provide a personalized approach to therapeutic procedures in the post-transplant period. The endpoint of efficacy should be to minimize the dose of drug immunosuppression without a donor organ rejection.

Thus, in the context of historical development, answering the question about the trend in the development of transplantation medical science in the choice of methods for achieving immunological tolerance,

one can see the convergence of two sections of its forming: central and peripheral ones. Obviously, tolerance is formed and functions as an interdependent single process. In this regard, the new treatment protocols that are being developed take into account all these components, although priority is given to the development of cell biotherapy methods aimed at immunological and immunometabolic modulation of regulatory mechanisms in the periphery.

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