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# Immunological tolerance in organ transplantation

M.Sh. Khubutiya, V.A. Gulyaev, V.B. Khvatov, V.L. Lemenev, S.A. Kabanova, M.S. Novruzbekov, K.N. Lutsyk, O.D. Olisov, S.V. Zhuravel', G.V. Bulava, D.Kh. Tsurova, N.V. Borovkova, A.S. Mironov, L.N. Zimina

*N.V. Sklifosovsky Research Institute for Emergency Medicine, Moscow, Russia*

Correspondence to: Mogeli Sh. Khubutiya, Acad. of RAS, Professor, President of N.V. Sklifosovsky Research Institute for Emergency Medicine, Moscow, Russia, e-mail: sklifos@inbox.ru

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*Modeling of immune tolerance will eliminate the need for taking medications to prevent rejection. This review of available literature covers the immune mechanisms of allograft rejection and the ways of tolerance induction. The role of mesenchymal stem cells and their use for tolerance development have been discussed. The authors also draw attention to the fact that blood transfusion from an organ donor leads to a decreased intensity of the immune response to donor cells in transplantation.*

**Keywords:** organ transplant, acute rejection, immunological tolerance

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Organ transplantation being one of the greatest achievements of the twentieth century has taken great strides in medicine as an alternative in the organ failure treatment that can save many patients who do not have other options to survive. More than 106,000 organ transplants were performed all

over the world in 2010 and that could serve an indicator of the medicine development in any state. Over the recent three decades, a one-year graft survival rate for the transplanted organs (kidneys, liver) has reached 90%, but the functional time span of transplanted organs have improved insignificantly due to the development of chronic graft rejection. An acute rejection, even after liver transplantation, was recorded in 1/3 of patients. Generally in most cases, it is coped with by using traditional therapeutics only; but with the treatment-resistant rejection or contraindications to such treatment, other methods should be used [1]. The prevention and treatment of acute rejection are efficient, but involve significant risks, including opportunistic infections, recipient intoxication, metabolic disorders, and malignant neoplasms. The development of new therapies that would not compromise the immune system, but specifically prevent damage to allogeneic tissues, is of primary importance for future transplant medicine. Induction of immunological tolerance will help to obviate the need for administering medications, avoiding a rejection and associated side effects [2].

In attempts to achieve immunological tolerance, researchers focused on studying the regulation of the immune response as the "cornerstone" of modern clinical transplantation. Observations of induced hematopoietic chimeras [3] in veterinary medicine and pioneer works of M. Hašek and V. Demikhov made in the 50s of the XX century contributed to the understanding of this issue [4, 5].

The purpose of this review was to describe the immune mechanisms of allograft rejection and ways of inducing tolerance, basing on the available literature data.

## Transplant rejection reaction

It is known that an allograft transplanted to a recipient from a genetically alien donor is not accepted and is inevitably rejected. Genetic differences in donor and recipient tissues play a key role in the development of the allogeneic graft rejection. Antigens that provide intraspecific differences are designated as the tissue compatibility (histocompatibility) antigens and refer to the major histocompatibility gene complex (MHC) [6]. In humans, MHC is called HLA (human leukocyte antigen). The biological role of MHC is to ensure the interaction between body cells, the recognition of native, alien, and native altered cells, the trigger and realization of the immune response against the foreign information carriers, the positive and negative selection of T cell clones, and the presentation of immune response targets.

The immunological nature of graft rejection was shown by Peter Medawar in the experiment on transplanting a genetically alien skin graft in rabbits [7]. Generally, both humoral and cellular mechanisms play a role in the graft rejection. Cellular mechanisms of rejection include the activation of T lymphocytes that become sensitized to donor-derived antigens. These lymphocytes cause damage to allogeneic tissue cells either by direct cytotoxicity, or by the secretion of lymphokines. The T-cell-mediated damage is characterized by necrosis of parenchymal cells, lymphocytic infiltration, and fibrosis. Humoral mechanisms are mediated by antibodies that can be present in the recipient's serum before transplantation or develop after allogeneic tissue transplantation. Humoral factors damage the transplanted tissue by the reactions that are equivalent to type II and III hypersensitivity reactions. The interaction of antibodies with antigen on the

surface of transplanted cells results in cell necrosis; and the accumulation of immune complexes in blood vessels activates the complement, which leads to the development of acute necrotizing vasculitis or chronic intimal fibrosis with vasoconstriction.

Three types of rejection reactions have been distinguished depending on how fast they can develop: a hyperacute rejection, an acute rejection, and a chronic graft dysfunction (or chronic graft nephropathy in case of kidney transplantation) [8, 9].

A hyperacute graft rejection occurs immediately after the blood perfusion has been resumed; its occurrence is associated with the presence of anti-donor antibodies in the recipient's circulation. The recipient antibodies bind to antigens expressed on the surface of the transplanted graft endothelium, form an antigen-antibody complex, and, in the presence of the complement, initiate the immune inflammation as in type II hypersensitivity. Fibrin deposition with thrombus formation occurs in the graft vessels; the blood flow derangement results in organ death.

An acute rejection, in turn, is classified into acute humoral and acute cellular rejection. The acute humoral rejection in typical cases occurs in a sensitized patient; the process usually begins in the period from several days to 4 weeks after transplantation. The acute cellular rejection can occur at almost any time, usually at 1 week to 6 months after transplantation. The chronic allograft dysfunction can develop in the period from 6 months to many years after transplantation. The chronic rejection of the allograft remains the main cause of failures in the long-term period after surgery. The organ failure occurs due to chronic inflammation, which causes the proliferation of the intimal smooth muscle cells and, as a result, vascular occlusions and ischemic damage. Pathogenesis involves the chronic

secretion of cytokines, the activation of T-lymphocytes, and the production of antibodies that are capable of activating the complement system, which, by classical type, results in chronic damage. Despite the advances in immunosuppressive therapy, this type of rejection persists, and new techniques are necessary to be developed to improve the graft survival.

## Immunological tolerance

The tolerance of the immune system is defined as a specific immunological non-response to antigens. Meanwhile, there is a characteristic non-response to a certain antigen, but keeping the response to any other one. According to a vivid expression by R.V. Petrova, "tolerance is an immunity with a minus sign". The immune system non-response to its native antigens protects the body from autoaggression [10]. When tolerance to alloantigens has been established, the transplanted tissue can be accepted. The tolerance to exogenous antigens entering the body from the outside can be induced both in the neonate period and at the age of puberty. The immune system mechanisms that allow blocking the aggression against the native or donor cells and tissues have been conditionally classified into central and peripheral mechanisms of tolerance induction. The central tolerance is induced in the central organs of immunogenesis: in the thymus and bone marrow, and limits the T- and B-lymphocyte autoreactivity.

The thymus is the major site of T-cell maturation and can be anatomically and functionally separated into two zones: the thymic cortex and medulla. The cortex contains densely packed immature thymocytes and represents the location where the positive selection takes place involving the selection of T lymphocytes that can bind to their own MHC molecules with low avidity. Thymocytes that do not react with their own MHC antigens are

subjected to apoptosis. The medulla contains loosely packed mature lymphocytes and is the site where the process of negative selection takes place. At this stage, the high avidity cells that react with the complex of their own MHC antigens are exposed to apoptosis. As a consequence of positive and negative selection, T cells that leave the thymus and populate peripheral lymphoid tissues are self-MHC restricted and tolerant to many auto-antigens.

Despite the central mechanisms of tolerance are highly efficient in deleting the auto-reactive lymphocyte clones, some of T cells are able to escape this control, to exit the thymus [9, 10] and induce autoimmune responses to inflammation, such as those in infection or trauma. So, there is a constant threat of potential autoimmune reactions due to the escape of auto-reactive T cell clones to the periphery. The control over these potentially dangerous cells is exercised by peripheral tolerance. There are four mechanisms of peripheral (post-thymic) tolerance: 1. T-cell ignorance of antigens. This phenomenon can be observed when the antigens are present in a very low amount that is insufficient for recognition, or when there is a shortage of the T cells that can develop an immune response. 2. T-cell anergy when T cells are made non-responsive to antigens. This may be due to an inadequate expression of the T-cell receptor or co-receptor molecules. 3. Clonal deletion of T cells is a mechanism similar to the processes occurring in the thymus with negative selection of T lymphocytes. 4. Negative activation with the development of apoptosis. An activated T lymphocyte expresses Fas-receptor and Fas-ligand on the membrane. Moreover, Fas-ligand is secreted in a soluble form. Apoptosis develops when the ligand and the receptor come in contact. This mechanism serves to control autoimmune reactions and maintain an optimal pool of lymphoid cells.

It is known that tolerance to the antigens entering the body can be induced both in the neonate and pubertate periods when the immune system unresponsiveness is establishing both at the level of the central organs of immunogenesis and at the peripheral organ level, i.e. the mechanisms of both central and peripheral tolerance are involved. One of the most popular ways of tolerance induction in clinical transplantation is the combined effect of antigen and immunosuppressant (the drug-induced tolerance). This strategy results in a donor-antigen-specific non-responsiveness and demonstrates the clonal nature of immunological tolerance. Currently there are no efficient mechanisms of the peripheral tolerance control that would have contributed to the deletion of activated effector T cells through the anergy induction, clonal depletion, or regulation of the effector T cell activation.

Clinically, tolerance is manifested by the existing well-functioning transplanted organ without histological signs of rejection in the absence of a destructive immune response in the recipient without immunosuppression with a fully preserved immune system [11]. The prevention of graft rejection may be achieved by the continuous use of immunosuppressive drugs that most likely have an effect on the entire immune system. Meanwhile, the target for immunosuppressants in their tolerance induction can be various chains of the immune system responsible for the presentation of the antigen, the development and regulation of the immune response to a foreign antigen.

The cellular therapy as a new therapeutic modality involves potential advantages, such as mastering the natural ability of cells to perform complex biological functions. But since these are living cells, there are certain difficulties in determining their identity, dosage, pharmacokinetics and interactions with other drugs. The knowledge of the cell functional

properties provides the basis for stem cell immune therapies, and adoptive transfusion of donor-specific regulatory T cells cultivated ex vivo, and the use of modified alloantigens [11].

## Antigen presentation

To induce the reactions of both cellular and humoral immunity, the presentation of MHC antigens to T lymphocytes by antigen presenting cells (APCs) is required. To date, there are three key mechanisms [12] that explain how alloantigens activate T cells.

The first mechanism is termed a direct presentation and denotes the recognition of whole unprocessed HLA molecules. This type of presentation is mediated by donor AICs, mostly the dendritic cells (DCs), being present in the allograft as "the passengers" that migrate to the draining lymph nodes and present the alloantigens to the recipient T cells. The activation of recipients cytotoxic T lymphocytes and T helpers by HLA class I and II molecules, respectively, is associated with the development of predominantly cellular rejection. But this mechanism is not permanent, and donor DCs leave the blood flow because of their natural death.

An indirect presentation (indirect pathway) implies the recognition of the processed MHC antigens and is carried out through the recipient AICs. This type of presentation induces the humoral and cellular immune responses determined by Th2 or Th1 T-helpers.

The third mechanism involved in the allograft recognition is termed a partial presentation when the fragments of donor membranes with HLA class I molecules are transferred, among others, to the AIC of the recipient. Partial presentation includes both the molecules of intercellular interactions, and the absorption of MHC small fragments by vesicles.

## Antigen presenting cells, their role in the rejection development and tolerance induction

Dendritic cells. Myeloid DCs are bone-marrow-derived cells and, along with macrophages and B lymphocytes, considered professional antigen-presenting cells playing an important role in generating the peripheral tolerance. DCs are located in the epithelium of the respiratory tract, bowel, and reproductive tract, near blood vessels and nerve endings, in the interstitium of almost all organs. In infection or tissue damage, immature DCs are activated by various pathogen-associated molecular receptors that bring about their maturation [11]. They migrate to the draining lymph nodes where they acquire the ability to activate intact T cells. Under normal physiological conditions of the body, peripheral DCs absorb apoptotic cells and corpuscles, cell fragments carrying their own antigens, and induce the state of tolerance that inhibits inflammatory or immune responses and thereby protects cells and body tissues from possible damage caused by pathogenic autoimmune reactions, and the immune reactions induced by viral or bacterial infection [13, 14].

At solid organ transplantation, DCs can act either as tolerogenic ones involved in the transplant acceptance, or as immunogenic ones playing a key role in the rejection reaction development [15]. This potential is directly related to the DC maturation. The mature DCs expressing high levels of APCs and costimulatory molecules contribute to generating the cell-mediated immunity, while the immature DCs expressing low levels of surface MHC class II and costimulatory molecules induce the development of T-cell tolerance. But, despite the fact that tolerance is mainly induced by immature DCs, even mature DCs can induce antigen-specific unresponsiveness, as well. Tolerogenic DCs have been characterized by low

levels of expression of CD86, CD40, PD-L2, and high levels of expression of PD-L1 and CD80 [16, 17]. Based on these data, a variety of pharmacological agents such as cytokines and growth factors (IL-10, TGF-β, GM-CSF, immunosuppressants (cyclosporine, mycophenolate mofetil, corticosteroids), as well as vitamin D3 and aspirin can be used in vitro to produce tolerogenic DCs [11]. So, in the presence of high doses of IL-10, DCs induce antigen-specific T-lymphocyte anergy, while low doses of GM-CSF lead to the development of immature DCs that induce alloantigen-specific T cell unresponsiveness in vitro and in vivo.

Thus, the researchers can consider DCs as the most attractive option for a targeted therapy aimed at inducing tolerance in organ transplantation.

**Macrophages**

Macrophages are the essential cells of the innate immune system that are the first to encounter the antigens and damaged native cells of the body, and also act as 'professional' antigen-presenting cells. Macrophages originate from the monocytes circulating in blood and, accordingly, they may be considered as cells having the bone-marrow origin. Thanks to having membrane receptors (such as TLR) and intracellular or cytosolic receptors (NOD), the macrophages are able to recognize extracellular and intracellular pathogens, and become activated. This results in the production of proinflammatory cytokines IL-12, TNF-α, IL-8, and chemokines by macrophages. Chemokines promote the migration of natural killers, neutrophils, and naive T lymphocytes (Th0) to the inflammation focus. Then, depending on the macrophage-produced cytokines, the adaptive immune response type (cellular, associated with lymphocytes Th1, or humoral, associated with Th2) is determined [18]. The classical pathway of

macrophage (M1) activation is associated with the production of proinflammatory cytokines IL-12, TNF-α, and IFN-γ, which facilitates the conversion of Th0 lymphocytes to Th1. Resulting from the effect of anti-inflammatory cytokines (IL-10, IL-4, TGF-beta), M2 macrophages potentiate the development of Th0 cells into Th2-cells (an alternative pathway of the activation). The function of macrophages, as a rule, is associated with the development of an inflammatory response and a rejection reaction. Meanwhile, a number of studies have shown the ability of macrophages to exhibit regulatory functions under certain culturing conditions and induce the immunological tolerance. So, B.G. Brem-Exner et al. demonstrated the ability of IFN-γ-activated macrophages cultured together with T cells CD4+ expressing CD40L ligand to enrich the T-lymphocyte population with the regulatory cells of CD4+, CD25+, FoxP3+ phenotypes and to lead to the caspase-dependent depletion of activated T cells. [19]. It means that macrophages in the state of such new activation produce a T-cell suppression effect. All these studies suggest that macrophages may be used as a therapy or immune conditioning for use in organ transplantation in the future.

**Activation of T- and B-lymphocytes**

The model of T-lymphocyte activation proposed by Lafferty and Cunningham remains relevant in the modern understanding of the immune response [20]. It was adapted by Bretscher and Cohn and presented as a two-signal model of the lymphocyte activation. The first signal leads to the T-cell recognition of the antigen on the APC surface: if the T cell is simultaneously receiving a second or "costimulatory" signal from the same APC, then the activation is switched on [21]. According to Lafferty/Cunningham model,

the immune system cells differ in their ability to stimulate T cells. DCs and B-cells are constitutively MHC class II-positive cells that are capable of presenting antigens to CD4+ T cells. At the same time, resting B cells are not capable to initiate a T-cell-mediated immune response [22]. However, when lightly irradiated, the resting B cells that present antigen can activate the CD4+ T-cell clone and even induce costimulatory signals [23]. If B-cells can present antigens to naïve T cells, but can not initiate the development of a cellular immune response, then, according to the two-signal model, they must induce tolerance. Fuchs and Matzinger [24] tested this hypothesis on the female intact mouse model by using a specific male minor antigen of histocompatibility HY, and suggested that the T-cell's choice between the activation and tolerance upon antigen encounter depended on two parameters: the differentiation state of the T cell (naive versus experienced) and the type of he APC. For example, if the antigen was first presented to intact T-cells by a B lymphocyte, then the tolerance to this antigen is induced, i.e. the immune system does not discriminate between self and non-self. The T-cell response in this case would be initiated only in case of tissue damage or pathological cell death.

Most likely, there is no period of "unique immune tolerance" either before or shortly after birth, in contrast to the claims of M.Burnet, P.Medawar, and J.Lederberg [25]. Probably, the tolerance-inducing ability arising in newborn mice after the injection of allogeneic spleen cells may be explained by the presence of mainly naive T lymphocytes in the bloodstream; the cells that, when first encounter the alloantigen on presenting naïve B cells, can not receive a full-blown signal 1 and are not provided with a costimulatory signal. And the DCs, capable of delivering both signals, are either absent during this period, or are critically few. Thus,

even a small number of naive T cells, when first encountering an alloantigen on a presenting but immature B cell, are able to induce tolerance to injected allogeneic spleen cells in newly born mice. This awareness became the first step towards understanding tolerance [26]. In this case, the T-cell response is initiated only if there is a damaged tissue or pathological death of self-cells.

**T-regulatory cells**

One of the main participants in the development of peripheral tolerance to antigens are the so-called T-regular (Treg) cells. The first mention of Treg cells dates back to late 90s of the last century, when a specific population of CD4+ T lymphocytes was described that, in contrast to T helpers, displayed a suppressive activity and inhibited the immunity reactions. In contrast to Th1 and Th2 cells, the Treg lymphocytes secrete IL-4, IL-10, and TGF-beta, but do not produce other cytokines, such as IL-2, IL-5, IL-13, and IFN-gamma. Treg cells are typically characterized by a high expression density on the surface of the CD25 molecule, CTLA-4 cytotoxic lymphocyte antigen, and GITR antigen. The most reliable marker of Treg cells is the transcription factor FoxP3 (the product of the FoxP3 gene). The expression density of this factor is crucial for the realization of the regulatory activity of CD4+ and CD25+ phenotype cells. Competitively binding to DNA, the FoxP3 gene products inhibit the production of pro-inflammatory cytokines and mediate the development of tolerance, thereby protecting the body from autoimmune diseases and chronic infection [27]. Thus, the most important functions of Treg cells are the suppression of autoaggression and the participation in the processes of establishing tolerance. The deficiency or dysfunction of these cells can cause

autoimmune diseases, impede the establishment of transplant tolerance; their excessive function has been noted in tumor diseases.

There are two types of Treg cells: natural and adaptive. Natural CD4+, CD25+, FoxP3+ Treg cells develop in thymus. Their suppressive activity results from a direct contact with the target-cells and is not mediated by cytokines, while the signals from the T-cell receptor play a decisive role in the formation of Treg cells [27]. An important factor for the formation of natural Treg cells includes also the costimulatory molecules CD28 interacting with CD80/CD86. The amount of a Treg cell pool depends on the costimulatory signal. Defects of CD28 and B7 are accompanied by a Treg cell deficiency and, as a result, contribute to the development of autoimmune diseases.

Adaptive Treg cells can develop during the immune response under the effect of antigen stimulus. They originate from the precursors common with T effector cells in conditions of the suboptimal presentation of the antigen and/or inadequate costimulation. Examples of the Treg cell development stimulation include the presentation of antigen by immature DCs, the blockade of CD40/CD40L costimulatory signal, and the presentation of CD4+ antigen to T lymphocytes in the presence of IL-10. The suppressive effect of this cell type is mediated through the cytokines these cells produce [27, 28].

Thus, the most promising current strategies to achieve a long-term graft acceptance in organ transplantation are those aimed at a tolerance induction. There may be distinguished several pathways of therapeutic effect. First of all, this is the preparation of such conditions for the antigen presentation to the T helpers, which would provide a tolerogenic rather than an immunogenic effect. These include, for example, blocking the

costimulatory signal delivery. Another important pathway is the enhancement of the Treg cell generation, or their transplantation.

**Tolerance induction by blocking the costimulatory signal**

As mentioned above, in addition to recognizing a specific antigen by a T cell receptor, the T lymphocyte activation requires a second nonspecific costimulatory signal generated by binding the CD80 and CD86 molecules expressed on APC to the CD28 receptor present on the T lymphocyte membrane. For a selective blockade of this costimulation pathway, a semisynthetic protein (belatacept) was developed [29]. As a result of the CTLA-4 fragment modification, belatacept actively binds to CD80 and CD86 molecules, which allows achieving the level of immunosuppression adequate to inhibit the graft rejection reaction and prevent the graft dysfunction. In vitro studies demonstrated that belatacept inhibits the T lymphocyte proliferation, and also contributes to the decrease in cytokine production. In pre-clinical studies on experimental animal models, the combination of belatacept with mycophenolate mofetil and steroids increased the graft life span compared to placebo, reducing the production of antibodies against the organ donor antigens. The use of this drug as a monotherapy made it possible to achieve a long-term graft function. However, the belatacept discontinuation was associated with the development of an acute renal allograft rejection. Thus, a long-term tolerance induction expected with using a costimulatory signaling blocker was not achieved.

The clinical efficacy and safety of belatacept as part of a combined immunosuppressive therapy compared to calcineurin inhibitors was assessed and summarized in 5 randomized trials [30-32]. P. Masson et al. [33] in their

study found no difference between the compared groups in the incidence of the acute renal allograft rejection and loss in the post-transplant period. At the same time, the patients receiving belatacept had a significantly better renal graft function as assessed by the glomerular filtration rate. Moreover, the recipients who received belatacept were 28% less likely to develop nephrosclerosis than the patients treated with calcineurin inhibitors. Donor-specific antibodies de novo were significantly less frequently formed with belatacept [32]. Other beneficial effects of belatacept included a 39% decrease in the diabetes incidence compared to calcineurin inhibitor therapy, a lower blood pressure in recipients and a better lipid profile, which reduced the risk of cardiovascular diseases in the post-transplant period. No significant differences between the recipients treated with belatacept vs. the calcineurin inhibitors were found in the rates of such a severe adverse drug reaction as post-transplant lymphoproliferative diseases.

### T-regulatory cells and tolerance induction

A new approach to the tolerance induction in autoimmune diseases and organ transplantation has been the development of protocols for cell therapy using Treg cells of CD4+, CD25+, FoxP3+ phenotypes that suppress alloreactive CD4+ and CD8+ lymphocytes and prolong the allograft survival. The efficacy of using Treg cells was demonstrated in the experiment in the treatment of the "graft versus host" reaction, however, the low blood content of these cells limited the use of the method [11]. Further studies demonstrated that alloantigene-specific Treg cells can be obtained from naive T cells by specific culturing, e.g., by adding B lymphocytes to the mixture of immature DCs, or by the exposure to retinoic acid, IL-2, or transforming growth factor beta (TGF-β). The cells obtained in this way

induced by Treg cells, retain the same immunosuppressive properties as the cells of natural origin, and can be used to induce tolerance to bone marrow allografts and solid organs.

The experimental studies on the kidney, liver and bone marrow transplantation models demonstrated the tolerance induction by using the Treg cell transfusion [34-43]. The study by M.Hu et al. [34] demonstrated that in transplantation of the kidney from DBA mice to C57Black mice if a spontaneous tolerance induction occurred, the Treg cell expansion was seen in allograft tissues, and in draining lymph nodes, as well. Elevated levels of TGF-β, IL-10 and IFN-γ were recorded in kidney tissues. The depletion of the Treg cell population resulted in a loss of tolerance.

Despite a rather large number of published reports on the Treg cell efficacy for creating tolerance, the mechanism of suppressing the effector T lymphocytes is not entirely clear. Different mechanisms for suppressing the antigen-specific response have been described; they can include a direct cell-to-cell contact, the secretion of anti-inflammatory cytokines that affect a wide range of cellular activities, the inhibition of T-memory-cell generation. However, it is not clear whether it is enough for Treg cells to migrate to a transplant and stay there in order to suppress the inflammatory process, or they are directly related to more complex mechanisms of an immunological tolerance induction [35-38].

In clinical transplantation, the following approaches to Treg cell therapy can be used: a pharmacologically stimulated generation of regulatory T cells in vivo, the therapy based on using their effector molecules (belatacept), the expansion of the isolated Treg cells ex vivo, and the infusion of the expanded Treg cells to the recipient [35]. In clinical practice, anti-thymocyte globulin has been actively used both for the

immunosuppression induction, and for the treatment of an acute cellular graft rejection. Its effect is based on the destruction of normal T lymphocytes, which leads to an increased proportion of Treg cells. A similar effect is produced by monoclonal antibodies against CD3, CD52 (Alemtuzumab), and rapamycin, which also promote the depletion of normal T cells and the decrease of their content in the bloodstream, which provides a higher "Treg/T effector" cell ratio [36]. Interestingly, the rapamycin therapy prevents the loss of CD25 and FoxP3, while tacrolimus keeps only the CD25 expression, but contributes to the loss of FoxP3 [36, 37].

There are two main trends to be distinguished in the development of therapeutic methods using isolated and expanded ex vivo Treg cells [35]: 1) the infusion of natural Treg cells; and 2) the infusion of Treg cells with in vitro-induced specificity for donor antigens [35, 40]. One of the most important factors of the efficacy and safety of using Treg cells is their survival, which is very difficult to be tracked in humans. The studies have confirmed that most infused Treg cells die quickly, and their effective directional migration and retention in the spleen or target organs such as the lungs, skin, and bowel have yet to be determined [44]. Besides the quantitative reduction of Treg cells, an additional negative consequence includes the loss of their identity. The infused Treg cells gradually lose their CD25 and FoxP3 markers. Rapamycin helps to retain their identity, but can not change the kinetics of the first phase disappearance of the cells. Perhaps, the Treg cell disappearance mechanisms and the instability of Treg cells represent different phenomena. Until now, not all the factors affecting the stability of Treg cells are known.

An important factor is the Treg cell syngeneic preference to the graft, rather than to the recipient. The use of cellular preparations from a living

donor is limited, but deceased donors can become the main source of graft-identical Treg cells. The sources of Treg cells can be the spleen, bone marrow, and peripheral blood. In an experimental study on rats Yute Abe et al. [41] showed that the donor blood transfusion at 1, 2, and 4 weeks before liver transplantation induces the tolerance development and contributes to the long-term allograft survival. And it was noted that tolerance to allografts develops only when the transfused blood is antigen-specific to the organ donor. Investigating the mechanism of long-term tolerance induction, the authors suggested that the donor blood transfusion before liver transplantation was associated with the increase in the level of FoxP3-expressing Treg cells in the recipient.

Thus, alloantigen-specific Treg cells exhibit an immunosuppressive activity and can be used as a specific cell therapy in combination with a reduced immunosuppression regimen. They are able to generate immunological tolerance to bone marrow allografts and solid organ transplants. Donor-derived Treg cells can be used to generate mixed chimerism with an unchanged peripheral recipient's T cell repertoire, which is crucial for an active suppression [45]. The development of new protocols will make it possible to use the Treg cell potential by increasing their number and regulatory functions aimed at the induction of transplantation tolerance.

## Mesenchymal stem cells and their application for the development of tolerance

Mesenchymal stem cells (MSCs) have a high potential for immunomodulatory therapy, which was recently presented as an encouraging way of tolerance induction. MSCs are multipotent progenitor

cells that can rapidly proliferate and differentiate in various different directions, and also serve as tolerance inducers. Currently, they are considered as a potential "homeostatic niche" for Treg cells and their replenishment. MSCs in combination with hematopoietic stem cell transfusion can be used to reduce the immune response to donor antigens in related kidney transplantation [46]. MSCs compete with other cell populations and suppress T cell proliferation.

Fibroblasts also have immunoregulatory properties, and this property is common to all stromal cells. MSCs, being bone marrow derivatives, can migrate to inflammation sites and regulate the function of most immune cells through a direct contact and/or through the cytokine secretion. MSCs of mice suppress a cardiac transplant rejection through the induction of FoxP3+ T cells, decrease the production of alloantibodies in vitro and on the models of diseases; and therefore they can be useful for patients suffering from autoimmune diseases [47, 48]. Most studies have shown that MSCs have a powerful immunomodulatory function, suppressing the proliferation and activation of T-cells and natural killers, modulate the maturation and function of APCs [47].

The therapeutic use of MSCs was studied in the treatment of "graft versus host" reaction and autoimmune diseases, as well as in improving the survival of transplanted hematopoietic stem cells [49-56]. M.J. Crop et al. found that donor MSCs in mixed cultures can significantly suppress the proliferation of recipients' T lymphocytes [49]. The pilot study demonstrated that the infusion of donor bone marrow MSCs in kidney transplantation can provide better graft acceptance results, and also allow a significant immunosuppressant dose reduction [50, 52]. In their study, J. Tan et al. used autologous MSCs in conjunction with a normal or reduced dose of

calcineurin inhibitors. The infusion of autologous MSCs resulted in a significant decrease in the incidence of acute rejection and contributed to a significant reduction in the incidence of opportunistic infections within 1 year in the patients who underwent kidney transplantation compared to the patients receiving a standard immunosuppressive therapy [51]. The mechanism of modulating the immune response by MSCs probably involves the expression of local factors, such as indoleamine 2,3-dioxygenase, inducing the nitric oxide synthesis, and the interaction with APCs or DCs.

MSCs can be obtained from bone marrow, adipose tissue and umbilical cord. MSCs can easily be isolated through their ability to adhere to plastics and then can be expanded in vitro without the loss of their potential for differentiation. In addition, MSCs are the actively secreting cells, produce cytokines and growth factors, thereby regulating hematopoiesis, favoring the survival of transplanted hematopoietic stem cells, and influencing the regeneration [53, 55]. Besides participating in hematopoiesis, MSCs can also exhibit bimodal immune functions, i.e. can have immunosuppressive and immunostimulating effects. The main antiproliferative effect has been found in culturing MSCs with lymphocytes in a mixed culture, even when third-party MSCs were added. The immunomodulatory effect of MSCs can be used to develop new treatment modalities for autoimmune diseases, such as ulcerative colitis, graft versus host disease, and for protecting the graft from rejection.

### Hemotransfusion to prevent rejection

Besides the use of the antibodies blocking the costimulatory signal delivery or directed against T-lymphocytes, a promising trend to the tolerance induction in solid organ transplantation includes, a cellular therapy

with MSCs or regulatory FoxP3-positive T-lymphocytes, which results in shifting their subpopulation balance towards the increase of the Treg cell content. The bone marrow and peripheral blood are the most accessible sources of such cells. The efficacy of blood transfusion for the tolerance induction in liver transplantation in rats is described above. The mechanisms of the immunomodulatory function in donor-specific blood transfusion include the clonal deletion/anergy, the regulatory cell generation, cytokine production, and the microchimerism promotion. The donor blood transfusion induces a decreased intensity of the immune response to donor cells during transplantation, provides the synergy with a costimulatory blockade of B7/CD154 signals and induces tolerance, can help to prevent a chronic allograft rejection and ensure a long-term allograft survival [57]. Stem cell transplantation and the donor-specific blood transfusion are useful for minimizing immunosuppression in transplantation. Stem cells have additional advantages in regulating the immune response to the allogeneic graft compared to donor blood, and provide a more stable generation of Treg cells [58]. The tolerance induction in donor blood transfusion develops through the above described mechanisms, and also through a mediated antigen presentation through phagocytosis of apoptotic donor cells [58].

Our experience of using the cellular blood components obtained while harvesting solid organs from a donor has been successfully applied in liver transplantation. The donor cellular blood component (CBC) was obtained at organ retrieval from heart-beating organ donors. That technique excluded negative impurities typical for the cadaveric blood proposed **for use** by Academician S.S. Yudin [59]. The obtained preparation contained erythrocytes, platelets, and various populations of T-lymphocytes, hematopoietic stem cells, and a small number of MSCs [60, 61] (Patent for

Invention No. 2452519). Our studies have shown that organ donor CBC infusion has an immunosuppressive effect and is accompanied by a 14.5% decrease in the CD3+ lymphocyte count and a 1.5-time decrease in CD8+ lymphocyte count compared to the control group. The most pronounced differences were revealed in studying the expression of activation markers. Among the recipients receiving the organ donor CBC (n=157), no cases of acute rejection crisis were recorded in the early postoperative period, while in the comparison group its incidence was 3%. Thus, the inclusion of organ donor CDC in the complex and transfusion therapy of liver transplant recipients reduced sensitization, produced an immunomodulatory effect, and reduced the risk of acute cellular rejection in the early postoperative period.

## Conclusion

The investigations of the immunological tolerance development, and the creation of efficient techniques to control the immune response, can completely prevent the graft rejection reaction. Today, the mechanisms of tolerance induction realized via blocking the costimulatory signal, or **by using** the effects of Treg cells and MSCs have been sufficiently studied. Research in this area is a hopeful trend in the transplantation development. The proposed method of the tolerance induction in solid organ transplantation using the transfusion of blood cellular components obtained from a postmortem heart-beating donor is a promising approach that requires further study.

## References

1. Raychaudhuri S.P., Kundu-Raychaudhuri S., Tamura K., et al. FR255734, a humanized, Fc-Silent, Anti-CD28 antibody, improves psoriasis

in the SCID mouse-psoriasis xenograft model. J Invest Dermatol. 2008;128:1969–1976. PMID:18337836 DOI:10.1038/jid.2008.38

2. Le Blanc K. Immunomodulatory effects of fetal and adult mesenchymal stem cells. Cytotherapy. 2003;5(6):485–489. PMID:14660044 DOI:10.1080/14653240310003611

3. Owen R.D. Immunogenetic consequences of vascular anastomoses between bovine twins. Science. 1945;102:400–401. PMID:17755278 DOI:10.1126/science.102.2651.400

4. Demikhov V.P. A new and simpler variant of heart-lung preparation of a warm-blooded animal. Bull Eksp Biol Med. 1950;(7):21–27.

5. Hašek M., Puza A. On the induction of immunological tolerance in adult recipients. Folia Biol (Praha). 1962;8:55–57. PMID:13905162

6. Marsh S.G., Albert E.D., Bodmer W.F., et al. Nomenclature for factors of the HLA system, 2010. Tissue Antigens. 2010;75(4):291–455. PMID:20356336 DOI:10.1111/j.1399-0039.2010.01466.x

7. Medawar P.B. A second study of the behaviour and fate of skin homografts in rabbits A Report to the War Wounds Committee of the Medical Research Council. J Anat. 1945;79(Pt 4):157–176.4. PMID:17104981

8. Khubutiya M.Sh., ed. Transplantation of organs and tissues in a multidisciplinary research center. Moscow: AirArt Publ., 2011. 424 p. (In Russian).

9. Ruiz P., Maldonado P., Hidalgo Y., et al. Transplant Tolerance: New Insights and Strategies for Long-Term Allograft Acceptance. Clin Dev Immunol. 2013;2013:210506. PMID:23762087 DOI:10.1155/2013/210506

10. Man'ko V.M., Devrishov D.A. Veterinary immunology. Fundamental fundamentals. Moscow: Agrovet Publ., 2011. 752 p. (In Russian).

11. Oluwole S.F., Oluwole O.O., Adeyeri A.O., DePaz H.A. New strategies in immune tolerance induction. Cell Biochem Biophys. 2004;40(3 Suppl):27–48. PMID:15289641

12. Nijagal A., Derderian C., Le T., et al. Direct and indirect antigen presentation lead to deletion of donor-specific T cells after in utero hematopoietic cell transplantation in mice. Blood. 2013;121(22):4595–4602. PMID:23610372 DOI:10.1182/blood-2012-10-463174

13. Voll R.E., Herrmann M., Roth E.A., et al. Immunosuppressive effects of apoptotic cells. Nature. 1997;390(6658):350–351. PMID:9389474 DOI:10.1038/37022

14. Williams C.A., Harry R.A., McLeod J.D. Apoptotic cells induce dendritic cell-mediated suppression via interferon-γ-induced IDO. Immunology. 2008;124(1):89–101. PMID:18067553 DOI:10.1111/j.1365-2567.2007.02743.x

15. Ezzelarab M., Thomson A.W. Tolerogenic dendritic cells and their role in transplantation. Semin Immunol. 2011;23(4):252–263. PMID:21741270 DOI:10.1016/j.smim.2011.06.007

16. Hu J., Wan Y. Tolerogenic dendritic cells and their potential applications. Immunology. 2011;132(3):307–314. PMID:21208205 DOI:10.1111/j.1365-2567.2010.03396.x

17. Lewis K.L., Reizis B. Dendritic Cells: Arbiters of Immunity and Immunological Tolerance. Cold Spring Harb Perspect Biol. 2012;4(8):a007401. PMCID:PMC3405856 DOI:10.1101/cshperspect.a007401

18. Monastyrskaya E.A., Lyamina S.V., Malyshev I.Yu. Ml and M2 phenotypes of activated macrophages and their role in immune response and pathology. Pathogenesis. 2008;6(4):31–39. (In Russian).

19. Sutterwala F.S., Noel G.J., Salgame P., Mosser D.M. Reversal of proinflammatory responses by ligating the macrophage Fcγ receptor type I. J Exp Med. 1998;188(1):217–222. PMID:9653099

20. Lafferty K.J., Prowse S.J., Simeonovic C.J., Warren H.S. Immunobiology of tissue transplantation: a return to the passenger leukocyte concept. Annu Rev Immunol. 1983;1:143–173. PMID:6443557 DOI:10.1146/annurev.iy.01.040183.001043

21. Bretscher P., Cohn M. A theory of self-nonself discrimination: paralysis and induction involve the recognition of one and two determinants on an antigen, respectively. Science. 1970;169:1042–1049. PMID:4194660

22. Lassila O., Vainio O., Matzinger P. Can B cells turn on virgin T cells? Nature. 1988;334:253–255. PMID:2969460 DOI:10.1038/334253a0

23. Ashwell J.D., Jenkins M.K., Schwartz R.H. Effect of gamma radiation on res-ting B lymphocytes. II. Functional cha-racterization of the antigen-presentation defect. J Immunol. 1988;141(8):2536–2254. PMID:2844902

24. Fuchs E.J., Matzinger P. B cells turn off virgin but not memory T cells. Science. 1992;258:1156–1159. PMID:1439825

25. Billingham R.E., Brent L., Medawar PB. Actively acquired tolerance of fo-reign cells. Nature. 1953;172: 603–606. PMID:13099277

26. Ranheim E.A., Kipps T.J. Activated T cells induce expression of B7/BB1 on normal or leukemic B cells through a CD40-dependent signal. J Exp Med. 1993;177(4):925–935. PMID:7681471

27. Freydlin I.S. Regulatory T–cells: origin and function. Medical Immunology (Russia). 2005;7(4):347-354. DOI:10.15789/1563-0625-2005-4-347-354 (In Russian).

28. Marino J., Paster J., Benichou G. Allorecognition by T Lymphocytes and Allograft Rejection. Front Immunol. 2016;7:582. PMID:28018349 DOI:10.3389/fimmu.2016.00582. eCollection 2016

29. Vincenti F., Dritselis A., Kirkpatrick P. Belatacept. Nat Rev Drug Discov. 2011;10(9):655–656. PMID:21878974 DOI:10.1038/nrd3536

30. Cohen J.B., Eddinger K.C., Forde K.A., et al. Belatacept Compared to Tacrolimus for Kidney Transplantation: A Propensity Score Matched Cohort Study. Transplantation. 2016; [Epub ahead of print] PMID:27941427 DOI:10.1097/TP.0000000000001589

31. Goring S.M., Levy A.R., Ghement I., et al. A network meta-analysis of the efficacy of belatacept, cyclosporine and tacrolimus for immunosuppression the-rapy in adult renal transplant recipients. Curr Med Res Opin. 2014;30(8):1473-1487. PMID:24628478 DOI:10.1185/03007995.2014.898140

32. Durrbach A., Pestana J.M., Florman S., et al. Long-Term outcomes in Belatacept- Versus Cyclosporine-Treated recipients of extended criteria donor kidneys: final results from BENEFIT-EXT, a Phase III Randomized Study. Am J Transplant. 2016;16(11):3192–3201. PMID:27130868 DOI:10.1111/ajt.13830

33. Masson P., Henderson L., Chapman J.R., et al. Belatacept for kidney transplant recipients. Cochrane Database Syst Rev. 2014;(11):CD010699. PMID:25416857 DOI:10.1002/14651858.CD010699.pub2

34. Hu M., Wang C., Zhang G.Y., et al. Infiltrating Foxp3(+) regulatory T cells from spontaneously tolerant kidney allografts demonstrate donor-specific tolerance. Am J Transplant. 2013;13(11):2819–2830. PMID:24102948 DOI:10.1111/ajt.12445

35. Shalev I., Selzner N., Shyu W., et al. Role of regulatory T cells in the promotion of transplant tolerance. Liver Transpl. 2012;18(7):761–770. PMID:22523007 DOI:10.1002/lt.23458

36. Juvet S.C., Whatcott A.G., Bushell A.R., Wood K.J. Harnessing regulatory T cells for clinical use in transplantation: the end of the beginning. Am J Transplant. 2014;14(4):750-763. PMID:24592900 DOI:10.1111/ajt.12647

37. Singh K., Stempora L., Donald Harvey R., et al. Superiority of rapamycin over tacrolimus in preserving nonhuman primate Treg half-life and phenotype after adoptive transfer. Am J Transplant. 2014;14(12):2691–2703. PMID:25359003 DOI:10.1111/ajt.12934

38. Fisher S.A., Lamikanra A., Dorée C. Increased regulatory T cell graft content is associated with improved outcome in haematopoietic stem cell transplantation: a systematic review. Br J Haematol. 2017;176(3):448–463. PMID:28094847 DOI:10.1111/bjh.14433

39. Alessandrini A., Turka L.A. FOXP3-Positive Regulatory T Cells and Kidney Allograft Tolerance. Am J Kidney Dis. 2017;69(5):667–674. PMID:28049555 DOI:10.1053/j.ajkd.2016.10.027

40. Noyan F., Zimmermann K., Hardtke-Wolenski M. Prevention of allograft rejection by use of regulatory T cells with a MHC-specific chimeric antigen receptor. Am J Transplant. 2017;17(4):917–930. PMID:27997080 DOI:10.1111/ajt.14175

41. Abe Y., Urakami H., Ostanin D., et al. Induction of Foxp3-Expressing Regulatory T-Cells by Donor Blood Transfusion Is Required for Tolerance to Rat Liver Allografts. PLoS One. 2009;4(11):e7840. PMID:19956764 DOI:10.1371/journal.pone.0007840

42. Dummer C.D., Carpio V.N., Gonçalves L.F., et al. FOXP3+ regulatory T cells: from suppression of rejection to induction of renal allograft tolerance. Transpl Immunol. 2012;26(1):1–10. DOI: 10.1016/j.trim.2011.08.009

43. Zhang L.F., Xia C.Q. Ex vivo expansion of regulatory T cells for clinical applications against graft-versus-host disease in allogeneic hematopoietic stem cell transplantation. Chin Med J (Engl). 2013;126(23):4575–4582. PMID:24286428

44. Tang Q. Pharmacokinetics of Therapeutic Tregs. Am J Transpl. 2014;14(12): 2679–2680. PMID:25358900 DOI:10.1111/ajt.12933

45. Pilat N., Klaus C., Gattringer M., et al. Therapeutic efficacy of polyclonal Tregs does not require rapamycin in a low-dose irradiation bone marrow transplantation model. Transplantation. 2011;92(3):280–288. PMID:21697774 DOI:10.1097/TP.0b013e3182241133

46. Vanikar A.V., Trivedi H.L., Feroze A., et al. Effect of co-transplantation of mesenchymal stem cells and hematopoietic stem cells as compared to hematopoietic stem cell transplantation alone in renal transplantation to achieve donor hyporesponsiveness. Int Urol Nephrol. 2011;43(1):225–232. PMID:20084457 DOI:10.1007/s11255-009-9659-1

47. Miyairi S., Hirai T., Ishii R., et al. Donor bone marrow cells are essential for iNKT cell-mediated Foxp3+ Treg cell expansion in a murine model of transplantation tolerance. Eur J Immunol. 2017;47(4):734–742. PMID:28127757 DOI: 10.1002/eji.201646670

48. Hsu W.T., Lin C.H., Chiang B.L., et al. Prostaglandin E2 potentiates mesenchymal stem cell-induced IL-10+IFN-γ+CD4+ regulatory T cells to control transplant arteriosclerosis. J Immunol. 2013;190(5):2372–2380. PMID:23359497 DOI:10.4049/jimmunol.1202996

49. Crop M.J., Baan C.C., Korevaar S.S., et al. Donor-derived mesenchymal stem cells suppress alloreactivity of kidney transplant patients. Transplantation. 2009;87(6):896–906. PMID:19300194 DOI:10.1097/TP.0b013e31819b3d72

50. Peng Y., Ke M., Xu L., et al. Donor-derived mesenchymal stem cells combined with low-dose tacrolimus prevent acute rejection after renal transplantation: A Clinical Pilot Study. Transplantation. 2013;95(1):161–168. PMID:23263506 DOI:10.1097/TP.0b013e3182754c53

51. Tan J., Wu W., Xu X., et al. Induction therapy with autologous mesenchymal stem cells in living-related kidney transplants: a randomized con-trolled trial. JAMA. 2012;307(11):1169–1177. PMID:22436957 DOI:10.1001/jama.2012.316

52. Popp F.C., Renner P., Eggenhofer E., et al. Dahlke mesenchymal stem cells as immunomodulators after liver transplantation. Liver Transpl. 2009;15;1192–1198. PMID:19790154 DOI:10.1002/lt.21862

53. Xu G., Zhang L., Ren G., et al. Immunosuppressive properties of cloned bone marrow mesenchymal stem cells. Cell Res. 2007;17:240–248. PMID:17325691 DOI:10.1038/cr.2007.4

54. Aggarwal S., Pittenger M.F. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood. 2005;105(4):1815-1822. PMID:15494428 DOI:10.1182/blood-2004-04-1559

55. Ryan J.M., Barry F., Murphy J.M., Mahon B.P. Interferongamma does not break, but promotes the immunosuppressive capacity of adult

human mesenchymal stem cells. Clin Exp Immunol. 2007;149(2):353–363. PMID:17521318 DOI:10.1111/j.1365-2249.2007.03422.x

56. Popp F.C., Eggenhofer E., Renner P., et al. Mesenchymal stem cells can induce longterm acceptance of solid organ allografts in synergy with low-dose mycophenolate. Transpl Immunol. 2008;20(1-2):55–60. PMID:18762258 DOI:10.1016/j.trim.2008.08.004

57. Kishimoto K., Yuan X., Auchincloss H.Jr., et al. Mechanism of action of donor-specific transfusion in indu-cing tolerance: role of donor MHC molecules, donor co-stimulatory mole-cules, and indirect antigen presentation. J Am Soc Nephrol. 2004;15:2423–2428. PMID:15339991 DOI:10.1097/01.ASN.0000137883.20961.2D

58. Dave S.D., Vanikar A., Trivedi H.L., et al. Stem cells versus donor specific transfusions for tolerance induction in living donor renal transplantation: a single-center experience. Transplantation. 2013;95(1):155-160. PMID:23263505 DOI:10.1097/TP.0b013e3182752bcc

59. Yudin S.S. Questions of military surgery and transfusion of posthumous blood. Moscow: Medgiz Publ., 1960. 309–393. (In Russian).

60. Nikulina V.P., Gulyaev V.A, Godkov M.A., Kobzeva E.N. Immunological aspects of the use of the erythrocyte mass of an organ donor for liver transplantation. In: Khubutiya M.Sh., ed. Topical issues of organ transplantation: materials of the city scientific-practical conference. Moscow: Triada Publ., 2008. 93–95. (In Russian).

61. Khubutiya M., Zhuravel S., Gulyaev V., et al. Using of Blood from Cadaveric Donor in Orthotopic liver Transplantation. Bull Georg Nat Acad Sci. 2014;8(3):108–115.