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Tolerance in Organ Transplantation

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Abstract

Transplantation is often the best option to treat organ end stage failure. Transplanted patients need to take long-term immunosuppressive drugs to inhibit rejection and maintain their graft. But those therapies have numerous important side effects such as cancer induction and opportunistic infections. Thus, the development of novel therapies to induce specific rather than general immunosuppression and therefore, tipping the balance between effector and regulatory functions to inhibit transplant rejection is a major goal in the field. One major approach is the blockade of costimulatory signals to abort effector T-cell activation following TCR engagement and to promote regulatory T cells. Here we summarized the research to date that details immune mechanisms involved in tolerance in organ transplantation and strategies toward tolerance.

Keywords: Transplantation, Tolerance, Costimulation, Tregs, Immunointervention

1. Introduction

The primary role of the immune system is to protect against foreign antigens without reacting against self-antigens. In this objective, some mechanisms of immune tolerance have been set up. Immune tolerance is defined in general by a total absence of specific reaction against antigens, and particularly self-antigens and a break in immune tolerance can lead to autoimmune disorders. Understanding the mechanisms of immunological tolerance is crucial for the development of strategies to manipulate the immune system in the context of organ transplantation. Here, we provide a comprehensive summary of the immune mechanisms involved in transplant rejection and tolerance induction and the research to date leading to new strategies toward tolerance, as well as their translation to the clinics.

2. Tolerance in organ transplantation

In the context of transplantation, the graft is recognized as foreign antigens and immune responses are triggered. Immunosuppressive treatments can repress total immune responses, including responses against the graft, but these drugs are well known for inducing side effects such as cardiovascular diseases or increased opportunistic infections and malignancies, and lead to high morbidity and mortality, even when avoiding excessive immunosuppression [1]. In addition, current immunosuppressive regimens have marginal effects on long-term allograft survival with for example a half-life of 10 years for kidney transplantation. In fact, these drugs can even be deleterious in the establishment of tolerance. Some cases of tolerance spontaneously acquired by patients who stopped their immunosuppressive treatments because of side effects and noncompliance have been reported [2, 3]. Tolerance in transplantation is defined by the following criteria: the graft must be definitely accepted without any lesions of chronic rejection, and the recipient should not be on treatment at the moment of analysis and should be able to develop immune responses against any other foreign antigen (i.e. immunocompetent), and thus represents the center of immunologist efforts working in the field of transplantation. The most commonly transplanted solid organ is the kidney, but the field of solid organ transplantation also includes the heart, liver, pancreas, lung or intestine. Nowadays, transplantation still remains the best solution for organ failure, even in the face of graft rejection. There are three types of solid graft rejection:

- The quickest mechanism of rejection, said hyperacute rejection, takes place between minutes to hours after transplantation. It is mediated by the presence of pre-existent circulating antibodies against A, B molecules expressed by red blood cells [4, 5] and acquired antibodies against the human leukocyte antigen (HLA) [6, 7]. Those antibodies allow the immune system of the grafted patients to strongly recognize the molecules present at the surface of graft endothelium and lead to the destruction of this endothelium through complement cascade, neutrophils, and monocytes. Nowadays, hyperacute rejection is no longer a concern due to the establishment of pre-transplantation tests as cross-match test, where closest HLA compatibility between donor and recipient is ensured (concerning mainly HLA-A, B, and DR) and blood group typing.
- The months following transplantation, acute rejection can occur. Two mechanisms can be involved in this process. The cell-mediated rejection is induced by presentation of alloreactive molecules by APCs (Antigen Presenting Cells) to recipient's T lymphocytes, leading such activated lymphocytes to infiltrate and destroy the grafted organ [8]. The humoral mechanism acts through generation of alloantibodies directed mainly against MHC (major histocompatibility complex) class I by activated alloreactive B lymphocytes leading to antibody-dependent cellular cytotoxicity of endothelial cells (ADCC) [9]. Prevention of acute rejection is now ensured in more than 85% of cases by the current repertoire of immunosuppressive drugs available [10].
- Even though, the main problem in transplantation which remains unsolved is the long-term allograft dysfunction or chronic rejection. This phenomenon is slow and progressive but irreversible and cannot be controlled by immunosuppressive drugs. It is characterized by

an increase of the thickness of the intima's layer of graft vessels, leading to the ischemia of the tissue and finally to graft loss. Chronic rejection is mediated by the antigen-specific cellular and humoral immune responses against the graft. These immune responses lead to the recruitment of inflammatory mediators to the graft through the activation of the endothelium, and the secretion of free radicals and damage signals which activate muscle vessels proliferation.

Thus, T cells play an important role in graft rejection. In solid organ transplantation, naive T cells from the recipient are activated by recognition of blood group (ABO), donor major histocompatibility complex (MHC), minor histocompatibility complex (mHC, MICA, MICB, H-Y), or nonpolymorphic peptides (collagen, angiotensin II receptor) presented by professional APCs from the recipient or the donor or directly from endothelial cells of the graft (mostly MHC class I) [11]. Thus, T cells can be primed by three distinct pathways.

The direct allorecognition: when the transplanted organ is reperfused, intact MHC/peptide (MHCp) at the surface of APCs from the donor travel to the lymphoid organs of the recipients where they interact with CD4⁺ and CD8⁺ alloreactive naive T cells and active them [8, 12]. This pathway is generally associated with acute rejection because donor's APCs persist a few months following transplantation. In addition, intact MHCp at the surface of endothelial cells of the graft can activate and maintain allogeneic CD8⁺ effector T-cell responses [13].

The indirect allorecognition: this pathway involves the presentation of allogeneic peptides derived from donor MHC molecules and presented by APCs of the recipient. Dominant peptides presented by this pathway are generally derived from hypervariable regions. This pathway involves capture, processing, and presentation of alloantigens and is predominantly used by CD4⁺ T cells and most of the alloantigens are presented by MHC class II. However, cross-presentation on MHC class I can occur and CD8⁺ T cells can also be activated [14–16]. The indirect allopresentation pathway is also necessary for B cells activation, since their activation depends on CD4⁺ T cells help [17]. The indirect allorecognition is involved in both acute and chronic rejection since the alloantigens are present during all the life of the allograft [18–21].

The semi-direct allorecognition: Lechler's team reported that APCs from the recipient can capture entire intact MHC/peptide complexes expressed at the surface of the donor's APCs. Those complexes are internalized, processed, and presented directly to CD8⁺ T cells or indirectly to CD4⁺ T cells. This presentation pathway involves the phenomenon of trogocytosis, i.e. the exchange of membrane fragments between cells in contact [22–24]. Brown and al have reported *in vivo*, in a model of transplantation in mice, the presence of intact donor MHC-I and MHC-II on the surface of DCs, B cells, and macrophages [25].

2.1. The immune tolerance

To date, two elaborated and complementary mechanisms of immune tolerance have been described as central tolerance and peripheral tolerance (see **Figure 1**).

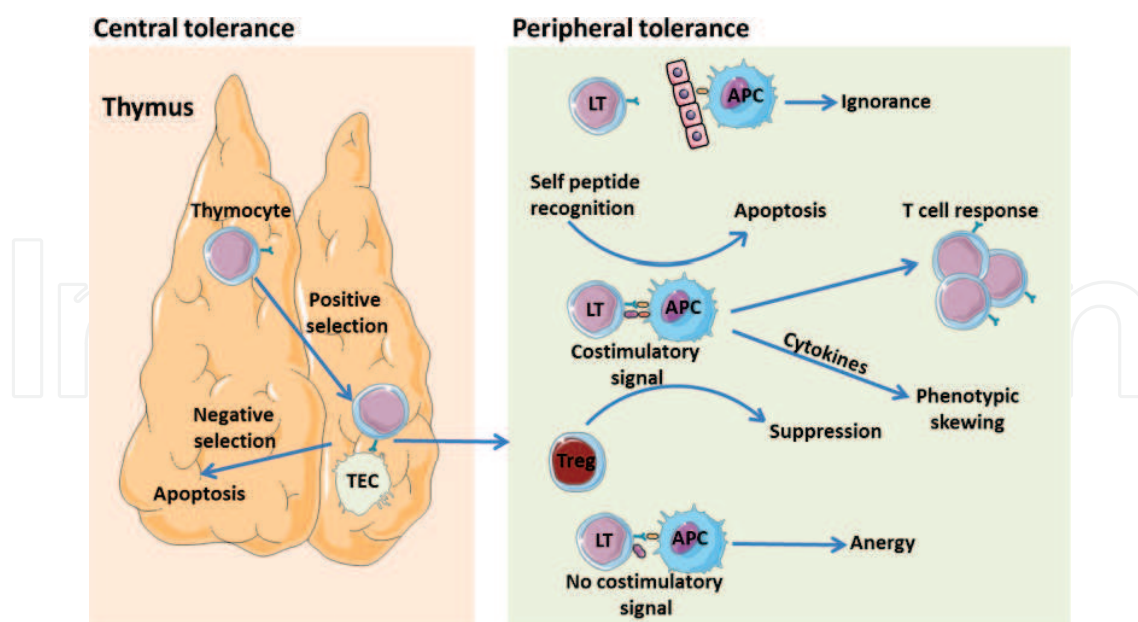


Figure 1. Central and peripheral tolerance. In the thymus, tight interactions between TCRs and self-antigens presented by thymic epithelial cells (TEC) induce thymocytes apoptosis. Four mechanisms induce peripheral tolerance: ignorance, apoptosis, phenotypic skewing and anergy. Appropriate combination of signals results in complete T-cell activation.

2.1.1. Central tolerance

In the context of immune tolerance, the T lymphocyte lineage is particularly important. T lymphocytes, B lymphocytes, and NK cells derive from a common hematopoietic precursor from foetal liver during embryogenesis or from adult bone marrow. The $CD3^+CD4^-CD8^-$ TCR $^-$ thymocytes colonize the thymus and undergo different stages of maturation leading to TCR rearrangement. During their migration to the cortex, cells also acquire expression of CD3, CD4, and CD8 molecules and undergo a step of positive selection. All $CD4^+CD8^+$ cells said double-positive (DP) thymocytes express a complete $\alpha\beta$ TCR but only 20–25% of them are able to interact with the MHC [26, 27]. Cells that strongly interact with MHC class I become $CD8^+$ simple positive (SP) and the one which interact with MHC class II becomes $CD4^+$ SP [28–30]. This interaction provides a survival signal to thymocytes that can pursue their education by migrating into the thymic medulla. In this medulla, SP thymocytes undergo the second step of immune central tolerance called negative selection. After positive selection, the TCR repertoire is very large and uncontrolled; thymocytes are able to recognize a broad range of foreign antigens but also self-antigens. The maturation of thymocytes expressing a TCR against self-antigens can lead to autoimmune disease in the periphery. Negative selection consists in the inhibition of potentially autoreactive thymocytes by clonal deletion or by induction of anergy if receptor editing fail. This selection is mediated by medullary thymic epithelial cells (mTECs) that are the only antigens presenting cells (APCs) expressing a panel of ectopic tissue-specific antigens (TSA). The expression of those TSA is mainly under control of a transcription regulator named Auto-Immune REgulator (AIRE) [31]. Two mechanisms of TSA presentations can occur in the medulla. The first one is the direct presentation by mTEC that can be sufficient

to induce negative selection of both CD4⁺ and CD8⁺ T cells [32, 33]. The second mechanism of presentation is mediated by thymic dendritic cells which are able to get TSAs from mTECs and to present them to thymocytes [34]. Thymocytes able to strongly recognize a TSA presented by mTECs or DCs receive a signal that leads to apoptosis [35]. After negative selection, approximately 5% of total thymocytes can finally go to the periphery.

2.1.2. *Peripheral tolerance*

Due to the absence of self-antigen presentation in the thymus [36], or the low affinity of T cells for self-antigens [37], autoreactive T cells escape sometimes from thymic negative selection. To complete the efficacy of central tolerance, the immune system developed many tools to neutralize these cells and avoid autoimmune diseases. These mechanisms are either passive, concerning antigen ignorance, T-cell anergy or apoptosis induction and phenotypic skewing, or active when mediated by regulatory cells [38].

Antigen ignorance allows autoreactive T cells to persist as functional circulating T cells while never primed by any antigen [39]. Indeed, antigens can be masked by anatomical barriers like lens proteins, spermatozooids, or nervous system protein protected by the meninges barrier. Besides, a low amount of antigens can be sufficient to activate cytotoxic T lymphocytes previously primed but not naive T lymphocytes [40].

On the contrary, a high amount of antigen can induce T-cell apoptosis. This mechanism called activation-induced cell death is induced by Fas signaling pathway [41]. Indeed, Fas expression deficiency results in autoimmunity [42]. Autoreactive T-cell peripheral deletion can also result from a lack of costimulatory signal or of growth factor. [43–45]. Likewise, the absence of costimulatory molecules induces a Fas-mediated apoptosis of autoreactive B cells [46], but anyway the autoreactive B cells escaping the clonal deletion are unlikely to meet the T cells specific to the same antigen they need to be completely primed [47, 48].

Autoreactive T cells can also be primed but functionally inactivated. This state of anergy is characterized by incapability to proliferate and to produce IL-2 following antigen stimulation [49, 50]. Antigens are required to maintain this inactive state [51], and large amounts of IL-2 or anti-OX40 antibodies can abrogate it [52]. Anergy results from either a lack of costimulatory signal by APCs, a low affinity of TCR for the antigen, or from CTLA4/B7 interaction. Indeed, interactions between CD28 expressed on T-cell surface and CD80-CD86 on antigen-presenting cells are essential for activation and proliferation of alloreactive T cells [53], allow generation of memory T cells and inhibit regulatory T (Tregs) suppressive activity [54, 55]. Without CD28/CD80-CD86 engagement, interactions between TCRs and alloantigens induce the anergy of T cells. CTLA-4 (CD152) has a large structural homology with CD28 and interacts with CD80-CD86 with better affinity than CD28 molecule and functions as a negative regulator of T-cell activation [56]. The expression of these two molecules regulates the balance between activation and inhibition of T cells and allows the control of an over-reaction of the immune system leading to inflammation or autoimmunity [57]. Similarly, the expression of Programmed Death-1 (PD-1) after antigenic stimulation and interaction with its ligand PDL-1 reduces IL-2 synthesis and induces T-cell anergy [58]. Another important co-stimulatory pathway is the CD40/CD40L co-stimulatory pathway. The CD40 molecule is a transmembrane protein that

belongs to the TNF receptor family. It is expressed on vascular endothelial cells [59], activated DCs [60], monocytes/macrophages, platelets [61] and B lymphocytes [62]. The CD40L, also called CD154, exists in soluble form or at the cell membrane [63]. It is expressed on activated CD4⁺ T cells, basophiles, eosinophils [64], DCs from the blood [65], endothelial cells, macrophages [66], and B lymphocytes [67]. The CD40-CD40L interaction is critical for T-cell-dependent effector functions [68]. Indeed, CD40/CD40L interaction acts with IL-12 to induce production of IFN- γ by human T lymphocytes stimulated by anti-CD3/anti-CD28, as well as IL-2 production by Th1 and IL-4, IL-5 and IL-10 by Th2 lymphocytes [69]. Besides, interaction with CD40 activates the expression of adhesion molecules by T lymphocytes [70]. Many other co-stimulation pathways are well described such as RANK/RANKL or ICOS/ICOSL. These costimulatory pathways are crucial for T-cell activation inducing rejection, so inhibition of one or more of these pathways may inhibit rejection. Moreover, anergized T cells can inhibit DCs function [71], become IL-10 producing Tregs [72], and induce T-cell apoptosis [73]. Autoreactive B cells also undergo anergy induced by chronic stimulation of BCR(B cell receptor) by antigens [74].

Activated autoreactive T cells can persist in a nonpathogenic state. Indeed, Th2 cytokines expression is linked to lower autoimmunity [75, 76] and tolerance in transplantation [77–79], sometimes through Tregs induction [80]. By contrast, Th1 and Th17 are linked to allograft rejection [81]. Therefore, immunosuppressive treatments, like glucocorticoides or sirolimus, aim to inhibit Th1 responses and promote Th2 responses [82, 83].

Peripheral tolerance is also maintained by regulatory cells. Immune cells can acquire a regulatory function during development, such as “natural” Tregs in the thymus, or in periphery under the influence of the microenvironment, such as “induced” Tregs in the allograft or draining lymph nodes. Almost all type of cells have a regulatory counterpart, including T cells, B cells, myeloid cells (MDSCs, M2 macrophages), DCs. These regulatory cells limit effector cell responses to pathogens and self-antigens, acting by contact or by secretion of suppressive cytokines. In transplantation, regulatory cells are targets for therapeutic strategies to control innate immune responses triggered by ischemia/reperfusion of the graft, and adaptive responses triggered by the allograft.

3. Induction of tolerance

3.1. Animal models of tolerance

The immune system complexity is due to a large number of possible interactions and activation pathways. So, *in vitro* experiments provide primary results but they need to be confirmed by *in vivo* studies to overview all the potential effects on the organism. There are lots of rodent models of allograft transplantation and numerous strategies have been used successfully to induce tolerance in these models.

Kidney transplantation is often used between MHC mismatched rodents [84, 85]. General physical condition is observed during all the experiments. Serum creatinine levels and urine

quality are measured after transplantation to evaluate kidney activity. Generally, this graft is realized in different steps. First, ablation of one recipient kidney and allograft of MHC mismatched kidney. The transplanted organ is not immediately efficient. So, ablation of the second recipient's kidney is realized few days after transplantation. At this time, the recipient has only one transplanted kidney. If the kidney is rejected, the rodent dies because of blood toxicity.

Cardiac allograft in rodent is also a good model [86–90]. In this case, it is a heterotopic graft. Recipients keep its heart and receive a MHC-mismatched heart in the abdomen. The heart is connected to recipient blood circulation. Heart allograft survival is evaluated by palpation through the abdominal wall and scoring of its beating. Just after transplantation, heart beats strongly. But, if there is activation of the recipient immune system against alloantigens, the heart tissue is stiffened and beating are less intense and frequent until their full arrest. The major advantage of this model is that the recipient survives even if rejection occurred, and thus mechanisms can be analyzed.

But, sometimes, results obtained in rodent models could not be reproduced in larger animal models, such as non-human primates (NHPs) or swine. Rodents are too different from human to serve as preclinical models. Indeed, rodents have 90% similarity with human genome while there are around 99% similarity between NHP and human. Thus, NHPs constitute a more relevant animal transplant model. But, using NHPs is considerably more expensive and restrictive than rodents. Indeed, NHPs need more time for reproduction and development and more place than rodents. Moreover, all protocols conducted on animals have to be approved by an ethical comity. NHPs are ethically largely more controlled than rodents. So, first experiments are generally conducted on rodent models to generate primary results and conclusion. Then, a model with immune system more reflective of the human immune system is essential for testing protocols before moving into clinical studies.

This was the case for anti-CD40L treatment. All results obtained in rodents have demonstrated a great potential for induction of transplant tolerance. However, similar experiments in primate and human have highlighted a major barrier. Indeed, CD40L is a molecule expressed on NHPs, and human platelets. So this treatment resulted in thrombosis in NHPs and could not be used in the clinic [91, 92]. New anti-CD40L antibodies are being engineered as alternatives given the potential of this strategy in multiple diseases [93–95].

An alternative possibility that has been developed in the last decade is the engineering of mouse with humanized immune system in which various types of human cells are engrafted and functional [96, 97]. These mice harbor a complete null mutation of the IL-2 receptor gamma chain, NOD/SCID IL-2r γ^{null} (NSG), or Rag 2^{-/-} γ^{null} and are characterized by an impaired development and function of murine T, B and NK cells. These mice can efficiently support the development of a functional human hemato-lymphopoiesis. There are different protocols, more and more efficient, to induce humanization in highly deficient mice, such as injection of CD34⁺ cells or PBMCs. Mice humanized with PBMCs represent the fastest model of graft versus host disease (GVHD), due to direct injection of adult PBMCs. Indeed, these models allow the analysis of human immunology *in vivo* [98, 99]. The mice are monitored for weight loss and tissue damages.

Another important model is skin transplantation [100–102]. It is possible to graft tailskin on the recipient lateral flank in rodent models. After removal of the bandage, grafts are observed to analyse their evolution. It's considered as rejected when skin dries and falls, indeed no viable skin remains. This strategy is even more relevant in humanized mice model [103]. In this case, mice are grafted with human skin obtained from abdominal surgery from patients. In this kind of model, skins are left to engraft at least 15 days before rejection is triggered by injection of allogeneic PBMCs.

3.2. Co-stimulation blockade

The aim of transplantation research is to exploit mechanisms of self-tolerance to generate specific tolerance after transplantation. One of the most promising approaches is to inhibit co-stimulatory pathways to abort activation of T cells following TCR engagement. T cells are an essential component of the immune response against allogeneic cells inducing allograft rejection. Interaction between TCR and MHC/antigen induces the first signal of stimulation. But this alloantigen recognition only is not sufficient for complete T cell activation. Co-stimulatory signals generated by the interaction between antigen and T cells and cytokine stimulation are also necessary for complete immune system activation [104]. There are 4 different co-stimulation molecules families: immunoglobulins superfamily, TNF receptors, integrin family, and T-cell immunoglobulin and mucin-containing domain (TIM) family.

Blockade of costimulatory pathways has been considered as a good strategy for the prevention of allograft rejection in transplantation by aborting activation following TCR engagement [52, 105]. These strategies have been studied in mice, rat, and NHPs and induces allograft tolerance [106, 107]. Blockade of both CD28/B7 and CD40/CD40L co-stimulatory pathways induces long-term allograft survival. In the '90s, the first CTLA-4Ig soluble molecule was generated [108]. This molecule corresponds to the fusion between CTLA-4 extracellular domain and a modified IgG1 constant fragment domain [109]. CTLA-4Ig interaction with CD80-CD86 inhibits T-cell activation and prevents rejection of cardiac and renal allografts in rodent models [87, 108, 110] and prolongs survival of human islets xenograft in mice [111]. But CTLA-4Ig is not sufficient to induce tolerance because all allograft were finally rejected. Besides, when this treatment is combined with donor-specific transfusion (DST), allograft tolerance is obtained in heart allograft rodent models [105, 112]. DST corresponds to injection of donor splenocytes during transplantation and induces a state of chimerism. CD28 blockade show interesting results in rodent models, but this strategy is not efficient enough in NHP models [113]. Furthermore, it has been described that CTLA-4 is important for Tregs functions [114] and also for induction of tolerance to allograft [115, 116]. Thus, it would be more benefic to target CD28 molecule on T cell than CD80/CD86. Different groups have demonstrated that specific targeting of CD28 prevented rejection and generated Tregs [115, 117, 118]. FR104 has been developed in our laboratory as a costimulatory inhibitor that target CD28 without altering CD80/CD86 [119]. There are important differences between murine and human CD28 in terms of expression and interactions [120]. Thus, FR104 has been studied in NOD/SCID mice and NHP models. FR104 is safe *in vitro* and *in vivo* on human cells and does not play agonistic

function on T cells [99]. This point is really important because another anti-CD28 molecule (TGN1412) studied in a phase 1 clinical trial induced important cytokine storm that caused life threatening effects [121].

CD40-CD40L blockade has also been studied for induction of transplantation tolerance. There are different strategies to block this pathway (monoclonal antibodies, CD40Ig). Treatment with monoclonal antibody anti-CD40L induces skin, renal, and cardiac allograft survival in mice [86, 122] and in NHP models [123, 124] but only with long-term repeated injections [123]. Some groups have shown that these monoclonal antibodies deplete activated T cells through cytotoxicity [125] and apoptosis [126], and induce Tregs [102]. These blockades of the CD40-CD40L pathway induce prolongation of allograft survival but without real allograft tolerance. Effect of anti-CD40L treatment has been improved by association with DST in islet graft in mice [127]. Different studies have demonstrated the importance of treatment associated with DST, such as anti-CD4, anti-thymocytes, or anti-CD40L [128–130]. Another strategy uses CD40Ig molecule as treatment in allograft models. Our team has demonstrated that treatment with CD40Ig molecule or gene transfer induces allograft acceptance in cardiac allograft rat models [131] mediated by CD8⁺CD45RC^{low} Tregs [132]. Several approaches use combined protocols. Indeed, anti-CD40L and CTLA-4Ig treatment synergies in heart and skin allograft in rodents [86, 101] and in renal allograft in NHPs [113].

ICOS-ICOSL pathway is another costimulatory pathway. ICOS presents around 40% similarity with CD28 and CTLA-4. ICOSL is constitutively expressed by B lymphocytes and monocytes and induced after T-cell activation [133]. Mice knocked out for ICOS have shown weak humoral responses [134–136]. ICOS-ICOSL blockade alone does not really induce significant effect on graft outcomes [137]. Therefore, our team have demonstrated that blockade of both ICOS-ICOSL and CD40-CD40L induce long-term heart allograft survival in rat model and decreases chronic rejection lesions [138]. During acute rejection, RANK and RANKL molecules are increased. Moreover, RANKL blockade induce long-term heart allograft survival in both rats and mice [139], and blockade effect is stronger when associated with CD40 pathway. PD1 is expressed by activated T lymphocytes, B and NK cells and macrophages, while PDL1 and PDL2 are expressed by activated APCs. PD-L1Ig alone does not improve allograft survival but synergizes with cyclosporine A, rapamycin, and anti-CD40L [140]. This pathway seems to play a role in the generation of some Tregs [141, 142].

3.3. Regulatory cells, crucial players in tolerance

In the context of organ transplantation, the findings of the last decades have put lights on regulatory cells as key players in the induction and maintenance of tolerance in organ transplantation. Although it has become evident that several distinct subsets of regulatory cells have the capacity to finely and tightly regulate the anti-donor immune responses in organ transplantation, one subset has attracted most of the research, the CD4⁺CD25^{high}CD127^{low}Tregs, thanks to the identification of a crucial master gene necessary for their development, identity, and function, the Foxp3 (Forkhead box P3) transcription factor [143–146].

3.3.1. Regulatory T cells (Tregs)

Tregs suppression of immune responses has been unravelled in the '70s [147]. Several subsets of Tregs have been evidenced including CD4⁺CD25^{high}CD127^{low} Tregs, but also CD8⁺ Tregs, among them the CD8⁺CD45RC^{low} and CD8⁺CD28⁻ Tregs are the most known, CD4⁻CD8⁻ Tregs, NKT cells and $\gamma\delta$ T cells. Tregs can further be subdivided in characteristics (phenotype, repertoire...) and potential in organ transplantation depending of their emergence from the thymus (nTregs) or from the periphery (iTregs). While nTregs developed as a distinct lineage in the thymus, iTregs can be generated from naive cells in the periphery and can be *in vitro* or *in vivo* induced in transplantation under some conditions such as donor-specific blood transfusion, blockade of the CD40/CD40L pathway, or donor MHC-derived peptides [148–150].

Since the identification of the CD25 and Foxp3 markers, several other markers have been proposed to better define Tregs, such as for CD4⁺ Tregs glucocorticoid-induced tumor necrosis factor receptor family-related protein (GITR), CTLA-4, CD62L, CD103 (alpha beta integrin), LAG-3 and CD127 (alpha chain of the IL-7 receptor) [151–155]. For CD8⁺ Tregs, the identification of relevant markers has been more difficult and several markers have been proposed such as CD122, CD28, CD45RC, CD103, and PD-1 [156–160]. A major discovery for Tregs biology was the identification of the Foxp3 and its role in CD4⁺ Tregs development, identity and function [161, 162]. Mutations of the Foxp3 gene lead to a lymphoproliferative pathology in mice and an immune dysregulation polyendocrinopathy enteropathy X linked (IPEX) syndrome in human [163]. To date, this gene remains the best marker to identify CD4⁺ Tregs, although in human it has been demonstrated that it can be transitory upregulated in T lymphocytes upon activation without providing regulatory capacity [164]. The Helios and neuropilin-1 (Nrp-1) markers have also been proposed to distinguish nTregs from iTregs, and also more recently as an important marker for CD8⁺ Tregs identity and function [165–167]. Indeed, the Cantor group demonstrated that Helios was a key transcription factor that stabilizes CD8⁺ Tregs in the context of an inflammatory response and that Helios-deficient Tregs developed an unstable phenotype (reduced Foxp3 and increased effector cytokines expression) during inflammatory responses. Antigen specificity has also been proposed to play an important role to distinguish Tregs origins [168]. Tregs originating from the thymus were selected for their specificity toward self-antigens, and thus are susceptible to be continuously stimulated by the self-antigens in periphery, potentially explaining their high expression of activation markers such as CD25, while iTregs generated by environmental conditions (inflammation, tolerance. . .) have a higher affinity toward exogenous antigens and thus are less stable once the antigens have been eliminated. In the context of organ transplantation, the antigens remain and we have demonstrated that CD8⁺CD45RC^{low} iTregs were maintained and stable for a long time [132, 150].

Tregs use different mechanisms to suppress anti-donor immune responses; they can mediate their activity through cell contact, cytokines secretion, or metabolic disturbance. Suppression through cell contact is mediated by the CTLA-4 and LAG-3 molecules expressed by Tregs. Interaction of CTLA-4 with B7 modulates CD80-86 expression by APCs and tryptophan catabolism in DCs, thus inhibiting T-cell activation [169]. Tregs can also induce effector cells

apoptosis by cytolysis mediated by cell contact and secretion of cytotoxic molecules such as granzyme B and perforin [170]. Suppression through metabolic disturbance consists in the modification of the biochemical and cytokine environment, leading to target cell death. Adenosine triphosphate (ATP), cyclic-adenosine-monophosphate (cAMP), or IL-2 deprivation has been shown has strong inhibitor of cell proliferation [171, 172]. Finally, immunosuppressive cytokines play a major role in Treg-mediated suppression. IL-10, TGF- β , IL-34, IL-35, FGL-2, and IFN γ are all involved in CD4⁺ and/or CD8⁺ Tregs function [89, 159, 173, 174]. We have demonstrated that IFN γ production by CD8⁺CD45RC^{low} Tregs in a model of cardiac allograft tolerance in rat resulted in indoleamine 2,3-dioxygenase (IDO) expression by DCs and endothelial cells of the graft [159]. We have shown that CD8⁺CD45RC^{low} iTregs also produced high levels of FGL-2 [89] and IL-34 in rat and we have demonstrated that IL-34 is a cytokine that is specifically expressed by human Foxp3⁺ CD4⁺ and CD8⁺ Tregs [173].

3.3.2. Other subsets of regulatory cells

Clinical and experimental observations have highlighted the role and potential of non-T cells with regulatory properties, defined as regulatory B cells (Bregs), tolerogenic dendritic cells (Tol-DCs), or regulatory macrophages (Mregs) or myeloid-derived suppressor cells (MDSCs) (nonexhaustive list) [89, 175–179].

The role of Bregs has been particularly investigated in allograft models of tolerance by our laboratory and in tolerant transplanted patients. In these patients, high levels of B lymphocytes and B markers have been observed, while these patients were tolerant and displayed an absence of donor-specific antibodies (DSAs) [3, 180, 181]. The phenotypic profile of Bregs remains unclear, in contrast to Tregs, although a few markers have been identified including CD1d, CD21, CD24, and IgM and it has been demonstrated that they mostly display an immature phenotype [182, 183]. A common feature of Bregs is their ability to secrete IL-10 and IL-35, two cytokines playing a major role in their activity and initially demonstrated in autoimmune diseases [184]. Other mechanisms of action resulting in Bregs activation and suppression involve their BCR engagement, cooperation with T lymphocytes, signaling via CD40/CD40L, TLR activation, IFN γ from tolerogenic DCs, or granzyme B secretion [185–189]. The engagement of their function results in inhibition of effector CD4⁺ T-cell proliferation, Th1 differentiation, APCs function, and monocytes activation. In addition, Bregs have been shown as able to induce Tregs and NKT cells [177, 190]. In the context of transplantation, the role of Bregs has been proven in a model of cardiac allograft tolerance in mice treated with anti-CD45RB [191]. In our laboratory, we have demonstrated that IgM⁺IgG⁻ B cells with a regulatory activity accumulated in the cardiac allograft of tolerant rat recipients and can adoptively transfer allograft tolerance to newly grafted recipients [183, 189]. We have shown that those Bregs had a partial defect in CD40 signaling and overexpressed granzyme B. In a similar model of cardiac allograft, we have demonstrated that administration of an adenovirus encoding fibroleukin-2 (FGL-2), a cytokine associated with Treg function, can induce tolerance to the allograft, thought generation of Bregs capable of infectious tolerance [89]. Finally, we have also demonstrated in a model of CD8⁺ Treg-mediated CD40Ig-induced allograft tolerance, deple-

tion of CD8⁺ Tregs resulted in maintenance of long-term survival induction through generation of Bregs and RegMCs [176].

Although DCs are mostly known to be immunogenic; they also have the capacity to be tolerogenic. They play a major role in central and peripheral tolerance since they are involved in clonal deletion of autoreactive T lymphocytes in the thymus and correlate with an increased risk of autoimmune diseases and decreased presence of CD4⁺ Tregs when depleted in periphery [192]. They are defined by their expression of tolerogenic molecules, such as IL-10, indoleamine 2,3-dioxygenase (IDO), TGF- β or heme-oxygenase 1 (HO-1), their low expression of immunostimulatory molecules, such as MHC molecules or CD80 and CD86, and in general their ability to generate Tregs and to inhibit effector T-cell responses. Their tolerogenic properties have been linked with their maturation, exposure to immunosuppressive, or anti-inflammatory treatments and their environment [193]. In the last year, a number of protocols have been developed to generate Tol-DCs as therapeutic tools to induce a specific tolerance to antigens in autoimmune diseases and transplantation, and our laboratory is involved in the first clinical trial in kidney transplanted patients using Tol-DCs in the context of a European "ONE STUDY" funding involving several centers [194, 195].

Initially, the focus was on the potential of immature conventional DCs (cDCs) expressing low levels of MHC and costimulatory molecules and their potential to induce transplant tolerance to a cardiac graft in mice [196, 197]. Our laboratory has demonstrated that autologous DC combination with suboptimal dose of immunosuppressive drugs induces long-term allograft survival in rat [198]. We have shown that they require TMEM176B, an intracellular protein identified in tolerant recipients, to cross-present donor antigens and induce Tregs and prolong allograft survival [199]. We have also shown that the molecule HO-1 can inhibit DC maturation, while preserving their production of IL-10, and thus leading to inhibition of pro-inflammatory and allogeneic immune responses [200].

Although their tolerogenic properties in transplantation are less well defined, the role of plasmacytoid DCs (pDCs) has been demonstrated for the regulation and the maintenance of bone marrow and organ transplantation [201]. As for cDCs, they can be immunogenic or tolerogenic according to their receptor engagement and maturation status. We have demonstrated their preferential interaction with CD8⁺ Tregs in a model of cardiac allotransplantation in rat treated with CD40Ig resulting in the superior suppressive potential of CD8⁺CD45RC^{low} Tregs [159, 202]. A group has shown their involvement in a model of cardiac allograft tolerance in mice treated with DST and anti-CD40L. In this model, pDCs induced tolerance through generation of alloantigen-specific CD4⁺CD25⁺Foxp3⁺ Tregs [203] and depletion of pDCs inhibited Treg development and tolerance induction. In human, pDCs stimulated through certain of their TLR (toll like receptor) or costimulatory molecules efficiently induced CD4⁺ and CD8⁺ Tregs inhibiting *in vitro* allogeneic T-cell stimulation [204–206]. Liver-transplanted patients tolerating their allograft displayed significantly more pDCs and CD4⁺ Tregs [207].

Finally, Tregs can generate tolerogenic DCs, by modifying molecules expression such as inducing tolerogenic molecules expression like IDO, ILT3 or ILT4, changing their function [208]. We have demonstrated in several models of allograft tolerance that DCs were essential for establishment and maintenance of allograft tolerance [202, 209–211].

In transplantation, although macrophages activation is often associated with allograft destruction and rejection in the early phases, the existence of an alternative population of Mregs contributing to tissue reparation, activated by Th2-type cytokine such as IL-4 and IL-13 and inhibiting pro-inflammatory cytokines secreted by macrophages [212]. Mregs can be induced by Treg interactions, Tregs depletions, and can even induce in turn Tregs via IL-10 secretion for example [213, 214]. The reduction of macrophages in mice receiving hematopoietic stem cells aggravated the GVHD, whereas expansion of macrophages with CSF-1 resulted in the opposite effect [215]. Mregs isolated from peripheral blood are characterized by their morphology, specific markers, although unstable, and their capacity to inhibit T-cell proliferation *in vitro* [216]. A clinical pilot study is administering donor-derived Mregs to kidney-transplanted patients, allowing graft survival under minimal immunosuppression one year following administration without clinical signs of graft rejection [217]. We and others have shown the potential of M-CSF and IL-34, two cytokines involved in monocytes survival and differentiation to induce Mregs *in vitro* and *in vivo* in human, mice, and rat transplantation models, and that those Mregs induced in turn Tregs capable of tolerance induction [173, 218].

MDSCs are a heterogeneous population of immature hematopoietic progenitor cells presenting numerous suppressive functions, including alloantigen tolerance induction in cardiac and islet allograft model in mice and kidney allograft in rat [219, 220].

3.4. Tipping the balance between effector and regulatory functions

Allograft outcome depends on the balance between effectors, which attack alloantigenic tissues, and regulators, which are essential for regulation/inhibition of alloresponses and induction of tolerance [221]. Induction of tolerance might be induced by the diminution of alloreactive T cells to allow Tregs to suppress the immune system activation. A large number of protocols have been studied with more or less efficacy.

Several approaches have been based on the expression amount of CD45 molecule on T cells allowing to distinguish effector and Tregs [222]. Strategies using monoclonal antibodies have been tested in animal models, notably in mice models of transplantation targeting CD45RB, as CD4⁺CD45RB^{high} T cells from untreated mice have been shown as capable of inducing colitis, diabetes, and thyroiditis [223]. In all cases where CD4⁺CD45RB^{high} T cells have been shown to cause autoimmunity, their counterpart CD4⁺CD45RB^{low} T cells have been shown to prevent the induction of the disease. Anti-CD45RB antibody (MB23G2) caused transitory decrease of circulating lymphocytes expressing CD45RB and induced allograft survival with normal kidney allograft function [85]. This antibody induces also upregulation of CTLA-4 on lymphocytes [224]. This treatment has been studied in kidney, pancreatic islets, and heart allograft models. Lazarovits et al. have studied two different CD45RB mAb (MB23G2 and MB4B4). They have shown that the nondepleting MB4B4 is therapeutically ineffective while MB23G2 depletes CD45RB^{high} lymphocytes and induces renal and islets allograft tolerance in mice model [85, 225].

Tipping the balance to favor regulatory functions is an interesting alternative to effector cells depletion. In our laboratory, we have developed and demonstrated the efficacy of different therapeutic strategies to modify the balance in favor of regulatory functions, such as CD40Ig,

anti-CD28 (FR104), HO-1, FGL-2, or IL-34, for example [55, 89, 131, 173, 226]. Our team studied CD40Ig treatment in a cardiac allograft rat model. We have demonstrated that this treatment induces long-term survival by generation of CD8⁺CD45RC^{low} [131]. CD45RC has been shown in rats, mice, and humans to be a marker of both CD4⁺ and CD8⁺ Tregs [98, 102–106]. Moreover, this cell population is able to transfer infectious tolerance to naive transplanted rats [159]. Anti-CD28 is a good candidate to prevent rejection in the clinic [115, 231]. Zhang and al have shown that anti-CD28 inhibits lymphocytes activation and increases the proportion of cells expressing Foxp3 in the allograft. Our team and others have proved that treatment with anti-CD28 acts through the increase of the proportion of Tregs [117, 118, 178]. Actually, FR104 is the most known molecule targeting CD28. This humanized molecule has no agonist activity on human T cells *in vitro* and it does not induce cytokine storm in NOD/SCID mice reconstituted with human PBMCs. Moreover, Poirier and al have demonstrated the potential of FR104 in GVHD humanized mice model. They have also administrated FR104 to NHPs. Their results indicated a good tolerance in NHP and excluded cytokines release [99]. Several nondepleting antibodies, such as anti-CD4 and anti-CD8 mAb, are efficient to induce tolerance [232] and have permitted the first proof of the possibility of infectious tolerance [233]. Moreover, different strategies should be combined to improve their effects. Indeed, anti-CD45RB mAb and anti-CD40L mAb synergized and improved long-term allograft survival in islets and skin allografts [234].

All these examples confirm the importance of the balance between effector and regulatory cells for graft outcome.

4. From the bench to the clinic

Regulatory cells are essential for tolerance in transplantation. Many animal models have highlighted the potential of such tools for preventing allograft rejection and GVHD development. Their human counterparts are a promising issue for following immune status and inducing tolerance in transplanted patients.

4.1. New biomarkers for a real-time adapted treatment

A biomarker is “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” [235]. Nowadays, the reliability of the prognosis to predict allograft survival/rejection is low. For pancreas and liver transplantation, the measurement of lipase/amylase and liver enzymes respectively is recommended as routine post transplantation monitoring. The monitoring of cardiac enzymes is not recommended because of the poor sensitivity of these markers in the diagnosis of acute rejection, thus patients have to suffer endomyocardial biopsies 15 times during the first year after transplantation [236]. Echocardiography, diastolic function analysis, and quantitative measurement of changes of the transverse relaxation time T2 in the myocardium by magnetic resonance imaging are considered to improve cardiac allograft rejection prediction [237]. For renal allograft, the prognosis is generally based on creatinemia evolution and glomerular filtration. This prediction can be improved by calculat-

ing the kidney transplant failure score (KTFS) considering not only the creatinemia at 3 and 12 months post graft, but also the proteinuria at 12 months, the number of previous transplantations, the age and sex of the donor, the creatinemia of the donor at the harvesting time, and the incidence of an acute graft rejection in first year post transplantation [238].

Noncompliant patients spontaneously developing an operational tolerance to their graft are useful to identify new biomarkers and adapt in real time the care of patients. By comparing phenotypes of cells and cytokines from tolerant patients with healthy volunteers and patients rejecting their graft, we should be able to identify markers correlating with the immune status toward the graft. However, studies are limited to blood and urine analysis because of the ethical question of performing a biopsy of a tolerated graft. Kidney-grafted spontaneous operational tolerant patients are defined as having ceased all immunosuppressive drugs for more than one year, with no increase in serum creatinine during the last 12 months (CRT < 10%). Using microarray analysis, Brouard, Newell, and Sagoo compared genes differentially expressed in tolerant recipients with patients exhibiting chronic rejection [181, 239, 240]. Tolerant patients showed a reduction of activation markers of proinflammatory T cells, a down-regulation of pro-inflammatory cytokines [239], a GATA3 upregulation suggesting a Th2 deviation [241], and an increase in CD4⁺CD45RA⁻Foxp3^{hi} memory Tregs versus patients with chronic rejection [3, 242, 243]. Interestingly, the three distinct studies and cohorts converge also with an increase of B cells in blood and CD20 transcript in urine in tolerant patients [180, 181, 240]. However, the phenotype of B cells named Bregs they described diverges [180, 181, 240, 244]. In liver-grafted spontaneous operational tolerant patients, higher numbers of CD4⁺CD25⁺CD127⁻ T cells, Vδ1⁺ T cells, and NK cells were detected [245, 246].

The heterogeneity of treatments, in terms of dose and type of immunosuppressors administered to patients during chronic rejection episodes, and the heterogeneity of the parameters selected to monitor regulatory cells activity in the recent trials, prevents comparison of the results. To define general tolerance signatures, consortiums as The ONE Study and EU COST Action "BM1305: action to focus and accelerate cell-based tolerance-inducing therapies," standardized immune monitoring of patients included in clinical trial [247]. Six panels of 7–9 markers designed are now standardized within 8 international laboratories to monitor T cells, B cells, and DCs [247].

Newly described cytokines associated with regulatory cells should be considered as prognostic markers. Recently, IL-34 has been closely associated with Tregs and M2 macrophages [173]. FGL2, produced by Tregs and generating Bregs, could also reflecting immune status of the graft [248]. Furthermore, nucleic acid analysis suggests new biomarkers for allograft rejection, such as donor-derived cell-free DNA (ddcfDNA) [249], OX40 [250], OX40L [250], PD-1 [250], Foxp3 [250] mRNA levels in urinary cells, and A20 [251], HO-1 [251], granzyme B [251], perforin [251], and Tim-3 mRNA [252] in both urinary cells and PBLs.

4.2. From patients observation to action

Many immunosuppressive drugs were developed these last decades. By targeting many cell types at different levels/pathways, their association largely contains the immune responses against the allograft. Briefly, glucocorticoids and calcineurin inhibitors (ciclosporin, tacroli-

mus) inhibit cytokine transcription in T lymphocytes and macrophages; mTOR inhibitors (rapamycin, everolimus [253]) inhibit expression of costimulatory molecules on APCs and T lymphocytes proliferation; cytostatics (cyclophosphamide, methotrexate, azathioprine) and purines inhibitors (mycophenolate mofetil) inhibit T and B cell divisions; antibodies targeting thymocytes (ATG, thymoglobulin), CD3 or CD52 (Campath-1H) deplete T lymphocytes, CD25 (basiliximab, daclizumab) inhibit T lymphocytes activation, and CD20 (rituximab) and CD52 deplete B lymphocytes; sphingosine 1 phosphate inhibitor FTY720 (Fingolimod) retain lymphocytes in lymphoid organs and decrease their circulation in blood; efalizumab inhibits LFA-1 functions. However, drawbacks and side effects they induce are still unresolved.

The identification of new immunoregulatory mediators and the recent findings regarding regulatory cells over-represented in tolerant patients whereas lacking in graft-rejecting ones suggest new therapeutic strategies to control the immune balance. Several cytokines and antibodies have shown promising results in animal models. It has recently been shown that the cytokine IL-34 is able to induce Tregs through conversion of regulatory macrophages [173]. Similarly, FGL2 can induce Bregs [248]. In addition, antibodies blocking costimulatory pathway like CD28 antagonist (FR104) or antibodies targeting TCR-associated signaling (CD45RC [254]) or DR3 [255] marker seem efficient to decrease T-cell function whereas promoting Tregs [55, 99, 256]. The promotion of regulatory cells is likely to induce lower drawback than classical broad-spectrum drugs.

Immunoregulatory cell therapy may be able to support peripheral tolerance and aims to induce a donor-specific unresponsiveness. This personalized method consists in harvesting blood cells from a patient, isolating and expanding *ex vivo* regulatory cells before re-infusing them to the patient in order to control allogeneic response against the graft in solid organ transplantation, or to control allogeneic response against the recipient infused with hematopoietic stem cells (HSCT) to avoid a GVHD reactions.

The first clinical trial was realized in 2009 by Trzonkowski et al, using 10^5 Tregs/kg expanded $CD4^+CD25^+CD127^-$ Tregs to reduce chronic GVHD symptoms [257]. In 2011, Brunstein began a phase I clinical trial and to date showed that until 3×10^6 expanded Tregs would be safe and efficient to reduce the incidence of grade II–IV aGVHD [258]. The same year, Di anni showed that freshly purified $CD4^+CD25^+$ Tregs counteracts the GVHD potential of a high number of donor Tcons in HLA-haploidentical HSCT [259]. In 2014, Martelli confirmed in a phase II clinical trial that co-infusion of freshly purified $CD4^+CD25^+$ Tregs significantly reduces GVHD incidence without affecting GVL (graft versus leukemia) effect [260]. In 2014, Bacchetta infused 10^5 donor T lymphocytes pretreated with IL-10/kg into recipient of HSCT and showed the protective effect of TR1 cells against GVHD [261]. Whereas these first clinical studies focused on Tregs in HSCT, several alternative regulatory cell types have been identified as potential sources for immunotherapies in solid organ transplantation. The EU-funded international ONE study consortium considers several immunoregulatory cell-based therapies for clinical management of solid organ transplant recipients and shares a common clinical protocol design. The ONE Study project titled “A Unified Approach to Evaluating Cellular Immunotherapy in Solid Organ Transplantation” aims to compare the feasibility and the potential of cell therapy by using MDSCs, Mregs, DC-10, Tol-DCs [262], rapa-DCs, monocytes conditioned with

mesenchymal stem cells [263], Tr1 [264], and CD4⁺ Tregs [265]. CD8⁺ Tregs are now approaching clinical tests [266–268] and Bregs are also considered as a tool for cell therapy [269].

Success of cell therapy to control allogeneic immune responses against the donor depends on regulatory properties of cells and on the number of regulatory cells infused into the recipient. Based on animal models and preclinical models of humanized mice, about 7 to 11 × 10⁸ Tregs/kg would be necessary to control allogeneic response [270]. However, we have to consider the *in vivo* proliferation of Tregs after infusion [271]. Thus, the maximal infusible dose approved by The ONE Study for safety is 10⁷ Tregs/kg [265]. Nowadays, Tregs from patients can be 100 to 1000 fold short-term expanded *ex vivo* while keeping their suppressive properties [272–274].

Importantly, clinical protocols also consider specificity of therapeutic cells against the graft donor. Indeed, antigen-specific Tregs have been shown more efficient in inhibiting anti-donor immune response [275]. The frequency of direct alloreactive Tregs has been estimated to 1–10% of total Tregs [276]. Injecting more donor-specific cells would amount to inject fewer cells, and to reduce nonspecific unwanted drawbacks. For Tang and Bluestone, the effect induced by 5 × 10⁹ polyclonal CD4⁺ Tregs would be equivalent to the effect induced by 1.5 × 10⁸ to 1 × 10⁹ allogeneic Tregs [277]. That is why processes have been developed to expand Tregs specifically with APCs or antigen derived from the HLA donor. Indeed, CD4⁺ Tregs expanded with donor DCs or B cells, or by indirect presentation of donor cell lysate antigen onto recipient APCs [276], showed a higher suppressive activity compared to polyclonally expanded Tregs [275, 278–280]. Similar protocols were used to generate donor-specific CD8⁺ Tregs [266]. Whereas TCR repertoire of donor APCs-expanded Tregs is still diverse, Tregs relatively efficiently reduce alloreactive T cell response without compromising general immunity according to mice models [266, 279]. Based on their capacity of infectious tolerance, Ag-specific Tregs can exert dominant tolerance to alloantigen *in vivo* by inducing regulatory properties in alloreactive T cells [281]. The identification of a unique shared peptide is of crucial interest today. Tregs with a unique antigen specificity can also be isolated and then expanded for cell therapy [282], or selected during the expansion by indirect presentation of one peptide [150, 267, 283]. Genetic engineering used to confer a TCR specificity to lymphocytes to redirect T cells in cancer immunotherapy [284, 285] offers new possibilities to obtain Tregs with an artificial specificity toward the graft donor Ag.

As cells will be re-infuse into the patient, all reagents used to culture cells have to be validated for “clinical grade,” and after expansion, cells are analyzed for purity and stability. Tregs are phenotypically characterized for expression of helios [167], DNA methylation status of the TSDR (Treg specific demethylated region) [286], while Foxp3 expression is no more sufficient [287], and tested in *in vitro* suppression assay.

Cell therapy strategy would allow reducing the use of conventional immunosuppression in organ transplant recipients; nevertheless, clinical trials are rarely totally free of drugs for patients' safety. This could affect regulatory cells survival and functions. Indeed, tacrolimus, mycophenolate mofetil (MMF), and methylprednisolone do not affect phenotype, function, or stability of Tregs, but reduce their proliferative capacity, whereas rapamycin did not [288–290]. Moreover, rapamycin is sometimes used to maintain regulatory properties of Tregs during expansion culture and also to convert conventional CD4⁺ T cells into Tregs *ex vivo* [291].

By targeting upstream IL-2 synthesis, cyclosporine A compromises the homeostatic behaviour of CD4⁺ Tregs in peripheral immune compartments [292]. On the contrary, FTY720 synergizes with rapamycin for the conversion of CD4⁺ Tregs [293]. Thus, the choice of drugs combined with cell therapy has to be considered.

5. Conclusion

Research in transplantation has made considerable progresses improving transplantation outcomes, but important obstacles remain. Induction of tolerance is considered as the key to reduce the impact of toxic side effects of general immunosuppressive drugs. Better definition of immune tolerance mechanisms in human should lead to a better understanding of the potential effects of targeting strategies.

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