

Transplantation Tolerance: From Bench to Bedside

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Abstract

During the past twenty years, solid organ transplantation has become the therapy of choice for many end-stage organ diseases. While short-term graft and patient outcome has greatly improved with the development of new immunosuppressive protocols, long-term results remain relatively disappointing, mainly because of the side effects associated with chronic drug exposure. Thus, the induction of donor-specific immune tolerance remains an important goal in transplantation. Here, we first describe the pathways underlying allograft rejection and the potential targets for immune intervention. We then review the strategies that could lead to the induction of transplantation tolerance based on experimental models and discuss the protocols that are currently evaluated in clinical transplantation. (Trends in Transplant. 2009;1:3-12)

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Introduction

Within the past twenty years, the successes achieved in clinical solid organ transplantation have been tightly linked to the use of powerful immunosuppressive drugs that can control the rejection process. Based on extensive data obtained in rodents and large-animal experimental transplantation models, newer immunosuppressive strategies have progressively been developed and transposed to routine clinical practice^{1,2}. Short-term outcomes such as patient and allograft survival, as well as rates of acute allograft rejection episodes in the first year after transplantation, have

steadily improved. However, the full potential of organ transplantation has not yet been realized because of the unwanted side effects and increased patient morbidity and mortality associated with chronic drug exposure³⁻⁵. Besides the complications related to nonspecific suppression of the immune response, there is an inexorable loss of transplanted organs due to chronic allograft dysfunction, a process involving immunologic (inadequate immunosuppression) and non-immunologic factors (drug-related toxicities)^{6,7}.

Because of the ever increasing number of potential transplant candidates and the shortage of donor organs⁸, the long-term outcome of clinical transplantation must be optimized. The ultimate goal in clinical transplantation remains, therefore, to safely achieve long-term allograft acceptance in the recipient, which implies sustained donor-specific T- and B-cell non-responsiveness with preserved allograft function, in the absence of (operational tolerance) or minimal (near-tolerance) chronic immunosuppression.

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Over the past 50 years, experimental models have become an invaluable tool for elucidating the mechanisms underlying the induction and maintenance of tolerance to alloantigens⁹. If these experimental protocols can be successfully transposed to clinical transplantation, the induction of donor-specific transplantation tolerance could result in long-term allograft survival while preserving the host from drug-related adverse effects.

Mechanisms of allograft rejection

Allorecognition

Adaptive immune responses to grafted tissues are the major obstacle to successful transplantation. The term "allorecognition" refers to T-cell recognition of genetically encoded polymorphisms between members of the same species, mainly major histocompatibility complex (MHC) molecules which are expressed on donor cells. Although MHC incompatibility can provoke a strong immune response, graft rejection can also occur in MHC-matched donor-to-recipient combinations due to the recognition of minor histocompatibility antigens¹⁰. These antigens are peptides derived from polymorphic proteins other than MHC molecules presented in the context of MHC class I and II molecules.

Tissues that are mismatched for MHC antigens can provoke antidonor immunity and allograft rejection via three distinct pathways, mediated primarily by T-cells: the direct, indirect, and semi-direct pathways¹¹. T-cells with direct allospecificity are activated after recognition of intact MHC alloantigens displayed at the surface of donor professional antigen-presenting cells (APC). T-cells with indirect allospecificity recognize donor alloantigens as processed peptides associated with recipient MHC molecules in a self-restricted manner. Both pathways were shown to help cytotoxic CD8⁺ T-cells, induce macrophage responses and allospecific antibody production by B-cells. The high frequency of T-cells with direct allospecificity and the relative low frequency of T-cells with indirect allospecificity in the T-cell repertoire has led to the concept that the direct alloresponse dominates the early posttransplantation period and is mainly involved in acute transplant rejection, while the indirect pathway plays a major role in later forms of alloresponses and in chronic transplant rejection¹¹⁻¹⁵. However, animal models also support a role for the indirect pathway in acute rejection as

this pathway has been shown to be sufficient to elicit allograft destruction in the absence of direct allorecognition. Thus, current data suggest that the indirect pathway might be the one to target to achieve long-term graft survival, particularly since experimental models have shown the importance of indirect allorecognition in the induction of transplantation tolerance¹¹⁻¹⁷. A third mechanism of allorecognition has recently been described (the semi-direct pathway), where trafficking recipient dendritic cells (DC) could acquire intact donor MHC:peptide complexes from donor DC or endothelial cells and induce proliferation of antigen-specific T-cells^{18,19}.

T-cell activation

The recognition by recipient T-cells of MHC-mismatched antigens is the primary event that ultimately leads to graft rejection. In the early stages after transplantation, proinflammatory signals produced as a result of initial ischemia/reperfusion tissue injury promote the maturation of donor DC and their migration out of the allograft towards secondary lymphoid organs to encounter recipient alloreactive T-cells^{20,21}. Once activated by donor DC (direct pathway), alloantigen-specific T-cells will home to the allograft and initiate the acute rejection process. At later stages, with the decline of donor-derived DC, the immune response against an allograft is maintained by recipient APC (DC and B-cells) that process and present allogeneic MHC molecules shed from the graft (indirect and semi-direct pathways).

In addition to antigen recognition, full T-cell activation requires a second distinct co-stimulatory signal. The first signal (signal 1) is delivered through the T-cell receptor (TCR) by recognition of peptide antigen presented in the context of MHC molecules on the APC. Co-stimulatory signals (signal 2) are delivered via constitutive or inducible receptors on the responding T-cell surface interacting with their ligands constitutively expressed or upregulated on the activated APC. A growing number of co-stimulatory receptor/ligand molecules have been identified. The CD28/B7.1(CD80)-B7.2 (CD86), CD40L(CD154)/CD40, ICOS/ICOSL positive activating co-stimulatory pathways have been best characterized so far in *in vivo* experimental transplantation models. These signals are balanced by inhibitory inducible signals such as CD152(CTLA-4)/B7 and PD-1/PD-L,

allowing a regulation of the T-cell response²²⁻²⁴. If partial activation occurs, as for instance in the absence of co-stimulation, T-cells become hypo-responsive to subsequent antigen-specific TCR signals (donor-specific anergy) or die by apoptosis^{25,26}. The engagement of the TCR/CD3 complex (signal 1) in the presence of signal 2 activates the intracellular calcium-calciurein signaling pathway and the induction of transcription factors, leading to the expression of new surface molecules, such as inducible co-stimulatory molecules and cytokine receptors, that deliver growth and proliferation signals (signal 3) via the downstream phosphatidylinositol 3-kinase (PI3K) and the mammalian target of rapamycin (mTOR) pathways.

T-cell effector function

The encounter of naive T-cells with DC modulates their differentiation into polarized T helper (Th) cells and thus is a major component in the regulation of T-cell responsiveness (Fig. 1)^{21,27-29}. Depending on the type of DC, the type and dose of antigens, and the local cytokine microenvironment, T-cells can differentiate into various subsets of effector T-cells, determining the outcome of the immune response towards immunity or tolerance. Mature DC and the presence of interleukin (IL)-12 promote the development of Th1 cells that secrete cytokines usually associated with inflammation (interferon- γ , IL-2 and tumor necrosis factor- α) and induce cell-mediated immune responses. Interleukin-4 skews towards Th2 cells, which are mainly involved in humoral immunity and allergic responses³⁰. More recently, a subset of IL-17-producing cells (Th17) has been identified in infectious and autoimmune disease models. *In vitro*, this subset of cells develops in the presence of transforming growth factor (TGF)- β and the proinflammatory cytokine IL-6³¹⁻³³.

These pathogenic effector T-cells are counterbalanced by a subset of naturally occurring regulatory T-cells, the CD4⁺CD25⁺ Foxp3⁺ T regulatory (T_{reg}) cells. This small population of CD4⁺ T-cells is selected in the thymus, and constitutively co-expresses the IL-2 receptor α -chain (CD25) and the forkhead box protein 3 transcription factor (Foxp3). These cells were shown to be crucial for the control of autoreactive T-cells in autoimmune-disease models, as well as being potent suppressors of the effector function of alloreactive T-cells in transplantation models *in vivo*³⁴⁻³⁶. There is increasing evidence that in the

peripheral immune system and under specific conditions, uncommitted naive T-cells can be skewed toward T-cells with regulatory potentials, also referred to as induced regulatory T-cells (iT_{reg}). The presence of "regulatory" cytokines such as TGF β or IL-10 and antigen presentation by immature DC have been shown to favor the generation of antigen-specific T-cells with regulatory properties *in vitro* and *in vivo*^{27,28}. Recent data suggest that naive T-cells could be induced to differentiate in the periphery into Th17 or iT_{reg} cells in a mutually exclusive manner³³. Thus, skewing of the immune response away from Th17 or Th1 proinflammatory cells (for example by blocking critical cytokines such as IL-6) and towards T_{reg} cells may prevent transplant rejection.

Immune tolerance in transplantation

Immune tolerance is defined as the ability of the immune system to distinguish between self and nonself harmful antigens, leading to a specific, protective, cell-mediated and humoral response. Experimental models have shown that the immunologic mechanisms that normally maintain immune homeostasis and tolerance to self-antigens were basically the same as those involved in the induction of tolerance to alloantigens. T-cell tolerance to self-antigens can be established centrally during lymphocyte development in the thymus, or in the periphery at sites of antigen recognition and processing. Self-tolerance is partly achieved by intra-thymic deletion of self-reactive lymphocytes from the immune repertoire (clonal selection). As not all self-antigens are expressed in the thymus, other mechanisms exist in the peripheral immune system to maintain a safe T-cell repertoire. Peripheral tolerance can be established and maintained by various mechanisms, including deletion of activated/effector T-cells, anergy induction, and active regulation of effector T-cells (Fig. 2)^{26,37}.

Central tolerance

Many experimental data support the role of the thymus in the induction of sustained robust tolerance to alloantigens^{38,39}. During the normal maturation process in the thymus, T-cells with high avidity for thymic-expressed self-antigens are deleted (negative selection) so that potentially deleterious antigen-reactive T-cells will not reach the periphery. This physiologic process

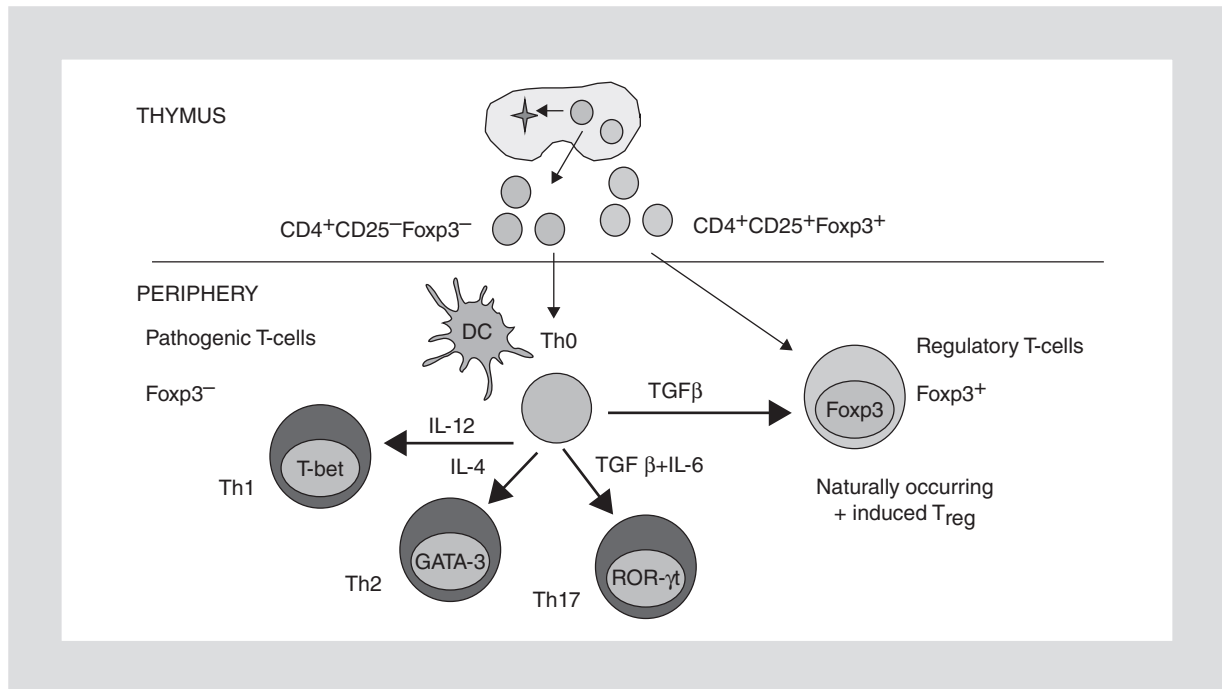


Figure 1. T-cell activation and differentiation into effector T-cell. The encounter of naive T-cells with antigen-presenting DC modulates their differentiation into polarized Th cells and thus is a major component in the regulation of T-cell responsiveness. The committed T-cells are characterized by their cytokine profile and the expression of specific transcription factors: T-bet for Th1, GATA-3 for Th2, FoxP3 for Treg cells and ROR- γ t for Th17 cells. To maintain immune homeostasis, the pathogenic Th1, Th2 and Th17 effector T-cells are counterbalanced by a subset of naturally occurring or induced Treg cells. DC: dendritic cells; Th: T helper; IL: interleukin; TGF: transforming growth factor; T_{reg} : regulatory T-cell.

could be exploited experimentally to induce specific tolerance to the allograft by the delivery of donor alloantigens to the thymus prior to organ transplantation. Experimentally, donor-derived allopeptides can be directly injected into the thymus⁴⁰. Another possibility, which is more appropriate for clinical application, is the induction of a state of hematopoietic mixed chimerism in the recipient's repertoire, that is to say the presence of cells from both recipient and donor origin. This implies the transfusion of donor whole bone marrow (BM) cells into a cell-depleted recipient. Experimental studies have demonstrated that after the transfer of donor hematopoietic cells, donor-derived APC migrate to the recipient's thymus and induce clonal deletion of donor-reactive T-cells^{9,38,41-43}. Thus, hematopoietic chimerism results in education of the immune system to recognize the donor as self. It was initially thought that to establish central tolerance in solid organ transplantation, total ablation of the preexisting, cross-reactive, peripheral T-cells and a state of hematopoietic donor-type full chimerism would be necessary. This implied myeloablation with total body irradiation or high-dose chemotherapeutic agents, similar to protocols used in clinical BM transplantation for malig-

nant disorders. It was reported that such recipients who achieved full chimerism could subsequently accept a kidney allograft from the original BM donor without immunosuppression⁴⁴. However, because of its toxicity, this approach could not be justified to achieve tolerance in solid organ transplant recipients without malignant diseases. Extensive work performed in small and large animal models have since demonstrated that sustained donor-specific transplantation tolerance can be induced through the generation of a state of hematopoietic mixed chimerism (reconstitution of the recipient with donor and recipient hematopoietic cells). Thus, high-intensity myeloablative preconditioning protocols could be successfully replaced by other less-toxic approaches (high-dose BM combined to co-stimulatory blockade and thymic irradiation)⁴⁵⁻⁴⁸.

Peripheral tolerance

Circulating alloreactive T-cells are crucial in the initiation and coordination of the immune response to an allograft, and, to promote tolerance, the alloreactive effector T-cell pool must be minimized while enhancing regulatory mech-

anisms³⁷. In addition, in the peripheral immune system, T-cells respond to alloantigens if they are presented in the context of appropriate co-stimulatory signals and within specialized structures of secondary or tertiary lymphoid organs⁴⁹⁻⁵¹. Experimental transplantation models have explored the following strategies to achieve peripheral transplantation tolerance:

- deletion of peripheral effector T-cells (lymphocyte-depleting protocols);
- inhibition of T-cell activation by blocking or modifying co-stimulatory signals (co-stimulatory blockade, manipulation of DC);
- interference with the effector function and homing of activated T-cells (anti-chemokines);
- active regulation of effector T-cells by antigen-specific T_{reg} cells.

After extensive studies in rodent and large-animal models, some of these approaches are now being progressively transposed to the clinic.

Translating tolerance into the clinic

Spontaneously tolerant transplant recipients

A number of clinical reports of occasional “tolerant” transplant recipients, characterized by prolonged allograft survival with minimal or no immunosuppression, suggest that immunologic tolerance may indeed be achievable in some patients⁵². Spontaneous operational tolerance is rare in kidney transplant recipients, but has been more frequently reported in liver transplant recipients, suggesting that various mechanisms may be involved such as the induction of mixed chimerism^{38,53} or the promotion of peripheral regulatory mechanisms⁵⁴⁻⁵⁶. Collaborative international studies are currently underway to pool these tolerant transplant recipients in order to determine more precisely how this state arises and to develop reliable tests to predict tolerance^{52,57,58}.

Mixed chimerism

The proof-of-concept of the clinical applicability of hematopoietic mixed-chimerism approaches to deliberately induce transplantation

tolerance was first established in a small number of patients with hematopoietic malignant disorders (e.g. end-stage renal failure secondary to refractory multiple myeloma). These highly selected recipients underwent HLA-matched BM and kidney transplantation from the same living donor, and had long-term acceptance of their renal allograft in the absence of ongoing immunosuppression^{44,59-62}. More recently, these protocols have been adapted to patients without concomitant malignancy. Following on their previous protocols developed in animal models and in small clinical trials⁶³, the Stanford group recently started a study on patients with end-stage renal disease without malignancy, undergoing combined HLA-matched kidney and hematopoietic cell transplantation, using a low-intensity conditioning regimen (total lymphoid irradiation and antithymocyte globulin)⁶⁴. Their first transplant recipient in this protocol had persistent mixed chimerism and normal kidney allograft function more than two years after discontinuation of all immunosuppressive drugs. Of interest, a few years ago the same group had attempted a similar protocol for the induction of allograft tolerance in HLA-mismatched recipients⁶³, but rejection generally developed following immunosuppression withdrawal. However, the recent report of the Massachusetts General Hospital group brings some hope of the possibility to extend these clinical protocols to HLA-mismatched patients⁶⁵. In their study, five patients with end-stage renal disease and no malignancy received combined BM and one-haplotype HLA-mismatched living-donor kidney transplantation, with the use of a non-myeloablative preconditioning regimen. Except for one patient who developed non-reversible humoral rejection, immunosuppression was withdrawn in the remaining patients at 9-14 months after transplantation, with stable kidney function and donor-specific T-cell unresponsiveness. Interestingly, all the recipients displayed only transient chimerism posttransplantation, suggesting that, while the induction of tolerance is dependent on central deletion of donor-reactive T-cells, peripheral mechanisms may be involved in the long-term maintenance of a tolerant state.

Clearly, because these trials have involved a relatively low number of patients, there is a need to confirm these results in larger groups in order to fully assess the clinical potential of the “mixed-chimerism strategy”. It also would be desirable to try to simplify this type of clinical protocol as, at least for now, these ap-

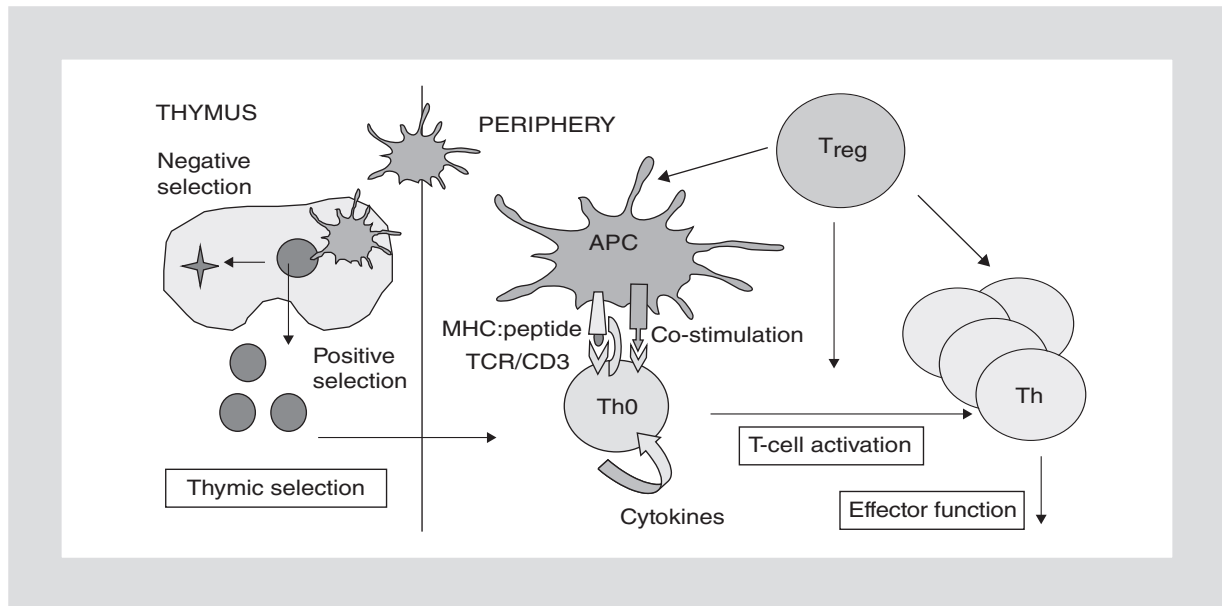


Figure 2. Mechanisms of T-cell activation and targets for the induction of transplantation tolerance. **(A) Central tolerance.** During T-cell maturation, T-cells with high or no affinity for thymically expressed antigens undergo intra-thymic deletion. As a result, only T-cells with moderate affinity to host MHC are selected and released to the periphery. In hematopoietic mixed chimerism, both host and donor type APC can migrate to the thymus, and dictate thymic selection. **(B) Peripheral tolerance.** Normal T-cell activation requires the interaction of MHC:peptide on the APC with the TCR, together with a co-stimulatory signal. The following peripheral mechanisms control effective T-cell activation, and could be exploited to induce immune tolerance: i) Co-stimulatory blockade, manipulation of dendritic cells. Antigen-recognition in the absence of an appropriate signal 2 leads to antigen-specific anergy. ii) T-cell depleting antibodies. All T-cells are depleted from the periphery, irrelevant of their specificity or activation state. iii) Anti-cytokines, anti-chemokines. Alloreactive T-cells are activated, but they cannot home to the allograft and exert their effector function. iv) T-cell regulation. T-cells are present, but their activation and effector function is controlled by T_{reg} cells. T_{reg} : regulatory T-cell; APC: antigen-presenting cells; MHC: major histocompatibility complex; TCR: T-cell receptor; Th: T helper.

proaches have required a high degree of technical expertise and medical “know how”, so that patients had to be referred to specific transplantation centers to be enrolled in such protocols.

T-cell depletion

The primary mediators of the immune response are T and B lymphocytes that have antigen-specific receptors, recognizing alloantigens and inducing donor-specific cellular and humoral responses. With the development of polyclonal and monoclonal antibodies (MAb), newer therapeutic strategies have been developed based on powerful cell-depletion at the time of transplantation (induction therapy) when immune activation is most intense. In various experimental rodent and nonhuman primate (NHP) models, anti-T-cell antibodies have been used either alone or in combination with other strategies that aim to limit the clonal expansion of effector T-cells such as co-stimulatory blockade or transfusion of donor-derived peptides⁶⁶. T-cell depletion strategies have been extensively studied in NHP transplantation models, and encouraging results have led

to clinical trials using polyclonal antithymocyte globulins, a humanized anti-CD52 MAb (alemtuzumab, Campath-1H), or anti-CD3 MAb.

Polyclonal antithymocyte globulins and Campath-1H induce profound and durable reduction of circulating leucocytes capable of mounting an alloresponse at a time when the allograft is already susceptible to inflammatory damage following the peri-transplantation ischemia/reperfusion injury. Lymphocytes will gradually repopulate the host weeks to months later when the innate immune response has resumed and the allograft is more quiescent. Calne, et al. first published interesting clinical data in deceased-donor kidney transplantation, using lymphocyte depletion with Campath-1H, which provided long-term, rejection-free allograft survival with minimal maintenance therapy (low-dose cyclosporine) in most patients. Because these recipients did not exhibit true operational tolerance, the authors coined the term “prope or near-tolerance” to describe their immunologic state⁶⁷. Other groups have extended this approach of drug minimization by combining polyclonal rabbit ATG or Campath-1H together with 15-deox-

yspergualin (DSG, a monocyte inhibitor), rapamycin, or tacrolimus. These subsequent clinical studies confirmed that, despite profound peri-transplant T-cell depletion, consistent transplantation tolerance could not be achieved when anti-T-cell Ab were used in monotherapy, and immunosuppressive therapy could rarely be completely withdrawn⁶⁸⁻⁷⁴. Of note, the use of Campath-1H alone or in combination with sirolimus or DSG was associated with an unusually high rate of early cellular rejection episodes, characterized by predominant monocytic infiltrates, as well as antibody mediated rejection episodes, so that minimal immunosuppression had to be maintained in later studies^{68,71-74}.

Non-activating humanized Fc-receptor-non-binding anti-CD3 MAb (teplizumab, ChAgly-CD3, visilizumab) are currently being tested in phase I and II clinical trials in autoimmune diseases settings (type 1 diabetes, Crohn's disease, arthritis) as well as in renal and pancreatic islet transplantation⁷⁵. Whether these antibodies will find a place in the transplant drug armamentarium still needs to be determined.

Co-stimulatory blockade

Current data suggest that the interruption of T-cell signaling pathways at specific steps prevents the differentiation of alloreactive T-cells into proliferating effector T-cells and may promote immune tolerance in some circumstances. By inhibiting T-cell activation rather than eliminating all T-cells, as in depleting protocols, the strategy of co-stimulatory blockade might more selectively target effector T-cells and spare beneficial T_{reg} cells. In many experimental rodent and NHP transplantation models, dual blockade of the CD154(CD40L):CD40 and the CD28:B7 pathways was shown to act synergistically to promote tolerance⁷⁶. Of the various co-stimulatory molecules that have been targeted in animal models, the most successful results were obtained using an anti-CD40L MAb⁷⁷. However, the administration of humanized anti-CD40L MAb in transplant recipients resulted in unexpected thromboembolic complications, possibly due to the expression of CD40L on platelets, and all clinical trials with this agent were therefore terminated⁷⁸.

CD28 is constitutively expressed on CD4⁺ T-cells and up to 50% of CD8⁺ T-cells, and its ligation by B7 molecules (CD80, CD86) expressed on

DC synergizes with TCR signaling to lower the activation threshold of T-cells. CD28 may also deliver a reverse signal to DC, inducing the production of IL-6^{79,80}. Cytotoxic T-lymphocyte-associated antigen (CTLA)-4 Ig, a fusion protein combining the extracellular binding domain of CTLA-4 with the Fc portion of IgG1 and with specificity for CD80/86 expressed on APC, was first used in experimental models with excellent outcomes. In NHP models, CTLA-4 Ig prolonged pancreatic islet survival and, in combination with anti-CD40L, induced indefinite acceptance of renal and heart allografts, while allowing prolonged skin-graft survival⁸¹⁻⁸³. Following these encouraging preclinical results, clinical trials were initiated with LEA29Y (belatacept), a high-affinity variant of CTLA-4 Ig⁸⁴. In a recent phase II renal transplantation clinical trial, after induction with anti-IL-2 receptor MAb (basiliximab), belatacept was administered every four weeks together with mycophenolate mofetil and steroids. The reported short-term results were promising in terms of safety and rates of biopsy-proven acute rejection within the first year, when compared to a "classical" cyclosporine-based maintenance regimen⁸⁵. But, co-stimulatory blockade has been so far used in the clinic more as an immunosuppressive agent and an alternative to calcineurin inhibitors. Longer follow-up data will have to establish if this drug can prevent chronic allograft dysfunction and/or favor the induction of transplantation tolerance, as was suggested in animal models.

Regulatory T-cells

The use of powerful induction therapies may promote regulatory mechanisms and the induction of peripheral transplantation tolerance by depleting or interfering with the activation and/or effector function of alloreactive T-cells at the time of transplantation. However, unlike in animal models, only a small proportion of patients can completely discontinue therapy (operational tolerance), or be kept on minimal immunosuppression (near-tolerance) safely, that is in the absence of donor-specific alloresponses and with long-term normal graft function. In the absence of the induction of robust, donor-specific tolerogenic mechanisms (as may occur following a state of donor-type mixed chimerism), some degree of immunosuppression must be maintained as long as the allograft is in place. Thus, transplant immunobiologists are now exploring newer individualized therapies based on

manipulated donor or recipient cells that specifically target donor alloantigens^{27,86}.

There is increasing evidence that in many experimental protocols where robust peripheral transplantation tolerance is achieved, immunoregulatory mechanisms dependent on donor-specific T_{reg} cells are critical in the induction and maintenance of the tolerant state^{35,87}. Based on these observations, strategies exploiting antigen-specific T_{reg} cells in the induction of transplantation tolerance are currently being explored. A first strategy to promote peripheral tolerance would be to manipulate host immune responses in order to delete peripheral, effector, alloreactive T-cells, and at the same time favor the development and expansion of donor-specific T_{reg} cells^{37,88,89}. In accordance with previous experimental results, induction regimens based on T-cell-depleting antibodies⁹⁰⁻⁹¹ or co-stimulatory blockade, as well as the use of mTOR inhibitors, appear to be promising protocols in clinical transplantation. In experimental transplantation models, mTOR inhibitors were shown to facilitate peripheral deletion of effector T-cells by promoting activation-induced cell death, leaving a small pool of residual alloreactive T-cells which could be regulated by T_{reg} cells⁸⁸. Furthermore, recent data have demonstrated that the mTOR inhibitor rapamycin could selectively expand T_{reg} cells *in vitro* and *in vivo*, while calcineurin inhibitors appear to have a deleterious effect⁹²⁻⁹⁴. Another strategy that is currently under investigation would be to achieve regulation of *in vivo* alloresponses by the transfer to the recipient of customized donor-specific T_{reg} cells, selected and expanded *ex vivo*^{86,95,96}.

Conclusion and perspectives

Encouraging results from experimental transplantation models have led to preliminary clinical trials with immunomodulatory therapies aiming at promoting donor-specific hyporesponsiveness and the induction of operational tolerance. However, the translation of these successful experimental tolerogenic protocols across species and into the clinic in human transplant recipients remains a major challenge. One of the major hurdle is the human T-cell repertoire that, compared to laboratory animals that have not been exposed to as many environmental antigens, contains a larger proportion of cross-reactive memory T-cells^{97,98}. Memory T-cells differ from naive T-cells by their activation requirements and

homing properties, and they have been shown to undergo homeostatic proliferation in T-cell-depleted hosts, thus rendering them more resistant to co-stimulatory blockade and anti-T-cell antibodies^{99,100}. Newer strategies are currently being investigated in experimental transplantation models to more efficiently target this population¹⁰¹. In addition, detailed characterization of rare, spontaneously "tolerant" transplant recipients, that are drug-free or only minimally immunosuppressed, may help in defining the mechanisms involved in clinical transplantation tolerance and designing new tolerogenic protocols.

A new era, therefore, has been opened in the field of immunomodulation in clinical transplantation. Ultimately, tolerance-inducing strategies will need to be compared to modern immunosuppressive drug regimens in order to define the optimal antirejection management for transplant recipients.

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