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REVIEW

From current immunosuppressive strategies to clinical tolerance of allografts

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Abstract

In order to prevent allograft rejection, most current immunosuppressive drugs nonspecifically target T-cell activation, clonal expansion or differentiation into effector cells. Experimental models have shown that it is possible to exploit the central and peripheral mechanisms that normally maintain immune homeostasis and tolerance to self-antigens, in order to induce tolerance to alloantigens. Central tolerance results from intrathymic deletion of T cells with high avidity for thymically expressed antigens. Peripheral tolerance to nonself-molecules can be achieved by various mechanisms including deletion of activated/effector T cells, anergy induction and active regulation of effector T cells. In this article, we briefly discuss the pathways of allorecognition and their relevance to current immunosuppressive strategies and to the induction of transplantation tolerance (through haematopoietic mixed chimerism, depleting protocols, costimulatory blockade and regulatory T cells). We then review the prospect of clinical applicability of these protocols in solid organ transplantation.

Introduction

In the past 50 years, immunosuppressive strategies have successfully been transposed from the experimental stage to routine clinical practice and have allowed solid organ transplantation to become the therapy of choice for end-stage organ diseases. However, most of the commonly used immunosuppressive drugs control the rejection process by targeting the immune response nonspecifically and, as lifelong administration is usually required, they often lead to unwanted side-effects including increased susceptibility to infections and development of tumours. Some of these drugs are also associated with nonimmunological complications including an increase in cardiovascular risk factors. Cardiovascular diseases are indeed now the leading cause of death following renal transplantation (in 30–40% of cases) with a high prevalence of hypertension (60–80%), hyperlipidemia (40–60%), as well as newly onset diabetes (10–15%) in the recipients. In addition, even in patients without complications because of their immunosuppressive drugs, there is an inexorable loss of transplanted organs because of chronic allograft rejection (3–5% annual rate of loss), a yet incompletely understood process involving immunological and nonimmunological factors [1–6]. While acute allograft rejection can be prevented or treated with current immunosuppressive treatment combinations, leading to more than 90% 1-year graft survival for most organs, optimal long-term graft survival still remains a problem. With the shortage of donor organs and the ever-increasing number of potential recipients, there is an urgent need to optimize the long-term outcome of clinical transplantation.

The ultimate goal in transplantation is, therefore, to avoid these complications by the induction of a sustained specific tolerance to donor alloantigens in the absence of chronic immunosuppressive therapy (operational

tolerance). Since the pioneering experiments of Medawar's group [7], research has thrived in the field of transplantation immunology and there is now data demonstrating that tolerance to allografts can be induced in experimental animal models and in human adult recipients. In this review, we describe the pathways of allorecognition leading to graft rejection and discuss current immunosuppressive strategies used to prevent rejection as well as potential new targets that may lead to the induction of transplantation tolerance.

Allorecognition

Pathways of allorecognition

The recognition of allograft major histocompatibility complex (MHC) antigens is the primary event that ultimately leads to graft rejection. T cells play a central role in the immune response to an allograft and can initiate rejection of MHC-mismatched tissues via three distinct pathways: the direct, indirect and the recently described semi-direct pathway (Fig. 1) [8–10]. It is now well

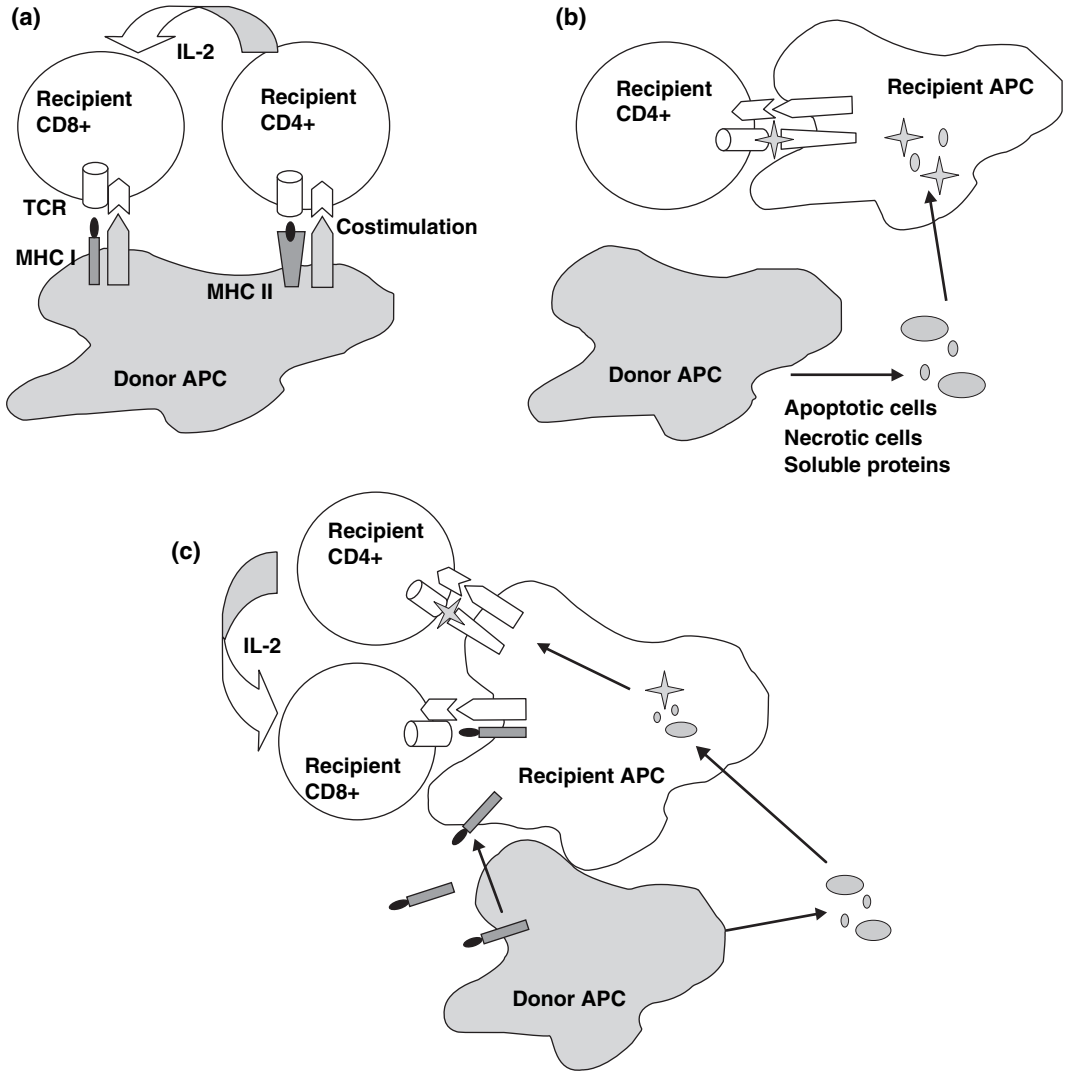

Figure 1 Pathways of allorecognition. (a) Direct pathway. Recipient T cells recognize intact allogeneic major histocompatibility complex (MHC) on donor antigen-presenting cells (APCs). Once primed by the donor APC, allospecific $CD4^+$ T cells can procure help for the effector function of $CD8^+$ T cells that have been activated by the same APC. (b) Indirect pathway. Allogeneic MHC molecules shed from the graft (soluble MHC molecules or dying/apoptotic cells) are taken up and processed by recipient APCs to be presented as peptides in the context of self-MHC molecules. (c) Semi-direct pathway. Recipient APCs acquire and present intact donor MHC I molecules to direct pathway $CD8^+$ T cells and simultaneously present internalized and processed donor MHC molecules to $CD4^+$ T cells with indirect allospecificity. As T cells with direct and indirect allospecificity are primed by the same APC, linked-help can occur.

established that immune responses to alloantigens can be induced by either recognition of intact allogeneic MHC molecules displayed at the surface of donor cells (direct pathway) or by peptides derived from polymorphic sequences of allogeneic MHC molecules associated with recipient MHC molecules in a self-restricted manner (indirect pathway) [11,12]. However, the relative contributions of these pathways to graft rejection are not completely defined yet. The high frequency of T cells with direct allospecificity and the relative low frequency of T cells with indirect allospecificity in the normal T-cell repertoire has led to the concept that the direct alloresponse dominates the early post-transplant 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 rejection [13–15]. 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 [13].

Collaboration between helper and effector T cells is required to ensure rejection and $CD4^+$ T cells play a central role by providing effector cytokines and cognate help for cytotoxic $CD8^+$, B-cell and macrophage responses. $CD4^+$ T cells can initiate allograft rejection through direct recognition of allogeneic MHC class II antigens as well as indirect recognition of allogeneic MHC peptides processed by self-antigen-presenting cells (APCs). Both pathways were shown to help $CD8^+$ T cells that eventually lyse allogeneic MHC class I-presenting target cells [16] and contribute to alloantibody production by B cells [17].

The first meeting point between host T cells and foreign transplant antigens, leading to the initiation of the immune response, is generally assumed to be in the secondary lymphoid organs rather than in the transplanted tissue itself [18,19]. In the early stages after transplantation, tissue-resident immature donor dendritic cells (DCs) migrate out of the graft via blood and/or lymph towards secondary lymphoid organs where they mature and encounter recipient naïve and resting memory T cells (direct pathway). The trafficking and maturation of DCs is triggered by proinflammatory signals produced as a result of tissue injury during the transplant surgery and is the cornerstone for the initiation of effective adaptive immune responses [20–22]. As migrating donor DCs are available only during the first few weeks after transplantation, the frequency of T cells with direct antidonor allospecificity is expected to decline with time as has been described in transplant recipients [23–25]. Recipient T cells will then be mainly activated in secondary lymphoid organs by self-DCs that have circulated through the graft and present processed donor antigens associated with self-MHC (indirect pathway) [26,27]. Elevated frequencies of T cells with indirect antidonor specificity detected in patients with chronic heart, kidney and lung transplant rejection [14,15,28–33] indeed suggest that with time after transplantation, the indirect pathway of allorecognition plays an important role.

The existence of these two distinct pathways of allorecognition suggests that T cells with direct and indirect allospecificity are activated by distinct APCs and cannot cross-regulate each other. A third pathway has been proposed based on the observation that recipient DCs can acquire substantial levels of intact MHC I and II molecules from donor DCs, endothelial cells or tissues and induce proliferation of antigen-specific T cells (semi-direct pathway) [10,34]. These data suggest that recipient DCs, because of acquisition of donor MHC:peptide complexes, may link T cells with direct and indirect allospecificity. Indeed, according to the semi-direct hypothesis, if the trafficking recipient DCs acquire allogeneic MHC class I molecules from donor tissues, they can simultaneously stimulate indirect pathway $CD4^+$ and direct pathway $CD8^+$ T cells, thus allowing $CD4^+$ T cell help to be effective for the generation of cytotoxic T cells (three-cell model, Fig. 1c) [35].

In clinical transplantation, acute allograft rejection can be successfully prevented or treated in most cases with current immunosuppressive regimens, but the loss of transplants because of chronic rejection remains a serious problem. A series of clinical data have indicated that the indirect pathway of alloresponse is the main driver for chronic rejection [14,28–33], thus the control of T cells with indirect antidonor allospecificity would help achieve transplantation tolerance.

T-cell activation

For full T-cell activation to occur, two distinct signals are required. The first signal (signal 1) is delivered through the T-cell receptor (TCR) by the recognition of peptide antigens presented in the context of MHC molecules on the APC. Costimulatory 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 [36]. There is a growing number of characterized costimulatory receptor:ligand molecules, including the followings: CD28:B7(CD80, CD86), ICOS:ICOSL, CD40L(CD154):CD40 and OX40(CD134):OX40L. These positive activating costimulatory signals are balanced by inhibitory inducible signals such as CD152(CTLA-4):B7 and PD1:PDL allowing a downregulation of the response after initial T-cell activation [37,38]. If partial activation occurs, T cells die by apoptosis or become unresponsive to proliferative signals, a state referred to as anergy [39]. In the context of

transplantation, bone marrow (BM)-derived professional APCs, mainly DCs of donor (direct pathway) or recipient (indirect and semi-direct pathway) origin, are responsible for the activation of recipient T cells.

Strategies to induce transplantation tolerance

In order to prevent the rejection process, most current immunosuppressive drugs nonspecifically target T-cell activation, clonal expansion or differentiation into effector T cells. The available agents include polyclonal or monoclonal antibodies (mAbs) against the TCR; calcineurin inhibitors such as cyclosporin or tacrolimus which block TCR-dependent T-cell activation; anti-interleukin-2 receptor (IL-2 R) mAbs; antiproliferative agents such as azathioprine, mycophenolate mophetil or enteric-coated mycophenolic acid, and the more recently introduced compounds sirolimus or everolimus, two inhibitors of the cell-cycle downstream the IL-2 R (Fig. 2) [6,40]. In the past decades, the use of these drugs has changed the outcome of organ transplantation and of some autoimmune diseases as well. However, the improved survival rates of allografts have come at a cost, with increased frequencies of drug-related adverse effects. Furthermore, the combination of these therapies has had little effect on chronic rejection [5,41]. Thus, one important goal for the transplant biologist is to investigate how to safely achieve long-term drug-free graft acceptance with normal organ function. Indeed, it has been shown in experimental models that the induction of tolerance can effectively prevent the development of chronic rejection; therefore, its successful application in clinical transplantation is expected to improve the long-term allograft survival [42].

Over recent years experimental models have shown that it is possible to exploit the mechanisms that normally maintain immune homeostasis and tolerance to self-anti-

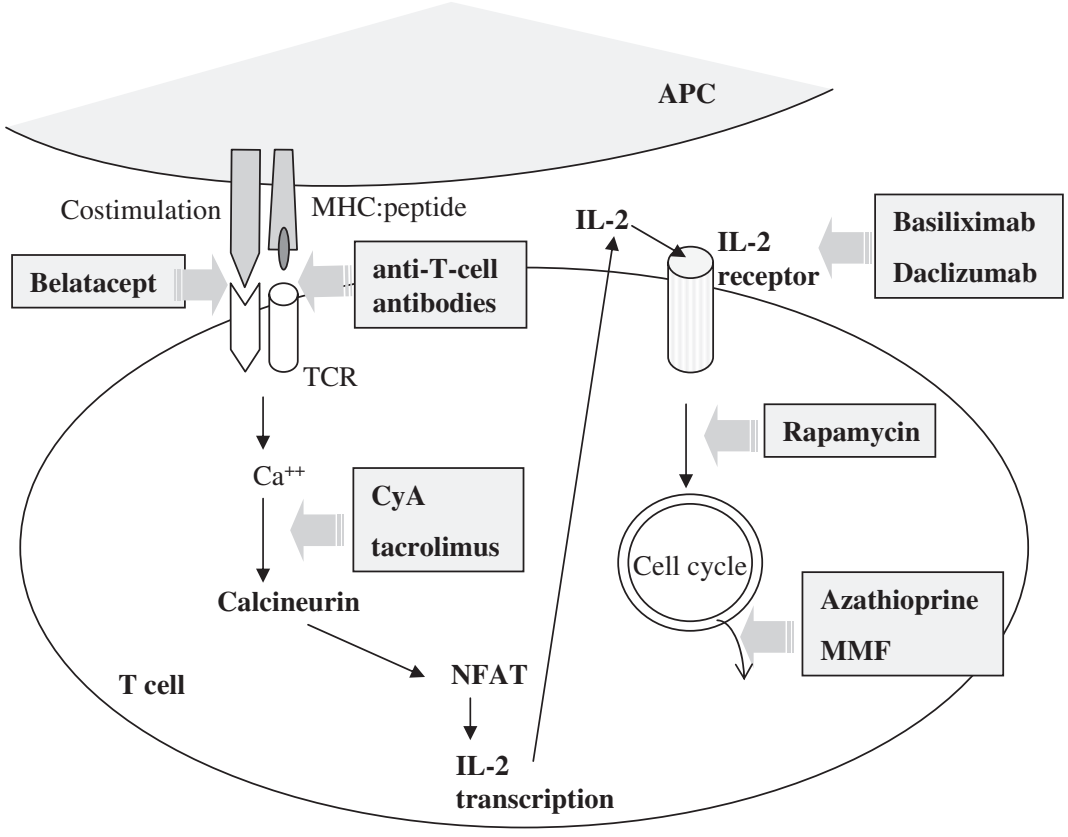

Figure 2 Targets for current immunosuppressive drugs. Signal 1 is delivered through the T-cell receptor (TCR) by recognition of peptide antigens presented in the context of major histocompatibility complex (MHC) molecules on the antigen-presenting cells. This stimulation results in calcineurin activation, a process blocked by cyclosporine A or tacrolimus. Activated calcineurin dephosphorylates the nuclear factor of activated T cells so that it can enter the nucleus and bind to the interleukin-2 (IL-2) promoter. Costimulatory signals (signal 2) are necessary for optimal IL-2 gene transcription in the T cell. IL-2 receptor stimulation induces the T cell to enter cell cycle and proliferate; this can be blocked by IL-2 receptor antibodies or by rapamycin which inhibits signalling induced by IL-2 receptor ligation. By blocking purine synthesis, azathioprine and mycophenolate mophetil MMF interrupt DNA replication and cell proliferation.

gens to induce tolerance to alloantigens. Indeed, the immune system is capable of mounting protective cell-mediated and humoral responses against foreign antigens, yet it remains unresponsive to self-antigens, resident bacteria or dietary proteins. It is now recognized that immunological tolerance involves central and peripheral mechanisms. Central tolerance results from intrathymic deletion of T cells with high avidity for thymically expressed antigens. Peripheral tolerance to nonself molecules can be achieved by various mechanisms including deletion of activated/effector T cells, anergy induction and active regulation of effector T cells (Fig. 3) [43,44]. Some clinical studies which have reported an association between donor-specific T-cell hyporesponsiveness and prolonged allograft survival with minimal immunosuppressive treatment suggest that immunological tolerance may be an achievable goal in clinical transplantation [45]. However, one should distinguish long-term graft acceptance with minimal immunosuppression from true transplantation tolerance, as the latter implies the absence of acute or chronic rejection, of donor-specific circulating alloantibody and of signs of subclinical rejection in allograft biopsies, in the absence of immunosuppressive drug therapy [46].

Central tolerance

The thymus plays an essential role in the maintenance of tolerance and although its size diminishes with age, it has been shown that the thymus remains functional throughout adult life [47]. Self-tolerance is partly achieved by intrathymic deletion of self-reactive lymphocytes from the immune repertoire (clonal selection). This mechanism can be exploited in transplantation by the delivery of donor antigens to the thymus of adult recipients leading to the central elimination of detrimental alloreactive T-cell

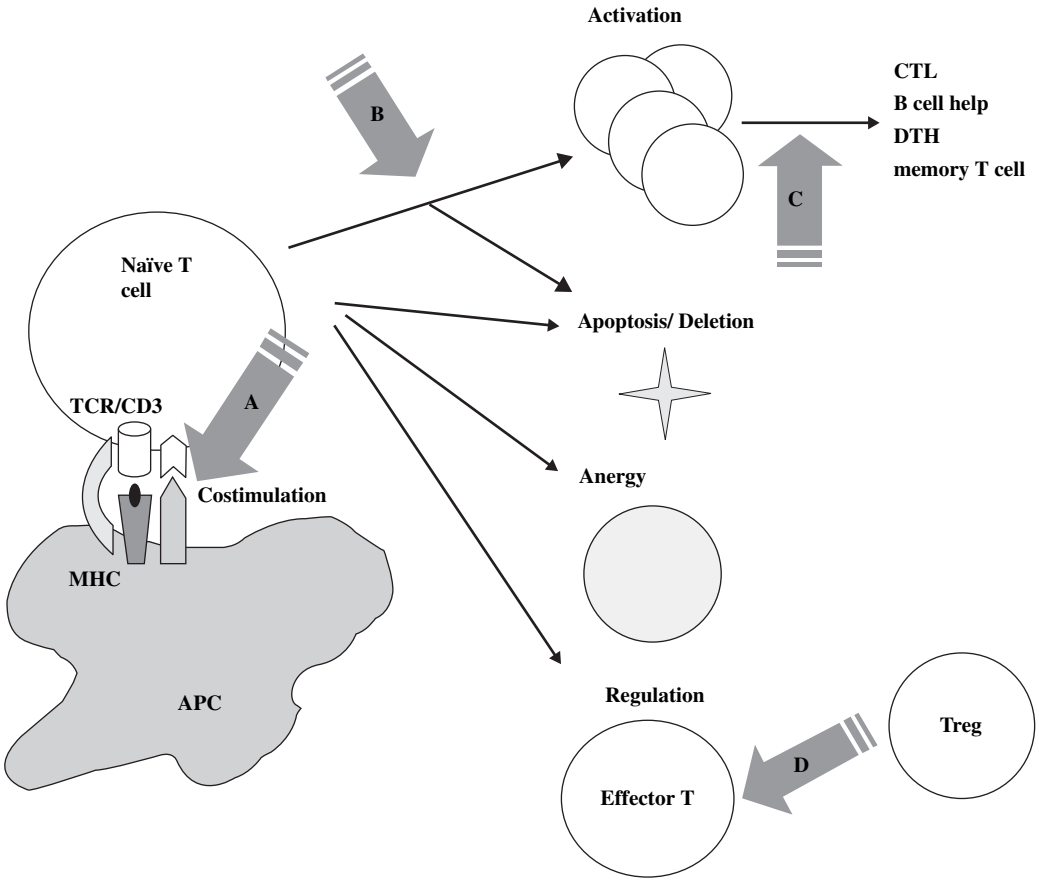

Figure 3 Strategies to induce peripheral tolerance. (A) Costimulatory blockade, manipulation of dendritic cells. In the absence of an appropriate costimulatory signal (signal 2), partially activated T cells become hyporesponsive to specific T-cell receptor signals (donor-specific anergy) or die by apoptosis. (B) Depleting mAbs. All T cells are depleted from the periphery, irrelevant to their specificity or activation state. (C) Anticytokines, anti-chemokines. Alloreactive T cells are activated but they cannot home to inflammatory sites and exert their effector function. (D) Regulation. Activated/effector T cells are present but their function is harnessed by regulatory T cells (Treg).

clones, resulting in specific tolerance to donor organs. This could either be performed experimentally by direct intrathymic injections of donor-derived allopeptides or by the induction in the recipient of haematopoietic mixed chimerism leading to the co-existence of cells from both recipient and donor origin [48].

Pioneering the concept of tolerance induction, Billingham, Brent and Medawar showed that infusion of donor allogeneic cells into newborn mice resulted in specific acceptance of skin allografts in the absence of added immunosuppression [7]. In their model, besides the immaturity of the newborn immune system, the observed tolerance to foreign antigens was mediated by the engraftment of donor cells and the migration of donor APCs to the recipient thymus where they induced negative selection of donor-reactive T cells, prior to release into the circulation. The induction of specific transplantation tolerance through the generation of a state of haematopoietic mixed chimerism in the recipient has been since studied extensively in rodent models as well as in nonhuman primates (NHP). A better understanding of this mechanism came with the work of Ildstad and Sachs. The authors used a myeloablative conditioning regimen in recipient animals, followed by infusion of combined T-depleted BM from donor and recipient before solid organ transplantation from the same BM donor was performed [49]. The conditioning regimen aimed at deleting pre-existing cross-reactive T cells that would reject the donor BM and grafted organ and created 'space' for the engraftment of infused BM cells. However, as the potential toxicities for the recipient of such conditioning protocols would render their clinical applicability difficult, other approaches were subsequently developed. Sykes' group reported successful induction of haematopoeitic-mixed chimerism and transplantation tolerance in a murine model combining the infusion of high-dose donor BM with costimulatory blockade but without the need of prior cytoreduction in the host [50–52]. By inoculating a larger amount of BM cells, they overcame the need of space for donor cells engraftment and thus the risks of myeloablation, while costimulatory blockade harnessed the peripheral T-cell alloresponse. Other similar approaches were used successfully in rodents as described by the Larsen group with low dose busulfan and costimulatory blockade [53]. In an NHP model, Myburgh *et al.* [54] achieved successful tolerance to hepatic allografts by combining donor BM infusion with antilymphocyte globulins. Further extensive work performed in recent years by the Massachusetts General Hospital (MGH) transplantation group showed that a donor lympho-haematopoietic chimerism in the peripheral blood over 1% and lasting for approximately 1 month was required to induce immune tolerance in NHP [55–58].

The importance of donor chimerism on immune tolerance was highlighted in 1991, by the report of two patients who had received a conventional BM transplant for treatment of acute leukaemia inducing a full donor chimerism. Several years later, as they had developed end-stage renal failure, they received a renal allograft from the original BM donor. These kidney grafts were accepted without immunosuppressive therapy as the recipients had reconstituted their immune system with donor cells [59].

Based on its extensive work performed in small and large animal models, the MGH transplantation group initiated a clinical trial of tolerance induction in the late 90s. Patients with co-existent multiple myeloma and chronic renal failure who were not accepted on regular kidney transplantation lists were treated by an conditioning regimen combining T-cell depletion and nonmyeloablative therapy including antilymphocyte globulin and cyclophosphamide. Subsequently, patients were transplanted with simultaneous BM and kidney from an human leukocyte antigen (HLA)-matched living donor. These patients indeed showed long-term acceptance of their renal allograft in the absence of ongoing immunosuppression and represent the first intentional and successful cases of clinical tolerance induction [60–62].

In another approach aiming at inducing clinical tolerance, Strober *et al.* [63,64] were able to wean off immunosuppression in three out of 25 patients treated at the time of transplantation with total lymphoid irradiation and rabbit antithymocyte globulin (ATG). Using a similar strategy, Millan *et al.* [65] established macrochimerism and donor specific hyporesponsiveness in renal transplant recipients of HLA-nonidentical donors. However, in this study, the tolerant state was not achieved as late rejection episodes occurred that required the reintroduction of immunosuppressive therapy.

The proof of concept that tolerance induction is feasible on a clinical level has been established in these highly selected patients. More studies are ongoing to confirm the efficacy of this approach in a wide range of patients, without concomitant malignancy, and across HLA-mismatch barriers.

Peripheral tolerance

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. In the transplant setting, circulating alloreactive T cells are crucial in the initiation and the co-ordination of the rejection response and, to promote tolerance, it is important to deplete or minimize the alloreactive effector T-cell pool while enhancing the regulatory mechanisms. Various strategies have been explored to achieve peripheral tolerance in experimental

protocols: targeting all peripheral T cells irrelevant to their specificity or activation state (depleting protocols), inhibiting T-cell activation by blocking or modifying costimulatory signals (costimulatory blockade, manipulation of DCs), interfering with the effector function or homing of activated T cells (anticytokines, antichemokines) or harnessing activated T cells by $CD4^+CD25^+$ antigen-specific regulatory T cells (Tregs) (Fig. 3). The description of the potential use of donor-specific Tregs or manipulated DCs to induce transplantation tolerance experimentally is beyond the scope of this review [66–69] and we will focus on some strategies that have already made their way in preclinical and clinical studies.

Depleting protocols

The advent of mAbs has allowed the development of various cell-depleting protocols in rodents, NHP models, as well as in clinical trials, in order to prevent acute rejection and possibly to promote transplantation tolerance. In various animal models, anti-T-cell antibodies, given at the time of transplantation (induction therapy), were used either alone or in combination with other strategies that aim to limit clonal expansion of effector T cells such as costimulatory blockade or transfusion of donor-derived peptides. By depleting T cells, and for some therapeutic combinations also B cells (anti-CD52, anti-CD45RB mAbs) and monocytes (deoxyspergualin, DSG), cell-depleting approaches result in a profound reduction of circulating leucocytes capable of mounting an alloresponse at a time when the allograft is already susceptible to inflammatory damage following the ischaemia/reperfusion injury [70–72]. Lymphocytes will gradually repopulate the host weeks to months later when the innate immune response has resumed and the allograft is more quiescent.

Depletion strategies have been extensively studied in NHP transplantation models. In these studies, encouraging results were obtained using rabbit ATG or anti-CD3-immunotoxin (monoclonal anti-Rhesus CD3 antibody with a modified diphtheria toxin) alone [73,74], or in combination with DSG (a monocyte inhibitor) [75] or rapamycin [76]. Indeed in these models, despite profound peritransplant T-cell depletion, consistent transplantation tolerance was not achieved with monotherapy as most treated animals eventually lost their grafts through chronic rejection [77].

Because the anti-CD3-immunotoxin did not cross-react with human CD3, and because of potential toxicity, such strategy was not easy to translate to clinical transplantation. However, the relative successes in NHP models paved the way towards human clinical trials using other T-cell-depleting reagents. Calne *et al.* [78,79] first published interesting data in humans using lymphocyte depletion with alemtuzumab, a humanized anti-CD52 mAb (CAMPATH-1H), as a means of minimizing immunosuppression (prope tolerance). Further studies have extended these results by combining CAMPATH-1H with DSG, or using polyclonal rabbit ATG together with rapamycin [80] or a combination of tacrolimus and mycophenolate mophetil [81]. The combined use of rapamycin in some tolerance inducing protocols may add a beneficial effect as this drug is thought to facilitate the peripheral deletion of effector T cells by promoting activation-induced cell death, while inducing Tregs in the periphery [82–84]. Other agents such as anti-CD45RB [85,86] and anti-CD4 mAbs [87] have been effective in murine models and await to be tested in NHP and future clinical trials.

Costimulatory blockade

As discussed previously, costimulation is required for full T-cell activation and the differentiation of naïve T cells into polarized effector T cells. In the absence of an appropriate second signal, partially activated T cells either become hyporesponsive to specific TCR signals (donor-specific anergy) or die by apoptosis [88]. Overall, by inhibiting T-cell activation rather than eliminating all T cells as in depleting protocols, this type of strategy might more selectively target effector T cells and thus spare beneficial Tregs [89]. In the past decade, key costimulatory molecules have been identified, the most important in T-cell stimulation and regulation possibly being the CD154:CD40 and the CD28:B7 pathways. In many experimental transplantation models, dual blockade of these costimulatory targets was shown to act synergistically to prevent rejection or induce tolerance.

CD154:CD40 targeted approaches

Various costimulatory molecules have been targeted in rodent models and the most successful results were obtained with the CD154:CD40 pathway blockade using MR1, an anti-CD40L mAb [90], which besides blocking signal 2 may also have a cytotoxic activity towards activated T cells [91]. The CD154:CD40 pathway plays a central role in effective antigen presentation. CD154 (CD40L) is expressed on T cells, B cells, eosinophils, natural killer (NK) cells, platelets and DCs; CD40 is mainly expressed on DCs, macrophages and endothelial cells and its ligation upregulates the expression of B7 and MHC molecules [92]. As all T-cell subsets are not as susceptible to blockade of costimulatory signals, in some models other additional strategies were needed to induce long-term graft acceptance [93].

Various clones of the anti-CD154 mAb have been used in monotherapy in NHP models, resulting in long-term acceptance of renal, heart and islet allografts. Unfortunately, development of alloantibody was not prevented in these experiments, resulting in cellular infiltrates in the biopsies of long-term surviving allografts and eventual graft loss [46,94–96]. Encouraged by the effect of CD154 blockade on prolonging allograft survival and even if true tolerance was not achieved in NHP models, a few trials were initiated. However, the administration of humanized anti-CD154 mAb in transplant recipients did not meet the expectations. Indeed, out of the seven patients enrolled in a clinical trial using the anti-CD154 hu5C8 clone, four patients developed early and three late acute rejections; in addition, three recipients presented with thromboembolic complications (two pulmonary emboli and one transient ischaemic attack) [97,98]. Subsequent analysis revealed that other anti-CD154 clones were also prothrombotic in NHP, this side-effect being possibly because of the expression of CD154 on platelets [98–100].

CD28:B7 targeted approaches

Blocking antibodies against the CD28 ligands CD80 (B7–1) and CD86 (B7–2) have been used but in monotherapy so far they have not significantly prolonged renal allograft survival in NHP models. Combined blockade of CD80 and CD86 led to prolonged survival in NHP, however, without resulting in tolerance as rejection occurred after therapy withdrawal [101–103]. Excellent outcomes were also observed first in small animal models using CTLA-4 Ig, a fusion protein with specificity to CD80/86 expressed on APCs [104]. CTLA-4 Ig was also used in NHP and was described to prolong pancreatic islet survival [105] and, when used in combination with anti-CD154, to induce indefinite acceptance of renal and heart allografts, while allowing prolonged skin graft survival [106,107].

Following these positive results in animal models, clinical trials were initiated and a promising agent in NHP models, LEA29Y (belatacept, a modified CTLA-4 Ig with higher affinity for B7 molecules), has now been used in phase II clinical trials in renal and islet transplantation [108–110]. As for most 'tolerogenic' therapies, while CTLA-4 Ig used alone has been effective in inducing transplantation tolerance in rodents, it had a limited efficacy when transposed to NHP models. This was in part attributed to the relatively low avidity of CTLA-4 Ig for CD86, thus achieving incomplete blockade *in vivo* in large animals. The new compound belatacept has two amino acid substitutions (L104E and A29Y) resulting in a slower dissociation rate from human CD80 and CD86 [111]. However, although the preliminary results obtained with belatacept in clinical trials look promising, one must consider that the combined blockade of CD80 and CD86 simultaneously prevents the ligation of CTLA-4 on T cells, which signalling may contribute to tolerance induction and the function of Tregs [112–114].

Barriers to tolerance in clinical transplantation

The availability of NHP models remains extremely important if not mandatory before the translation of tolerance induction protocols to clinical transplantation. It is worth considering the differences that exist between rodents and larger animals; differences that may indeed explain the disappointing outcomes of some of the most robust animal approaches when applied to humans. Unlike in rodents, memory T cells account for a bigger proportion of the alloreactive T-cell repertoire in larger animals and in humans living in nongerm-free environments. The pool of pre-existing memory T cells in the adult human recipient may therefore play a greater role in allograft rejection even if the transplanted organ differ in MHC from the sensitization alloantigens, because of the cross-reactivity in the T-cell repertoire between antiviral, antibacterial, environmental and transplantation antigens [115–118]. Furthermore, T-cell subsets represent a nonhomogeneous target for immunotherapy as memory T cells are less dependent on costimulatory signals for their activation and may therefore be more resistant to some tolerance induction strategies. Importantly, recent studies have shown that depleting regimens are less effective at eliminating memory T cells that can undergo homeostatic expansion and increase the pool of potential effector T cells [119]. Thus, pre-existing memory T cells and 'heterologous immunity' are considered to be a major barrier in the induction of tolerance in humans [120,121], and strategies have to be developed that could more efficiently target this population without compromising host normal defences to environmental pathogens. As often in biology and medicine, the more we learn, the more we realise that systems are more complex than previously estimated. The immune response indeed involves multiple mechanisms and factors that intervene at different time points and levels. It might therefore not be reasonable to want to limit an immunotherapeutic intervention to only one target and, especially in stringent models and across major MHC barriers, combined strategies appear to have more chances of success.

Conclusions and future prospects

In the past 50 years, transplant biologists have studied new strategies to harness normal mechanisms involved in immune tolerance to promote acceptance of allografts

and thus allow the minimization of potentially harmful immunosuppressive treatments. Encouraging results have emerged from many experimental models and so far relatively limited clinical trials in transplant recipients. To evaluate the efficacy and safety of these new protocols for a wider clinical application, it will be important to be able to monitor individual host immune responses after transplantation. This implies the possibility to detect hyporesponsive or tolerant patients as well as rejectors and follow the evolution of their alloresponses, using a panel of validated biological parameters [122]. Furthermore, detailed immunological studies of the rare 'spontaneous tolerant' patients may bring insights into the mechanisms responsible for the specific silencing of the immune system in a transplant setting [123–125]. Eventually, if tolerance induction becomes a clinical (and reproducible) reality, future prospective trials would probably have to be conducted to compare the new tolerogenic approaches to modern immunosuppressive drug regimens. It is only then that we might be able to better define the optimal antirejection strategies for recipients of organ allografts.

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