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REVIEW

Depleting T-cell subpopulations in organ transplantation

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Summary

T-cell depletion strategies are an efficient therapy for the treatment of acute rejection after organ transplantation and have been successfully used as induction regimens. Although eliminating whole T cells blocks alloreactivity, this therapy challenges the development of regulatory mechanisms because it depletes regulatory cells and modifies the profile of T cells after homeostatic repopulation. Targeting T-cell subpopulations or selectively activated T cells, without modifying Treg cells, could constitute a pro-tolerogenic approach. However, the perfect molecular target that would be totally specific probably still needs to be identified. In this study, we have reviewed the biological activities of broad or specific T-cell depletion strategies as these contribute to the induction of regulatory cells and tolerance in organ transplantation.

Given that allograft rejection is mainly a T-lymphocyte-mediated process, the depletion of recipient T lymphocytes has been an obvious approach to counteract acute rejection in rodents, in nonhuman primate trials and in humans. However, total T-cell depletion might not be favorable for the induction of immunologic tolerance. Hereunder, we have reviewed certain aspects of the depletion of T cells and their subpopulations defined by the expression of target antigens, with a special focus on the induction of regulatory mechanisms in experimental organ transplantation (summarized in Table 1).

Mechanisms of action of depleting antibodies

The dominant parameters influencing the cytotoxicity of antibodies include the isotype and affinity of the antibody, the surface antigenic density and the antigen modulation or internalization of the antigen-antibody complex. The latter can lead to a reduction in the ability of the antibody to produce cell death [1]. The expression of complement regulatory proteins by the target cell [2] is also of considerable significance. In most cases, complement-dependent cytotoxicity and antibody-dependent cell-mediated cytotoxicity are believed to represent the dominant mechanisms of action of the unconjugated Mab, although the induction of apoptosis or cell-cycle arrest could also be highly relevant in other cases [3,4]. Therapeutic antibodies use a combination of these mechanisms to deplete target cells [5]. As regards organ transplantation, whatever the mechanism, it appears that the therapeutic effect not only relates to the efficacy of the depletion but also to the immune reactivity of residual cells that might expand and either contribute to tolerance or to rejection.

T-cell reconstitution and homeostatic proliferation

Given that thymic function declines every year after adolescence, naive T-cell reconstitution is impaired after severe lymphopenia. However, T lymphocytes that previously escaped depletion undergo a homeostatic proliferation to fill free 'space' (for review [6]). This process is under control of interleukin (IL)-7, IL-15 and IL-21 cytokines (for review [7]). IL-15 is released in large quantities after severe depletion of T cells. IL-15 and IL-21 have little impact on naive T-cell proliferation but are important for memory CD8 T-cell function, expansion and survival. Independently of cytokines, post-transplant exposure to alloantigens also contributes to the expansion of memory CD4 and CD8 T cells and to the modification of naive T cells that acquire an effector-memory 'like' phenotype. These cells progressively lose CD62L expression, overex-

Table 1. Antibody-mediated T-cell depletion and transplantation outcomes.

Molecular target/reagent	Mechanism of action	Impact on transplant tolerance
Whole T cells		
Thymocyte (ATG)	Rapid and profound lymphopenia CDC/ADCC/opsonization/phagocytose	Expansion of Treg in human kidney allografts [16]
CD52 (Campath-1)	Cytotoxicity	Expansion Treg and shift from myloïd to regulatory plasmacytoid DC in human kidney allografts [24,29]
CD3	Complement activation/ADCC/apoptosis	Inhibition of acute but not humoral rejection [33]. High frequency of $CD4^+CD25^+$ Treg in kidney NHP allografts [34,35]. Tolerance to heart but not to skin grafts in rodents [30]
CD2	Transient but profound T-cell depletion Inhibition of mitogenic and allogenic responses	Long-term survival of heart grafts in rodents [36] Prolonged kidney allograft survival in NHP [37] High level of FoxP3 mRNA in human kidney allografts [39]
TCR$\alpha\beta$	Transient T-cell depletion	Tolerance in rodents [40]
T-cell subpopulations		
CD4	Profound CD4 T-cell depletion	Tolerance to heart, skin and islet grafts in rodents [41–43,50] No tolerance; modest prolongation in kidney and liver models (mice, dog and NHP) [46–49] Prolongation of pancreas and xeno islet grafts in rodents [51–53]
CD8	Transient but profound CD8 T-cell depletion of donor-specific memory CD8 T cells	No effect on heart grafts in miniature swine and mice [60–62] Moderate prolongation if kidney graft survival in dogs [47] Nondonor-specific tolerance in a small bowel allograft model in mice [8] Tolerance with low dose total body irradiation, thymic irradiation, antithymocyte globulin, anti-CD154 antibody and a brief course of calcineurin inhibitor plus donor bone marrow transplantation in kidney transplantation in primates [59]
CD28	Apoptosis induced cell death	Prevention of graft-versus-host disease [67]
T-cell activation markers		
CD154 (Hu5C8)	Apoptosis of activated T cells	Tolerance possible to kidney grafts in primates and skin grafts in mice [75,76]
LAG-3	Complement dependant cell cytotoxicity	Prolongation of heart graft survival in the rat but prevention of tolerance [78]
IL-2/CD25 (PC61/immunotoxin)	Depletion/immunotoxicity	Prevention of tolerance to heart and liver allografts in mice [84]
CD45 (MB23G2/6G3)	Enrichment in CD45RBlow T cells	Tolerance to kidney allografts in mice [93] Prolongation of survival and tolerance with rapamycin in heart transplantation in mice [91] Prolongs kidney graft survival in primates [98]

press CD44 and are less sensitive to CD28 costimulation. Their cytotoxic activity, proliferative capacities and cytokine production are also enhanced. In contrast with activated T cells, effector-memory-like T cells do not overexpress the CD25 and CD69 activation markers (for review [8]). Therefore, memory T cells disproportionately expand after severe lymphodepletion and become the dominant cell type in humans [9] or experimental models [10]. In the case of rodents, homeostatic proliferation and memory 'like' phenotype are responsible for a resistance to tolerance induction after severe lymphodepletion. In addition, regulatory T cells (Tregs) are depleted as efficiently as naive T cells by current depleting strategies, but might be less suitable for homeostatic proliferation than memory T cells [8]. As predicted, the predominant T-cell type that is present after antibody-mediated T-cell depletion in humans is an activated memory-like T cell. Patients induced with depleting agents without mainte-

nance immunosuppression experienced rejection within 1 month despite 97% T-cell depletion and essentially as a result of the action of residual activated memory-like T cells that predominated peripherally as well as in the allograft during rejection [11]. Thus, the homeostatic proliferation that follows lymphodepletion might hinder the development of transplant tolerance. As described hereafter, regulatory cells can, however, equally expand in the repletion phase after massive T-cell depletion with a possible beneficial effect that needs to be evaluated.

Targeting all T cells

Anti-lymphocyte globulins

The first antibody preparation used since the 1960s is polyclonal anti-thymocyte globulin (ATG, rabbit and horse). ATG induces a rapid and profound lymphocytopenia classically attributed to complement-dependent cytolysis, cell-mediated antibody-dependent cytolysis, opsonization and subsequent phagocytosis by macrophages. In addition, ATG generates various transduction signals to the target cells which interfere with activation signals and can trigger an activation-induced cell death phenomenon [12]. After MOG immunization in the murine model EAE, ATG treatment depleted effector T cells, enhanced the expansion of MOG-specific Tregs ($CD4^+Foxp3^+$) and skewed an auto-antigen-specific immune reaction from a pathogenic T-cell response to a potentially protective T-reg response. Therefore the therapeutic effects of ATG may not only occur because of lymphocyte depletion but also because of the enhanced Treg cell number and function [13]. Equally *in vitro*, rabbit ATG can induce the expansion of functional Treg by converting $CD4^+CD25^-$ T cells through transcriptional enhancement of NFAT1 expression, in turn conferring FOXP3 expression and regulatory activity [14,15]. However, no study in kidney transplantation is available to show tolerance in nonhuman primates or humans following administration of ATG, with the exception of the studies that combined ATG with total lymphoid irradiation and hematopoietic stem-cell transplantation [16]. In clinical practice, outcomes after ATG treatment in kidney transplantation are not different from the use of nondepleting induction treatments such as anti-CD25 monoclonal antibodies, suggesting no clear benefit in terms of a potential pro-tolerogenic effect of ATG [17].

Anti-CD52 (Campath-1)

The CD52 antigen is highly expressed on lymphocytes, monocytes and eosinophils. CAMPATH-1 (alemtuzumab) is a strongly cytotoxic anti-CD52 Mab that has been used to treat lymphoid malignancies for many years [18]. It has also been used for the treatment of several autoimmune diseases such as arthritis, MS, vasculitis, autoimmune cytopenias, etc., and as part of the preparative regimes for allogeneic hematopoietic stem cell transplantation [19]. This antibody has been used in kidney transplantation with low dose cyclosporine A (CsA) monotherapy in the hope of establishing 'prope' or near tolerance [20]. However, there has been no long-term, prospective, randomized study to date that has determined the optimal immunosuppressive regimen to be used with Campath-1. The differing surface expression of CD52 on T-cell subtypes suggests that complement and noncomplement-mediated mechanisms of cytotoxicity by Campath-1 might not equally mediate the killing of all T-cell subtypes *in vivo*. The phenotypic transformation of CD52-positive to CD52-negative T cells can also modulate the action of anti-CD52 cytotoxic antibodies [21]. Although the precise role of CD52 is still unknown, it does not play an essential co-stimulatory role in normal T-cell activation. When cross-linked, anti-CD52 Mabs can transduce an activation signal in resting T cells in a calcineurin-dependent manner [22]. Recently, it has been demonstrated that CD52 signaling by Campath-1 also induces Treg cells that could be expanded by culture with IL-2 and is able to reverse the xenogeneic graft-versus-host disease reactions in SCID mice caused by human PBMC [23]. An increase in $FOXP3^+$ Tregs in Campath-1 treated kidney transplant patients was indeed observed, which was not fully explained by their homeostatic proliferation in the repletion phase, increased thymic output, or Treg-sparing, suggesting a de novo generation/expansion [24]. In Campath-1-depleted kidney transplant recipients that received a reduced dose of mycophenolate mofetil and tacrolimus, there was additionally reported to be a repopulation by immunosenescent T cells of the $CD28^-CD8^+$ phenotype. These cells suppressed the proliferation of $CD4^+$ T cells *ex vivo*. As a result, expanded $CD28^-CD8^+$ T cells might compete for 'immune space' with $CD4^+$ T cells, suppressing their proliferation and therefore delaying $CD4^+$ T-cell recovery [25]. The depletion of effector cells, direct interference with T-cell signaling and upregulation of Treg cells might not account for all the mechanisms of action of anti-CD52 antibodies. An induction with Campath-1 in kidney transplant recipients also caused a sizeable and sustained reduction in the total number of peripheral DC and a significant shift from myeloid to immunoregulatory plasmacytoid DC subsets as early as 1 month post-transplantation [26].

Anti-CD3

After binding to target T cells, anti-CD3 Mab induce only 20–50% T-cell depletion, depending not only on comple-

ment activation and antibody-dependent cell-mediated cytotoxicity (ADCC) but also on the induction of apoptosis through direct signal transduction, independently of the Fc part of the antibody. *In vitro*, it has been established that activated T cells preferentially undergo apoptosis whereas resting T cells are resistant to the action of the original mouse OKT3 antibody or of humanized anti-CD3 antibodies [27,28]. Other target cells that are not depleted *in vivo* lose their CD3 expression as a result of antigen down-modulation [29]. Initially recognized as a nonspecific immunosuppressant, for many years anti-CD3 antibodies have demonstrated their capacity to induce tolerance to heart grafts but not to skin grafts in rodents [30]. That regulatory cells arise after anti-CD3 administration has been shown in a NOD mouse model of spontaneous diabetes, where regulatory $CD4^+CD25^+CD62L^+$ T cells producing high levels of TGF-β increased in number and were able to transfer protection to diabetes [31]. The reason for this might be that anti-CD3 Mabs mimic altered peptide ligands, which can also induce tolerance [32]. Depletion by anti-CD3 antibodies in the kidney grafts of monkeys inhibited the acute cellular but not the humoral rejection [33]. Although a correlation with longer survival could not be proven in primates, the use of anti-CD3 antibodies nevertheless induced a high frequency of $CD4^+CD25^+$ T regulatory cells [34,35].

Other pan-T targets (TCRαβ, CD2, CD45, CD7…)

Targeting CD2 with depleting antibodies resulted in long-term survival in rat cardiac allograft recipients by inducing a transient but profound T-cell depletion and local immunoregulatory mechanisms that are seemingly involved in maintaining long-term graft acceptance [36]. In primates, a rat anti-CD2 Mab inhibiting mitogenic and allogeneic responses *in vitro* provided a rapid peripheral T-cell depletion and slightly prolonged renal allograft survival [37]. Recent insight into patients receiving non-myeloablative haploidentical hematopoietic cell transplantation treated with CD2 antibodies pointed to an expansion of the $CD4^+CTLA4^+FoxP3^+$ Treg cell compartment [38]. In fact, there were high levels of FOXP3 mRNA in a small cohort of kidney transplant recipients from HLA single-haplotype mismatched donors who received CD2 antibody combined with bone marrow transplantation. In these patients, it was possible to discontinue all immunosuppressive therapy 9–14 months after the transplantation and renal function remained stable for many years [39]. T-cell depletion induced by targeting TCRαβ [40] in rat heart transplantation also resulted in similar graft acceptance, suggesting that T-cell depletion *per se* is important rather than the specificity of the molecular target.

Targeting T-cell subpopulations

CD4

As helper CD4 T lymphocytes orchestrate humoral and cellular responses, their depletion using anti-CD4 antibodies appeared to be the best strategy to achieve tolerance to heart [41] or skin allografts in mice [42] and rats [43], alone or in combination with pretransplant intrathymic donor-specific transfusion [44,45]. The depletion of CD4 positive T cells in kidney allotransplantation was less effective: in rats [46], dogs [47] or in monkeys [48], the prolongation of survival was modest and tolerance could not be attained. Similar results were obtained for liver allotransplantation in rats [49]. However, tolerance was achieved in rodents by depleting CD4 helper T cells in islet allotransplantation [50], pancreas allotransplantion and islet xenotransplantion [51–53]. Furthermore, depleting anti-CD4 antibodies cured new-onset diabetes, prevented recurrent autoimmune diabetes, and delayed islet allograft rejection in NOD mice [54]. Therefore, organ specificity is seemingly important and strain specificities in mice is equally a factor of importance [53]. Interestingly, the humoral response to alloantigens that occurred after CD4 depletion in heart allotransplantation was modified from the formation of IgG to IgM alloantibodies only [55]. This might be related to the observation that $CD4^+$ T-cell depletion prevented the development of chronic allograft vasculopathy (CAV) in mice [56]. Finally, whereas depletion of natural Tregs ($CD4\ CD25^{high}$) by anti-CD4 antibodies was considered as a major problem, Yi *et al.* [57] published recently that depleting CD4 antibodies depletes Tregs but not as efficiently as $CD4^+CD25^-$ cells, resulting in an enhanced peripheral $CD4^+CD25^+/CD4^+CD25^-$ ratio and thus promoting tolerance.

CD8

Cytotoxic CD8 T lymphocytes with a memory phenotype ($CD45RO^+CD45RA^-CD25^-$) are highly aggressive towards allografts [58] and resistant to immune regulation [59]. Therefore it appeared useful to specifically deplete these cells in transplantation. Surprisingly, however, the depletion of cytotoxic T cells by CD8 antibodies was not efficient to prevent or treat acute rejection in miniature swine [60], dogs [47] or mice [51,61]. Albeit protective against rejection, the depletion of murine CD8 T cells modifies intragraft cytokine production from Th1 to Th2 and enhances eosinophil, large mononuclear cell and fibroblast-like cell infiltration [61]. In miniature swine, even though the depletion of CD8 T cells did not significantly prolong graft survival in combination with CsA, an inhibition of intimal proliferation in these grafts

was observed, suggesting that the depletion of CD8 T cells could protect from CAV [60]. However, the depletion of CD8 T cells had no effect on CAV in rats [62] and counter-productively increased the severity of rejection of liver allotransplants [49]. In a human pilot study, the depletion of CD8 T cells completely reversed acute rejection in two patients and delayed rejection or was ineffective in four others [63]. In contrast, the depletion of $CD8^+$ T cells induced a nondonor-specific tolerance [8] in the context of small bowel transplantation in mice, suggesting that CD8 T cells indeed play a greater role in the rejection of intestinal transplants. More recently, it has been described that depletion of memory T cells by anti-CD8 antibodies in combination with a low dose of total body irradiation, thymic irradiation, ATG, anti-CD154 antibody, a brief course of calcineurin inhibitor plus donor bone marrow transplantation, could induce tolerance of a previously transplanted kidney allograft in the nonhuman primate. In this model, the depletion of $CD8^+$ T cells was necessary to achieve tolerance [59].

CD28

CD28 is constitutively expressed on most $CD4^+$ T cells and on 50% of $CD8^+$ T cells. Although most anti-CD28 antibodies have been used either to stimulate [64] or to antagonize [65] T cells, certain antibodies can induce target-cell depletion. In fact, although a physiological role of CD28 is to upregulate anti-apoptotic genes in T lymphocytes after antigenic challenge, strong CD28 signaling can also lead to T-cell apoptosis. This is borne out by the observation that CD28 null human T cells manifest resistance to apoptosis in patients with arthritis or sclerosis [66]. Yu *et al.* have looked at the effect of an agonist anti-CD28 antibody (clone 37.51) in mice and found that it surprisingly inhibited donor T-cell expansion. They also found that the effect prevented graft-versus-host disease by selectively depleting alloantigen-activated donor T cells through apoptosis, in an IFN-γ-dependent manner, but spared the T cells that did not recognize recipient alloantigens [67]. One drawback to eliminating $CD28^+$ T cells might be the blockade of immune regulation as CD28 is expressed by a subset of Treg cells and is paramount in their expansion and function [68]. However, certain regulatory cells are controlled by ICOS and not by CD28 [69] and another subset of Tregs, the $CD8^+CD28^-$ cells, can function independently of CD28 [70,71]. Therefore, $CD28^+$ T-cell depletion might still favor or at least spare the subsequent development of Treg cell subsets. In contrast with rodents and primates, agonist anti-CD28 antibodies cannot be used in humans because they caused a massive cytokine storm and a multiorgan failure in six healthy human volunteers in a phase I study [72]. One hypothesis that could explain the different reactivity of human T cells towards stimulation by agonist anti-CD28 antibodies is the differential expression of molecules of the Siglec family that carry ITIMs motifs in the intracytoplasmic domain and actively dephosphorylate tyrosine residues in other signaling molecules. Rodents and monkey T cells express various members of these molecules whereas they are barely detectable in man. The signaling threshold required to activate the intracellular machinery in humans therefore appears much lower and more sensitive to the CD28 signaling [73].

Targeting T-cell activation markers

The selective depletion of activated T lymphocytes as an immunosuppressive induction treatment may result in the development of regulatory cells able to support the long-term survival of allogeneic organs. The proof of concept has been obtained in mice engineered such that their T cells express a viral thymidine kinase suicide gene metabolizing the nontoxic prodrug ganciclovir into a metabolite that is toxic only to dividing cells. After transplantation, this approach therefore depleted alloreactive dividing T cells. The result was a significant delay in the rejection of skin and heart grafts and the induction of an immune tolerance in a fraction of the recipient mice [74]. The therapeutic translation of this strategy requires the targeting of an antigen that would be highly specific for activated T cells. So far, the perfect target is still to be ascertained.

CD154 (CD40Ligand)

CD40 ligand is a co-stimilatory molecule member of the TNF family of membrane receptors expressed mainly on activated $CD4^+$ T lymphocytes. It is also expressed at different levels by mast cells, macrophages, basophils, NK cells, B lymphocytes, as well as nonhematopoietic cells. CD40 ligand binds to CD40 on antigen-presenting cells (APC) and induces APC activation. It also regulates B-cell function by engaging CD40 on the B-cell surface and is expressed by resting platelets in a cryptic way and is rapidly exposed after stimulation. In fact, platelets account for over 95% of the CD40L molecules in the blood. This molecule serves as a receptor for the integrin αIIbβ3 also expressed on stimulates platelets. CD40L/αIIbβ3 interactions are involved in the stabilization of arterial thrombi. The significance of this interaction was underscored by the observation that administration of a humanized CD154 antibody in patients induced thrombosis, an adverse event which halted pending additional clinical evaluation. In addition, thrombotic troubles were found in four animals out of nine treated with anti-CD154

antibodies (5C8.33) [75] in a primate kidney graft model that involved a protocol including nonmyeloablative total body irradiation, thymic irradiation, anti-thymocyte globulin, donor bone marrow infusion and a 1-month course of CsA. The administration of heparin, however, could reduce the incidence of thromboembolic complications. Experiments showing that short courses of CD40L antibody therapy could achieve long-term graft survival in mice and primates [76,77] have been initially interpreted as an effect of the co-stimulation blockade. However, Monk *et al.* [76] showed that much of the efficacy of anti-CD40L therapy derives not from a co-stimulation blockade, but from the destruction of activated T cells. The outcome is a selective purging of potentially aggressive T cells that have experienced antigen. Anti-CD40L also seems to spare Tregs that, although expressing CD40L, might expose fewer antigens or have an enhanced function after CD40L blockade [20].

CD223 (lymphocyte activated gene-3)

Lymphocyte activated gene-3 (LAG-3) is expressed in activated $CD4^+$ and $CD8^+$ lymphocytes residing in inflamed secondary lymphoid organs or tissues, such as human tumors, but not in the spleen, thymus or blood. It is also expressed by graft infiltrating lymphocytes in acutely rejected hearts allografts [78]. LAG-3 is a negative regulator of activated human CD4 and CD8 T cells inhibiting early events in primary activation [79]. Although expressing high levels of LAG-3 mRNA, unstimulated murine $CD4^+CD25^+$ T reg cells, do not express LAG-3 protein on their cell surfaces. However, they do so after activation [80]. Complement-activating anti-LAG-3 polyclonal antibodies have been used in a model of rat cardiac allotransplantation and induced a specific depletion of activated LAG-3^+ T cells without modification of the whole T-cell count. The treatment could reverse an ongoing acute rejection and prolonged graft survival from 5 days in controls to a median of 30 days [78]. However, the same treatment prevented the development of the tolerance otherwise induced by pretransplant donor blood transfusions. Strikingly, it also induced the rejection of tolerated heart allografts 100 days after tolerance induction, by depleting Treg cells [78]. Therefore anti-LAG-3 antibodies can be used to treat acute rejection but do not promote graft acceptance, where Treg cells are instrumental.

IL-2Rα/CD25

The IL-2 receptor is composed of three proteins: the α, β and γ chains, the first being the CD25 antigen. CD25 is not expressed on normal or unstimulated lymphocytes, but it is rapidly transcribed and expressed on activated T cells [81]. The administration of anti-CD25 antibodies in rodents synergized with subtherapeutic administration of Cyclosporine to induce tolerance to pancreatic islet allografts [82]. Tolerance could be achieved in several experimental transplant models (reviewed by Strom *et al.* [83], presumably because many IL-$2R^+$ activated T cells are depleted. However, CD25 is also expressed on Treg cells at very high levels and therefore killing $CD25^+$ T cells will also affect Treg cells. The administration of a depleting anti-CD25 antibody (PC61 clone) reduced the ratio of $CD4^+CD25^+$ Treg cells in the liver and recipient spleen and induced acute rejection [84] in a mouse model where the liver allograft is accepted spontaneously. A similar effect was observed in the bm12 cardiac grafts in the C57Bl/6 recipient mouse model where administration of the PC61 antibody induced a significant decrease in the percentage of $CD4^+CD25^+$ cells in the spleen and broke the tolerance otherwise installed after administration of anti-CD4 antibodies [85]. In addition, the depletion of $CD25^+$ T cells induced rejection [85] in the model, where male CBA/Ca skin grafts are spontaneously accepted in female CBA/Ca recipients expressing a transgenic anti-HY T-cell receptor. In clinical practice, the two available anti-CD25 Mabs (Daclizumab and Basiliximab; IL-2 receptor antagonists) show a diminished capacity to directly kill $CD25^+$ T cells as compared with their murine counterparts and do not interfere with the T reg compartment in kidney [86] and heart [87] transplantation. Moreover, Daclizumab was shown to induce a gradual decline in circulating $CD4^+$ and $CD8^+$ T cells and the expansion of regulatory $CD56^{bright}$ NK cells in multiple sclerosis patients. These regulatory cells negatively regulate activated T cells and might participate in the therapeutic effect of the antibody [88].

CD45 isoforms

CD45 is a protein tyrosine phosphatase involved in signal transduction and early activation by IL-2, IFN-γ and TNF-α. Multiple CD45 isoforms are expressed at varying densities on hematopoietic cells, according to the differentiation status [89]. Cytotoxic T cells, helper T cells and most thymocytes express CD45RB. $CD4^+$ cells that express a high density of CD45RB (in the mouse) and CD45RC (in the rat) on their surface are naive cells that have been shown to cause a number of autoimmune disorders. In contrast, autoimmunity caused by the $CD45RB^{high}$ cells is inhibited by $CD4^+CD45RB^{low}$ cells. Importantly, CD45RBhigh cells have been associated with pancreas transplant rejection [90]. By contrast, $CD45RB^{low}$ cells express FoxP3 [91], exert a regulatory activity and inhibit allograft rejection [92]. Mouse kidney

transplant recipients treated with an induction consisting of anti-CD45RB antibodies (clone MB23G2) acquired a normal kidney graft function for their natural lifespan [93]. At the cellular level, MB23G2 caused a significant drop in the number of circulating lymphocytes, which returned to normal after 1 week. These cells then presented an increased tyrosine phosphorylation of PLCγ1, which is a property of anergic T cells [94]. In a heart transplant model, the same CD45RB antibody induced an enrichment of the $CD45RB^{low}$ population, prolonged survival in monotherapy and induced tolerance if associated with rapamicin. Therefore, the $CD45RB^{low}/CD45RB^{high}$ balance is of critical importance in the induction of tolerance by this treatment [91]. Anti-CD45RB antibodies also induced tolerance to allogenic pancreatic islets [95]. The peri-islet infiltrate from treated animals showed a slight increase in CD4 cells, a decrease in CD8 cells, and a reduced intensity of CD45RB expression, associated with an increase in the intragraft expression of transcripts for IL-4 and IL-10. This was consistent with the emergence of a distinct immunoregulatory T-cell subset [95]. The CD45RA and CD45RO isoforms are used in humans to differentiate between naïve and primed/memory T cells, respectively [96,97]. In peripheral blood, $CD45RA^{+}$ cells also express high levels of CD45RB, whereas CD45R0 cells express little CD45RB. A mouse anti-human CD45 antibody (clone 6G3) has been tested as a monotherapy in primates where it delayed the rejection of kidney grafts for more than 200 days in two out of six animals (median survival time = 27 days) [98]. In these assays, the $CD45RB^{high}/CD45RB^{low}$ ratio decreased during treatment and returned to normal after 1 month. In bitherapy with tacrolimus, the median survival time was prolonged to 72 days [98].

Conclusion

Whether T-cell depletion promotes or precludes the development of immune tolerance is still unclear as on the one hand, it might deplete regulatory cells, but on the other hand, these cells can secondarily dominate as a result of a selective expansion. Further investigations will be needed to understand whether the selective depletion of effector T-cell subpopulations, initially sparing existing regulatory cells, might be a better strategy. The ideal molecular target, however, expressed by alloreactive effector cells but not by resting and Tregs, still needs to be defined.

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