Regulatory T cells and transplantation tolerance: 
Emerging from the darkness?
Herman Waldmann

Sir William Dunn School, University of Oxford, Oxford, OX13RE, UK

The field of tissue transplantation has revolutionized the treatment of patients with failing organs. Its success, thus far, has depended on combinations of immunosuppressive drugs that damp host immunity, while also imposing numerous unwanted side-effects. There is a longstanding recognition that better treatment outcomes, will come from replacing these drugs, fully or in part, by taking advantage of tractable physiological mechanisms of self-tolerance. The past 50 years have seen many advances in the field of self-tolerance, but perhaps, the most tractable of these has been the more recent discovery of a subset T-cells (Treg) whose role is to regulate or damp immunity. This article is intended to first provide the reader with some historical background to explain why we have been slow to identify these cells, despite numerous clues to their existence, and also to indicate how little we know about how they achieve their regulatory function in averting transplant rejection. However, as is often the case in immunology, the therapeutic needs often dictate that our advances move to translation even before detailed explanations of the science are available. The final part of the article will briefly summarize how Treg are being harnessed as agents to interface with or perhaps, replace current drug combinations.

Keywords: Regulatory T cells · Tolerance · Transplantation

Introduction

In order for the reader to understand how we have arrived at our current thinking on Treg, their role in self-tolerance, possible utility in reversing autoimmune disease, and in enabling transplant acceptance, it is important to reflect on the impact of the accumulating historical evidence in the context of technologies as they became available, the derivative dogmas that biased the direction of research and the identification of therapeutic needs to which that knowledge could be applied. Once this is understood, it is then possible to look at the more recent developments and promising avenues for future research which are covered later in this review.

The field of Immunology has made spectacular progress over the past 70 years in parallel with remarkable technological advances. Many of the early breakthroughs involved serological measures of responses to simple antigens and, following the discoveries of thymic derived and Bursa equivalent lymphocytes, more detailed cellular insights. The availability of inbred mouse strains, initially as a biproduct of recreational mouse fanciers, itself led to the discovery of the MHC, and opened up new avenues of investigation into the diversity of T-cell responses, and opportunities to investigate mechanisms underlying graft rejection where foreign tissue grafts, structurally organized, often large, packages of antigens, offered novel challenges to the immunologist. Immune responses became amenable to improved quantitation, so providing enormous opportunities for basic research and its translation. Transplant immunology moved a little more slowly, being somewhat dependent on less quantitative yes/no decisions of graft rejection, and the assumption that grafts were just passive victims to a reactive immune system.

Throughout the early days, scientists were operating on the premise that the immune system must have built-in mechanisms to ensure no attack on self. This belief became dogma following the classical experiment of Medawar and his colleagues [1] in which they observed that newborn mice of one strain could be rendered tolerant of the antigens of a different donor strain.
by the injection of donor splenocytes, as demonstrated by the long-term acceptance of donor skin transplanted later. With the general acceptance of the clonal selection theory [2], clonal deletion had become the widely accepted explanation, although Medawar himself had observed the sluggish performance of infused syngeneic naive lymphocytes in breaking tolerance [3]. Scattered publications over the years [4], suggested that Medawar’s classical transplantation-tolerance induced between different strain combinations might not depend solely on clonal purging, yet these exceptions did not divert thinking away from the dogma of clonal deletion. Publications from Gershon on “infectious tolerance” mediated by murine Lyt-2+ (CD8+) T-cells [5, 6] temporarily raised the possibility of fail-safe regulatory mechanisms, and indeed a transient flurry of papers on the subject, soon followed; however, for one reason or another, perhaps as a result of a shortage of adequate reagents, publications on T-cell suppression” soon fell out of fashion.

Another anomaly influencing the sanctity of clonal deletion as the sole mechanism of self-tolerance was the diverse descriptions of immune privilege in transplantation that had also attracted Medawar’s attention as noted in Ref. [7]. This was a term adopted for the unusual acceptance of foreign grafts without the need for any added immunosuppression. Over the years, there have been many descriptions of tissues exhibiting forms of privilege, although disguised by different descriptors [8–13].

The mAb revolution [14] combined with the rapid evolution of genetic technologies, provided the necessary array of reagents to probe and monitor the immune system. It was, therefore, inevitable that the issue of immune regulation in self-tolerance would resurface. This reawakening came by identifying circumstances in which the lack of supposed Treg in lymphopenic rodent models generated immunopathology in diverse organs [15, 16]. Such immunopathology could be prevented by infusion of a CD4 T-cell subset defined by membrane expression of a particular CD45 isoform [15], or CD25, the α–chain of the IL-2 receptor [17]. These early models did not study the role of antigen in evoking suppression, but rather antigen as a target of attack by effector T cells.

The lack of a meaningful in vitro readout for suppression delayed the resolution of mechanisms and, although suppression of T-cell proliferation has provided one useful correlate of in vivo suppression [18], it has not enabled a deeper understanding of the mechanisms, nor provided information on the antigen specificities used by Treg. This could be for many reasons, for example, the lack of relevant components found in the tissue microenvironment studied, or the formulation of standard tissue culture ingredients to enable optimal immune responses and so, perhaps, less useful for studying suppression. The involvement of antigen in driving suppression became clearer with the identification of a role for suppressive CD4 T cells in certain forms of acquired transplantation tolerance following short-term immunosuppression with cyclosporin A[19] and anticoceptor (CD4/CD8) antibodies [20, 21]. In short, tolerance through regulation could be induced to predefined antigens, enabling research on the roles for antigen from the outset of its induction.

Fast forward then to the description of the clinical multiorgan autoimmune IPEX syndrome [22], the scurfy mouse [23, 24] and ectopic expression of FoxP3 [25], all implicating FoxP3 as a key element in driving CD4+ T cells to become suppressive/regulatory. FoxP3 expression now provided a more precise marker for a set of CD4 T cells that could regulate immune responses. Insertion of a membrane-localizing tag in the Foxp3 locus has since enabled detection, isolation, and ablation of FoxP3+ T cells in vivo [26–28].

The isolation of a TCR used by CD4 T cells to reject a graft [29] made it possible to create TCR transgenic mice-bearing T cells expressing that receptor only, and enabled research into how a whole population of such cells could be therapeutically tolerated. This paved the way for the demonstration that FoxP3 could be induced in peripheral antigen-specific T cells as a route to tolerance [30].

Finally, the discovery that cancers evolve in conjunction with modifications in their tissue microenvironment [31–33], and in which Tregs might play an important part in minimizing immune elimination, has drawn more attention to the notion of suppressive microenvironments, and their role in immunological privilege and tissue graft acceptance.

**Dogma, language, and publication profile**

In the past few years a great deal has been written about Tregs. As has been the case for much of the history of immunology, our use of language and acronyms can sometimes unwittingly mislead. For example, the nomenclature for T cells as either helper or cytotoxic encouraged the assumption that graft rejection is mediated by CD8+ cytotoxic cells, only for it to be later discovered that CD4+ Thelper cells could also mediate rejection [34, 35]. Similarly by referring to Tregs, we make the assumption that regulation might be their only function and that immune regulation is solely mediated by them, both still contentious issues. It is becoming clear that the context in which cell function is assessed may lead to cells having regulatory powers in transplantation (e.g. CD8 T cells [36], Tr1 cells [37], exhausted T cells [38, 39], B cells [40], and so on [41] depending on the context of the graft-host interaction).

It has also become clear that regulation may often require interactions with a subordinate network of recruited compliant cell types. I suggest that this may be more apparent with tissues as targets (as in transplantation) than with simple soluble protein antigens and peptides traditionally used [42] in experimental cellular immunology.

Our vehicles for publication may also, unwittingly, distort the importance of particular discoveries. For example, articles using elegant and technically sophisticated transgenic mice seem to find favor with the “elite” journals, even in the absence of a defined experimental inductive antigen, whereas transplant models, offering a precise package of antigens in a defined location, tend to be published in more specialist journals.
Of course, the field of experimental transplantation is no less liable to premature conclusions than any other area of immunology, with its own special challenges, ranging from the variety of cellular transplants investigated, each with its own relative abundance of foreign antigens, to the surgical damage involved in transplant placement, and the extent to which the different tissues can protect themselves (their natural privilege) from immune attack. For all these reasons, it is sometimes hard to generalize about the regulatory mechanisms in transplantation tolerance.

I hope I have offered the reader some explanation of why research in the field of immune regulation has lagged somewhat behind other immunological areas outside of transplantation, and why research on T-cell mediated regulation (Treg) in preventing graft rejection can make a useful contribution both to understanding mechanisms underlying regulation and their potential for therapeutic applications.

Regulatory T cells in transplantation tolerance

Ever since the Medawar experiment [1], achieving transplantation tolerance with minimal and short-term intervention became valued as a goal for better understanding mechanisms and for enabling drug minimization [3]. The more recent concession that active T-cell-mediated suppression may contribute to self-tolerance, as described above provided the incentive, if that was ever needed, to ask whether such active tolerance could be exploited therapeutically in transplantation.

One obvious strategy was to assess whether known immunosuppressive agents could, when given as a short pulse, achieve graft tolerance through harnessing regulation. This was initially demonstrated with ectopic heart grafts in rats given cyclosporin A [19], and marrow [20, 43, 44] and skin grafts [21, 45] in mice exploiting coreceptor (anti-CD4 and CD8) and costimulation blockade (CD40L) [46, 47], or a CD40L/CD8 combination of both [48, 49]. As is the case for foreign proteins [45], persistent exposure to the tolerizing antigen seems crucial in this form of transplantation tolerance, as its withdrawal resulted in eventual loss of the tolerance [50]. A common, but not widely acknowledged, attribute of that tolerant state was the difficulty experienced in breaking it with lymphocyte transfusions. This resistance was not seen where tolerance had been created by clonal deletion [43, 45, 50, 51], and was shown to be mediated by host T cells [45, 50]. Most likely resistance was not, as first thought, to be an issue of theoretical space for cells to occupy, but almost certainly a demonstration of T-cell-mediated suppression, one that probably explains Medawar's observations where tolerance was not easily broken by injection of naive lymphocytes [3].

Transplantation tolerance induction in adult rodents proved easier to induce to MHC mismatched heart grafts than to skin grafts [52], and, for the latter, easier to induce across mismatches for multiple minor than major histocompatibility antigens [43]. This means that much of our mechanistic data have been selectively derived from the models where tolerance was feasible, but that need not be a reason to assume that the findings are not relevant to the tougher situations.

Tolerance could be shown to involve host processing of donor alloantigens within host APCs, and once induced, could spread to include third-party antigens, when these were presented in a subsequent challenge graft that simultaneously expressed the tolerated antigens (linked suppression) [53, 54] (Fig. 1). One possible explanation for linked suppression was that Treg somehow decommissioned APC such that any third-party antigens within them were only presentable for tolerance. In studies with conventional peptide antigens, Treg demonstrated an ability to strip specific peptide-MHC nominal antigen off the presenting-cell surface (trogocytosis) [55, 56], but did not do so for a third-party antigen. APC so interrogated by Treg could still induce proliferative responses to the third-party peptide [56]. It seems unlikely then, that decommissioning by trogocytosis is sufficient to explain linked-suppression. As we know that host DC must process donor antigens to generate regulatory activity and tolerance [54] one alternative explanation, is that the Treg operate within the graft to provide it with some form of acquired immunological privilege. Acquired privilege generated through linked suppression has potential benefits for regenerative medicine where grafts of developmentally immature tissue cells may come to express new differentiation antigens as they mature, yet still avoid rejection [57].

There is indeed compelling evidence that antigen-specific Treg can localize to the transplanted donor tissues. This derives from
INFECTIONOUS TOLERANCE

Figure 2. Resistance to the breakdown of tolerance by infusions of naïve T-cells, and the acquisition of resistance through infectious tolerance (21). Strain A mice whose T cells were tagged with an accessible (here shown in red) marker (human CD2) were tolerized to strain B skin allografts using coreceptor blockade. Two months later T cells from the same (untagged) strain (here shown in green) were injected into the tolerized recipients, and till could not reject the B-type skin. Cohabitation with the tagged cells and the tolerated graft for 2 weeks, after which tagged T cells were depleted, led to the (green) untagged cells becoming unable to reject the same graft, and resisting breakdown of a further infusion of naive T cells (here shown as pink).

How is the longevity of tolerance maintained?

It is remarkable that a short course of antibody treatment can give lifelong tolerance. Does that mean that the Treg co-opted in the tolerizing phase need to survive and function for the lifetime of the recipient? By exploiting genetically marked T cells, it seems that new Treg could be continuously recruited into the suppressive process. In other words, under the influence of the first cohort of Treg, new ones can be enlisted into action throughout the life of the grafts [21, 27, 64]. Such infectious tolerance (Fig. 2) (distinct from the one-generation suppression seen by Gershon) can also operate in conjunction with linked suppression [64], so that the host can in sequential stages, even become tolerant of a tissue graft from the third party. The mechanisms underlying infectious tolerance are poorly understood, but recently it has been suggested that one way that Treg do this is by releasing exosomes laden with tolerance enhancing cytokines and other immune-inhibitory entities [65].

Nature of Treg operating in transplantation tolerance

The low Treg cell frequencies within the lymphocyte pool has logistic consequences for studying their function after transfer in vivo. Inevitably popular readouts for in vivo suppression have required use of lymphopenic recipients [19, 21]. This may have biased the determination of antigen specificity of Treg induced by antibody therapy [66, 67] by encouraging homeostatic expansion, so favoring expansion of thymic (t)Treg with specificity to self [62, 68] rather than any induced by antigen. CD8+ T-cells mediating suppression have also been described in such lymphopenic systems [36].

This could mean that many of our conclusions, currently based on lymphopenic recipients, may not inform adequately on Treg responses to foreign antigens, nor predict their behavior in lymphocyte-replete systems.

Is this all about tTreg, or are peripherally induced (p)Treg involved?

Although it has been widely assumed that the Treg responsible for therapeutic tolerance are derived from so called natural (t)thymic Treg, there is compelling evidence that peripherally induced (p)Treg are also involved. TCR transgenic mice whose only naïve CD4+ lymphocytes did not naturally express FoxP3, were rendered tolerant by coreceptor blockade to grafts bearing the target antigen [69]. Tolerized recipients were then shown to gain expression of FoxP3 in some of their T cells, especially within...
the tolerated tissue (Fig. 3). That Foxp3 expression was relevant to the tolerance was demonstrated by the failure to tolerate FoxP3-deficient TCR transgenic mice, and by the demonstration that ectopic expression of Foxp3 in TCR transgenic cells rendered them suppressive in vivo [70].

Following the in vitro demonstration that CD4 T cells could be induced to express Foxp3 by crosslinking TCR in the presence of TGF-β [71], evidence for a role for TGF-β in coreceptor antibody-mediated tolerization was provided by the demonstration that induced (i) Treg generated in vitro could also be suppressive and prevent graft rejection in TCR transgenic mice [72]; that neither tolerance nor linked suppression could be induced by a TGF-β neutralizing antibody; and that tolerance could not be induced in TCR transgenic mice genetically defective in TGF-β signaling to T cells, nor in mice lacking a functioning Foxp3 gene [58, 70].

In short, all these studies point to TGF-β dependent pTreg contributing to this type of Treg-dependent experimental transplantation tolerance. Moreover, they suggest that functional Treg do not need to acquire that function in the thymus, but may acquire it in the periphery under appropriate circumstances. Recent studies have suggested that limiting CD28 signaling of naive peripheral human CD4+ T cells can direct them to this function [73], as can exposure of naive CD4+CD25− T cells [74], and γδ T cells to appropriate mediators [75], with obvious therapeutic implications.

A connection between regulatory T cells and those mediating resistance can be inferred by the finding that resistance can be overridden by neutralizing TGF-β [58].

The case for pTreg involvement in infectious tolerance could be strengthened if there were proven markers to distinguish tTreg from pTreg. In the absence, however, of such a fool-proof marker [76], a necessary role for pTreg in the generation of therapeutic tolerance remains to be unequivocally proven.

In relation to the infectiousness of tolerance, one can ask whether the Treg, which have succeeded in preventing graft rejection, can recruit pTreg to help ensure tolerance maintenance. The answer is that this can be seen in TCR transgenic models even in the absence of coreceptor antibody blockade (Fig. 4) [27], with the implication that this could happen in normal mice. This all suggests that once tolerance has been initiated through therapy, it might be self-sustaining by continuous recruitment (infectiousness) of further Treg, both tTreg and pTreg into the antigen-focused microenvironments (Fig. 5).

Cancers as models of privileged microenvironments

If one regards cancers as slowly evolving “foreign” cells creating their own tolerogenic microenvironments, then one could likely identify common themes with tolerated tissue grafts [77]. In that context, it is quite likely that many of the immune checkpoints uncovered in cancer immunology, have relevance in therapeutic graft privilege. First as tumors evolve from single cells, their upfront immunogenicity is minimal. As they grow they have scope, in the absence of strong inflammatory signals, to reshape their local microenvironment so as to let some tumor cells pass through particular immunological checkpoints [31]. By analogy, the successful “blockading” antibody therapies effectively blindfold the immune system so that inflammation and innate immunity are minimized. Effective blockading antibody therapy does not immediately tolerize host T cells (as shown by cell-transfer studies), but this can take some weeks [50]. We interpret this
iTreg enable pTreg to develop in vivo: an example of infectious tolerance.

How might Treg contribute toward creating privileged microenvironments?

Perhaps the simplest way to think about this, is how Treg may be given an advantage over potential T-effectors in any particular location. This could relate to the actual numbers of Treg, as well as their maturity, stability, ability to survive conditions adverse to T-effector, as well as interacting with other necessary cell partners in that tissue. In that context, what can the tissue offer in terms of access to appropriate immunosuppressive partners? There is no reason to think that only one network of interacting cellular partners can deliver effective privilege, and indeed different tissues might well make use of different combinatorial sets.

In search for distinct functional targets to enable privileged microenvironments, a number of possible candidate areas have emerged. For example,

(a) The high expression of CD25 on Treg [17] has raised the possibility that some engineered form of IL-2 might be used to selectively expand Treg in vivo and enable therapeutic benefit in preclinical studies [78–83].

(b) Distinctive metabolic features of Treg in use of glycolysis, oxidative phosphorylation, and lipid metabolism have also attracted much interest if suitable drug candidates could be identified [84–93].

(c) Potential roles for amino acid metabolism and sensing were initiated by the pioneering work of Munn and Mellor who first identified a role for IDO-mediated tryptophan catabolism in preventing a maternal immune attack of the allogeneic fetus [11]. Although this role for IDO has been partially explained by GCN2 sensing [94] and generation of immunosuppressive kynurenines [95–97], an additional explanation is that of nutrient sensing giving rise to inhibition of the mTOR inhibition [98–100]. Such nutrient sensing of tryptophan [101, 102] and other essential amino acids...
with resultant inhibition of mTOR could constitute a significant component of immunologically privileged microenvironments. Mice with mTOR inactivation within their T cells have enhanced generation of FoxP3 \( ^ {+} \) Treg cells, and TORC1 inactivation within FoxP3 \( ^ {+} \) Treg cells appears to break self-tolerance resulting in an IPEX-like syndrome [103]. In line with this, the mTOR inhibitor rapamycin synergises with IL-2 expanded Treg to enable graft acceptance in rodent models, where the “tolerogenic” effect is emphasized locally within the tolerated grafts [104].

(d) One of the early observations for potentially suppressive molecules on Treg was the identification of highly expressed CD39 and CD73, able to generate immunosuppressive adenosine from ATP [105–107]. As TGF-\( \beta \) has been shown to upregulate both molecules on T cells and other nonlymphoid cell types, there is a possibility that ubiquitous adenosine generation may be conducive to generation of disseminated acquired privilege in sites of TGF-\( \beta \) activity.

(e) Another attractive, and potentially translational, theme has been to build on epigenetic studies which favor the expression of diverse immunosuppressive genes by Treg [73, 75, 108–116].

The challenge for clinical translation

The rationale for applying Treg as therapeutic modalities in transplantation, is that current immunosuppressive drugs need to be given long term, tend to penalize the whole immune system risking infections and cancers, and have many unwanted side-effects. Notwithstanding that, they are now relatively cheap, and can be efficacious for many years.

The attraction of exploiting antigen-specific Treg, is that their effects may be localized to the tissue concerned, and hopefully over time through their tolerance-promoting actions, allow drug minimization and even total weaning.

From what we have learned from the animal studies above, it seems that we would need to select and amplify the immunosuppressive function of Treg, and where possible harness the accessory mechanisms of privilege that the transplanted tissue may have. This could, in principle, be achieved by combined use of appropriate drugs/biotherapeutics administered to the patient, or by empowering Treg and/or graft in vitro before their transplantation into recipients.

This latter goal is given some credence by preliminary studies in liver transplantation where complete drug weaning proved possible after adoptive therapy with Treg enriched cells. [117]. However, adoptive cell therapy in transplantation has significant challenges [118], particularly in relation to heterogeneity of T cells from different donors, the logistics of manufacture, personalization, and cost. Also, given the relative dominance and effectiveness of immunosuppressive drugs, clinical trials to show their comparative benefit over drugs are likely to be complex and the ultimate therapy expensive.

Therapeutic success will likely depend on creation of an immunological “ceasefire” for a sustained period following graft placement, one sufficient to control all rejection mechanisms, limiting “danger” signals, and yet allowing tissue healing to complete. It is by no means established that the current best drugs for the inductive stage of transplantation are also the best at sparing those cell functions crucial to creation of a tolerogenic microenvironment. In the case of the preclinical studies with coreceptor and costimulation-blocking antibodies, it was likely important they did not prevent the Treg, from creating a local tolerogenic microenvironment in the transplant, a feature that might distinguish these agents from the current drug options.

Empowering Treg from within

Given the extensive preclinical data available, there has been a somewhat limited effort to apply coreceptor and costimulatory antibodies as used in the preclinical models. The reasons are likely complex, and include concerns on possible toxicities, the high costs of clinical trials to demonstrate clinical benefit over conventional drug combinations, and pharma anguish at the level of income that will accrue for “biologics” that might only be given for a limited period.

One approach we have advocated is to use lymphocyte-depleting antibodies as induction therapy to substantially reduce the number of effector T cells in the body, and thereafter apply appropriate drugs to ensure that the subsequent lymphocyte reconstitution favors regulatory T-cells — a strategy we have referred to as Physician Aided Reconstitution of the Immune System (PARIS for short) [119]. Along those lines, one elegant study that merits mention is that of combining lymphocyte depletion at the time of transplant with the use of a costimulatory inhibitor (belatacept) and sirolimus as induction therapy in renal transplantation, with successful weaning to monotherapy with belatacept in 12 of 19 patients, so sparing unwanted calcineurin and steroid adverse effects and good 5-year outcomes [120].

At a much earlier stage are the studies based on using a monoclonal antibody that selectively targets effector T cells and spares Tregs [121], or the use of low-dose IL-2 to override the inhibitory effect of calcineurin inhibitors on IL-2 secretion [122].

As discussed earlier, one can anticipate in the near future the clinical testing of Histone Deacetylase inhibitors and other approaches to epigenetically stabilize Treg. Agents that could selectively drive TGF-\( \beta \) signaling pathways to convert naive CD4+ T cells to Treg may also have potential [123–126].

Harnessing in vitro expanded Treg from without

Impressive advances have been made in generating GMP protocols for expanding purified Treg in vitro to a point now that allows innovative early studies for their use in transplantation. These
provide useful resources for studies to further amplify the numbers, potency, and stability of Treg for their clinical use. While awaiting comprehensive evidence for their potential utility, initial preclinical data, and safety trials look very encouraging [127–131]. We now await trial efficacy outcomes with creative drug combinations en route to drug minimization.

The lack of information about alloantigen specificity has led to efforts to confer such specificities onto Treg with chimeric antigen receptors (CAR) [112, 132–137]. As CARs in this case are directed to alloantigens expressed on diverse cells in the donor tissue, including endothelia as the first portal of contact, it will be interesting to establish which target locations are necessary for their effects. As insertion of CAR increases the frequency of antigen reactive T cells, there is enthusiasm at gene editing approaches to improve CAR potency [138]. However, likely it is that these can improve CAR Treg function, there remains the constant dilemma of the need to personalize and manufacture these cells. Perhaps, if it was possible to develop universal nonrejectable CAR-T cells then perhaps such “super” Treg would have a therapeutic future. Efforts to create hypoimmunogenic cells are too early in their development [139] to be considered as offering a probable solution in the short term.

Preparing the transplant to develop a beneficial synergy with Treg

Much of the history of transplant immunosuppression has treated the graft as a potential victim waiting to be rejected. The evidence for the existence of natural and acquired privilege has taught us that grafted issues can undergo some sort of remodeling that enables them to resist attack. We certainly need to categorize the key elements of immune privilege, and use this knowledge to enable the grafts to have more efficient liaisons with Treg. This may offer new opportunities for creative premedication of grafts with intelligently chosen perfusates [140].

Conclusions

It seems quite remarkable how regulatory T cells have emerged as important players in immune homeostasis. Given the initial uncertainty about their existence they have, become a major growth industry in advancing knowledge on immunological tolerance and how to harness its mechanism clinically. Although we still remain very much in the dark on what antigens best drive Treg, and how they interact with tissues to achieve suppression, it is remarkable how much effort has gone into exploiting them clinically. The arena of transplant protection has provided a source of antigen around which the biology of these cells can be unraveled. The widespread faith in their curative potential can gain much from a fuller understanding of acquired immunological privilege and tolerance-enhancing microenvironments orchestrated by Treg, and may provide simpler routes to their clinical exploitation that have been contemplated thus far. For me, the remaining big question is whether injected therapeutic Treg can persist for the lifetime of the graft, or whether the host will need to replenish the “sites of action” with further cohorts of regulatory cells through infectious tolerance (Fig. 5). There we find ourselves undoubtedly very much in the dark!

Acknowledgments: HW is now retired, and no funding source need be acknowledged.

Conflict of interest: The author declares no commercial or financial conflict of interest.

References

1 Billingham, R. E., Brent, L. and Medawar, P. B., Actively acquired tolerance of foreign cells. Nature 1953. 172: 603–606.

2 Forsdyke, D. R., The origins of the clonal selection theory of immunity as a case study for evaluation in science. PASEB J. 1995. 9: 164–166.

3 Simpson, E., Medawar’s legacy to cellular immunology and clinical transplantation: a commentary on Billingham, Brent and Medawar (1956) ‘Quantitative studies on tissue transplantation immunity. III. Actively acquired tolerance’ Philos. Trans. R. Soc. Lond. B Biol. Sci. 2015. 370 https://doi.org/10.1098/rstb.2014.0382.

4 van Twuyver, E., Kast, W. M., Mooijaart, R. J., Melief, C. J., De Waal, L. P., Induction of transplantation tolerance by intravenous injection of allogeneic lymphocytes across an H-2 class II mismatch. Different mechanisms operate in tolerization across an H-2 class I vs. H-2 class II disparity. Eur. J. Immunol. 1990. 20: 441–444.

5 Gershon, R. K., Kondo, K., Infectious immunological tolerance. Immunology 1971. 21: 903–914.

6 Gershon, R. K., Kondo, K., Cell interactions in the induction of tolerance: the role of thymic lymphocytes. Immunology 1970. 18: 723–737.

7 Medawar, P. B., Immunity to homologous grafted skin; the fate of skin homografts transplanted to the brain, to subcutaneous tissue, and to the anterior chamber of the eye. Br. J. Exp. Pathol. 1948. 29: 58–69.

8 Bach, F. H., Ferran, C., Hechenleitner, P., Mark, W., Koyamada, N., Miyatake, T., Winkler, H. et al., Accommodation of vascularized xenografts: expression of “protective genes” by donor endothelial cells in a host Th2 cytokine environment. Nat. Med. 1997. 3: 196–204.

9 Streilein, J. W., Niederkorn, J. Y., Induction of anterior chamber-associated immune deviation requires an intact, functional spleen. J. Exp. Med. 1981. 153: 1058–1067.

10 Miyajima, M., Chase, C. M., Alessandri, A., Faroksh, E. A., Delia Pelle, P., Benichou, G., Graham, J. A. et al., Early acceptance of renal allografts in mice is dependent on foxp3(+) cells. Am. J. Pathol. 2011. 178: 1635–1645.

11 Munn, D. H., Zhou, M., Attwood, J. T., Bondarev, I., Conway, S. J., Marshall, B., Brown, C. et al., Prevention of allogeneic fetal rejection by tryptophan catabolism. Science 1998. 281: 1191–1193.

12 Meister, M., Papastasioullou, M., Nordstrom, V., Kumar, V., Ludwig, J., Lui, K. O., Boyd, A. S. et al., Dickkopf-3, a tissue-derived modulator of local T-cell responses. Front. Immunol. 2015. 6: 78.
tions of FOXP3. Organ-specific autoimmune disease induced in mice by elimination of T cell subsets. V. Neonatal administration of cyclosporin A causes autoimmune disease. J. Immunol. 1989. 142: 471–480.

17 Sakaguchi, S., Sakaguchi, N., Asano, M., Itoh, M., Toda, M., Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J. Immunol. 1995. 155: 1151–1164.

18 Thornton, A. M., Shevach, E. M., CD4+CD25+ immunoregulatory T cells suppress polyclonal T cell activation in vitro by interfering with interleukin 2 production. J. Exp. Med. 1998. 188: 287–296.

19 Hall, B. M., Jefford, B. S., Dors, E. D., Specific unresponsiveness in rats with prolonged cardiac allograft survival after treatment with cyclosporine. Mediation of specific suppression by Thelper/inducer cells. J. Exp. Med. 1985. 163: 1633–1649.

20 Qin, S., Cobbold, S., Benjamín, R., Waldmann, H., Induction of classical transplantation tolerance in the adult. J. Exp. Med. 1989. 169: 779–794.

21 Qin, S., Cobbold, S. P., Pope, H., Elliott, J., Klioussis, D., Davies, J., Waldmann, H., “Infectious” transplantation tolerance. Science 1993. 259: 974–977.

22 Bennett, C. L., Christie, J., Ramsdell, F., Brunekow, M. E., Ferguson, P. J., Whitesell, L., Kelly, T. E. et al., The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat. Genet. 2001. 27: 20–21.

23 Clark, L. B., Appleby, M. W., Brunekow, M. E., Wilkinson, J. E., Ziegler, S. F., Ramsdell, F., Cellular and molecular characterization of the scurfy mouse mutant. J. Immunol. 1999. 162: 2546–2554.

24 Fontenot, J. D., Gavin, M. A., Rudensky, A. Y., Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat. Immunol. 2003. 4: 330–336.

25 Hori, S., Nomura, T., Sakaguchi, S., Control of regulatory T cell development by the transcription factor Foxp3. Science 2003. 299: 1057–1061.

26 Miyao, T., Floess, S., Setoguchi, R., Luche, H., Fehling, H. J., Waldmann, H., Hueh, J. et al., Plasticity of Foxp3(+) T cells reflects promiscuous Foxp3 expression in conventional T cells but not reprogramming of regulatory T cells. Immunity 2012. 36: 262–275.

27 Kendal, A. R., Chen, Y., Regateiro, F. S., Ma, J., Adams, E., Cobbold, S. P., Hori, S. et al., Sustained suppression by Foxp3+ regulatory T cells is vital for infectious transplantation tolerance. J. Exp. Med. 2011. 208: 2043–2053.

28 Kim, J. M., Rasmussen, J. P., Rudensky, A. Y., Regulatory T cells prevent catastrophic autoimmunity throughout the lifespan of mice. Nat. Immunol. 2007. 8: 191–197.

29 Scott, D., Addey, C., Ellis, P., James, E., Mitchell, M. J., Saut, N., Jurcevic, S. et al., Dendritic cells permit identification of genes encoding MHC class II-restricted epitopes of transplantation antigens. Immunity 2000. 12: 711–720.

30 Zelenika, D., Adams, E., Mellor, A., Simpson, E., Chandler, P., Stockinger, B., Waldmann, H. et al., Rejection of H-Y disparate skin grafts by monospecific CD4+ Th1 and Th2 cells: no requirement for CD8+ T cells or B cells. J. Immunol. 1998. 161: 1868–1874.

31 Hanahan, D., Weinberg, R. A., Hallmarks of cancer: the next generation. Cell 2011. 144: 646–674.

32 Woo, E. Y., Chu, C. S., Goetzl, T. J., Schlienger, K., Yeh, H., Coukos, G., Rubin, S. C. et al., Regulatory CD4(+)CD25(+) T cells in tumors from patients with early-stage non-small cell lung cancer and late-stage ovarian cancer. Cancer Res. 2001. 61: 4766–4772.

33 Curiel, T. J., Coukos, G., Zou, L., Alvarez, X., Cheng, P., Mottram, P., Evdemon-Hogan, M. et al., Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat. Med. 2004. 10: 942–949.

34 Sprent, J., Schafer, M., Lo, D., Kornfeld, R., Functions of purified L3T4+ and Lyt-2+ cells in vitro and in vivo. Immuno. Rev. 1986. 91: 195–218.

35 Cobbold, S. P., Martin, G., Lovat, P. E., Waldmann, H., Immunosuppression with monoclonal antibodies—rules for effective serotherapy. Adv. Exp. Med. Biol. 1985. 186: 789–795.

36 Guillonneau, C., Picarda, E., Anego, I., CD8+ regulatory T cells in solid organ transplantation. Curr. Opin. Organ Transplant. 2010. 15: 751–756.

37 Roncarolo, M. G., Bacchetta, R., Bordignon, C., Narula, S., Levings, M. K., Type 1 T regulatory cells. Immunol. Rev. 2001. 182: 68–79.

38 Gallimore, A., Glihero, A., Godkin, A., Tisot, A. C., Pluckthun, A., Elliott, T., Hengartner, H. et al., Induction and exhaustion of lymphocytic choriomeningitis virus-specific cytotoxic T lymphocytes visualized using soluble tetrameric major histocompatibility complex class I-peptide complexes. J. Exp. Med. 1998. 187: 1383–1393.

39 Zajac, A. J., Blattman, J. N., Murali-Krishna, K., Sourdive, D. J., Suresh, M., Altman, D. J., Ahmed, R., Viral immune evasion due to persistence of activated T cells without effector function. J. Exp. Med. 1998. 188: 2205–2213.

40 Rosser, E. C., Mauri, C., Regulatory B cells: origin, phenotype, and function. Immunity 2015. 42: 607–612.

41 Graca, L., Transplantation tolerance: context matters. Eur. J. Immunol. 2015. 45: 1921–1925.

42 Shevach, E. M., Foxp3(+) regulatory cells: still many unanswered questions—a perspective after 20 years of study. Front. Immunol. 2018. 9: 1048.

43 Waldmann, H., Cobbold, S. P., Qin, S., Benjamín, R. J., Wise, M., Tolerance induction in the adult using monoclonal antibodies to CD4, CD8, and CD11a (LFA-1). Cold Spring Harb. Symp. Quant. Biol. 1989. 54: 885–892.

44 Remelman, F., Honey, K., Adams, E., Cobbold, S., Waldmann, H., Bone marrow transplantation induces either clonal deletion or infectious tolerance depending on the dose. J. Immunol. 1998. 160: 2645–2648.

45 Qin, S. X., Wise, M., Cobbold, S. P., Leong, L., Kong, Y. C., Parnes, J. R., Waldmann, H., Induction of tolerance in peripheral T cells with monoclonal antibodies. Eur. J. Immunol. 1990. 20: 2737–2745.

46 Taylor, P. A., Friedman, T. M., Kornfeld, R., Noelle, R. J., Blazar, B. R., Tolerance induction of alloreactive T cells via ex vivo blockade of the CD40-CD40L costimulatory pathway results in the generation of a potent immune regulatory cell. Blood 2002. 99: 4601–4609.

47 Taylor, P. A., Noelle, R. J., Blazar, B. R., CD4(+)CD25(+) immune regulatory cells are required for induction of tolerance to alloantigen via costimulatory blockade. J. Exp. Med. 2001. 193: 1311–1318.

48 Honey, K., Cobbold, S. P., Waldmann, H., CD40 ligand blockade induces CD4+ T cell tolerance and linked suppression. J. Immunol. 1999. 163: 4805–4810.
Akeus, P., Scully, R., Cobbold, S., Waldmann, H. Mechanisms in CD4 antibody-mediated transplantation tolerance: kinetics of induction, antigen dependency and role of regulatory T cells. Eur. J. Immunol. 1994. 24: 2383–2392.

Kong, Y. M., Giraldo, A. A., Waldmann, H., Cobbold, S. P., Fuller, B. E., Resistance to experimental autoimmune thyroiditis: L3T4+ cells as mediators of both thyroglobulin-activated and TSH-induced suppression. Clin. Immunol. Immunopathol. 1989. 51: 38–54.

Waldmann, H., Cobbold, S., Regulating the immune response to transplants: a role for CD4+ regulatory cells? Immunity 2001. 14: 399–406.

Davies, J. D., Leong, L. Y., Mellor, A., Cobbold, S. P., Waldmann, H., T cell suppression in transplantation tolerance through linked recognition. J. Immunol. 1996. 156: 3602–3607.

Wise, M. P., Remelman, F., Cobbold, S. P., Waldmann, H., Linked suppression of skin graft rejection can operate through indirect recognition. J. Immunol. 1998. 161: 5813–5816.

Hou, T. Z., Qureshi, O. S., Wang, C. J., Baker, J., Young, S. P., Walker, L. S., Sansom, D. M., A transendoctyosismodel of CTLA-4 function predicts its suppressive behavior on regulatory T cells. J. Immunol. 2015. 194: 2148–2159.

Akkaya, B., Oya, Y., Akkaya, M., Al Souz, J., Holstein, A. H., Kamenyeva, O., Kabat, J. et al., Regulatory T cells mediate specific suppression by depleting peptide-MHC class II from dendritic cells. Nat. Immunol. 2019. 20: 218–231.

Lui, K. O., Howie, D., Ng, S. W., Liu, S., Chien, K. R., Waldmann, H., Tolerance induction to human stem cell transplants with extension to their differentiated progeny. Nat. Commun. 2014. 5: 5629.

Daley, S. R., Ma, J., Adams, E., Cobbold, S. P., Waldmann, H., A key role for TGF-beta signaling to T cells in the long-term acceptance of allografts. J. Immunol. 2007. 179: 3648–3654.

Graca, L., Cobbold, S. P., Waldmann, H., Identification of regulatory T cells in tolerated allografts. J. Exp. Med. 2002. 195: 1641–1646.

Lopez-Ocasio, M., Buszko, M., Blain, M., Wang, K., Shevach, E. M., T follicular regulatory cell suppression of T follicular helper cell function is context-dependent in vitro. Front. Immunol. 2020. 11: 637.

Piao, W., Xiong, Y., Li, L., Saxena, V., Smith, K. D., Hippen, K. L., Paluskievic, C. et al., Regulatory T cells condition lymphatic endothelia for enhanced transendothelial migration. Cell Rep. 2020. 30: 1052–1062.

Fu, H., Kishore, M., Gittens, B., Wang, G., Cee, D., Komarowska, I., Infante, E. et al., Self-recognition of the endothelium enables regulatory T-cell trafficking and defines the kinetics of immune regulation. Nat. Commun. 2014. 5: 3436.

Akeus, P., Szeponik, J., Ahlmann, F., Sundstrom, P., Alsen, S., Gustavsson, B., Sparwasser, T. et al., Regulatory T cells control endothelial chemokine production and migration of T cells into intestinal tumors of APC(mini−) mice. Cancer Immunol. Immunother. 2018. 67: 1067–1077.

Chen, Z. K., Cobbold, S. P., Waldmann, H., Metcalfe, S., Amplification of natural regulatory immune mechanisms for transplantation tolerance. Transplantation 1996. 62: 1200–1206.

Sullivan, J. A., Tomita, Y., Jankowska-Gan, E., Lema, D. A., Arvedson, M. F., Nair, A., Bracamonte-Baran, W. et al., Treg-cell-derived IL-35-coated extracellular vesicles promote infectious tolerance. Cell Rep. 2020. 30: 1039–1051.

Graca, L., Le Moine, A., Lin, C. Y., Fairchild, P. J., Cobbold, S. P., Waldmann, H., Donor-specific transplantation tolerance: the paradoxical behavior of CD4+CD25+ T cells. PNAS 2004. 101: 10122–10126.

Waldmann, H., Graca, L., Infectious tolerance. What are we missing? Cell. Immunol. 2020. 354: 104152.

Maganto-Garcia, E., Bu, D. X., Tarrio, M. L., Alcaide, P., Newton, G., Griff, H. K., Croce, K. J. et al., Foxp3+-inducible regulatory T cells suppress endothelial activation and leukocyte recruitment. J. Immunol. 2011. 187: 3521–3529.

Cobbold, S. P., Castejon, R., Adams, E., Zelenika, D., Graca, L., Humm, S., Waldmann, H., Induction of Foxp3+ regulatory T cells in the periphery of T cell receptor transgenic mice tolerant to transplants. J. Immunol. 2004. 172: 6003–6010.

Regateiro, F. S., Chen, Y., Kendal, A. R., Hilbrands, R., Adams, E., Cobbold, S. P., Ma, J. et al., Foxp3 expression is required for the induction of therapeutic tissue tolerance. J. Immunol. 2012. 189: 3947–3956.

Chen, W., Wahl, S. M., TGF-beta: the missing link in CD4+CD25+ regulatory T cell-mediated immunosuppression. Cytokine Growth Factor Rev. 2003. 14: 85–89.

Hilbrands, R., Chen, Y., Kendal, A. R., Adams, E., Cobbold, S. P., Waldmann, H., Howie, D., Induced Foxp3(+) T cells colonizing tolerated allografts exhibit the hypomethylation pattern typical of mature regulatory T cells. Front. Immunol. 2016. 7: 124.

Mikami, N., Kawakami, R., Chen, K. Y., Sugimoto, A., Ohkura, N., Sakaguchi, S., Epigenetic conversion of conventional T cells into regulatory T cells by CD28 signal deprivation. PNAS 2020. 117: 12258–12268.

MacMillan, M. L., Hippen, K. L., McKenna, D. H., Kadidlo, D., Sumstad, D., DeFor, T. E., Brunstein, C. G. et al., First-in-human phase 1 trial of induced regulatory T cells for graft-versus-host disease prophylaxis in HLA-matched siblings. Blood Adv. 2021. 5: 1425–1436.

Koukanou, L., Peters, C., Sun, Q., Floess, S., Bhat, J., Huehn, J., Kabelitz, D., Vitamin C supports conversion of human gamma delta T cells into FOXP3-expressing regulatory cells by epigenetic regulation. Sci. Rep. 2020. 10: 6550.

Thornton, A. M., Shevach, E. M., Helios: still behind the clouds. Immunol. 2019. 158: 161–170.

Li, C., Jiang, P., Wei, S., Xu, X., Wang, J., Regulatory T cells in tumor microenvironment: new mechanisms, potential therapeutic strategies and future prospects. Mol. Cancer 2020. 19: 116.

Webster, K. E., Walters, S., Kohler, R. E., Mrkvan, T., Boyman, O., Surh, C. D., Grey, S. T. et al., In vivo expansion of Treg cells with IL-2-mAb complexes: induction of resistance to EAE and long-term acceptance of islet allografts without immunosuppression. J. Exp. Med. 2009. 206: 751–760.

Permanyer, M., Bosnjak, R., Glage, S., Friedrichsen, M., Floess, S., Huehn, J., Patzer, G. E. et al., Efficient IL-2R signaling differentially affects the stability, function, and composition of the regulatory T-cell pool. Cell Mol. Immunol. 2021. 18: 388–414.

Sockolosky, J. T., Trotta, E., Parisi, G., Picton, L., Su, L. L., Le, A. C., Chhabra, A. et al., Selective targeting of engineered T cells using orthogonal IL-2 cytokine-receptor complexes. Sci. Rep. 2018. 8: 1037–1042.

Peterson, L. B., Bell, C. J. M., Howlett, S. K., Pekalski, M. L., Brady, K., Hinton, H., Sauter, D. et al., A long-lived IL-2 mutein that selectively activates and expands regulatory T cells as a therapy for autoimmune disease. J. Autoimmun. 2018. 95: 1–14.

Khoryati, L., Pham, M. N., Sherve, M., Kumari, S., Cook, K., Pearson, J., Bogdani, M. et al., An IL-2 mutein engineered to promote expansion of
regulatory T cells arrests ongoing autoimmunity in mice. Sci Immunol. 2020. 5: eaaz2564.

83 Pilat, N., Sprent, J. Treg therapies revisited: tolerance beyond deletion. Front. Immunol. 2020. 11: 622810.

84 Kurniawan, H., Soriano-Bagueu, L., Brenner, D. Regulatory T cell metabolism at the intersection between autoimmune diseases and cancer. Eur. J. Immunol. 2020. 50: 1626–1642.

85 Gerriets, V. A., Kishton, R. J., MO, J., Cohen, S., Siaka, P. J., Nicholas, A. G., Warmoes, M. O. et al., Foxp3 and Toll-like receptor signaling balance Treg cell anabolic metabolism for suppression. Nat. Immunol. 2016. 17: 1459–1466.

86 Tanimine, N., Turka, L. A., Priyadharshini, B., Navigating T-cell immunometabolism in transplantation. Transplantation 2018. 102: 230–239.

87 Howie, D., Cobbold, S. P., Adams, E., Ten Bokum, A., Necula, A. S., Zhang, W., Huang, H. et al., Foxp3 drives oxidative phosphorylation and protection from lipotoxicity. JCI Insight 2017. 2: e89160.

88 Howie, D., Ten Bokum, A., Necula, A. S., Cobbold, S. P., Waldmann, H. The role of lipid metabolism in T lymphocyte differentiation and survival. Front. Immunol. 2017. 8: 1949.

89 Howie, D., Ten Bokum, A., Cobbold, S. P., Yu, Z., Kessler, B. M., Waldmann, H., A novel role for triglyceride metabolism in Foxp3 expression. Front. Immunol. 2019. 10: 1860.

90 Michalek, R. D., Gerriets, V. A., Jacobs, S. R., Macintyre, A. N., MacVyer, N. J., Mason, E. F., Sullivan, S. A. et al., Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4+ T cell subsets. J. Immunol. 2011. 186: 3299–3303.

91 Michaels, A. J., Campbell, C., Bou-Puerto, R., Rudensky, A. Y., Nuclear receptor LXRbeta controls fitness and functionality of activated T cells. J. Exp. Med. 2021. 218:e20201311.

92 Metallo, C. M., Gameiro, P. A., Bell, E. L., Mattaini, K. R., Yang, J., Hillier, K., Jewell, C. M. et al., Reductive glutamine metabolism by IDH1 mediates lipogenesis under hypoxia. Nature 2011. 481: 380–384.

93 Weinberg, S. E., Singer, B. D., Steiner, E. M., Martinez, C. A., Mehta, M. M., Martinez-Reyes, I., Gao, P. et al., Mitochondrial complex III is essential for suppressive function of regulatory T cells. Nature 2019. 565: 495–499.

94 Munn, D. H., Sharma, M. D., Baban, B., Harding, H. P., Zhang, Y., Ron, D., Mellor, A. L., GCN2 kinase in T cells mediates proliferative arrest and energy induction in response to indoleamine 2,3-dioxygenase. Immunity 2005. 22: 633–642.

95 Puccetti, P., Grohmann, U., IDO and regulatory T cells: a role for reverse signalling and non-canonical NF-kappaB activation. Nat. Rev. Immunol. 2007. 7: 817–823.

96 Belladonna, M. L., Orabona, C., Grohmann, U., Puccetti, P., TGF-beta and kynurenines as the key to infectious tolerance. Trends Mol. Med. 2009. 15: 41–49.

97 Pallarino, F., Grohmann, U., You, S., McGrath, B. C., Cavener, D. R., Vacca, C., Orabona, C. et al., Tryptophan catabolism generates autoimmune-preventive regulatory T cells. Transpl. Immunol. 2006. 17: 58–60.

98 Laplante, M., Sabatini, D. M., mTOR signaling at a glance. J. Cell Sci. 2009. 122: 3589–3594.

99 Cobbold, S. P., Adams, E., Farquhar, C. A., Nolan, K. F., Howie, D., Lui, K. O., Fairchild, P. J. et al., Infectious tolerance via the consumption of essential amino acids and mTOR signaling. PNAS 2009. 106: 12055–12060.

100 Condon, K. J., Sabatini, D. M., Nutrient regulation of mTORC1 at a glance. J. Cell Sci. 2019. 132:jcs222570.
1. Waldmann, H., Hilbrands, R., Howie, D., Cobbold, S., Harnessing FOXP3+ regulatory T cells for transplantation tolerance. *J. Clin. Invest.* 2014. 124: 1439-1445.

2. Schmitz, R., Fitch, Z. W., Xu, H., Ghali, A., Mehta, A. K., Guausch, A., Kirk, A. D., Kidney transplantation using alemtuzumab, belatacept, and sirolimus: five-year follow-up. *Am. J. Transplant.* 2020. 20: 3609-3619.

3. Boucault, L., Lopez Robles, M. D., Thiolat, A., Bezie, S., Schmuck-Heneresse, M., Braudeau, C., Vimond, N. et al., Transient antibody targeting of CD45RC inhibits the development of graft-versus-host disease. *Blood Adv.* 2020. 4: 2501-2515.

4. Hu, M., Hawthorne, W. J., Nicholson, L., Burns, H., Qian, Y. W., Liuwanta, D., Jimenez Vera, E. et al., Low-dose interleukin-2 combined with rapamycin led to an expansion of CD4(+)CD25(+)Foxp3(+) regulatory T cells and prolonged human islet allograft survival in humanized mice. *Diabetes* 2020. 69: 1735-1748.

5. Ogawa, C., Tone, Y., Tsuda, M., Peter, C., Waldmann, H., Tone, M., TGF-beta-mediated Foxp3 gene expression is cooperatively regulated by Stat5, Ceb, and AP-1 through CNS2. *Cold Spring Harb. Perspect. Med.* 2013. 4: m3734.

6. Edinger, M., Regulatory T cells for the prevention of graft-versus-host disease: professionals defeat amateurs. *Eur. J. Immunol.* 2009. 39: 2966-2968.

7. Harden, P. N., Game, S., Sawitzki, B., Van der Net, J., Hester, J., Bushell, A., Issa, F. et al., Feasibility, long-term safety and immune monitoring of regulatory T cell therapy in living donor kidney transplant recipients. *Am. J. Transplant.* 2020. 21: 1603-1611.

8. Roemhild, A., Otto, N. M., Moll, G., Abou-El-Enein, M., Kaiser, D., Bold, G., Schachtner, T. et al., Regulatory T cells for minimising immune suppression in kidney transplantation: phase I/IIa clinical trial. *BMJ* 2020. 371: m3734.

9. Tang, Q., Bluestone, J. A., Regulatory T-cell therapy in transplantation: moving to the clinic. *Cold Spring Harb. Perspect. Med.* 2013. 3: a019552.

10. Sawitzki, B., Harden, P. N., Reinke, P., Moreau, A., Hutchinson, J. A., Game, D. S., Tang, Q. et al., Regulatory cell therapy in kidney transplantation (The ONE Study): a harmonised design and analysis of seven non-randomised, single-arm, phase 1/2A trials. *Lancet* 2020. 395: 1627-1639.

11. Sicard, A., Lamarche, C., Speck, M., Wong, M., Rosado-Sanchez, I., Blois, M., Glaichenhaus, N. et al., Donor-specific chimeric antigen receptor Tregs limit rejection in naive but not sensitized allograft recipients. *Am. J. Transplant.* 2020. 20: 1562-1573.

12. Dawson, N. A. J., Rosado-Sanchez, I., Novakovsky, G. E., Fung, V. C. W., Huang, Q., McIver, E., Sun, G. et al., Functional effects of chimeric antigen receptor co-receptor signaling domains in human regulatory T cells. *Sci. Transl. Med.* 2020. 12: eaaz3866.

13. Boardman, D. A., Philippeos, C., Fruhwirth, G. O., Ibrahim, M. A., Hannen, R. F., Cooper, D., Marelli-Berg, F. M. et al., Expression of a chimeric antigen receptor specific for donor HLA class I enhances the potency of human regulatory T cells in preventing human skin transplant rejection. *Am. J. Transplant.* 2017. 17: 931-943.

14. Boardman, D., Maher, J., Lechner, R., Smyth, L., Lombardi, G., Antigen-specificity using chimeric antigen receptors: the future of regulatory T-cell therapy? *Biochem. Soc. Trans.* 2016. 44: 342-348.

15. Pierini, A., Ilipoulo, B. P., Peiris, H., Perez-Cruz, M., Baker, J., Hsu, K., Gu, X. et al., T cells expressing chimeric antigen receptor promote immune tolerance. *JCI Insight* 2017. 2:e92865.

16. Amini, L., Greig, J., Schmuck-Heneresse, M., Volk, H. D., Bezie, S., Reinke, P., Guillonneau, C. et al., Super-Treg: toward a new era of adoptive Treg therapy enabled by genetic modifications. *Front. Immunol.* 2020. 11: 611638.

17. Deuse, T., Hu, X., Gravina, A., Wang, D., Tediashvili, G., De, C. et al., Hypoimmunogenic derivatives of induced pluripotent stem cells evade immune rejection in fully immunocompetent allogeneic recipients. *Nat. Biotechnol.* 2019. 37: 252-258.

18. Tietjen, G. T., Blois, M., Hutchinson, J. A., Cui, J., Deep, D., Song, E., Kraehling, J. R. et al., Nanoparticle targeting to the endothelium during normothermic machine perfusion of human kidneys. *Sci. Transl. Med.* 2017. 9: eaam6764.

Abbreviation: CAR: chimeric antigen receptors

**Full correspondence:**

e-mail: Herman.waldmann@path.ox.ac.uk

Received: 18/1/2021
Revised: 29/3/2021
Accepted: 5/5/2021
Accepted article online: 7/5/2021