Transplant International

REVIEW

Endogenous memory T cells with donor-reactivity: early post-transplant mediators of acute graft injury in unsensitized recipients

Erik H. Koritzinsky[1,2]*, Hidetoshi Tsuda[1]* & Robert L. Fairchild[1,2,3]

1 Department of Inflammation and Immunity, Lerner Research Institute, Cleveland Clinic, Cleveland, OH, USA
2 Department of Pathology, Case Western Reserve University School of Medicine, Cleveland, OH, USA
3 Transplant Center, Cleveland Clinic, Cleveland, OH, USA

Correspondence
Erik H. Koritzinsky and Hidetoshi Tsuda, NB3-30, Cleveland Clinic, 9500 Euclid Avenue, Cleveland, OH 44195, USA.
Tel.: 216-444-3416;
fax: 216-444-3854;
e-mails: koritze@ccf.org (E.H.K.) and tsudah@ccf.org (H.T.)

*These authors contributed equally, and each should be considered primary authors.

SUMMARY

The pretransplant presence of endogenous donor-reactive memory T cells is an established risk factor for acute rejection and poorer transplant outcomes. A major source of these memory T cells in unsensitized recipients is heterologously generated memory T cells expressing reactivity to donor allogeneic MHC molecules. Multiple clinical studies have shown that the pretransplant presence of high numbers of circulating endogenous donor-reactive memory T cells correlates with higher incidence of acute rejection and decreased graft function during the first-year post-transplant. These findings have spurred investigation in preclinical models to better understand mechanisms underlying endogenous donor-reactive memory T-cell-mediated allograft injury in unsensitized graft recipients. These studies have led to the identification of unique mechanisms underlying the activation of these memory T cells within allografts at early times after transplant. In particular, optimal activation to mediate acute allograft injury is dependent on the intensity of ischaemia–reperfusion injury. Therapeutic strategies directed at the recruitment and activation of endogenous donor-reactive memory T cells are effective in attenuating acute injury in allografts experiencing increased ischaemia–reperfusion injury in preclinical models and should be translatable to clinical transplantation.

Transplant International 2021; 34: 1360–1373

Key words
acute T-cell-mediated rejection, costimulation blockade-resistant rejection, ischaemia–reperfusion injury, memory T cells

Received: 7 December 2020; Revision requested: 15 March 2021; Accepted: 3 May 2021;
Published online: 29 June 2021

Introduction

Transplantation is often the only effective treatment for end-stage organ disease. The current standard of calcineurin inhibitor-based immunosuppression has substantially extended the survival of organ transplants. However, long-term graft survival continues to be limited for most transplant patients, with current median survival rates of 12.4 years for kidney, 9.5 years for heart and 11.6 years for liver transplants [1]. Factors undermining current organ transplant survival include immunosuppressive drug-mediated tissue toxicity and the post-transplant de novo appearance of donor-reactive T cells and donor-specific antibodies. There are also several important pretransplant conditions that are acknowledged risk factors exacerbating graft tissue injury and undermining transplant longevity. These include the ischaemic time imposed on grafts prior to transplant that increases ischaemia–reperfusion injury (IRI), and the pretransplant presence of donor-specific

doi:10.1111/tri.13900

antibodies and/or endogenous donor-reactive memory T cells. This review will focus on the source of such donor-reactive memory T cells in unsensitized recipients, how these endogenous memory T cells are activated within allografts to mediate acute graft injury that undermines early- and late-graft outcomes, and potential strategies to obviate this risk factor.

Where do endogenous memory T cells come from in unsensitized individuals?

In general, naïve T cells become activated to differentiate into effector T cells following cognate recognition of foreign peptide/MHC complexes and the delivery of costimulation signals on antigen-presenting cells. After immune-mediated clearance of the antigen, most effector cells undergo apoptosis to contract the reactive repertoire, but a small proportion of the effector cells differentiate into long-lived antigen-reactive memory T cells. The mechanisms directing effector to memory T-cell differentiation during primary T-cell responses remain unclear, with several different proposed models under investigation and have been reviewed elsewhere [2–5].

Allogeneic HLA-reactive memory T cells can be generated by exposure to allogeneic tissue and/or cells following blood transfusions, a prior transplant, or multiple pregnancies [6]. In clinical transplantation, such allosensitized patients have a much higher risk for graft rejection and are more difficult to manage [7–9]. However, the presence of donor-reactive memory T cells in unsensitized recipients raises the obvious question of where and how such memory T-cell originate. Studies in mice have revealed two sources of memory CD8 T cells that are generated in the absence of antigen recognition: innate memory T cells and virtual memory T cells. Innate memory CD8 T cells are generated in the thymus through a process that depends on NK T-cell production of IL-4 prior to their release into the periphery [10,11]. In contrast, virtual memory CD8 T cells are generated in the periphery of naïve mice from precursors expressing high levels of CD5, indicating T-cell receptor (TcR) engagement with self-peptide/self-class I MHC complexes [12]. One mechanism generating virtual memory CD8 T cells is via homeostatic proliferation in lymphodeficient environments by T-cell receptor interaction with self-peptide/self-class I MHC complexes and stimulation with cytokines such as IL-7 [13–16]. Peripheral maintenance of both innate and virtual memory CD8 T-cell populations is dependent on IL-15, and both populations can be activated to produce IFN-γ in response to cytokine or TcR stimulation [17]. Following such activation, virtual memory CD8 T cells can also mediate antigen nonspecific bystander killing activity [12]. Whether these memory T-cell populations are also present and impact ongoing immune responses in humans is unclear, although CD8 T cells expressing similar phenotypes and ex vivo functions have been described [17–19]. Although it is unlikely that innate memory T cells play a role in transplant rejection, it is possible that virtual memory T cells are involved, especially when lymphoablative induction is used.

With relevance to transplant recipients, systemic T-cell depletion induced by polyclonal (e.g. rabbit antithymocyte globulin, ATG) or monoclonal (e.g. anti-CD52 antibody, such as alemtuzumab) depleting antibody affects naïve T cells to a much greater extent than memory T cells [20–23]. The subsequent rapid expansion of T cells by homeostatic proliferation leads to an increase in T cells expressing a memory phenotype and function. Ayasoufi and colleagues have reported that CD40–CD154 (CD40L) interactions between memory CD4 T cells and B cells are required for CD8 T-cell reconstitution and proliferation after ATG-mediated T-cell depletion in mouse heart transplantation models [24]. Their subsequent studies revealed that B-cell-derived IL-1β and IL-6 also regulate this T-cell recovery [25]. Whether these memory CD8 T cells express the phenotype of virtual or conventional T cells has not been closely examined to date. Nevertheless, further understanding the mechanisms of T-cell homeostatic proliferation and reconstitution after lymphoablative induction therapy could be instructive for devising strategies to diminish the generation of donor-reactive memory T cells and improve the efficacy of this induction strategy in transplantation.

Donor-reactive memory T cells can also arise from prior exposure to unrelated environmental antigens, an occurrence termed heterologous immunity. The low affinity/degenerate nature of T-cell receptor binding to peptide/self-MHC complexes coupled with the lower threshold of epitope and costimulation expression required to elicit memory T-cell responses often results in memory T-cell responses to peptide/MHC complexes that are distinct from those that originally generated the memory T cells. Selin and coworkers showed that prior T-cell memory generated to viral exposure can enhance the primary response to a different virus through recognition and activation to the novel pathogen [26]. Such heterologous memory CD8 T-cell immunity often plays a role in responses to infectious agents although the protection afforded is usually not as complete as that observed to the primary infection.

Heterologous memory T-cell immunity is often an important component of immune responses to MHC-disparate allografts in unsensitized recipients and can undermine transplant survival. In vitro studies of human memory T cells generated in response to Epstein–Barr virus, cytomegalovirus, varicella zoster virus and influenza virus infection showed that 45% of virus-specific T-cell clones demonstrated cross-reactivity with allogeneic HLA molecules [27]. These and other studies demonstrating reactivity of pathogen-induced memory T cells to allogeneic MHC molecules predict that such heterologous immunity in transplant recipients might generate responses to an allograft and compromise the transplant. In support of this potential response, Adams and colleagues have used mouse models to demonstrate that memory T cells generated by viral infection bind tetramers of allogeneic class I MHC molecules and produce IFN-γ upon stimulation with MHC-mismatched donor splenocytes [28]. They further showed that the presence of these virally generated memory T cells accelerated skin allograft rejection and prevented tolerance induction. Several other groups have also shown that the T-cell repertoires generated in response to a variety of pathogens display cross-reactive alloreactivity that impedes the development of transplant tolerance [29–38].

Endogenous memory T cells expressing donor-reactivity in clinical transplantation

The presence of endogenous memory T cells with donor-reactivity in transplant patients significantly impacts graft function and survival. Seminal studies by Heeger *et al.* [39–43] reported that the pretransplant presence of high numbers of donor-reactive T cells in the peripheral blood of kidney transplant patients was associated with an increased incidence of acute graft rejection and poorer early graft function during the first-year post-transplant. The donor-reactive T cells were identified by ELISPOT assays to detect IFN-γ producing cells during overnight co-culture of recipient peripheral blood cells and donor stimulator cells. Several subsequent studies used similar IFN-γ-producing T-cell ELISPOT approaches to confirm the association of pretransplant endogenous memory T cells with increased acute rejection and decreased kidney graft function [44–47]. Recent work has shown that the composition of circulating memory CD8 T cells one-year postkidney transplant, particularly the frequency of terminally differentiated effector memory CD8 T cells, can also predict the risk of graft failure [48]. In addition, other groups have reported that the presence of $CD45RO^+$ memory T cells in biopsies of heart and kidney allograft patients correlates with the severity of graft rejection [49–52]. Collectively, these observations sparked efforts to better understand the pathogenic role of pre-existing donor-reactive memory T cells in undermining transplant outcomes.

Mechanisms of endogenous memory T-cell-mediated graft injury

Clinical studies indicating that the pretransplant presence of higher numbers of endogenous donor-reactive memory T cells correlates with worse transplant outcomes raised questions about the identity of these memory T cells and how they mediate graft injury. It was assumed that laboratory mice housed under conventional-specific pathogen-free (SPF) conditions would lack these populations of heterologous endogenous memory T cells and would not be useful for studying endogenous memory T-cell reactivity to allografts in unsensitized recipients. This assumption spurred many studies in rodent models using an alternative approach to investigate the impact of memory CD4 and CD8 T cells on allografts by directly priming memory T cells with skin allografts or by immunization with allogeneic cells and then testing the activation and function of the donor-primed memory T cells in response to a graft from the sensitizing donor. For the most part, these studies produced results similar to those observed for memory T cells generated in response to viral, bacterial and model antigens.

Initial studies from our laboratory indicated that CD8 T cells with a memory phenotype infiltrated heterotopically transplanted heart allografts in unsensitized SPF mouse recipients within 12 h of graft vascularization and their numbers within the allografts increased with time after transplant [53,54]. This early post-transplant allograft CD8 T-cell infiltration was accompanied by their rapid production of IFN-γ and increased infiltration of neutrophils, findings that were not observed in isografts. Histologically, the allografts had obvious pockets of tissue necrosis that were dependent on IFN-γ and on CD8 T-cell and neutrophil infiltration. This histopathology occurred several days prior to detection of the primary (i.e. naïve) donor-reactive T-cell response in the recipient spleen, implicating memory CD8 T cells as a key mechanism underlying the allograft inflammation. Memory CD8 T-cell activation to produce IFN-γ was dependent on allograft expression of allogeneic class I MHC and memory T-

Transplant International 2021; 34: 1360–1373

cell expression of ICOS that was induced during their proliferation within the allografts [55]. Notably, memory CD8 T-cell activation to expand and produce IFN-γ within the allografts during the first 1–2 days after transplantation was not diminished by the absence of recipient CD4 T cells or by blocking CD154- or CD28-mediated costimulatory signals [56].

The conspicuous activation of memory CD8 T cells to express effector functions within heart grafts expressing allogeneic class I MHC raised questions about the ability of the memory CD8 T cells to mediate acute rejection of the allografts. Peri-transplant treatment with anti-LFA-1 mAb inhibited memory CD8 T-cell infiltration into allografts but also inhibited the de novo priming of donor-reactive T cells, preventing the ability to distinguish the impact of the endogenous donor-reactive memory CD8 T cells and the de novo primed donor-reactive T cells on rejection of the allografts [57]. To separate these effects, recipients were treated with anti-LFA-1 mAb on days 3 and 4 post-transplant, which allowed early memory CD8 T-cell infiltration and activation within the allografts while maintaining inhibition of the de novo donor-reactive T-cell response. This strategy indicated that activation of allograft infiltrating memory CD8 T cells alone could not mediate sufficient acute graft injury to provoke rejection. In support of this, peri-transplant treatment with CTLA-4Ig had no effect on the infiltration and activation of endogenous memory CD8 T cells but did inhibit de novo donor-reactive T-cell priming and resulted in long-term survival of the allografts. Overall, these results indicated that the endogenous memory CD8 T-cell activation within allografts transplanted to unsensitized recipients increased graft inflammation at early times post-transplant but not to the intensity required to mediate acute graft injury and rejection.

In clinical transplantation, organs from deceased donors are typically subjected to several hours of cold ischaemic storage (CIS) prior to transplant. Importantly, increasing times of CIS for clinical transplants have been shown to correlate with poorer graft outcomes [58–61]. Prolonged CIS leads to greater intensity of IRI after vascularization through increased graft production of reactive oxygen species and pro-inflammatory cytokines and increased recipient leukocyte infiltration (Fig. 1). In contrast, transplant procedures in rodent models are typically performed as quickly as possible, subjecting grafts to minimal (approximately 30–60 min) CIS time and do not account for the increased inflammation and oxidative stress caused by longer CIS time in the clinical setting. This realization led us to test a more clinically relevant model of transplantation where grafts are subjected to prolonged (6–8 h) CIS prior to transplantation in anticipation that graft vascularization in the recipient would be followed by more intense IRI and possibly by increased activation of the endogenous donor-reactive memory CD8 T cells within the allografts.

When compared to minimal CIS, imposition of longer CIS on allografts prior to transplant led to marked increases in both acute phase cytokine (IL-1β, IL-6 and TNF-α) production and in the intensity of macrophage, neutrophil and memory CD4 and CD8 T-cell infiltration into allografts during the first 2 days post-transplant [62]. The longer CIS times also induced slight increases in these inflammatory characteristics in isografts, but not to the magnitude observed in allografts. Peri-transplant deletion of recipient CD8 T cells attenuated the increased early post-transplant inflammatory response in allografts, indicating the key role of endogenous donor-reactive memory CD8 T cells in elevating the intensity of the ongoing IRI. The increased inflammation in allografts subjected to prolonged versus minimal CIS was associated with marked increases in memory CD8 T-cell proliferation and expression of IFN-γ, perforin and granzyme B. In recipients conditioned with peri-transplant CTLA-4Ig, allografts subjected to prolonged CIS were rejected between 15 and 25 days post-transplant whereas those subjected to minimal CIS survived beyond 50 days. CTLA-4Ig conditioning of recipients of high ischaemic allografts inhibited the appearance of de novo primed donor-reactive T cells in the spleen at the time of allograft rejection, implicating increased endogenous donor-reactive CD8 T-cell activation in directly mediating the allograft rejection. In support of this, CTLA-4Ig-resistant rejection of high ischaemic allografts was abrogated by depletion of recipient CD8 T cells prior to transplant. These results linked the activation of the heterologous donor-reactive CD8 T-cell response within the allograft to the increased CIS and intensity of IRI following graft vascularization, two important risk factors undermining graft outcomes in clinical transplantation.

The presence of donor-reactive memory CD8, and CD4, T cells within the endogenous memory T-cell repertoire of unsensitized recipients suggested that expansion of those T cells reactive to donor allogeneic class I and class II MHC molecules would be required to achieve the pathogenic response to the allograft. Further study revealed important differences in endogenous memory CD8 T-cell proliferation observed within allografts subjected to prolonged versus minimal CIS. Unlike the CD4 T cell- and CD154-independent low

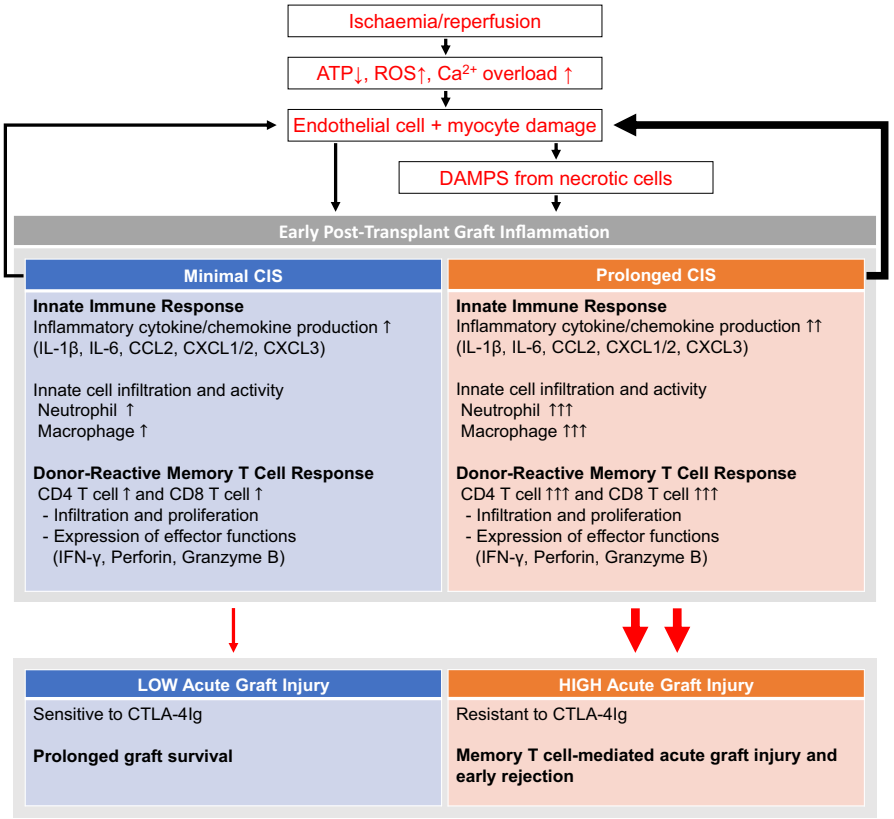

Figure 1 Prolonged cold ischaemic storage exacerbates ischaemia–reperfusion-mediated acute graft injury leading to CTLA-4Ig-resistant rejection and worse outcomes. Ischaemia–reperfusion injury disrupts energy and ion homeostasis in cells and is characterized by decreased ATP production, increased ROS generation and intracellular Ca^{2+} accumulation. These stressors cause cell damage and death with release of DAMPS that perpetuate acute graft injury. Early after graft reperfusion in the recipient, prolonged CIS causes increased pro-inflammatory cytokine and chemokine production, innate immune cell infiltration, and CD4+ and CD8+ T-cell activation and differentiation. This more aggressive early post-transplant immune response leads to worse acute graft injury and CTLA-4Ig-resistant rejection.

level activation of memory CD8 T cells observed in low ischaemic allografts, the increased proliferation of endogenous memory donor-reactive CD8 T cells within high ischaemic allografts required help generated by graft infiltrating endogenous memory CD4 T-cell recognition of allogeneic class II MHC molecules plus delivery of costimulation through CD154 interactions with CD40 expressed by graft cells [63]. One key product of this help was revealed by experiments showing that high ischaemic allografts have increased mRNA and protein expression of the p40 subunit of the IL-12 family of cytokines without increases in the p35 subunit of IL-12 or the p19 subunit of IL-23. The absence or neutralization of p40 but not p35 or p19 abrogated the increased memory CD8 T-cell proliferation observed in high ischaemic allografts, suggesting a role for p40 homodimers (p40HD) in driving the proliferation. In support of this hypothesis, high ischaemic allografts had increased levels of p40HD protein but not p70/IL-12 (p40 + p35) heterodimers when compared with levels in low ischaemic allografts 24–48 h after transplant. However, increased memory CD8 T-cell proliferation within low ischaemic allografts is induced by peri-transplant administration of p40HD, but not IL-12 or IL-23 heterodimers (H Tsuda, manuscript submitted). Despite the role of p40 HD in this proliferation, in vitro studies indicated the failure of p40HD to directly induce proliferation of memory CD8 T cells sorted from either the high ischaemic allografts or the spleens of naive mice, suggesting that p40HD might promote memory CD8 T-cell proliferation through an indirect mechanism. This was further suggested when administration of p40HD to recipients of allo- or isografts subjected to 30 min of CIS stimulated increased graft levels of IL-15 but not IL-2. These results are consistent with many studies indicating the key role of IL-15 in memory CD8 T-cell and NK cell homeostasis and proliferation [64–66].

Treatment with blocking antibodies to CD122 (shared IL-2/IL-15 receptor β chain), but not to CD25

Transplant International 2021; 34: 1360–1373

(IL-2 receptor-specific α chain), inhibited p40HD-driven memory CD8 T-cell proliferation in both low and high ischaemic allografts, directly supporting a role for p40HD-induced IL-15 in memory CD8 T-cell proliferation within the allografts (H Tsuda, manuscript submitted). It is important to note that administration of p40HD to recipients of isografts also induced IL-15 but low levels of graft infiltrating memory CD8 T-cell proliferation, indicating the importance of alloantigen in IL-15-mediated intra-graft proliferation. While the production and role of p40HD in allografts had not been previously identified, the importance of IL-15 signalling in alloreactive memory T-cell activation has been shown in both humans [67] and animal models [68–70]. Consistent with the critical roles of p40HD and IL-15 in endogenous memory CD8 T-cell activation within highly ischaemic allografts, recipient treatment with anti-CD122 or anti-p40 mAb abrogated the CTLA-4Ig-resistant rejection of high ischaemic allografts (H Tsuda, manuscript submitted). Furthermore, the increased memory CD8 T-cell proliferation and CTLA-4Ig-resistant rejection of high ischaemic allografts from wild type donors was not observed in IL-15Rα$^{-/-}$ donors, suggesting that the source of the IL-15 required for endogenous donor-reactive memory CD8 T-cell activation is allograft- and not recipient-derived. The need for donor IL15Rα suggests that IL-15-mediated endogenous donor-reactive memory CD8 T-cell activation occurs via trans-presentation and/or release of soluble IL-15/IL15Rα complexes by donor-derived cells, but not via IL-15 cis signalling [66, 71]. These results are in line with studies by Adams and colleagues indicating that anti-CD122 mAb plus costimulatory blockade with anti-CD154 mAb plus CTLA-4Ig promotes long-term skin allograft survival in mice and that anti-CD122 mAb plus CTLA-4Ig extends complete MHC mismatched kidney allograft survival in rhesus macaques [68].

The findings in mouse models testing endogenous memory CD8 T-cell activation within allografts subjected to minimal vs. prolonged CIS prior to transplant indicate the role of increased ischaemia and alloantigen in promoting optimal proliferation of endogenous memory CD8 T cells within the allografts. These increases are dependent on the helper signals generated in high, but not low, ischaemic allografts, suggesting that the activation and/or function of at least some components of this help require a high ischaemic environment. A similar approach using prolonged CIS prior to transplant of heterotopically transplanted heart allografts indicated that the increased ischaemia increases graft infiltrating CD4 T-cell alloreactivity through increased IL-6 signalling and allograft dendritic cell immunogenicity through increased TLR4 signalling [72,73]. Importantly, administration of p40HD to recipients of low ischaemic allografts induces greater endogenous memory CD8 T-cell proliferation but is not sufficient to cause CTLA-4Ig-resistant allograft rejection (H Tsuda, manuscript submitted). The memory CD8 T cells in this environment do not express IFN-γ, perforin and granzyme B at the high levels observed within high ischaemic allografts, indicating the need for the increased ischaemic environment to achieve expression of the effector functions that mediate acute allograft injury. It is possible that in addition to p40HD production, other CD4 T-cell-derived helper signals are generated in the highly ischaemic environment that are required for the endogenous memory CD8 T cells to express the increased levels of effector function. Overall, these findings link the high ischaemic environment with the increases in effector function expression and the ability of the endogenous donor-reactive memory CD8 T cells to mediate acute allograft injury.

It is likely that endogenous memory CD8 T cells with donor-reactivity also function to mediate graft injury following resolution of IRI. Certainly the pretransplant presence of such T cells has been identified as a risk factor for increased acute rejection during the course of the first year post-transplant [39–47]. In such cases, the activation of these memory T cells would be predicted to be resistant to standard of care immunosuppression and possibly to costimulatory blockade. The BENEFIT trial tested the impact of replacing the calcineurin-inhibitor cyclosporine A with CTLA4-Ig (belatacept) in a large cohort of kidney transplant patients [74,75]. The rationale for this trial was to test an immunosuppressive regimen that avoids the known nephrotoxic and other side effects that accompany calcineurin-inhibitor use. After seven years of follow-up, mean eGFR, graft survival and overall survival were higher among patients in the belatacept treated cohort compared to those treated with cyclosporine A [75]. Despite these improvements, there was a higher incidence of acute cell-mediated rejection with higher Banff grade classification in patients in the belatacept treatment cohort compared to those who received cyclosporine A [74]. Although not directly tested, these results suggested the emergence of a CTLA-4Ig-resistant donor-reactive T-cell response in this subset of patients. These results spurred many studies into CTLA-4Ig-resistant activation of human memory CD4 and CD8 T-cell populations. Interestingly, in vitro activation of memory CD28^{-} T cells requires exogenous IL-15 [67,76]. The identification of these T

cells and the development of targeted therapies to mitigate the increased risk of acute T-cell-mediated rejection events at later times post-transplant as well as in belatacept treated patients will be important to facilitating broader adoption of this treatment approach.

Potential therapies targeting endogenous donor-reactive memory T cells

Current standard of care transplant immunosuppression is effective at limiting the activation of de novo donor-reactive T cells but may not be as effective for endogenous donor-reactive T cells. Thus, the risk presented by endogenous donor-reactive memory T cells to graft function and survival raises the need to develop strategies to inhibit this mechanism of allograft injury. There are many approaches to targeting memory T cells currently under investigation (summarized in Table 1) that can be broadly classified as blocking memory T-cell recruitment to the graft or blocking their activation within the graft. Because of the relationship between the intensity of IRI and endogenous memory T-cell activation, therapies directed at attenuating IRI and its effects should also blunt memory T-cell activation. Work from our laboratory indicates that recruitment and activation of alloreactive endogenous memory T cells, especially in a prolonged CIS environment, occurs early after graft revascularization. This suggests that novel treatment strategies to block endogenous memory T-cell-mediated allograft injury should focus on this early post-transplant window of opportunity and, unlike many other approaches in transplant immunosuppression, will likely not require chronic treatment.

Directed at attenuating IRI

IRI is an inherent and important mediator of tissue injury in solid organ transplantation, and it is well known that increased IRI correlates with worse graft outcomes. IRI causes direct injury through the generation of reactive oxygen species (ROS), pro-inflammatory cytokines and various damage-associated molecular patterns (DAMPs), such as mitochondrial DNA and fragments of extracellular matrix proteins [77,78]. Subsequent cell death, endothelial cell dysfunction and immune system activation act synergistically to enhance this pathology. Studies from this and other laboratories have indicated the relationship between increased IRI and endogenous memory T-cell activation, suggesting that approaches to attenuate IRI should also blunt the early donor-reactive memory T-cell response in transplantation.

There is significant interest in developing strategies to attenuate IRI in transplantation and other pathologies. Although not always feasible, the most straightforward approach to reduce IRI in transplantation is the minimization of CIS time, which is known to improve outcomes in deceased-donor transplantation [58–61]. Although preclinical studies that target ROS in transplant IRI models have shown some benefit [79–82], findings from clinical trials studying the use of antioxidants or ROS scavengers in clinical transplantation have been mixed [83–88]. Despite these results, there remains interest in targeting ROS in transplantation, with newer, more specific approaches under investigation [89,90]. Another approach to reduce the damage caused by IRI is to block the key pro-inflammatory cytokines generated during this process. These include TNF-α, IL-6 and IL-1β, each of which can be blocked with monoclonal antibodies, soluble receptors or small molecules that are already in use to treat autoimmune diseases. Ex vivo organ perfusion is also known to reduce graft IRI upon revascularization in the recipient [91–93]. Additionally, in vitro studies have shown that the Steen solution used as perfusate for ex vivo lung perfusion has antioxidant effects on lung parenchymal cells and is likely to further attenuate IRI [94]. Adoption of ex vivo organ perfusion for deceased-donor organs is steadily increasing and its use should help attenuate the extent of IRI-induced donor-reactive memory T-cell activation in these transplants.

Directed at recruitment of endogenous memory CD4 and CD8 T cells

Multiple groups have shown the promise of targeting integrins and other adhesion molecules upregulated specifically on memory T cells to block their early infiltration into allografts. Preclinical and clinical studies have focused on CD2 [95,96], lymphocyte function-associated antigen-1 (LFA-1) [57,97] and very late antigen-4 (VLA-4) [98,99], which interact with lymphocyte function-associated antigen-3 (LFA-3), intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), respectively, expressed on activated endothelium. In addition to their critical roles in T-cell and NK cell adherence, ligand engagement of CD2, LFA-1 and VLA-4 deliver costimulatory signals to the expressing cell. Therefore, targeting these molecules may inhibit both memory T-cell recruitment and activation to express the effector functions mediating graft injury.

Immunosuppressive agents targeting each of these adhesion molecules have been used clinically for other

Table 1. Pharmacological approaches to targeting endogenous memory T cells.

	Function	Drug type	Drug name(s)	Studies in solid organ transplantation (clinical and NHP)	Approved clinical uses for other indications	Key barriers to clinical use
Targeting memory T-cell recruitment						
Lymphocyte function associated antigen-1 (LFA-1)	Adhesion molecule on T cells, binds ICAM-1 on activated endothelium	Monoclonal antibody	Efalizumab	Clinical: [107] NHP: [97,128]	Psoriasis (withdrawn from market)	Withdrawn from market due to cases of PML with chronic use
Very late antigen-4 (VLA-4)	Adhesion molecule on T cells, binds VCAM-1 on activated endothelium	Monoclonal antibody	Natalizumab	Clinical: NHP:	Multiple sclerosis, Crohn's disease	Increased risk of PML
CD2	Adhesion molecule on T cells, binds LFA-3 on activated endothelium	Fusion protein	Alefacept	Clinical: [129] NHP: [96]	Psoriasis	Discontinued after supply chain disruption
		Monoclonal antibody	Siplizumab	Clinical: [100–102] NHP: [104]		Human-specific, limits ability to study in nonhuman primates
Targeting memory T-cell activation and proliferation						
CD40	Costimulatory molecule, binds CD-154	Monoclonal antibody	Bleselumab/ ASKP1240/4D11, Iscalimab/CFZ-533	Clinical: [118,119] NHP: [130–133]		
CD154/CD40L	Costimulatory ligand, binds CD-40	1st generation: Monoclonal antibody	Ruplizumab/ hu5C8, toralizumab, ABI793	Clinical: NHP: [134–138]		Increased risk of thromboembolism, development halted
		2nd generation: Fab' (antigen-binding fragment)	Dapirolizumab pegol	Clinical: NHP: [117]		
		Fc-modified monoclonal antibody	Letolizumab/BMS-986004			
		Fusion protein	VIB4920/ MEDI4920			
p40	Subunit of IL-12 cytokine family members	Monoclonal antibody	Ustekinumab, Briakinumab	Clinical: NHP:	Psoriasis, Crohn's disease, Ulcerative colitis	
CD122	Shared IL-2 and IL-15 receptor β-chain	Monoclonal antibody	HuABC2, Hu-Mik-Beta-1	Clinical: NHP: [68]		

indications. Alefacept, a fusion protein that blocks CD2 on T cells, was originally approved as a treatment for psoriasis [52] and showed promise as an adjunct to costimulatory blockade in a nonhuman primate renal transplant study [96]. Its production was voluntarily discontinued in 2011 due to supply chain disruptions. A monoclonal antibody targeting CD2 has been used in early phase clinical trials in transplant patients [100–102], and in vitro studies using human PBMCs have shown its ability to selectively deplete donor-reactive memory T cells while promoting Treg expansion [103]. Its development for use in transplant patients has been slowed by its specificity for human CD2, which limits the ability to perform mechanistic studies in nonhuman primate models of transplantation [104]. Efalizumab, a monoclonal antibody targeting a subunit of LFA-1, was originally approved as a treatment for psoriasis [105,106] and was tested in a phase I/II clinical trial in renal transplantation [107]. The drug was eventually withdrawn from the market in 2009 after three confirmed cases of progressive multifocal leukoencephalopathy (PML), a rare but severe viral infection of the brain, were reported in psoriasis patients who received chronic efalizumab treatment [108,109]. Natalizumab, a monoclonal antibody targeting a subunit of VLA-4, was first approved to treat multiple sclerosis and Crohn's disease [110,111]. Like efalizumab, natalizumab has also been associated with an increased risk of PML [112]. This prompted a withdrawal of natalizumab from the market, but it was later reapproved following regulatory review of longer-term safety and efficacy data.

Directed at activation of memory CD8 T cells

Activation of donor-reactive memory CD8 T cells is a multi-faceted process involving interactions between donor and recipient immune cells (Fig. 2). Endogenous memory CD4 T cells expressing CD154 stimulate graft dendritic cells via CD40 to produce both IL-15, which drives memory CD8 T-cell activation and proliferation, and p40HD, which further enhances IL-15 production by graft dendritic cells. Each of these steps represents a potential target for blocking endogenous donor-reactive memory T-cell-mediated allograft injury.

Blockade of CD40–CD154 costimulation has been tested in several inflammatory and autoimmune conditions. Monoclonal antibodies to CD154 have been tested in humans in conditions ranging from transplantation, to lupus nephritis and immune thrombocytopenic purpura (ITP) [113]. All trials of anti-CD154 monoclonal antibodies have been halted due to an

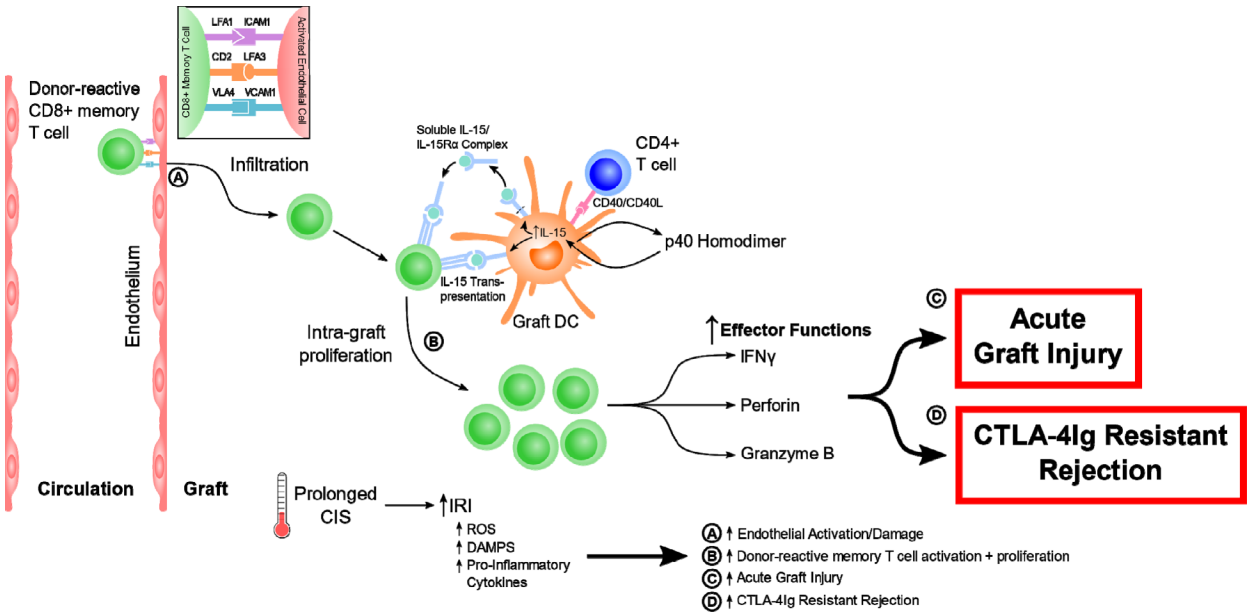

Figure 2 Endogenous memory T cells with donor reactivity mediate allograft injury. Prolonged cold ischaemic storage causes increased ischaemia–reperfusion injury, leading to accumulation of ROS, DAMPS, and pro-inflammatory cytokines. Upregulation of key integrins on activated endothelial cells promotes infiltration of donor reactive endogenous CD8+ memory T cells early after graft reperfusion (A). Graft dendritic cells produce p40 homodimers causing increased IL-15 production. Direct trans-presentation and/or release of soluble IL-15/IL-15Rα complex by graft DCs stimulate activation and proliferation of donor-reactive memory T cells within the allograft (B), leading to increased effector functions including release of IFNγ, granzyme B and perforin which results in worse allograft injury (C) and CTLA-4Ig-resistant rejection (D).

Transplant International 2021; 34: 1360–1373

increased risk of thromboembolism observed with multiple candidates in this drug class in both patients and nonhuman primate models [113–115]. Despite this setback, interest in CD40–CD154 blockade remains strong and a new generation of antibodies designed to reduce thromboembolism risk while maintaining efficacy in blocking the interactions are in development. Early-stage human trials in lupus and ITP using these candidates are ongoing [116]. To date, no studies have been published testing these newer anti-CD154 antibodies in transplant patients, but clinical trials are ongoing and results from preclinical studies in nonhuman primates have been promising [117]. Although attempts to block CD40–CD154 costimulation have primarily focused on blocking CD154, anti-CD40 antibodies are also under development, with one candidate, bleselumab, recently successfully completing a phase two trial in kidney transplant recipients [118] and another, iscalimab/CFZ-533 currently in phase two trials [119].

In line with our mouse transplant studies indicating the important role of p40HD in endogenous donor-reactive memory CD8 T-cell activation within highly ischaemic allografts, an anti-p40 mAb, ustekinumab, is approved for use in humans to treat multiple autoimmune disorders [120]. Targeting IL-15 represents another possible strategy to reducing alloreactive memory T-cell activity. Approaches to blocking IL-15 signalling include antagonist peptides/proteins and monoclonal antibodies. Multiple studies in transplantation [121–123] and other conditions [124–126] have used IL-15 antagonist proteins to block IL-15 signalling, but this work has only been performed in preclinical models. A monoclonal antibody targeting CD122, a subunit of the IL-15 and IL-2 receptors, has been tested in multiple small phase I trials for T-cell lymphoid malignancies [70,127]. Of note, this anti-CD122 mAb showed a favourable safety profile and decreased CD8 T-cell IFN-γ expression.

Ongoing work by multiple groups is focused on further clarifying the factors that drive activation and proliferation of alloreactive memory T cells in transplantation. A deeper understanding of the mechanisms by which these cells mediate allograft rejection should lead to the discovery of additional potential targets for pharmacological prevention and/or treatment of CTLA4-Ig-resistant allograft rejection.

Conclusion

The pretransplant presence of circulating donor-reactive memory T cells is an important prognostic factor linked to worse graft function and outcomes. Donor-reactive memory T cells are typically generated through three main processes, allo-sensitization, heterologous immunity, and homeostatic proliferation. Numerous preclinical model studies have shown the promise of targeting early infiltration and activation of memory T cells to prevent acute allograft injury and rejection. Recent studies have provided further insight into how endogenous donor-reactive memory T cells infiltrate allografts and are activated to cause injury, identifying key molecular mechanisms including CD40–CD154 interactions, p40 homodimers, and IL-15 signalling. The intensity of IRI is another key driver of endogenous donor-reactive memory T-cell activation and represents an additional target that could be included in treatment strategies designed to attenuate donor-reactive memory T-cell-mediated graft injury. Although no memory T-cell-specific therapy has reached clinical use in transplantation, this remains an important area of ongoing research. The development of memory T-cell-specific therapies will be vital to further improving transplant outcomes in both sensitized and unsensitized recipients, particularly those who receive deceased donor grafts, where increased IRI is likely to increase the proliferation and effector function of donor-reactive memory T cells to mediate acute graft injury that decreases short and long-term graft survival.

Funding

This work was supported by NIH grants R01-AI40459 and P01-AI087586 (RLF) and T32-GM007250 and T32-AI089474 (EHK).

Conflict of interest

The authors report no conflicts of interest.

REFERENCES

1. Rana A, Gruessner A, Agopian VG, *et al.* Survival benefit of solid-organ transplant in the United States. *JAMA Surg* 2015; **150**: 252.
2. Chang JT, Wherry EJ, Goldrath AW. Molecular regulation of effector and memory T cell differentiation. *Nat Immunol* 2014; **15**: 1104.
3. Kaech SM, Cui W. Transcriptional control of effector and memory CD8+ T cell differentiation. *Nat Rev Immunol* 2012; **12**: 749.

4. Kaech SM, Wherry EJ. Heterogeneity and cell-fate decisions in effector and memory CD8+ T cell differentiation during viral infection. *Immunity* 2007; **27**: 393.
5. Kaech SM, Wherry EJ, Ahmed R. Effector and memory T-cell differentiation: implications for vaccine development. *Nat Rev Immunol* 2002; **2**: 251.
6. Hartigan CR, Sun H, Ford ML. Memory T-cell exhaustion and tolerance in transplantation. *Immunol Rev* 2019; **292**: 225.
7. Orandi BJ, Garonzik-Wang JM, Massie AB, *et al*. Quantifying the risk of incompatible kidney transplantation: a multicenter study. *Am J Transplant* 2014; **14**: 1573.
8. Scornik JC, Bromberg JS, Norman DJ, Bhanderi M, Gitlin M, Petersen J. An update on the impact of pre-transplant transfusions and allosensitization on time to renal transplant and on allograft survival. *BMC Nephrol* 2013; **14**: 217.
9. Obrador GT, Macdougall IC. Effect of red cell transfusions on future kidney transplantation. *Clin J Am Soc Nephrol* 2013; **8**: 852.
10. Weinreich MA, Odumade OA, Jameson SC, Hogquist KA. T cells expressing the transcription factor PLZF regulate the development of memory-like CD8+ T cells. *Nat Immunol* 2010; **11**: 709.
11. Lee YJ, Jameson SC, Hogquist KA. Alternative memory in the CD8 T cell lineage. *Trends Immunol* 2011; **32**: 50.
12. White JT, Cross EW, Burchill MA, *et al*. Virtual memory T cells develop and mediate bystander protective immunity in an IL-15-dependent manner. *Nat Commun* 2016; **7**: 11291.
13. Schluns KS, Kieper WC, Jameson SC, Lefrancois L. Interleukin-7 mediates the homeostasis of naive and memory CD8 T cells in vivo. *Nat Immunol* 2000; **1**: 426.
14. Tan JT, Dudl E, LeRoy E, *et al*. IL-7 is critical for homeostatic proliferation and survival of naive T cells. *Proc Natl Acad Sci USA* 2001; **98**: 8732.
15. Guimond M, Veenstra RG, Grindler DJ, *et al*. Interleukin 7 signaling in dendritic cells regulates the homeostatic proliferation and niche size of CD4+ T cells. *Nat Immunol* 2009; **10**: 149.
16. Napolitano LA, Grant RM, Deeks SG, *et al*. Increased production of IL-7 accompanies HIV-1-mediated T-cell depletion: implications for T-cell homeostasis. *Nat Med* 2001; **7**: 73.
17. White JT, Cross EW, Kedl RM. Antigen-inexperienced memory CD8 (+) T cells: where they come from and why we need them. *Nat Rev Immunol* 2017; **17**: 391.
18. Jacomet F, Cayssials E, Basbous S, *et al*. Evidence for eomesodermin-expressing innate-like CD8(+) KIR/NKG2A(+) T cells in human adults and cord blood samples. *Eur J Immunol* 2015; **45**: 1926.
19. Min HS, Lee YJ, Jeon YK, *et al*. MHC class II-restricted interaction between thymocytes plays an essential role in the production of innate CD8+ T cells. *J Immunol* 2011; **186**: 5749.
20. Goldrath AW, Bogatzki LY, Bevan MJ. Naive T cells transiently acquire a memory-like phenotype during homeostasis-driven proliferation. *J Exp Med* 2000; **192**: 557.
21. Cho BK, Rao VP, Ge Q, Eisen HN, Chen J. Homeostasis-stimulated proliferation drives naive T cells to differentiate directly into memory T cells. *J Exp Med* 2000; **192**: 549.
22. Sener A, Tang AL, Farber DL. Memory T-cell predominance following T-cell depletional therapy derives from homeostatic expansion of naive T cells. *Am J Transplant* 2009; **9**: 2615.
23. Pearl JP, Parris J, Hale DA, *et al*. Immunocompetent T-cells with a memory-like phenotype are the dominant cell type following antibody-mediated T-cell depletion. *Am J Transplant* 2005; **5**: 465.
24. Ayasoufi K, Fan R, Valujskikh A. Depletion-resistant CD4 T cells enhance thymopoiesis during lymphopenia. *Am J Transplant* 2017; **17**: 2008.
25. Ayasoufi K, Zwick DB, Fan R, *et al*. Interleukin-27 promotes CD8+ T cell reconstitution following antibody-mediated lymphoablation. *JCI Insight* 2019; **4**: 1.
26. Selin LK, Nahill SR, Welsh RM. Cross-reactivities in memory cytotoxic T lymphocyte recognition of heterologous viruses. *J Exp Med* 1994; **179**: 1933.
27. Amir AL, D'Orsogna LJA, Roelen DL, *et al*. Allo-HLA reactivity of virus-specific memory T cells is common. *Blood* 2010; **115**: 3146.
28. Adams AB, Williams MA, Jones TR, *et al*. Heterologous immunity provides a potent barrier to transplantation tolerance. *J Clin Invest* 2003; **111**: 1887.
29. Burrows SR, Silins SL, Khanna R, *et al*. Cross-reactive memory T cells for Epstein-Barr virus augment the alloresponse to common human leukocyte antigens: degenerate recognition of major histocompatibility complex-bound peptide by T cells and its role in alloreactivity. *Eur J Immunol* 1997; **27**: 1726.
30. Landais E, Morice A, Long HM, *et al*. EBV-specific CD4+ T cell clones exhibit vigorous allogeneic responses. *J Immunol* 2006; **177**: 1427.
31. Brehm MA, Pinto AK, Daniels KA, Schneck JP, Welsh RM, Selin LK. T cell immunodominance and maintenance of memory regulated by unexpectedly cross-reactive pathogens. *Nat Immunol* 2002; **3**: 627.
32. Yang H, Welsh RM. Induction of alloreactive cytotoxic T cells by acute virus infection of mice. *J Immunol* 1986; **136**: 1186.
33. Welsh RM, Markees TG, Woda BA, *et al*. Virus-induced abrogation of transplantation tolerance induced by donor-specific transfusion and anti-CD154 antibody. *J Virol* 2000; **74**: 2210.
34. Pantenburg B, Heinzel F, Das L, Heeger PS, Valujskikh A. T cells primed by Leishmania major infection cross-react with alloantigens and alter the course of allograft rejection. *J Immunol* 2002; **169**: 3686.
35. Macedo C, Orkis EA, Popescu I, *et al*. Contribution of naive and memory T-cell populations to the human alloimmune response. *Am J Transplant* 2009; **9**: 2057.
36. Stapler D, Lee ED, Selvaraj SA, *et al*. Expansion of effector memory TCR Vbeta4+ CD8+ T cells is associated with latent infection-mediated resistance to transplantation tolerance. *J Immunol* 2008; **180**: 3190.
37. Williams MA, Tan JT, Adams AB, *et al*. Characterization of virus-mediated inhibition of mixed chimerism and allospecific tolerance. *J Immunol* 2001; **167**: 4987.
38. Beus JM, Hashmi SS, Selvaraj SA, *et al*. Heterologous immunity triggered by a single, latent virus in Mus musculus: combined costimulation- and adhesion- blockade decrease rejection. *PLoS One* 2013; **8**: e71221.
39. Heeger PS, Greenspan NS, Kuhlenschmidt S, *et al*. Pretransplant frequency of donor-specific, IFN-gamma-producing lymphocytes is a manifestation of immunologic memory and correlates with the risk of posttransplant rejection episodes. *J Immunol* 1999; **163**: 2267.
40. Augustine JJ, Siu DS, Clemente MJ, Schulak JA, Heeger PS, Hricik DE. Pre-transplant IFN-gamma ELISPOTs are associated with post-transplant renal function in African American renal transplant recipients. *Am J Transplant* 2005; **5**: 1971.

41. Poggio ED, Clemente M, Hricik DE, Heeger PS. Panel of reactive T cells as a measurement of primed cellular alloimmunity in kidney transplant candidates. *J Am Soc Nephrol* 2006; **17**: 564.
42. Poggio ED, Augustine JJ, Clemente M, *et al*. Pretransplant cellular alloimmunity as assessed by a panel of reactive T cells assay correlates with acute renal graft rejection. *Transplantation* 2007; **83**: 847.
43. Augustine JJ, Poggio ED, Clemente M, *et al*. Hemodialysis vintage, black ethnicity, and pretransplantation antidonor cellular immunity in kidney transplant recipients. *J Am Soc Nephrol* 2007; **18**: 1602.
44. Nather BJ, Nickel P, Bold G, *et al*. Modified ELISPOT technique–highly significant inverse correlation of post-Tx donor-reactive IFNgamma-producing cell frequencies with 6 and 12 months graft function in kidney transplant recipients. *Transpl Immunol* 2006; **16**: 232.
45. Nickel P, Presber F, Bold G, *et al*. Enzyme-linked immunosorbent spot assay for donor-reactive interferon-gamma-producing cells identifies T-cell presensitization and correlates with graft function at 6 and 12 months in renal-transplant recipients. *Transplantation* 2004; **78**: 1640.
46. Bestard O, Nickel P, Cruzado JM, *et al*. Circulating alloreactive T cells correlate with graft function in long-standing renal transplant recipients. *J Am Soc Nephrol* 2008; **19**: 1419.
47. Kim SH, Oh EJ, Kim MJ, *et al*. Pretransplant donor-specific interferon-gamma ELISPOT assay predicts acute rejection episodes in renal transplant recipients. *Transplant Proc* 2007; **39**: 3057.
48. Jacquemont L, Tilly G, Yap M, *et al*. Terminally differentiated effector memory CD8(+) T cells identify kidney transplant recipients at high risk of graft failure. *J Am Soc Nephrol* 2020; **31**: 876.
49. Salom RN, Maguire JA, Esmore D, Hancock WW. Analysis of proliferating cell nuclear antigen expression aids histological diagnosis and is predictive of progression of human cardiac allograft rejection. *Am J Pathol* 1994; **145**: 876.
50. Ibrahim S, Dawson DV, Sanfilippo F. Predominant infiltration of rejecting human renal allografts with T cells expressing CD8 and CD45RO. *Transplantation* 1995; **59**: 724.
51. Azzawi M, Hasleton PS, Geraghty PJ, *et al*. RANTES chemokine expression is related to acute cardiac cellular rejection and infiltration by CD45RO T-lymphocytes and macrophages. *J Heart Lung Transplant* 1998; **17**: 881.
52. Lebwohl M, Christophers E, Langley R, *et al*. An international, randomized, double-blind, placebo-controlled phase 3 trial of intramuscular alefacept in patients with chronic plaque psoriasis. *Arch Dermatol* 2003; **139**: 719.
53. Schenk AD, Nozaki T, Rabant M, Valujskikh A, Fairchild RL. Donor-reactive CD8 memory T cells infiltrate cardiac allografts within 24-h post-transplant in naive recipients. *Am J Transplant* 2008; **8**: 1652.
54. El-Sawy T, Miura M, Fairchild R. Early T cell response to allografts occurring prior to alloantigen priming up-regulates innate-mediated inflammation and graft necrosis. *Am J Pathol* 2004; **165**: 147.
55. Schenk AD, Gorbacheva V, Rabant M, Fairchild RL, Valujskikh A. Effector functions of donor-reactive CD8 memory T cells are dependent on ICOS induced during division in cardiac grafts. *Am J Transplant* 2009; **9**: 64.
56. El-Sawy T, Belperio JA, Strieter RM, Remick DG, Fairchild RL. Inhibition of polymorphonuclear leukocyte-mediated graft damage synergizes with short-term costimulatory blockade to prevent cardiac allograft rejection. *Circulation* 2005; **112**: 320.
57. Setoguchi K, Schenk AD, Ishii D, *et al*. LFA-1 antagonism inhibits early infiltration of endogenous memory CD8 T cells into cardiac allografts and donor-reactive T cell priming. *Am J Transplant* 2011; **11**: 923.
58. Banner NR, Thomas HL, Curnow E, *et al*. The importance of cold and warm cardiac ischemia for survival after heart transplantation. *Transplantation* 2008; **86**: 542.
59. Russo MJ, Chen JM, Sorabella RA, *et al*. The effect of ischemic time on survival after heart transplantation varies by donor age: an analysis of the United Network for Organ Sharing database. *J Thorac Cardiovasc Surg* 2007; **133**: 554.
60. Debout A, Foucher Y, Trébern-Launay K, *et al*. Each additional hour of cold ischemia time significantly increases the risk of graft failure and mortality following renal transplantation. *Kidney Int* 2015; **87**: 343.
61. Postalcioglu M, Kaze AD, Byun BC, *et al*. Association of cold ischemia time with acute renal transplant rejection. *Transplantation* 2018; **102**: 1188.
62. Su CA, Iida S, Abe T, Fairchild RL. Endogenous memory CD8 T cells directly mediate cardiac allograft rejection. *Am J Transplant* 2014; **14**: 568.
63. Tsuda H, Su CA, Tanaka T, *et al*. Allograft dendritic cell p40 homodimers activate donor-reactive memory CD8+ T cells. *JCI Insight* 2018; **3**: 1.
64. Waldmann TA. The biology of interleukin-2 and interleukin-15: implications for cancer therapy and vaccine design. *Nat Rev Immunol* 2006; **6**: 595.
65. Guo Y, Luan L, Patil NK, Sherwood ER. Immunobiology of the IL-15/IL-15Ralpha complex as an antitumor and antiviral agent. *Cytokine Growth Factor Rev* 2017; **38**: 10.
66. Waldmann TA, Miljkovic MD, Conlon KC. Interleukin-15 (dys)regulation of lymphoid homeostasis: implications for therapy of autoimmunity and cancer. *J Exp Med* 2020; **217**: 1.
67. Traitanon O, Gorbachev A, Bechtel JJ, *et al*. IL-15 induces alloreactive CD28 (-) memory CD8 T cell proliferation and CTLA4-Ig resistant memory CD8 T cell activation. *Am J Transplant* 2014; **14**: 1277.
68. Mathews DV, Dong Y, Higginbotham LB, *et al*. CD122 signaling in CD8+ memory T cells drives costimulation-independent rejection. *J Clin Invest* 2018; **128**: 4557.
69. Smith XG, Bolton EM, Ruchatz H, Wei X, Liew FY, Bradley JA. Selective blockade of IL-15 by soluble IL-15 receptor alpha-chain enhances cardiac allograft survival. *J Immunol* 2000; **165**: 3444.
70. Waldmann TA, Conlon KC, Stewart DM, *et al*. Phase 1 trial of IL-15 trans presentation blockade using humanized Mikbeta1 mAb in patients with T-cell large granular lymphocytic leukemia. *Blood* 2013; **121**: 476.
71. Bergamaschi C, Bear J, Rosati M, *et al*. Circulating IL-15 exists as heterodimeric complex with soluble IL-15Ralpha in human and mouse serum. *Blood* 2012; **120**: e1.
72. Jurewicz M, Takakura A, Augello A, *et al*. Ischemic injury enhances dendritic cell immunogenicity via TLR4 and NF-kappa B activation. *J Immunol* 2010; **184**: 2939.
73. Uehara M, Solhjou Z, Banouni N, *et al*. Ischemia augments alloimmune injury through IL-6-driven CD4(+) alloreactivity. *Sci Rep* 2018; **8**: 2461.
74. Vincenti F, Charpentier B, Vanrenterghem Y, *et al*. A phase III study of belatacept-based immunosuppression regimens versus cyclosporine in renal transplant recipients (BENEFIT study). *Am J Transplant* 2010; **10**: 535.

75. Vincenti F, Rostaing L, Grinyo J, *et al.* Belatacept and long-term outcomes in kidney transplantation. *N Engl J Med* 2016; **374**: 333.
76. Dedeoglu B, Litjens NHR, Klepper M, *et al.* CD4(+) CD28(null) T cells are not alloreactive unless stimulated by interleukin-15. *Am J Transplant* 2018; **18**: 341.
77. de Groot H, Rauen U. Ischemia-reperfusion injury: processes in pathogenetic networks: a review. *Transplant Proc* 2007; **39**: 481.
78. Akhtar MZ, Henderson T, Sutherland A, Vogel T, Friend PJ. Novel approaches to preventing ischemia-reperfusion injury during liver transplantation. *Transplant Proc* 2013; **45**: 2083.
79. Amersi F, Nelson SK, Shen XD, *et al.* Bucillamine, a thiol antioxidant, prevents transplantation-associated reperfusion injury. *Proc Natl Acad Sci USA* 2002; **99**: 8915.
80. Plin C, Tillement JP, Berdeaux A, Morin D. Resveratrol protects against cold ischemia-warm reoxygenation-induced damages to mitochondria and cells in rat liver. *Eur J Pharmacol* 2005; **528**: 162.
81. Ben Mosbah I, Roselló-Catafau J, Franco-Gou R, *et al.* Preservation of steatotic livers in IGL-1 solution. *Liver Transpl* 2006; **12**: 1215.
82. Inci I, Zhai W, Arni S, Hillinger S, Vogt P, Weder W. N-acetylcysteine attenuates lung ischemia-reperfusion injury after lung transplantation. *Ann Thorac Surg* 2007; **84**: 240; discussion 6.
83. Fang JC, Kinlay S, Beltrame J, *et al.* Effect of vitamins C and E on progression of transplant-associated arteriosclerosis: a randomised trial. *Lancet* 2002; **359**: 1108.
84. Hilmi IA, Peng Z, Planinsic RM, *et al.* N-acetylcysteine does not prevent hepatorenal ischaemia-reperfusion injury in patients undergoing orthotopic liver transplantation. *Nephrol Dial Transplant* 2010; **25**: 2328.
85. Land W, Schneeberger H, Schleibner S, *et al.* The beneficial effect of human recombinant superoxide dismutase on acute and chronic rejection events in recipients of cadaveric renal transplants. *Transplantation* 1994; **57**: 211.
86. Schramm L, La M, Heidbreder E, *et al.* L-arginine deficiency and supplementation in experimental acute renal failure and in human kidney transplantation. *Kidney Int* 2002; **61**: 1423.
87. Danilovic A, Lucon AM, Srougi M, *et al.* Protective effect of N-acetylcysteine on early outcomes of deceased renal transplantation. *Transplant Proc* 2011; **43**: 1443.
88. Orban JC, Quintard H, Cassuto E, Jambou P, Samat-Long C, Ichai C. Effect of N-acetylcysteine pretreatment of deceased organ donors on renal allograft function: a randomized controlled trial. *Transplantation* 2015; **99**: 746.
89. Minami K, Bae S, Uehara H, *et al.* Targeting of intragraft reactive oxygen species by APP-103, a novel polymer product, mitigates ischemia/reperfusion injury and promotes the survival of renal transplants. *Am J Transplant* 2020; **20**: 1527.
90. Martin JL, Costa ASH, Gruszczyk AV, *et al.* Succinate accumulation drives ischaemia-reperfusion injury during organ transplantation. *Nat Metab* 2019; **1**: 966.
91. Cypel M, Yeung JC, Liu M, *et al.* Normothermic ex vivo lung perfusion in clinical lung transplantation. *N Engl J Med* 2011; **364**: 1431.
92. Nasralla D, Coussios CC, Mergental H, *et al.* A randomized trial of normothermic preservation in liver transplantation. *Nature* 2018; **557**: 50.
93. Liu Q, Hassan A, Pezzati D, *et al.* Ex situ liver machine perfusion: the impact of fresh frozen plasma. *Liver Transpl* 2020; **26**: 215.
94. Pagano F, Nocella C, Sciarretta S, *et al.* Cytoprotective and antioxidant effects of steen solution on human lung spheroids and human endothelial cells. *Am J Transplant* 2017; **17**: 1885.
95. Lo DJ, Weaver TA, Stempora L, *et al.* Selective targeting of human alloresponsive CD8+ effector memory T cells based on CD2 expression. *Am J Transplant* 2011; **11**: 22.
96. Weaver TA, Charafeddine AH, Agarwal A, *et al.* Alefacept promotes co-stimulation blockade based allograft survival in nonhuman primates. *Nat Med* 2009; **15**: 746.
97. Badell IR, Russell MC, Thompson PW, *et al.* LFA-1-specific therapy prolongs allograft survival in rhesus macaques. *J Clin Invest* 2010; **120**: 4520.
98. Iida S, Miyairi S, Su CA, *et al.* Peritransplant VLA-4 blockade inhibits endogenous memory CD8 T cell infiltration into high-risk cardiac allografts and CTLA-4Ig resistant rejection. *Am J Transplant* 2019; **19**: 998.
99. Isobe M, Suzuki J, Yagita H, *et al.* Immunosuppression to cardiac allografts and soluble antigens by anti-vascular cellular adhesion molecule-1 and anti-very late antigen-4 monoclonal antibodies. *J Immunol* 1994; **153**: 5810.
100. Pruett TL, McGory RW, Wright FH, Pescovitz MD, Yang H, McClain JB. Safety profile, pharmacokinetics, and pharmacodynamics of siplizumab, a humanized anti-CD2 monoclonal antibody, in renal allograft recipients. *Transplant Proc* 2009; **41**: 3655.
101. Kawai T, Cosimi AB, Spitzer TR, *et al.* HLA-mismatched renal transplantation without maintenance immunosuppression. *N Engl J Med* 2008; **358**: 353.
102. Kawai T, Sachs DH, Sprangers B, *et al.* Long-term results in recipients of combined HLA-mismatched kidney and bone marrow transplantation without maintenance immunosuppression. *Am J Transplant* 2014; **14**: 1599.
103. Podestà MA, Binder C, Sellberg F, *et al.* Siplizumab selectively depletes effector memory T cells and promotes a relative expansion of alloreactive regulatory T cells in vitro. *Am J Transplant* 2020; **20**: 88.
104. Berglund E, Alonso-Guallart P, Danton M, *et al.* Safety and pharmacodynamics of anti-CD2 monoclonal antibody treatment in cynomolgus macaques – an experimental study. *Transpl Int* 2020; **33**: 98.
105. Gordon KB, Papp KA, Hamilton TK, *et al.* Efalizumab for patients with moderate to severe plaque psoriasis: a randomized controlled trial. *JAMA* 2003; **290**: 3073.
106. Lebwohl M, Tyring SK, Hamilton TK, *et al.* A novel targeted T-cell modulator, efalizumab, for plaque psoriasis. *N Engl J Med* 2003; **349**: 2004.
107. Vincenti F, Mendez R, Pescovitz M, *et al.* A phase I/II randomized open-label multicenter trial of efalizumab, a humanized anti-CD11a, anti-LFA-1 in renal transplantation. *Am J Transplant* 2007; **7**: 1770.
108. Kothary N, Diak IL, Brinker A, Bezabeh S, Avigan M, Dal Pan G. Progressive multifocal leukoencephalopathy associated with efalizumab use in psoriasis patients. *J Am Acad Dermatol* 2011; **65**: 546.
109. Schwab N, Ulzheimer JC, Fox RJ, *et al.* Fatal PML associated with efalizumab therapy: insights into integrin alphaLbeta2 in JC virus control. *Neurology* 2012; **78**: 458; discussion 65.
110. Polman CH, O'Connor PW, Havrdova E, *et al.* A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. *N Engl J Med* 2006; **354**: 899.
111. Sandborn WJ, Colombel JF, Enns R, *et al.* Natalizumab induction and

maintenance therapy for Crohn's disease. *N Engl J Med* 2005; **353**: 1912.
112. Bloomgren G, Richman S, Hotermans C, *et al*. Risk of natalizumab-associated progressive multifocal leukoencephalopathy. *N Engl J Med* 2012; **366**: 1870.
113. Law CL, Grewal IS. Therapeutic interventions targeting CD40L (CD154) and CD40: the opportunities and challenges. *Adv Exp Med Biol* 2009; **647**: 8.
114. Kawai T, Andrews D, Colvin RB, Sachs DH, Cosimi AB. Thromboembolic complications after treatment with monoclonal antibody against CD40 ligand. *Nat Med* 2000; **6**: 114.
115. Koyama I, Kawai T, Andrews D, *et al*. Thrombophilia associated with anti-CD154 monoclonal antibody treatment and its prophylaxis in nonhuman primates. *Transplantation* 2004; **77**: 460.
116. Karnell JL, Rieder SA, Ettinger R, Kolbeck R. Targeting the CD40-CD40L pathway in autoimmune diseases: Humoral immunity and beyond. *Adv Drug Deliv Rev* 2019; **141**: 92.
117. Kim SC, Wakwe W, Higginbotham LB, *et al*. Fc-silent anti-CD154 domain antibody effectively prevents nonhuman primate renal allograft rejection. *Am J Transplant* 2017; **17**: 1182.
118. Harland RC, Klintmalm G, Jensik S, *et al*. Efficacy and safety of bleselumab in kidney transplant recipients: a phase 2, randomized, open-label, noninferiority study. *Am J Transplant* 2020; **20**: 159.
119. Espié P, He Y, Koo P, *et al*. First-in-human clinical trial to assess pharmacokinetics, pharmacodynamics, safety, and tolerability of iscalimab, an anti-CD40 monoclonal antibody. *Am J Transplant* 2020; **20**: 463.
120. Benson JM, Peritt D, Scallon BJ, *et al*. Discovery and mechanism of ustekinumab: a human monoclonal antibody targeting interleukin-12 and interleukin-23 for treatment of immune-mediated disorders. *mAbs* 2011; **3**: 535.
121. Ferrari-Lacraz S, Zheng XX, Kim YS, *et al*. An antagonist IL-15/Fc protein prevents costimulation blockade-resistant rejection. *J Immunol* 2001; **167**: 3478.
122. Zheng XX, Gao W, Donskoy E, *et al*. An antagonist mutant IL-15/Fc promotes transplant tolerance. *Transplantation* 2006; **81**: 109.
123. Ferrari-Lacraz S, Zheng XX, Fueyo AS, Maslinski W, Moll T, Strom TB. CD8(+) T cells resistant to costimulatory blockade are controlled by an antagonist interleukin-15/Fc protein. *Transplantation* 2006; **82**: 1510.
124. Kim YS, Maslinski W, Zheng XX, *et al*. Targeting the IL-15 receptor with an antagonist IL-15 mutant/Fc gamma2a protein blocks delayed-type hypersensitivity. *J Immunol* 1998; **160**: 5742.
125. Ruchatz H, Leung BP, Wei XQ, McInnes IB, Liew FY. Soluble IL-15 receptor alpha-chain administration prevents murine collagen-induced arthritis: a role for IL-15 in development of antigen-induced immunopathology. *J Immunol* 1998; **160**: 5654.
126. Ferrari-Lacraz S, Zanelli E, Neuberg M, *et al*. Targeting IL-15 receptor-bearing cells with an antagonist mutant IL-15/Fc protein prevents disease development and progression in murine collagen-induced arthritis. *J Immunol* 2004; **173**: 5818.
127. Morris JC, Janik JE, White JD, *et al*. Preclinical and phase I clinical trial of blockade of IL-15 using Mikbeta1 monoclonal antibody in T cell large granular lymphocyte leukemia. *Proc Natl Acad Sci USA* 2006; **103**: 401.
128. Samy KP, Anderson DJ, Lo DJ, *et al*. Selective targeting of high-affinity LFA-1 does not augment costimulation blockade in a nonhuman primate renal transplantation model. *Am J Transplant* 2017; **17**: 1193.
129. Rostaing L, Charpentier B, Glyda M, *et al*. Alefacept combined with tacrolimus, mycophenolate mofetil and steroids in de novo kidney transplantation: a randomized controlled trial. *Am J Transplant* 2013; **13**: 1724.
130. Okimura K, Maeta K, Kobayashi N, *et al*. Characterization of ASKP1240, a fully human antibody targeting human CD40 with potent immunosuppressive effects. *Am J Transplant* 2014; **14**: 1290.
131. Cordoba F, Wieczorek G, Audet M, *et al*. A novel, blocking, Fc-silent anti-CD40 monoclonal antibody prolongs nonhuman primate renal allograft survival in the absence of B cell depletion. *Am J Transplant* 2015; **15**: 2825.
132. Imai A, Suzuki T, Sugitani A, *et al*. A novel fully human anti-CD40 monoclonal antibody, 4D11, for kidney transplantation in cynomolgus monkeys. *Transplantation* 2007; **84**: 1020.
133. Aoyagi T, Yamashita K, Suzuki T, *et al*. A human anti-CD40 monoclonal antibody, 4D11, for kidney transplantation in cynomolgus monkeys: induction and maintenance therapy. *Am J Transplant* 2009; **9**: 1732.
134. Kirk AD, Burkly LC, Batty DS, *et al*. Treatment with humanized monoclonal antibody against CD154 prevents acute renal allograft rejection in nonhuman primates. *Nat Med* 1999; **5**: 686.
135. Kenyon NS, Chatzipetrou M, Masetti M, *et al*. Long-term survival and function of intrahepatic islet allografts in rhesus monkeys treated with humanized anti-CD154. *Proc Natl Acad Sci USA* 1999; **96**: 8132.
136. Schuler W, Bigaud M, Brinkmann V, *et al*. Efficacy and safety of ABI793, a novel human anti-human CD154 monoclonal antibody, in cynomolgus monkey renal allotransplantation. *Transplantation* 2004; **77**: 717.
137. Cho CS, Burkly LC, Fechner JH Jr, *et al*. Successful conversion from conventional immunosuppression to anti-CD154 monoclonal antibody costimulatory molecule blockade in rhesus renal allograft recipients. *Transplantation* 2001; **72**: 587.
138. Kanmaz T, Fechner JJ Jr, Torrealba J, *et al*. Monotherapy with the novel human anti-CD154 monoclonal antibody ABI793 in rhesus monkey renal transplantation model. *Transplantation* 2004; **77**: 914.