Memory T Cells in Transplantation

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Abstract Following infections and other environmental exposures, the resulting primary immune response generates memory T cells that provide long-term protective immunity. Compared to their naïve T cell counterparts, memory T cells possess unique characteristics that endow them with the ability to quickly and robustly respond to foreign antigens. While such memory T cells are beneficial in protecting hosts from recurrent infection, memory cells reactive to donor antigens pose a major barrier to successful transplantation and tolerance induction. Significant progress has been made over the past decade contributing to our understanding of memory T cell generation, their distinct biology, and their detrimental impact in clinical and animal models of transplantation. This review focuses on the unique features that make memory T cells relevant to the transplant community and discusses potential therapies targeting memory T cells which may ameliorate allograft rejection and improve graft outcomes.

Keywords Memory T cells · Transplant tolerance · Homeostatic proliferation · Heterologous immunity · Animal models · Transplantation

Introduction

Lymphocyte development to memory cells is a keystone feature of the adaptive immune response to protect animals

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through the unique ability to rapidly recognize and effectively eliminate previously encountered pathogens. An individual's repertoire of T and B memory cells essentially provides a historical log of infections and environmental exposures that have been encountered over the course of one's life. While the ability of memory T cells to quickly mobilize and mount a potent recall response is beneficial in protective immunity against recurrent pathogens, memory T cells are a significant barrier to successful transplantation. Pre-existing donor antigen-primed memory T cells, or memory T cells generated during responses to infectious or environmental antigens that cross-react with donor allogeneic MHC molecules, are quickly activated in response to allografts, and express functions mediating graft tissue injury that can directly lead to graft failure, or promote sufficient injury to jeopardize patient survival. In this review we will describe fundamental characteristics of memory T cells and their generation, discuss their role in clinical transplantation and animal models of allograft rejection, and explore the development of therapeutic agents that target memory T cells.

Memory T Cell Generation

Memory CD4⁺ and CD8⁺ T cells are generated during the course of primary immune responses, such as during infections and vaccinations. Viral and bacterial infection models and models immunizing with model protein antigens have indicated clonal proliferation of antigen-responsive primary effector T cells and the development of some of these proliferating T cells into memory T cells expressing a specific phenotype, CD127^{hi} (IL-7 receptor α chain), CD27^{hi}, Bcl-2^{hi}, KLRG1^{low} [1••]. At what point during primary activation antigen-responsive T cells commit to memory T cell development remains unclear, with several models being tested. Discussion of

this commitment is beyond the focus of this review and sources detailing these models are referenced for interested readers [1•, 2–4].

Another mechanism of memory T cell generation involves the homeostasis of the peripheral T cell repertoire. In adults the size and composition of the peripheral lymphocyte pool is tightly regulated and, in the absence of disease, is maintained at relatively constant levels [5]. Homeostatic proliferation of lymphocytes, as the name implies, is a physiological process triggered by lymphopenia to maintain a constant level of T cell numbers. With normal involution of the thymus, which contributes new naïve T cells through early adulthood, homeostatic proliferation of mature peripheral T cells becomes the predominant mechanism sustaining T cell numbers in adulthood [6]. In response to insults that decrease peripheral T cell numbers such as viral infections, cancer chemotherapy, or the peritransplant depletion therapies commonly utilized in higher risk transplant patients, homeostatic proliferation stimulates replenishment of lymphocyte numbers until threshold numbers are re-achieved. Mechanistically, homeostatic proliferation of T cells typically occurs in the absence of any exogenous antigen, but instead is supported by self-peptide-MHC complexes and cytokines, especially IL-7 and IL-1 [7, 8]. Interestingly, this spontaneous expansion has a side-effect of converting naïve T cells directly to memory cells, especially those of an effector-memory phenotype [9]. As would be expected, this conversion of naïve T cells to memory T cells through homeostatic proliferation is detrimental to transplant recipients. Studies in animal models and human patients have demonstrated that such memory cells generated by homeostatic proliferation vigorously respond to transplanted allografts and mediate accelerated rejection that is highly resistant to tolerization [10–12].

Memory T Cell Biology

Memory T cells possess several inherent characteristics that differentiate them from their naïve counterparts and allow them to robustly respond to foreign antigens such as microbial pathogens and allografts (Table 1). A key characteristic that memory T cells possess is that upon re-encounter with antigen, memory T cells are able to multiply and differentiate into effector T cells much more quickly and effectively than their naïve counterparts. It has been estimated that virus-specific memory CD8⁺ T cells require only hours to express cytolytic function, compared to the several days needed by naïve CD8⁺ T cells to differentiate into cytotoxic effector cells [13, 14]. Moreover, on a per cell basis, memory T cells give rise to roughly fivefold more effector T cells than naïve T cells do during a primary immune response [15]. The ability of memory T cells to mount such a vigorous response stems from both quantitative and qualitative advantages over naïve T cells. Quantitatively, clonal populations of antigen-specific memory cells can exist for many years and far outnumber the precursor frequencies of rare antigen-reactive naïve T cells [16, 17]. Antigen-reactive memory T cells are not only more abundant within the host, but qualitatively exhibit intrinsic antigen hyper-responsiveness compared to naïve T cells. The mechanisms of this hyper-responsiveness are incompletely understood, but are thought to include enhanced T cell receptor signaling, affinity maturation, and a reduced activation threshold [18]. Whereas naïve T cells require CD28/B7-mediated co-stimulation provided by professional antigen presenting cells (APCs), memory T cells may be fully activated in the absence of co-stimulation via CD28/B7 and/or CD40/154 pathways, and by many nonprofessional APC types such as B cells and macrophages [19, 20]. This heightened propensity to activation, coupled with a shortened lag time to enter cell cycle and acquire effector functions, allows memory T cells to quickly and robustly mount an effective recall response.

Table 1 Characteristics of memory versus naïve T cells

Cell type	Naïve T cell	Central memory T cell	Effector memory T cell
Phenotype (human)	CD45RA ^{hi} CD45RO ^{low} CD62L ^{hi} CCR7 ^{hi}	CD45RA ^{low} CD45RO ^{hi} CD62L ^{hi} CCR7 ^{hi}	CD45RA ^{low} CD45RO ^{hi} CD62L ^{low} CCR7 ^{low}
Phenotype (mouse)	CD44 ^{low} CD62L ^{hi} CCR7 ^{hi}	CD44 ^{hi} CD62L ^{hi} CCR7 ^{hi}	CD44 ^{hi} CD62L ^{low} CCR7 ^{low}
Location	Spleen, lymph nodes	Spleen, lymph nodes, bone marrow	Spleen, blood, peripheral tissues
Response time after antigen	Days	Hours	Hours
Co-stimulation requirements	+++	-/+	-/+
Proliferative potential	-	+++	++
Cytokine production	-	+++ (IL-2)	+++ (IFN-γ, TNF-α)
Effector functions/ cytotoxicity	-	+	+++
Sensitivity to immuno-suppression	+++	-/+	-/+

Moreover, CD8⁺ effector memory T cells in particular may constitutively express cytolytic functions, further decreasing the response time between antigen re-exposure and immune reaction [21].

An additional advantage that memory T cells possess over naïve T cells is in their distribution and accessibility to antigens. While the circulation of naïve T cells is restricted to secondary lymphoid tissues, memory T cells circulate through both lymphoid and peripheral non-lymphoid tissues [22]. Consistent with these properties, memory CD8⁺ and CD4⁺ T cells express distinct cell surface homing receptors and comprise heterogeneous populations of CCR7⁺/CD62L^{high} and CCR7⁺/CD62L^{low} subsets characterized as central memory and effector memory, respectively [21]. The high expression of CCR7 and CD62L promotes central memory T cell localization within secondary lymph nodes, whereas effector memory T cells express integrins and chemokine receptors that specifically promote their localization to distinct peripheral tissue sites such as the lungs, skin or intestinal mucosa. Unlike naïve T cells which require secondary lymphoid organs for full activation by professional APCs, the activation of memory T cells does not require secondary lymphoid organs. Memory CD8⁺ T cells in peripheral tissues have been demonstrated to spontaneously lyse target cells upon antigen re-encounter, without the need for clonal expansion and differentiation, and effectively mediate cardiac allograft rejection in rodent models lacking secondary lymphoid tissue [22, 23]. Thus, memory T cells have an anatomic advantage not only in their access to antigen, but are also well poised to initiate immediate function at the site of antigen reencounter, bypassing the need for formal antigen presentation in the draining lymph node.

Finally, memory T cells possess unique survival advantages when compared to naïve T cells. By definition, memory cells must provide their hosts with memory of past antigenic experiences and in order to provide protection for future re-exposures, memory T cells must be long-lived and persist, often for the lifetime of the individual. The long-term maintenance of memory T cells is dependent on constant division in the periphery, and the intrinsic ability of memory T cells to survive in a relative resting state for an extended duration. Consequently, memory T cells have been demonstrated to survive lymphoablative therapies with polyclonal antithymocyte globulin (ATG) or monoclonal anti-CD52 antibody (Campath), therapies used in clinical transplantation that very effectively eliminate naïve T cells [24–27].

Alloreactive Memory T Cell Generation

Alloreactive memory T cells are generated through three major mechanisms. First, target antigen stimulation of naïve T cells provides the most direct source of memory T cells

during sensitization of an individual. In the transplant setting, donor antigen sensitization occurs in individuals through prior blood transfusion, previous transplant, or pregnancy which all generate donor-specific memory T cells [28]. In support of this, a study of chronic renal failure patients revealed primed alloreactive T cells in all patients that had received a prior blood transfusion or transplant [29]. However, even in the absence of prior alloantigen sensitization, individuals may contain alloreactive precursors within the memory T cell population generated through homeostatic proliferation or via heterologous immunity.

As mentioned earlier, alloreactive memory T cells may also be generated in instances when a lymphopenic environment is imposed on transplant recipients. Current induction therapies include the use of T cell depleting agents such as ATG and Campath. Although these strategies are very efficient in decreasing the number of T cells and increasing the efficacy of standard immunosuppression, the T cells that remain following administration of T cell depletion therapies can be activated to undergo homeostatic proliferation, and those with alloreactive TCRs will clonally expand to become donor-reactive memory T cells. Memory T cells surviving the administration of these depletion strategies are associated with acute rejection episodes in treated transplant patients [24, 30]. Importantly, elegant studies in mouse models have shown that homeostatically generated memory T cells confer resistance to the co-stimulatory blockade strategies that promote long term survival of complete MHC mismatched heart allografts in the absence of donor-reactive memory T cells [10–12].

Memory T cells generated from prior immunological exposures have the capacity to influence the course of future immune responses to unrelated pathogens, a phenomenon termed heterologous immunity. Despite being highly specific for their priming antigen, memory T cells maintain a varied TCR repertoire and exhibit degeneracy in their ability to recognize and cross-react with a number of antigens [31]. This diversity and degeneracy in memory TCRs is thought to be evolutionarily beneficial to allow the immune system to respond to mutations in previously encountered pathogens. Unfortunately, a major implication of heterologous immunity is that many of the pathogen-specific memory T cells generated during an individual's history are potentially reactive to previously unseen transplant antigens. Indeed, it has been estimated that while the frequency of pathogen specific naïve T cells is quite rare (about 1:200,000), the proportion that can directly recognize foreign MHC is substantially greater, about 1 to 10 % of the total T cell repertoire [32, 33]. The molecular basis of this TCR degeneracy has been explained by observations that while hundreds of peptides in any pathogenic infection have the appropriate sequence to bind MHC class I; data from studies using crystal structures of peptide-MHC complexes suggest that only a few contact residues of the embedded peptide actually interact with the TCR to stimulate an

immunogenic response [31, 34]. Thus, a TCR may tolerate certain amino acid substitutions in the peptide sequence and still become activated. Aside from this “molecular mimicry”, the degeneracy of the T cell response has also been attributed to the possibility that different regions of the same TCR may interact with different targets and that a T cell may express two different TCRs, due to incomplete allelic exclusion of the TCR alpha chain. Together, these unique features of memory cells make them formidable obstacles in transplantation.

Memory T Cells in Clinical Transplantation

In humans, alloreactive memory cells generated by previous donor antigen sensitization through prior blood transfusion, previous transplant, pregnancy, or through cross-reactive heterologous immunity contribute to both acute rejection episodes and the development of chronic graft injury. Seminal work by Heeger and colleagues demonstrated that the frequency of donor-reactive memory T cells in the peripheral blood of renal transplant patients prior to transplant correlates with the risk of developing acute rejection episodes and with poor renal allograft function at 1 year [35, 36]. Such alloreactive memory cells are detectable by their production of IFN- γ in ELISPOT assays within 24 hours of allostimulation, a technique which has been confirmed to predict post-transplant renal graft function, and even the development of chronic allograft nephropathy [37, 38]. Additionally, the detection of memory-phenotype (CD45RO⁺) cells within biopsies and peripheral blood of heart and kidney allograft patients has been shown to correlate with the incidence and severity of transplant rejection [39–42].

Given the intrinsic advantages that memory T cells possess over their naïve counterparts in terms of survival and activation thresholds, it is perhaps unsurprising that donor-reactive memory T cells are often detectable in organ transplant recipients receiving induction therapy to deplete T cells and/or high levels of immunosuppression [35, 43]. Importantly, in a recent phase 3 clinical trial of Belatacept, a second-generation CTLA4-Ig that blocks CD28-mediated co-stimulation, an increased incidence and severity of acute renal allograft rejection episodes was observed compared to cyclosporine treatment, despite overall improved renal function in patients receiving Belatacept [44]. One possible mechanism contributing to this increased frequency of acute rejection in some Belatacept-treated patients may be the presence of memory T cells resistant to co-stimulatory blockade [45]. In recent *in vitro* studies, we have observed the ability of IL-15, but not IL-2 or IL-7, to provoke the proliferation of human alloreactive CD28⁺ memory CD8⁺ T cells and their expression of effector functions including production of TNF α and IFN- γ and expression of CD107a indicating cytolytic function [46••]. Furthermore, IL-15 conferred CTLA4-Ig

resistance to alloreactive CD28⁺ memory CD8⁺ T cells. These results are consistent with the ability of a pro-inflammatory cytokine that is likely to be produced during inflammation in grafts to promote the proliferation and effector function of donor-reactive memory CD8⁺ T cells, whether CTLA4-Ig is administered or not.

Studying Memory T Cells in Animal Models

While a correlation between high numbers of alloreactive memory T cells and poor transplant outcomes has been observed clinically, studies in numerous animal models have been able to directly demonstrate a detrimental impact of memory T cells on allograft function and survival. Several approaches have been used to study the impact of donor-reactive memory T cells on allograft outcome in rodent transplant models. The most commonly used approach has involved priming naïve animals directly to donor antigens with a donor graft such as skin allograft to generate reactive T cells that develop into memory T cells 6–8 weeks later. The sensitized animals are then either challenged with a second allograft or the memory T cells are isolated from the sensitized animal and transferred to a naïve animal that is then itself challenged with the allograft. A complementary approach has been to utilize memory T cells expressing a transgenic TCR with known reactivity to graft alloantigens. Memory T cells generated by such means have all been demonstrated to be capable of mediating transplant rejection. It is well established that mice that have previously rejected an allograft develop donor-specific memory T cells that reject a second graft from the same donor with accelerated kinetics, a phenomenon known as second set rejection [47]. The potency of these memory T cells alone in mediating rejection is further supported by the demonstration that accelerated rejection of secondary allografts in such donor-antigen primed animals can occur in the complete absence of B cells and circulating antibody [48, 49]. Moreover, unlike naïve T cells, memory T cells are able to exert their effector functions and cause allograft rejection without first homing to secondary lymphoid tissues [23].

The generation of alloreactive memory T cells by homeostatic proliferation and cross-reactive heterologous immunity has also been used to study the impact of donor-reactive memory T cells in animal models of transplantation. Experimentally, the most commonly used approach to induce homeostatic proliferation utilizes the transfer of purified naïve CD4⁺ and/or CD8⁺ T cells into immunodeficient mice, such as RAG-1^{-/-} or RAG-2^{-/-} mice, which induces a robust expansion of memory phenotype cells, including those with alloreactive TCRs. When T cell receptor (TCR) transgenic or polyclonal congenically marked T cells are harvested, following homeostatic proliferation within such lymphopenic hosts, they can be

shown to express the functions of antigen-driven memory T cells. An additional approach to study alloreactive memory T cells in rodents has been to sensitize candidate recipients through infection with a virus or other infectious entity that induces antigen-specific memory CD4⁺ or CD8⁺ T cells that cross-react with the target allogeneic MHC molecules expressed by the allograft. Following recovery from the infection and the development of memory T cells, the sensitized animals are then challenged with the target allograft, or an allograft to which the generated memory T cells do not react as a control. This approach has been important in directly demonstrating the generation and consequence of heterologous immunity on the immune response to an allograft. Memory T cells generated through homeostatic proliferation or parasitic or virally induced heterologous immunity are similarly capable of directly mediating rejection of cardiac and skin allografts [19, 49, 50].

Memory T cells are increasingly being appreciated as significant barriers to the induction of donor-specific immunologic tolerance which remains the ultimate goal in transplantation. While numerous strategies have been developed to significantly prolong allograft survival or induce tolerance in rodent models, few if any have been successfully translated to non-human primate (NHP) models or human patients [51, 52]. One of the factors postulated to contribute to the success of tolerance induction strategies in pathogen-free lab mice versus their failure in larger animals is the level of environmental exposure and consequent development of alloreactive memory T cells [53]. In support of this, co-stimulation blockade of the CD154/CD40 pathway in the presence of donor-specific transfusion (DST) has been extremely successful in promoting long-term or permanent survival of various allografts in rodent models of transplantation [54]. However, this strategy fails to prevent allograft rejection in mice with high levels of donor-reactive memory T cells. Specifically, prior donor antigen sensitization of recipient animals, transfer of donor-specific memory T cells into naïve mice, and induction of cross-reactive memory T cells by viral infection all render recipient animals refractory to tolerance induction strategies that are extremely effective in naïve animals [55–58].

This manipulation of rodent recipients, to induce high numbers of donor-specific memory T cells, has clearly demonstrated that memory T cells are able to directly mediate aggressive rejection of transplanted organs that is resistant to co-stimulatory blockade therapies; and is reminiscent of the increased acute allograft rejection observed clinically. The antithesis of these observations is that the rejection of allografts in unsensitized rodent recipients takes longer, and that such rejection can be obviated using many different co-stimulatory blockade strategies and other tolerance inducing strategies. The observation that such strategies are not efficacious when applied to NHP or clinical transplant patients has emphasized the importance of memory T cells induced

through supposed greater environmental antigen exposure. This implies that the numbers of these memory T cells, in rodents housed in Specific Pathogen Free environments in research facilities, are not sufficient to mediate rejection of an allograft independently of the primary effector T cells developing *de novo* in response to an allograft; and are not sufficient to confer resistance to the co-stimulatory blockade strategies that fail in donor alloantigen sensitized recipients.

It has been previously demonstrated that naïve unmanipulated adult laboratory mice possess a repertoire of endogenous memory T cells which are naturally generated from environmental exposures. While these endogenous memory CD8⁺ T cells have not been previously primed to donor antigens, a proportion of this endogenous memory T cell repertoire in naïve mice are reactive to donor class I MHC molecules, and are able to rapidly detect and infiltrate cardiac allografts within just hours of graft reperfusion [59]. Endogenous memory CD4⁺ T cells also rapidly infiltrate the allografts but their activation to express function is unclear at this time. While further studies detailed the ability of these endogenous memory CD8⁺ T cells to undergo proliferation, and produce IFN- γ within the allograft upon specifically reacting against donor class I MHC [59], their expression of effector functions were found to be insufficient to directly mediate allograft rejection, findings consistent with prior observations [60]. This discrepancy, in the ability to effect transplant rejection between naturally generated endogenous memory T cells, and those deliberately generated to be donor-antigen reactive, suggests that the priming strategies used to generate the latter, disproportionately bias the endogenous T cell receptor repertoire to strong reactivity to donor antigens (Fig. 1). While these rodent models, inducing high numbers of donor-reactive memory T cells, yield accelerated allograft rejection and resistance to co-stimulatory blockade induced tolerance, the extent to which the endogenous memory T cell repertoire has been skewed is rarely seen naturally in NHPs and human patients.

We realized however, that cardiac transplantation performed in most laboratories, including our own, is performed as quickly as possible with cold ischemic storage time minimized. One reason for this approach is to optimize results testing allograft-prolonging strategies such as co-stimulatory blockade. Ischemia-reperfusion injury is an inherent pathological challenge of transplantation, and numerous studies have demonstrated that organs from deceased donors, and grafts subjected to longer periods of ischemia, correlate with poorer graft function and survival following transplantation [61–68]. With these studies and our results in mind, we hypothesized that increasing the duration of cold ischemic storage would generate an inflammatory environment within the allograft, to promote endogenous memory CD8⁺ T cell activation to sufficient levels that increased effector functions, and provoked allograft failure in “naïve” unprimed mice. Increasing the duration of cold ischemic storage in a clinically relevant

Fig. 1 Repertoires of donor-reactive memory T cells in unprimed vs. donor-antigen primed individuals. A hypothetical representation of the endogenous memory T cell repertoire (blue) within (a) unmanipulated animals and (b) following priming by strategies typically used to induce high numbers of donor-reactive memory T cells. While such priming approaches certainly expand the population of memory T cells reactive to donor-MHC (green), it is likely that the frequency of strongly donor-reactive memory T cells (i.e., those with high affinity TCRs for donor antigen) (red) is also significantly increased

manner resulted in heightened early infiltration of endogenous memory CD8⁺ T cells into the allograft and their increased expression of effector functions [69]. Furthermore, allografts subjected to prolonged ischemia conferred endogenous CD8⁺ memory T cells resistance to the effects of CTLA4-Ig, and the ability to reject the allograft independently of donor-reactive T cells that developed *de novo* from naïve precursor T cells. While further work is needed to define the impact of the post-ischemic inflammatory environment on the clonal expansion and/or function of endogenous memory CD8⁺ T cells; these initial findings recapitulate the detrimental impact of endogenous alloreactive memory T cell responses observed in NHP recipients and clinical transplant patients in a mouse model without prior recipient priming to bias the endogenous memory T cell repertoire to strong donor reactivity. In light of these studies, an interesting point to consider is whether the priming strategies currently used to generate high frequencies of donor-antigen reactive or cross-reactive memory T cells in rodent models are physiologic recapitulations of the clinical impact of endogenous memory T cells in transplantation.

Targeting Memory T Cells in Transplantation

Considering the negative impact of memory T cells in transplantation, and their unique characteristics hindering tolerance induction, strategies to selectively target alloreactive memory cells should improve transplant outcomes. The need for memory T cell-directed therapies in transplantation has arisen from observations that immunosuppressive agents that are effective against naïve T cells often have minimal effects against memory T cells [70]. In particular, treatments directed at traditional T cell activation and depletion therapies have been found to be less effective in preventing allograft rejection in the presence

of alloreactive memory T cells. However, studies in several experimental transplant models have suggested that targeting the trafficking and infiltration of memory T cells into the allograft, and their subsequent activation and proliferation, may be promising therapeutic approaches.

Once within the graft, alloreactive memory T cells proliferate, and are capable of mediating significant injury; and thus inhibiting their initial trafficking into the transplanted organ itself has been demonstrated to be beneficial to allograft survival. The administration of FTY720, a sphingosine-1 phosphate receptor agonist, prevents lymphocyte migration from the thymus and peripheral lymphoid tissues, effectively sequestering T cells in the lymph nodes and inhibiting them from trafficking to peripheral graft sites. In mouse models, treatment with FTY720 has been shown to sequester memory CD4⁺ T cells in the lymph nodes, and delay heart allograft rejection [71]. However, this sequestration does not affect the ability of donor antigen-reactive CD4⁺ T cells to provide the helper signals needed to drive a donor-specific antibody response, and it is this antibody that underlies the rejection of the allograft in such FTY720-treated recipients. Numerous studies have also shown that disruption of adhesion molecules, notably leukocyte integrins such as LFA-1 and VLA-4, is quite effective in inhibiting memory T cell infiltration into grafts. Mouse models of transplantation have shown that anti-LFA-1 or anti-VLA-4 monoclonal antibodies result in attenuation of donor-reactive memory recall responses and decreased T cell trafficking to the allograft, ultimately resulting in prolonged survival of allografts [69, 72, 73]. While testing anti-LFA-1 mAb in NHP models of transplantation was also promising [74], clinical testing of the anti-LFA-1 agent efalizumab in renal transplantation suggested that this agent evoked a higher rate of EBV-associated malignancy [75]. Thus, although anti-LFA-1 antibody is not currently clinically

available, these findings in animal models suggest that targeting other trafficking molecules on memory T cells, such as VLA-4, may be similarly efficacious and warrant further investigation.

While there is a growing body of evidence to suggest that memory T cells are relatively resistant to traditional co-stimulation blockade, some studies have suggested that memory T cells may use unique alternative co-stimulatory pathways for activation and effector functions. For CD8⁺ T cells, the engagement of 4-1BB (CD137) by its ligand has been shown to provide both CD28-dependent and CD28-independent signals that result in proliferation, cytokine production, augmented cytotoxic effector activity, and enhanced cell survival. Blockade of the 4-1BB co-stimulatory pathway in mouse models has shown some efficacy in prolonging survival of intestinal, skin, and cardiac allografts [76, 77]. Another co-stimulatory pathway that may be important for the recall of memory T cells, particularly the CD4⁺ population, is the OX40/OX40L pathway. Deficiency or blockade of OX40 has been shown to result in impaired memory CD4⁺ T cell formation, and in transplantation models was found to prolong survival of heart and skin allografts in recipients possessing donor-antigen primed or memory T cells [19, 78, 79].

Targeting the proliferation of memory T cells induced by cytokine and TCR signaling is another potential therapeutic approach. Janus kinases (JAK) are a family of kinases which transduce cytokine mediated signals via the JAK-STAT pathway. JAK3, expressed primarily in hematopoietic cells, is downstream of the common γ chain (CD132) which binds a broad array of cytokines including IL-2, IL-7, IL-15, and IL-21. These cytokines have been demonstrated to have important roles in the generation, maintenance, and proliferation of memory T cells, making JAK inhibitors a potential molecular target for blockade of cytokine signals. A highly selective and potent JAK3 inhibitor, tofacitinib (CP-690550), has been shown to prevent allograft rejection in both murine and NHP models, and is currently finishing phase II clinical trials in renal transplantation [80–82]. Signals for cell proliferation and survival may also be induced through TCR signaling and attempts to inhibit nuclear factor- κ B (NF- κ B), a protein complex that regulates DNA transcription, have also been promising. Inhibition of NF- κ B has been shown to prevent activation of donor-specific memory CD8⁺ T cells, and synergize with co-stimulatory blockade to prolong skin allograft survival in rodent models, and suppress the proliferation of rapamycin-resistant memory T cell populations in NHPs [83, 84].

Conclusion

The unique characteristics that make memory T cells so effective and beneficial in protective immunity against recurrent

pathogens also endow them to be formidable barriers to successful transplantation. The anatomic advantages to accessing antigen, increased frequency, hyper-responsiveness, and ability to exhibit heterologous immunity are critical features, enabling memory T cells to vigorously defend their hosts; which unfortunately subvert strategies directed at inducing long-term allograft survival and transplant tolerance. Further advances in our understanding of the mechanisms of memory T cell generation, activation, function, and trafficking to allografts are necessary to develop clinically translatable approaches to target donor-reactive memory T cells in order to eliminate or attenuate allograft rejection.

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Compliance with Ethics Guidelines

Conflict of Interest Charles A. Su and Robert L. Fairchild declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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