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The evolving roles of memory immune cells in transplantation

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Abstract

Memory cells are the products of immune responses but also exert significant impact on subsequent immunity and immune tolerance, thus placing them in a unique position in transplant research. Memory cells are heterogeneous, including not only memory T cells but also memory B cells and innate memory cells. Memory cells are a critical component of protective immunity against invading pathogens, especially in immunosuppressed patients, but they also mediate graft loss and tolerance resistance. Recent studies suggest that some memory cells unexpectedly act as regulatory cells, promoting rather than hindering transplant survival. This functional diversity makes therapeutic targeting of memory cells a challenging task in transplantation. In this article we highlight recent advances in our understanding of memory cells, focusing on diversity of memory cells and mechanisms involved in their induction and functions. We also provide a broad overview on the challenges and opportunities in targeting memory cells in the induction of transplant tolerance.

Introduction

Memory cells are part of the immune repertoire, and they are indispensable in protective immunity. But memory cells do represent a unique challenge in transplantation. On one hand, they are needed for protection against a myriad of pathogens, and without them patients would be in grave danger. On the other hand, memory cells endanger transplant survival and contribute to graft loss. This duality of memory cells constitutes a significant paradox in management of transplant survival in transplant patients. Interestingly, the topic of memory is constantly evolving. Memory cells are thought to be a feature adaptive immunity in which naïve T and B cells are transformed into memory phenotypes during immune responses to confer long term protection. However, recent studies suggest that certain types of innate immune cells, such as NK cells and macrophages, can also acquire memory features in that they can mediate robust recall responses.(1,2) Furthermore, in certain transplant models, subsets of memory cells have been recently shown to function as regulatory cells, and contrary to the initial belief, such memory T cells actually promote transplant survival.(3,4) Additionally, not all memory cells are committed to memory functions at all times. For example, central memory T cells can be converted to Foxp3⁺ T

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cells, while effector memory T cells fail to do so.(5) Furthermore, central memory T cells, but not effector ones, can be readily tolerized. Together, these new findings clearly put memory cells to a new level of complexity, and certainly invite re-assessment of the role of memory cells in transplant outcomes. In this context, the topic of memory cells remains timely.

In this review, we highlight the latest findings in these areas and discuss potential implications in transplant tolerance. We focus on memory T cells, highlighting their generation, unique features, and challenges and emerging approaches in targeting memory T cells toward desired outcomes. Memory B cells in transplantation have been recently reviewed by Chong and Sciammas(6), and will be briefly mentioned here. We also discuss the recently discovered innate memory cells, their relationships with adaptive memory cells, and their contributions to immunity and immune pathology.

What are memory cells and what do they do?

Memory cells are a unique cell population in the immune system; they are extremely diverse and dynamic, consisting of multiple cell types (T cells, B cells, NK cells, macrophages, etc.), and even within each cell type there are also many different subsets. These diverse memory cell types and subsets collectively form the memory pool in the body. Generally speaking, memory cells are long-lived cells and exhibit heightened responses to recall antigens; they are progenies of naïve lymphocytes and developed after productive responses upon antigen stimulation. Consequently, the memory pool differs among individuals due to differences in immune history, pathogen exposures, and age. Memory cells are traditionally believed to be key features of adaptive immune cells (i.e., T cells and B cells), but recent studies have challenged this belief and further suggested that certain innate immune cells, such as NK cells and macrophages, can also acquire certain memory properties(1,2), a finding that is of great significance in transplantation.

Memory cells distinguish themselves from naïve ones with several outstanding features. Phenotypically, memory cells express distinct surface markers, and in the case of T cells in the mouse, memory T cells express high levels of CD44, and therefore, they are identified as CD44^{high} cells, whereas naïve T cells are CD44^{low}. In rats, naïve T cells are contained in the CD45RC^{high} population while memory T cells are present in both CD45RC^{high} and CD45RC^{low} populations. Interestingly, regulatory T (Treg) cells, which are specialized in immunosuppression, often have memory phenotypes.(7,8) In humans, naïve T cells and memory T cells are segregated by surface expression of different CD45 isoforms in that memory T cells express the CD45RO isoform, whereas naïve T cells express the CD45RA or RB isoform. Moreover, additional expression of CCR7 (a chemokine receptor for CCL21) further divides memory T cells into central memory T cells (Tcm) and effector memory T cells (Tem).(9) In both humans and mice, Tcm cells are CCR7⁺, whereas Tem cells are CCR7⁻. Also, CD62L (also called L-selectin) is another surface maker that is commonly used to differentiate Tcm from Tem cells. It should be noted that Tcm and Tem cells are found in both the CD4 and the CD8 compartments. Anatomically, Tcm and Tem cells are circulating cells but they preferentially reside at different sites in vivo; Tcm cells are primarily located in the secondary lymphoid organs, while Tem cells are present in both

the lymphoid and non-lymphoid organs. Functionally, Tem cells exhibit much better effector functions than Tcm cells, while Tcm cells show greater proliferation potential than Tem cells.(10) Furthermore, based on their cytokine profiles, memory T cells are divided into Th1, Th2, Th9, Th17 subsets, and different subsets often accumulate in different tissues during immune responses, suggesting that memory T cells are likely to be compartmentalized in vivo.(11) More recently, tissue-resident memory T (Trm) cells have been identified as a new memory T cell subset.(12) Trm cells express CD69 and CD103 (integrin α E), and are non-recirculating cells and located at common portals of pathogen entry sites, such as mucosa and skin.(12) Trm cells not only provide rapid control upon pathogen reinfection, but also trigger immunity against antigenically unrelated pathogens. (13,14) Some CD69⁺ Trm cells are also present in lymphoid tissues and may guard pathogen entry points in these tissues.(15,16)

Memory T cells also possess other features to fulfill their protective functions. Memory T cells, as a population, are long-lived cells and have self-renewing capability in vivo. Once developed, memory cells persist long-term, providing lifelong protection against pathogens. Mechanistically, memory T cells replenish themselves through slow cell divisions, without losing their memory identities, a process that is driven primarily by homeostatic cytokines. (17) Memory T cells have a clear survival advantages over their naïve counterparts, and they are often resistant to lymphocyte depleting therapies. This is best exemplified by the finding that in both humans and mice anti-lymphocyte antibodies readily deplete naïve T cells, but not memory T cells in the periphery.(18) Importantly, memory T cells exhibit a much lower activation threshold than naïve T cells, primarily due to their reduced reliance on CD28 and CD154 costimulatory signals for activation.(19) Thus, conditions that fail to activate naïve T cells often trigger robust activation of memory T cells. Because of this, memory T cells respond to recall antigens more vigorously than naïve T cells, and at a per cell basis, memory T cells give rise to far more effector cells than their naïve counterparts. Furthermore, in contrast to naïve T cells, which are confined to secondary lymphoid tissues, memory T cells reside in both the lymphoid and the non-lymphoid tissues (e.g., the liver, lung, and the gut).(10,12) Thus, they are well positioned to initiate protective immunity in the body. In addition, activation of memory T cells does not require the presence of secondary lymphoid organs, places where activation of naïve T cells occurs.(20) Taken together, memory T cells are well equipped, strategically positioned, and extremely efficient in responding to antigens in the immune system.

In the B cell lineage, B cell memory provides long-lasting humoral immunity against many infectious diseases and is a key component of successful vaccination. B cell memory consists of long-lived plasma cells, which continuously produce antibodies in the periphery (the serological memory), as well as long-lived memory B cells.(21) Memory B cells are responsible for the rapid burst of antigen-specific antibodies following antigen re-encounter. Phenotypically, CD27, a member of the tumor necrosis factor receptor family, has been used to distinguish between memory (CD27⁺) and naïve (CD27⁻) B cells in humans, though CD27⁻ memory B cells have also been reported. CD27⁺ memory B cells mainly consist of somatically mutated IgM⁺IgD⁺ and isotype switched (IgG⁺ or IgA⁺) cells.(21) Plasma cells are characterized by expression of CD138 (syndecan 1). Moreover, plasma cells lose the expression of pan-B cell markers, CD19 and CD20, but retain CD27.(22) Murine memory B

cells do not have a definitive marker. Tomayko et al. have identified several murine memory B cell subsets according to the differential expression of CD80, PD-L2, and CD73.(23) The function and relationship between these subsets remain unclear.

One of the most exciting findings recently is that some innate immune cells can also acquire memory features. It has been reported that about 50% murine NK cells express the Ly49H receptor, which recognizes the mouse cytomegalovirus (MCMV)-encoded m157 antigen. Upon MCMV infection, Ly49H⁺ NK cells underwent clonal expansion and contraction, followed by formation of memory-like NK cells. Adoptive transfer of these MCMV-experienced NK cells into naïve mice provided protection against MCMV challenge.(1) This memory behavior of NK cells has also been reported in mice infected with vaccinia and influenza viruses.(24,25) Moreover, evidence of NK cell memory has been shown in response to haptens. Following sensitization with a hapten, *Rag2*^{-/-} mice (lacking T and B cells), but not *Rag2*^{-/-}*Il2rg*^{-/-} mice (lacking T, B and NK cells), developed substantial contact hypersensitivity responses to the re-challenge with the same hapten. Hence, NK cells can mediate memory recall responses independent of B cells and T cells.(26) Intriguingly, *Candida albicans* infection and Bacille Calmette-Guerin vaccine promote functional reprogramming of monocytes that affords protection from re-infection.(27,28) The term “trained immunity” has thus been used to describe how the initial infection increases the monocyte response to the secondary infection. Recent studies show that trained immunity is associated with significant epigenetic changes during monocyte-to-macrophage differentiation, which in turn modulate monocyte/macrophage function by affecting cellular metabolism and signaling pathways.(29,30) However, surface markers and lifespan of “trained” innate immune cells remain to be determined. It should be noted that in many aspects, such “innate memory” remains very different from the “adaptive memory” in that innate memory cells often exhibit heightened responses in an antigen non-specific manner.

Clearly, research on innate memory cells is an emerging area. In the context of transplantation, we reported that NK cells can mediate skin allograft rejection upon IL-15-driven maturation, and monocytes/macrophages can be induced to an alloreactive state where they readily recognize and reject allogeneic cells.(2,31) In the case of alloreactive macrophages, activated CD4⁺ T cells are involved in licensing such macrophages via CD40/CD40L engagement.(2) Others also reported alloreactive potential of monocytes/macrophages in a murine heart transplant model.(31)

How are memory cells generated and controlled?

The memory cell pool is established by multiple mechanisms. Antigen sensitization remains the primary source of memory T cells, and such memory T cells exhibit well-defined antigen specificities. In transplant settings, donor antigen exposure via blood transfusion, pregnancy, and prior transplant all lead to the induction of donor specific memory T cells.(32) Recent studies have demonstrated other mechanisms whereby memory T cell are induced, and such mechanisms include homeostatic cell proliferation and heterologous immunity.(33) Homeostatic proliferation occurs under lymphopenic conditions in which naïve T cells are directly converted to memory cells without foreign antigen stimulation, and this type of cell proliferation is primarily driven by excess of cytokines (e.g., IL-7, IL-15) in the

lymphopenic hosts.(34) Additionally, memory T cells generated in response to one antigen can react to other unrelated antigens, a process called heterologous immunity.(33) A major implication of heterologous immunity in transplantation is that pathogen specific memory T cells, which are numerous in humans, can respond to alloantigens, and therefore they are alloreactive. The exact contribution of homeostatic proliferation and heterologous immunity to the overall alloreactive memory pool is unclear, but memory T cells generated by either mechanisms can mediate allograft rejection.(33)

Another potential mechanism is the presence of dual TCRs on a subset of mature T cells. During T cell development in the thymus, a second TCR α chain may be expressed by developing T cells with a “non-selectable” TCR. This leads to the presence of mature T cells expressing dual TCRs, comprising of two α chains paired with the same β chain. In the periphery, the non-selectable TCR is functional and exhibits protective properties by responding to the foreign antigens. Once dual TCR T cells are activated through the non-selectable TCR by viral infection, they can trigger autoimmunity using the other TCR that recognizes self-antigen.(35) Strikingly, antigen stimulation through one TCR on dual TCR T cells facilitate the generation of memory T cells, which are capable of giving a response to an unrelated antigen through the other TCR.(36) It will be interesting to explore whether this property of dual TCR T cells is one of the reasons for generating memory T cell response in transplantation.

The homeostasis of memory T cell in vivo is controlled primarily by cytokines and costimulatory molecules. IL-7 is important for CD4⁺ and CD8⁺ memory T cell survival, while IL-15 supports intermittent proliferation of CD8⁺ memory T cells. Expression of IL-7R α is used as a marker for memory precursor effector cells (MPECs), which favors a memory T cell fate during the contraction phase. By contrast, short lived effector cells (SLECs) express high level of KLRG1 but not IL-7R α .(9) Pro-apoptotic Bim mediates the massive contraction phase and apoptosis of KLRG1^{high}IL-7R α ^{low} SLECs (Figure 1). In IL-7R α ^{high} MPECs, Bcl-2 induced by IL-7 (or IL-15) binds to and counteracts Bim, which permits MPEC survival and transition into a memory state.(37) Costimulatory molecules, especially those in the TNFR superfamily, act synergistically with cytokines to support memory T cell generation and homeostasis. This notion is supported by findings that simultaneous blockade of both OX40 and IL-7 signaling completely inhibit CD4⁺ Tem cell proliferation in lymphopenic mice.(38) Moreover, IL-15-induced 4-1BB expression maintains CD8⁺ memory T cell survival in the absence of antigen.(39)

Additional mechanisms for memory T cell generation are also emerging. Upon immunization, a single CD8⁺ naïve T cell produces progeny with diverse effector and memory cell fates.(40) The asymmetric cell division model suggests that the fate decision between effector and memory T cells can arise from a single T cell as early as the first cell division after TCR stimulation, which is due to unequal protein inheritance in daughter T cells.(41) During the process of activation, expansion and contraction phases of T-cell response, multiple signaling pathways and transcriptional programs are involved in controlling SLEC and memory T cell fates.(9) We include a simplistic model to illustrate distinct fates of effector T cells (Figure 1), and will briefly discuss the molecular basis of memory T cell formation.

Cytokine signals instruct memory T cell development during the early phase of T cell activation. Within the first asymmetric division of primed CD8⁺ T cells, IL-2Rα^{low} cells exhibit a greater capacity to give rise to Tcm cells than that of IL-2Rα^{high} cells.(42) IL-2 induces the expression of the transcriptional repressor Blimp-1 and represses the transcriptional repressor Bcl-6 and the cytokine receptor IL-7Rα. Blimp-1 then promotes the terminal differentiation of CD8⁺ effectors and inhibits Tcm cell formation. Conversely, Bcl-6, a well-characterized antagonist of Blimp-1, supports Tcm cell generation.(9) These findings reflect the molecular basis of IL-2 in fate decision between effector and memory T cells. IL-12 signal and subsequent IFN-γ production also hinder the formation of CD8⁺ memory T cells.(9) IL-21 and IFN-γ are known to induce the transcription factor T-bet. Although T-bet and another transcription factor Eomes coordinately control CD8⁺ CTL function, their expression is somewhat reciprocal. Recent studies have shown that accumulating amounts of T-bet in SLECs drives their terminal effector differentiation; whereas high levels of Eomes in MPECs enable them to compete for the memory cell niche. (9) Taken together, IL-2, IL-12, and IFN-γ facilitate the generation of fully activated effector cells and limit long-term T cell memory via transcriptional regulation (e.g., Blimp-1 vs. Bcl-6, T-bet vs. Eomes) (Figure 1). By contrast, IL-10/IL-21/Stat3 pathway has been shown to commit CD8⁺ effectors into memory T cells. In the absence of IL-10/IL-21 or Stat3, CD8⁺ effector cells retain a terminal differentiation state and fail to mature into functional memory T cells. Stat3 is required to sustain expression of suppressor of cytokine signaling 3 (SOCS3), BCL-6, and Eomes. The reduced expression of SOCS3 in Stat3^{-/-} CD8⁺ T cells may render them hyperresponsive to IL-12.(43)

Memory T cell generation also involves several other aspects. For example, not all antigen-primed T cells exhibit equal potential to form memory T cells. T memory stem cells (Tscm), a population of self-renewal cells displaying both naïve (e.g., CD45RA⁺CD62L⁺) and memory (e.g., IL-2Rβ⁺CXCR3⁺) markers, represent the least differentiated memory T cell subset and display a multipotent ability to derive Tcm, Tem, and effector T cells.(44) These properties of Tscm cells may be critical for maintaining long-term T cell memory. It has been suggested that memory T cells may also develop without going through a “full” effector phase. Vaccination with heat-killed *Listeria monocytogenes* induces a substantial population of CD8⁺ memory T cells without an overt effector phase, though these memory cells are less potent at providing protection from *L. monocytogenes* infection.(45) Moreover, each memory T cell subset may have a unique developmental pathway. An in vitro study shows that CD45RA⁺ effector memory (TEMRA; CD45RA⁺CCR7⁻) cells are derived from Tcm cells upon stimulation with homeostatic cytokines IL-7 and IL-15, but not antigen.(46)

The recent discovery of metabolic pathways in the control of memory T cell generation is significant, and such pathways are related to energy production in different phases of T cell response.(47) Naïve-to-effector and effector-to-memory T cell transitions present distinct metabolic demand. In essence, naïve T cells are quiescent and rely on fatty acid oxidation (FAO) as an energy source. Upon activation T cells quickly switch to glucose glycolysis and glutaminolysis to meet their energy demands for proliferation and differentiation to effector cells.(47) This is mediated primarily through TCR triggered activation of the PI3K-Akt-mTOR axis.(48) For the activated T cells to become memory cells, they must switch back to

FAO as an energy source. Otherwise they remain as effector T cells and die of apoptosis. Thus, transition from glycolysis to FAO is a critical checkpoint in memory cell generation, and pathways regulated by AMP-activated protein kinase (AMPK) and TNFR-associated factor 6 (TRAF6) play a critical role in this transition. This is supported by recent reports that generation of memory T cells is impaired in AMPK or TRAF6 deficient mice, as activated T cells fail to make the transition to FAO.(49,50) Interestingly, in certain experimental settings, inhibition of the Akt/mTOR pathway during the contraction phase of a T cell response can markedly enhance memory T cell generation by limiting glucose glycolysis.(48) Inhibition of mTOR also enhances activity of the forkhead transcription factor FOXO1, which represses terminal effector differentiation and establishes the memory program in MPECs.(51) These findings undoubtedly open new avenues of investigation in manipulating memory T cells in various disease settings.

The revelation that memory T cells can acquire an exhausted phenotype suggests that there might be other approaches to control memory T cells in transplantation.(52) In general, exhausted T cells are known to progressively lose their effector activities.(53) This is followed by physical deletion from the T cell repertoire. Exhausted T cells express cell surface receptors that usually transduce inhibitory signals, such as PD-1, Tim-3, LAG-3, CD160, CTLA-4 and many others.(53,54) Some of these inhibitory receptors (e.g., PD-1) are critical to the exhausted phenotype. In addition, mechanisms that control telomere erosion in memory T cells may also be involved in regulating memory exhaustion.(55) The exact mechanisms of T cell exhaustion are unclear, but several factors favor the induction of the exhausted phenotype. Antigen persistence is a key contributing factor. Furthermore, an inhibitory process, both intrinsic and extrinsic, is also required for the induction of T cell exhaustion. The inhibitory receptors on the cell surface provide an intrinsic mechanism, while the production of suppressive cytokines such as TGF- β , IL-10, IL-35, as well as the presence of regulatory T cells may constitute an extrinsic mechanism. In certain models, lack of help from CD4⁺ T cells or disruption of secondary lymphoid structures also favors T cell exhaustion.(52) Intriguingly, Blimp-1 maintains CD8⁺ effector terminal differentiation and represses memory T cell formation,(9,56) which may lead to a “restless” cell state and eventually drive CD8⁺ T cell exhaustion.(57) BATF, a transcription factor that is required for Th17 and Tfh cell differentiation, is involved in transcribing Blimp-1 during CD8⁺ effector differentiation.(58) PD-1 has been shown to up-regulate BATF expression in exhausted HIV-specific CD8⁺ T cells. Silencing BATF reverses this exhausted state.(54) All of these factors are well represented in transplant settings, and whether memory T cells could be pushed to exhaustion in favor of graft survival deserves more attention.

Much less is known about B cell memory in transplantation. The presence of donor-specific anti-HLA antibodies (DSA) in sensitized patients likely indicates the existence of long-lived plasma cells specific for donor antigens. Moreover, DSA levels post-transplant are promptly increased in sensitized patients(59), suggesting that alloreactive memory B cells exist and promptly differentiate into high-affinity plasma cells. Both long-lived plasma cells and memory B cells are derived from naïve B cells, though through distinct differentiation pathways and transcriptional regulation.(60) Naïve B cells acquire antigens at the boundary between the follicle and the subcapsular sinus of lymph nodes. Primed B cells migrate to the B cell-T cell borders where they interact with Tfh cells that recognize the same antigen, and

this interaction induces antibody class switch recombination (CSR) and plasma cell development via an extra-follicular pathway.(61) This initial Tfh-B cell contact also facilitates the formation germinal centers (GCs), where B cells further scan antigens presented by DCs, contact again with Tfh cells, undergo CSR and somatic hypermutation, and develop into plasma and memory B cells.(62)

Various cytokines influence B cell survival, differentiation, and isotype switch. For instance, T cells help B cell response not only through cell to cell interaction (e.g., CD40-CD40L), but also through producing IL-4 and IL-21. IL-4 promotes CSR to IgG1 and IgE, whereas IL-21 enhances CSR to IgG of all types. Because IL-21 is a potent inducer for both BCL-6 and Blimp-1, it plays a complex role in B and Tfh cell differentiation.(63) Moreover, B cell activating factor (BAFF), a TNF family cytokine, maintains B cell homeostasis by supporting survival of naïve and memory B cells. BAFF also promotes the generation of rapidly-dividing plasmablasts (Ig-secreting cells) from activated memory B cells by enhancing their survival.(64) In a clinical study that enrolled 143 patients with stable kidney graft function for more than 5 years, elevated serum BATF levels predicted generation of de novo DSA and elevated BAFF-R transcripts represented a higher risk of developing graft dysfunction.(65) Further studies are required to delineate the roles of IL-21 and BAFF in antibody-mediated transplant rejection (AMR).

Why do memory cells matter in transplantation?

Besides providing protective immunity, memory T cells are also capable of mediating transplant rejection. It is well recognized that donor specific memory T cells mediate “second-set” rejection that is extremely difficult to inhibit(66), and all measures are used now to avoid such scenario in clinical transplantation. In animal models, memory T cells alone are sufficient to trigger rejection; they are among the first cell types infiltrating the grafts, and memory T cells don't need the secondary lymphoid tissues to gain effector functions.(20,67) Furthermore, Tcm and Tem cells appear to be equally potent in mediating the rejection response.(68)

Memory T cells generated through homeostatic proliferation or heterologous immunity are also capable of mediating transplant rejection. It has been shown that memory T cells post homeostatic proliferation are potent effector cells in rejection of heart and skin allografts. (19,69) Similarly, heterologous immunity also results in the generation of memory T cells that are alloreactive in transplant settings. For example, infection of B6 mice with parasitic or viral antigens resulted in the generation of memory T cells that are directly reactive to alloantigens, and therefore, heart transplant grafted onto such pathogen challenged mice were rejected in an accelerated fashion.(70) In selected models, memory T cells are shown to be key mediators in chronic allograft rejection, suggesting a possibility that memory T cells and chronic rejection may be intimately linked. Thus, regardless of memory phenotypes or mechanisms by which they develop, memory T cells are capable of mediating rejection. In humans, a large proportion of alloreactive T cells (~50%) reside in the memory compartment, as recently shown by Macedo et al.(71), suggesting that heterologous immunity may contribute significantly to the overall alloreactive memory T cells in healthy individuals.

A significant issue is that memory T cells are not as amendable as their naïve counterparts to tolerizing therapies. In a variety of animal models, memory T cells are found to be highly resistant to tolerance induction. Thus, tolerizing therapies that produce allograft tolerance in naïve animals often fail to do so in memory-rich animals. For example, donor specific transfusion (DST) plus anti-CD154 (MR1) is very effective at inducing allograft tolerance in rodents. However, this protocol completely fails to prevent rejection in donor antigen pre-sensitized mice.(72) Similarly, transferring donor-specific memory T cells into naïve mice breaks allograft tolerance induced by DST and anti-CD154 treatment. Similarly, memory T cells developed through heterologous immunity are also barriers to tolerance induction. This is elegantly demonstrated by Adams et al that virally induced memory T cells prevented the induction of mixed bone marrow chimerism and donor specific tolerance.(73) Similar findings have also been reported in kidney transplantation in the clinic. Patients with higher frequency of memory T cells are associated with worse transplant outcomes under conventionally immunosuppression.(74)

Memory T cells developed by homeostatic proliferation are also resistant to tolerization. For example, treatment of naïve B6 mice with depleting anti-CD4 and anti-CD8 mAbs induces profound depletion of peripheral T cells. However, a subset of residual T cells undergoes extensive homeostatic proliferation in the treated mice, and memory T cells post homeostatic proliferation are highly resistant to tolerization by DST plus CD154 blockade treatment.(69) Similarly, in models where memory T cells are generated in immunodeficient mice through homeostatic proliferation, CD28 and CD154 costimulatory blockade also fails to tolerize such memory cells.(19) Clearly, memory T cells are inherently resistant to tolerization.

Another significant issue is that memory T cells often evade Foxp3⁺ Treg-mediated suppression.(75) Thus, in transplant settings where tolerance is mediated through Tregs, memory T cells may break tolerance and trigger graft rejection. Earlier work by Yang et al showed in an adoptive transfer model that Foxp3⁺ Tregs effectively inhibited rejection triggered by naïve CD4⁺ T cells but not by memory CD4⁺ T cells.(76) Tregs were similarly found to be unable to suppress alloreactive memory CD8⁺ T cells. A study in humans showed that memory T cells are also resistant to suppression by Tregs.(77) Thus, tolerance strategies that boost Tregs as a way to achieve tolerance are unlikely to be effective against memory T cells. What mediates resistance of memory T cells to Tregs and whether memory T cells can be rendered sensitive to Tregs are important areas of investigation.

Can memory cells be therapeutically targeted?

It is generally believed that memory T cells are hurdles to tolerance induction. Thus significant efforts have been devoted to therapeutically inhibit memory T cells, especially allospecific memory cells, while leaving non-alloreactive ones unaltered. However, recent studies in the identification of regulatory memory T cells that support transplant survival present further challenges in targeting memory T cells in transplant settings.(3,78)

Current immunosuppressive drugs that are effective at inhibiting naïve T cells have minimal effects at preventing memory T cell-mediated rejection(79), confirming that the activation

requirement of memory T cells is quite different from that of naïve T cells in rejection. In fact, there is a strong correlation between the presence of pre-transplant alloreactive memory T cells and acute rejection episodes that occurred despite tacrolimus- and sirolimus-based therapies. Moreover, depletion therapies (e.g., anti-thymocyte globulin or ATG) are less effective at eliminating pre-existing memory T cells.(18,80) In fact, following depletion therapies, memory CD4⁺ T cells are a dominant cell type remaining and are capable of initiating rejection episodes. In some studies, the commonly used immunosuppressive drugs inhibit the ability of memory T cells to respond to alloantigens in vitro.(18,79) However, the poor transplant outcomes in patients with high memory T cell frequency suggest that the in vivo efficacy of conventional immunosuppression drugs in containing memory T cells is limited.

Clearly, alternative approaches are needed to targeting alloreactive memory T cells in transplant settings. Much attention is devoted to prevent activation and survival of memory T cells and ways to block their accumulation in the grafts. Memory T cells appear to use alternative costimulatory pathways for activation and effector functions.(81) For example, 4-1BB/4-1BBL interactions have been shown to be important in CD8⁺ T-cell recall responses. Additionally, OX40/OX40L pathway is pivotal for the generation of CD4⁺ memory cells. In certain models, OX40 deficiency or OX40 blockade resulted in greatly impaired memory CD4⁺ T cells.(82) In transplant models in which rejection is dominated by memory T cells, blocking OX40/OX40L pathway facilitated survival of heart and skin allografts.(19) Thus, memory T cells require different signals to develop or function in transplant rejection, which suggests new opportunities in therapeutic intervention of memory T cells in transplant settings. However, the validity of these pathways awaits further testing in large animal models.

There have been other attempts to control memory T cells in transplant models, and progress in this area is equally exciting. For example, the NF- κ B blocker LF15-0195, an analog of 15-deoxyspergualin, prevented activation of donor specific memory CD8 T cells and synergized with costimulatory blockade to induce heart allograft survival in donor skin-presensitized mice.(83) Administration of the sphingosine-1 phosphate receptor agonist FTY720 resulted in sequestration of donor-specific memory CD4 T cells in the peripheral lymph nodes and delayed heart allograft rejection in mice.(84) Peritransplant anti-LFA-1 mAb effectively inhibited early allograft infiltration of donor-reactive memory CD8 T cells in murine models.(85) When combined with costimulatory blockade, anti-LFA-1 mAb also attenuated memory T-cell recall effector function.(86) Additionally, disruption of the integrin LFA-1 pathway showed promising effect in blocking memory T cells in non-human primate models.(87)

Memory T cells are heterogeneous populations with distinct functions and locations of action, which should be taken into account when designing therapeutic strategies. For instance, CD2^{hi} Tem cells have been shown to mediate costimulation blockade-resistant transplant rejection. CD2 adhesion molecule blockade with a LFA-3-Ig fusion protein selectively eliminated memory T cells and, when combined with co-stimulation blockade, prevented renal allograft rejection in nonhuman primates.(88) By contrast, in a murine lung allograft acceptance model induced by costimulatory blockade, a surprising observation has

been reported that CD8⁺ T cell deficiency or depletion precluded graft acceptance. Thus, a “tolerogenic” CD8⁺ T cell subpopulation may be responsible for the costimulatory blockade-mediated lung allograft acceptance. Accordingly, the authors indicated that CD8⁺ Tcm cells with unknown origin infiltrated in the lung allografts and protected them by producing IFN- γ and NO.(3) In heart allografts, early infiltration of CD8⁺ memory T cells accelerates rejection.(67) The apparent discrepancy in the functions of infiltrating CD8⁺ memory cells might be due to the difference in cell subsets (e.g., Tem vs. Tcm) and local inflammatory environments, as the lung is exposed to a variety of foreign substances and is a highly immunogenic organ.

Not all Tcm cells exhibit a tolerogenic or suppressive function. The phenotype plasticity of CD8⁺ Tcm cells has recently been addressed. In *Rag1*^{-/-} mice transplanted with islet allografts, co-transfer of CD8⁺ Tcm cells has also been shown to delay naïve T cell-mediated transplant rejection.(89) CD8⁺ Tcm cells contain a CD122⁺PD-1⁺ population. CD8⁺CD122⁺PD-1⁺ Tcm cells, but not their PD-1⁻ counterparts, exhibit a suppressive phenotype.(90) These suppressive Tcm cells depend on IL-15 for proliferation and IL-10 for suppressor function.(4,89,90) Tcm cells also display “lineage” plasticity, which is the ability to differentiate themselves into a variety of memory cell subsets.(91) CD4⁺ Tcm cells can even differentiate into Foxp3⁺ Treg cells in the presence of TGF- β .(5) Hence, the plasticity of Tcm cells account for their functional difference in rejecting or tolerizing allografts in a variety of transplant models.(3,4,67,68,89,90) This represents a challenge but also an opportunity in preventing memory T cell-mediated transplant rejection.

Regarding B cell memory in transplantation, advances in detecting DSA facilitate the identification of donor-antigen sensitized candidates, to whom transplantation may not be considered an option or appropriate desensitization protocols need to be applied. DSAs can also develop de novo at any time after transplantation. Patients with complement C1q-binding DSAs after kidney transplantation exhibit the lowest 5-year rate of graft survival, as compared with patients with non-complement-binding DSAs or without detectable DSAs.(92) Eculizumab, an anti-C5 mAb that inhibits terminal complement cascade, significantly decreased the incidence of AMR in kidney recipients who had a positive crossmatch against their living donor.(93) Undoubtedly, AMR poses a significant challenge to successful transplantation. Advances in detecting “deleterious” DSAs, together with insights into factors maintaining humoral immunity (e.g., IL-21 and BAFF), may provide new opportunities to control B cell memory and treat AMR in transplantation.

Where do we stand now?

Considerable progress has been made in the past few years on various aspects memory cells, including not only memory T cells, but also memory B cells and innate memory cells. Multiple targets and pathways have been identified, and some of which are under vigorous resting in various models including organ transplantation. However, challenges remain in this area. By all means, transplant tolerance in the clinic demands selective inhibition of allospecific memory cells while leaving behind protective ones, and those with suppressive functions should be preserved, or even better, stimulated. In this context, the following questions deserve special attentions. 1) What controls alloreactive versus non-alloreactive

memory T cells? 2) Why are memory T cells resistant to the suppressive effects of Tregs? Can they be rendered susceptible to regulation? 3) What are the mechanisms that confer survival advantage of memory T cells in the periphery? 4) What is the role of innate memory cells in transplant outcomes? 5) What are the consequences of altering the memory pool in transplant patients? The goal in the future is to tolerize memory T cells in an antigen-specific manner. Otherwise, the risk of compromised protective immunity or altered T cell repertoire will be significant in transplant patients.(94) Many of the above issues are also relevant to B cell memory in transplantation. Current desensitization methods are far from ideal with respect to specific elimination of DSA producing plasma cells and alloreactive memory B cells. Transplant research needs to embrace complexity of memory responses to a transplanted organ. Accumulating evidence indicates the allo-specificity of innate immune cells as well as the existence of innate memory cells in certain transplant model.(2,31,95) The bottom line is that memory cells are functionally diverse, approaches to deal with memory cells have to be more subtle and more specific, as inhibition of memory cells en masse carries considerable risks to transplant patients. Also, the identification of regulatory memory cells in various transplant models is exciting, but their relationships with other types of regulatory T cells, especially Foxp3⁺ Tregs, and their exact mechanisms of suppression remain to be better defined.

Summary and future perspectives

Memory cells represent a major challenge in transplantation in that they are required for protection against invading pathogens, but their presence also endangers transplant survival. (77) It is imperative that tolerization of memory cells in transplant recipients should not drastically alter patients' protective immunity. This is a significant issue in clinical transplantation, but resolving this issue will have major impact on transplant outcomes. The growing awareness of memory B cells in transplant rejection will facilitate the discovery of new strategies in the induction of B cell tolerance, and the recent identification of innate memory cells and regulatory memory T cells will open new areas of investigation and certainly will broaden the prospects of tolerance or tolerance resistance. Knowledge gained through this inquiry will help not only the design of the greatly improved tolerance induction protocols but also stratification of donor recipient selections to reduce the risk of graft damage inflicted by memory cells.

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References

1. Sun JC, Beilke JN, Lanier LL. Adaptive immune features of natural killer cells. *Nature*. 2009; 457:557. [PubMed: 19136945]
2. Liu W, Xiao X, Demirci G, Madsen J, Li XC. Innate NK cells and macrophages recognize and reject allogeneic nonself in vivo via different mechanisms. *J Immunol*. 2012; 188:2703. [PubMed: 22327074]
3. Krupnick AS, Lin X, Li W, et al. Central memory CD8⁺ T lymphocytes mediate lung allograft acceptance. *J Clin Invest*. 2014; 124:1130. [PubMed: 24569377]

4. Li S, Xie Q, Zeng Y, et al. A naturally occurring CD8+CD122+ T-cell subset as a memory-like Treg family. *Cell Mol Immunol*. 2014; 11:326. [PubMed: 24793406]
5. Zhang X, Chang Li X, Xiao X, Sun R, Tian Z, Wei H. CD4+CD62L+ central memory T cells can be converted to Foxp3+ T cells. *PLoS One*. 2013; 8:e77322. [PubMed: 24155942]
6. Chong AS, Sciammas R. Memory B cells in transplantation. *Transplantation*. 2015; 99:21. [PubMed: 25525921]
7. Guillonnet C, Hill M, Hubert FX, et al. CD40Ig treatment results in allograft acceptance mediated by CD8CD45RC T cells, IFN-gamma, and indoleamine 2,3-dioxygenase. *J Clin Invest*. 2007; 117:1096. [PubMed: 17404623]
8. Picarda E, Bezie S, Venturi V, et al. MHC-derived allopeptide activates TCR-biased CD8+ Tregs and suppresses organ rejection. *J Clin Invest*. 2014; 124:2497. [PubMed: 24789907]
9. Kaech SM, Cui W. Transcriptional control of effector and memory CD8+ T cell differentiation. *Nat Rev Immunol*. 2012; 12:749. [PubMed: 23080391]
10. Masopust D, Vezys V, Marzo A, Lefrancois L. Preferential localization of effector memory cells in nonlymphoid tissue. *Science*. 2001; 291:2413. [PubMed: 11264538]
11. Sallusto F, Lanzavecchia A. Heterogeneity of CD4+ memory T cells: functional modules for tailored immunity. *Eur J Immunol*. 2009; 39:2076. [PubMed: 19672903]
12. Shin H, Iwasaki A. Tissue-resident memory T cells. *Immunol Rev*. 2013; 255:165. [PubMed: 23947354]
13. Ariotti S, Hogenbirk MA, Dijkgraaf FE, et al. T cell memory. Skin-resident memory CD8+ T cells trigger a state of tissue-wide pathogen alert. *Science*. 2014; 346:101. [PubMed: 25278612]
14. Schenkel JM, Fraser KA, Beura LK, Pauken KE, Vezys V, Masopust D. T cell memory. Resident memory CD8 T cells trigger protective innate and adaptive immune responses. *Science*. 2014; 346:98. [PubMed: 25170049]
15. Sathaliyawala T, Kubota M, Yudanin N, et al. Distribution and compartmentalization of human circulating and tissue-resident memory T cell subsets. *Immunity*. 2013; 38:187. [PubMed: 23260195]
16. Schenkel JM, Fraser KA, Masopust D. Cutting edge: resident memory CD8 T cells occupy frontline niches in secondary lymphoid organs. *J Immunol*. 2014; 192:2961. [PubMed: 24600038]
17. Schluns KS, Lefrancois L. Cytokine control of memory T-cell development and survival. *Nat Rev Immunol*. 2003; 3:269. [PubMed: 12669018]
18. 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. [PubMed: 15707400]
19. Vu MD, Clarkson MR, Yagita H, Turka LA, Sayegh MH, Li XC. Critical, but Conditional, Role of OX40 in Memory T Cell-Mediated Rejection. *J Immunol*. 2006; 176:1394. [PubMed: 16424166]
20. Chalasani G, Dai Z, Konieczny B, Baddoura FK, Lakkis FG. Recall and propagation of allospecific memory T cells independent of secondary lymphoid organs. *Proc Natl Acad Sci U S A*. 2002; 99:6175. [PubMed: 11983909]
21. Lanzavecchia A, Sallusto F. Human B cell memory. *Curr Opin Immunol*. 2009; 21:298. [PubMed: 19497721]
22. Robillard N, Willems S, Moreau P, Bene MC. Immunophenotype of normal and myelomatous plasma-cell subsets. *Front Immunol*. 2014; 5:137. [PubMed: 24744760]
23. Tomayko MM, Steinel NC, Anderson SM, Shlomchik MJ. Cutting edge: Hierarchy of maturity of murine memory B cell subsets. *J Immunol*. 2010; 185:7146. [PubMed: 21078902]
24. van Helden MJ, de Graaf N, Boog CJ, Topham DJ, Zaiss DM, Sijts AJ. The bone marrow functions as the central site of proliferation for long-lived NK cells. *J Immunol*. 2012; 189:2333. [PubMed: 22821961]
25. Gillard GO, Bivas-Benita M, Hovav AH, et al. Thy1+ NK [corrected] cells from vaccinia virus-primed mice confer protection against vaccinia virus challenge in the absence of adaptive lymphocytes. *PLoS Pathog*. 2011; 7:e1002141. [PubMed: 21829360]
26. O'Leary JG, Goodarzi M, Drayton DL, von Andrian UH. T cell- and B cell-independent adaptive immunity mediated by natural killer cells. *Nat Immunol*. 2006; 7:507. [PubMed: 16617337]

27. Quintin J, Saeed S, Martens JH, et al. *Candida albicans* infection affords protection against reinfection via functional reprogramming of monocytes. *Cell Host Microbe*. 2012; 12:223. [PubMed: 22901542]
28. Kleinnijenhuis J, Quintin J, Preijers F, et al. *Bacille Calmette-Guerin* induces NOD2-dependent nonspecific protection from reinfection via epigenetic reprogramming of monocytes. *Proc Natl Acad Sci U S A*. 2012; 109:17537. [PubMed: 22988082]
29. Saeed S, Quintin J, Kerstens HH, et al. Epigenetic programming of monocyte-to-macrophage differentiation and trained innate immunity. *Science*. 2014; 345:1251086. [PubMed: 25258085]
30. Cheng SC, Quintin J, Cramer RA, et al. mTOR- and HIF-1 α -mediated aerobic glycolysis as metabolic basis for trained immunity. *Science*. 2014; 345:1250684. [PubMed: 25258083]
31. Oberbarnscheidt MH, Zeng Q, Li Q, et al. Non-self recognition by monocytes initiates allograft rejection. *J Clin Invest*. 2014; 124:3579. [PubMed: 24983319]
32. Valujskikh A, Li XC. *Frontiers in Nephrology: T Cell Memory as a Barrier to Transplant Tolerance*. *J Am Soc Nephrol*. 2007; 18:2252. [PubMed: 17634436]
33. Taylor DK, Neujahr D, Turka LA. Heterologous immunity and homeostatic proliferation as barriers to tolerance. *Curr Opin Immunol*. 2004; 16:558. [PubMed: 15341999]
34. Kieper WC, Tan JT, Bondi-Boyd B, et al. Overexpression of Interleukin (IL)-7 Leads to IL-15-independent Generation of Memory Phenotype CD8⁺ T Cells. *J Exp Med*. 2002; 195:1533. [PubMed: 12070281]
35. Ji Q, Perchellet A, Goverman JM. Viral infection triggers central nervous system autoimmunity via activation of CD8⁺ T cells expressing dual TCRs. *Nat Immunol*. 2010; 11:628. [PubMed: 20526343]
36. Lee WT, Shiledar-Baxi V, Winslow GM, Mix D, Murphy DB. Self-restricted dual receptor memory T cells. *J Immunol*. 1998; 161:4513. [PubMed: 9794376]
37. Kurtulus S, Tripathi P, Moreno-Fernandez ME, et al. Bcl-2 allows effector and memory CD8⁺ T cells to tolerate higher expression of Bim. *J Immunol*. 2011; 186:5729. [PubMed: 21451108]
38. Yamaki S, Ine S, Kawabe T, et al. OX40 and IL-7 play synergistic roles in the homeostatic proliferation of effector memory CD4⁺ T cells. *Eur J Immunol*. 2014; 44:3015. [PubMed: 25103720]
39. Pulle G, Vidric M, Watts TH. IL-15-dependent induction of 4-1BB promotes antigen-independent CD8 memory T cell survival. *J Immunol*. 2006; 176:2739. [PubMed: 16493029]
40. Stemmerger C, Huster KM, Koffler M, et al. A single naive CD8⁺ T cell precursor can develop into diverse effector and memory subsets. *Immunity*. 2007; 27:985. [PubMed: 18082432]
41. Chang JT, Palanivel VR, Kinjyo I, et al. Asymmetric T lymphocyte division in the initiation of adaptive immune responses. *Science*. 2007; 315:1687. [PubMed: 17332376]
42. Arsenio J, Kakaradov B, Metz PJ, Kim SH, Yeo GW, Chang JT. Early specification of CD8⁺ T lymphocyte fates during adaptive immunity revealed by single-cell gene-expression analyses. *Nat Immunol*. 2014; 15:365. [PubMed: 24584088]
43. Cui W, Liu Y, Weinstein JS, Craft J, Kaech SM. An interleukin-21-interleukin-10-STAT3 pathway is critical for functional maturation of memory CD8⁺ T cells. *Immunity*. 2011; 35:792. [PubMed: 22118527]
44. Gattinoni L, Lugli E, Ji Y, et al. A human memory T cell subset with stem cell-like properties. *Nat Med*. 2011; 17:1290. [PubMed: 21926977]
45. Lauvau G, Vijn S, Kong P, et al. Priming of memory but not effector CD8 T cells by a killed bacterial vaccine. *Science*. 2001; 294:1735. [PubMed: 11721060]
46. Geginat J, Lanzavecchia A, Sallusto F. Proliferation and differentiation potential of human CD8⁺ memory T-cell subsets in response to antigen or homeostatic cytokines. *Blood*. 2003; 101:4260. [PubMed: 12576317]
47. Wang R, Green DR. Metabolic checkpoints in activated T cells. *Nat Immunol*. 2012; 13:907. [PubMed: 22990888]
48. Araki K, Turner AP, Shaffer VO, et al. mTOR regulates memory CD8 T-cell differentiation. *Nature*. 2009; 460:108. [PubMed: 19543266]

49. O'Neill LA, Hardie DG. Metabolism of inflammation limited by AMPK and pseudo-starvation. *Nature*. 2013; 493:346. [PubMed: 23325217]
50. Pearce EL, Walsh MC, Cejas PJ, et al. Enhancing CD8 T-cell memory by modulating fatty acid metabolism. *Nature*. 2009; 460:103. [PubMed: 19494812]
51. Rao RR, Li Q, Gubbels Bupp MR, Shrikant PA. Transcription factor Foxo1 represses T-bet-mediated effector functions and promotes memory CD8+ T cell differentiation. *Immunity*. 2012; 36:374. [PubMed: 22425248]
52. Valujskikh A, Li XC. Memory T cells and their exhaustive differentiation in allograft tolerance and rejection. *Curr Opin Organ Transplant*. 2012; 17:15. [PubMed: 22186090]
53. Wherry EJ. T cell exhaustion. *Nat Rev Immunol*. 2011; 12:492.
54. Quigley M, Pereyra F, Nilsson B, et al. Transcriptional analysis of HIV-specific CD8+ T cells shows that PD-1 inhibits T cell function by upregulating BATF. *Nat Med*. 2010; 16:1147. [PubMed: 20890291]
55. Jin HT, Anderson AC, Tan WG, et al. Cooperation of Tim-3 and PD-1 in CD8 T-cell exhaustion during chronic viral infection. *Proc Natl Acad Sci U S A*. 2010; 107:14733. [PubMed: 20679213]
56. Rutishauser RL, Martins GA, Kalachikov S, et al. Transcriptional repressor Blimp-1 promotes CD8+ T cell terminal differentiation and represses the acquisition of central memory T cell properties. *Immunity*. 2009; 31:296. [PubMed: 19664941]
57. Shin H, Blackburn SD, Intlekofer AM, et al. A role for the transcriptional repressor Blimp-1 in CD8+ T cell exhaustion during chronic viral infection. *Immunity*. 2009; 31:309. [PubMed: 19664943]
58. Kurachi M, Barnitz RA, Yosef N, et al. The transcription factor BATF operates as an essential differentiation checkpoint in early effector CD8+ T cells. *Nat Immunol*. 2014; 15:373. [PubMed: 24584090]
59. Burns JM, Cornell LD, Perry DK, et al. Alloantibody levels and acute humoral rejection early after positive crossmatch kidney transplantation. *Am J Transplant*. 2008; 8:2684. [PubMed: 18976305]
60. Nutt SL, Taubenheim N, Hasbold J, et al. The genetic network controlling plasma cell differentiation. *Semin Immunol*. 2011; 23:341. [PubMed: 21924923]
61. Shulman Z, Gitlin AD, Weinstein JS, et al. Dynamic signaling by T follicular helper cells during germinal center B cell selection. *Science*. 2014; 345:1058. [PubMed: 25170154]
62. Victora GD, Mesin L. Clonal and cellular dynamics in germinal centers. *Curr Opin Immunol*. 2014; 28:90. [PubMed: 24681449]
63. Spolski R, Leonard WJ. IL-21 and T follicular helper cells. *Int Immunol*. 2010; 22:7. [PubMed: 19933709]
64. Avery DT, Kallal SL, Ellyard JJ, et al. BAFF selectively enhances the survival of plasmablasts generated from human memory B cells. *J Clin Invest*. 2003; 112:286. [PubMed: 12865416]
65. Thibault-Espitia A, Foucher Y, Danger R, et al. BAFF and BAFF-R levels are associated with risk of long-term kidney graft dysfunction and development of donor-specific antibodies. *Am J Transplant*. 2012; 12:2754. [PubMed: 22883025]
66. Lakkis FG, Sayegh MH. Memory T cells: a hurdle to immunologic tolerance. *J Am Soc Nephrol*. 2003; 14:2402. [PubMed: 12937320]
67. Schenk AD, Nozaki T, Rabant M, Valujskikh A, Fairchild RL. Donor-reactive CD8 memory T cells infiltrate cardiac allografts within 24-h posttransplant in naive recipients. *Am J Transplant*. 2008; 8:1652. [PubMed: 18557725]
68. Oberbarnscheidt MH, Ng YH, Chalasani G. The roles of CD8 central and effector memory T-cell subsets in allograft rejection. *Am J Transplant*. 2008; 8:1809. [PubMed: 18671680]
69. Wu Z, Bensinger S, Zhang J, Chen C, et al. Homeostatic proliferation is a barrier to transplantation tolerance. *Nature Medicine*. 2004; 10:21.
70. 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. [PubMed: 12244161]
71. 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. [PubMed: 19624567]

72. Chen Y, Heeger PS, Valujskikh A. In vivo helper functions of alloreactive memory CD4+ T cells remain intact despite donor-specific transfusion and anti-CD40 ligand therapy. *J Immunol.* 2004; 172:5456. [PubMed: 15100287]
73. Adams AB, Williams MA, Jones TR, et al. Heterologous immunity provides a potent barrier to transplantation tolerance. *J Clin Invest.* 2003; 111:1887. [PubMed: 12813024]
74. 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. [PubMed: 10438971]
75. Li XC, Turka LA. An update on regulatory T cells in transplant tolerance and rejection. *Nat Rev Nephrol.* 2010; 6:577. [PubMed: 20683480]
76. Yang J, Brook MO, Carvalho-Gaspar M, et al. Allograft rejection mediated by memory T cells is resistant to regulation. *Proc Natl Acad Sci U S A.* 2007; 104:19954. [PubMed: 18042727]
77. Krummey SM, Ford ML. Heterogeneity within T Cell Memory: Implications for Transplant Tolerance. *Front Immunol.* 2012; 3:36. [PubMed: 22566919]
78. Dai Z, Zhang S, Xie Q, et al. Natural CD8+CD122+ T cells are more potent in suppression of allograft rejection than CD4+CD25+ regulatory T cells. *Am J Transplant.* 2014; 14:39. [PubMed: 24219162]
79. Page AJ, Ford ML, Kirk AD. Memory T-cell-specific therapeutics in organ transplantation. *Curr Opin Organ Transplant.* 2009; 14:643. [PubMed: 19779342]
80. Minamimura K, Sato K, Yagita H, Tanaka T, Arai S, Maki T. Strategies to Induce Marked Prolongation of Secondary Skin Allograft Survival in Alloantigen-Primed Mice. *Am J Transplant.* 2008; 8:761. [PubMed: 18261171]
81. Li XC, Rothstein DM, Sayegh MH. Costimulatory pathways in transplantation: challenges and new developments. *Immunol Rev.* 2009; 229:271. [PubMed: 19426228]
82. Salek-Ardakani S, Song J, Halteman BS, et al. OX40 (CD134) Controls Memory T Helper 2 Cells that Drive Lung Inflammation. *J Exp Med.* 2003; 198:315. [PubMed: 12860930]
83. Ge W, Jiang J, Liu W, et al. Regulatory T cells are critical to tolerance induction in presensitized mouse transplant recipients through targeting memory T cells. *Am J Transplant.* 2010; 10:1760. [PubMed: 20636455]
84. Zhang Q, Chen Y, Fairchild RL, Heeger PS, Valujskikh A. Lymphoid Sequestration of Alloreactive Memory CD4 T Cells Promotes Cardiac Allograft Survival. *J Immunol.* 2006; 176:770. [PubMed: 16393960]
85. 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. [PubMed: 21466654]
86. Kitchens WH, Haridas D, Wagener ME, et al. Integrin antagonists prevent costimulatory blockade-resistant transplant rejection by CD8+ memory T cells. *Am J Transplant.* 2012; 12:69. [PubMed: 21942986]
87. Badell IR, Russell MC, Thompson PW, et al. LFA-1-specific therapy prolongs allograft survival in rhesus macaques. *J Clin Invest.* 2010; 120:4520. [PubMed: 21099108]
88. Weaver TA, Charafeddine AH, Agarwal A, et al. Alefacept promotes co-stimulation blockade based allograft survival in nonhuman primates. *Nat Med.* 2009; 15:746. [PubMed: 19584865]
89. Wan N, Dai H, Wang T, Moore Y, Zheng XX, Dai Z. Bystander central memory but not effector memory CD8+ T cells suppress allograft rejection. *J Immunol.* 2008; 180:113. [PubMed: 18097010]
90. Dai H, Wan N, Zhang S, Moore Y, Wan F, Dai Z. Cutting edge: programmed death-1 defines CD8+CD122+ T cells as regulatory versus memory T cells. *J Immunol.* 2010; 185:803. [PubMed: 20548035]
91. Graef P, Buchholz VR, Stemmerger C, et al. Serial transfer of single-cell-derived immunocompetence reveals stemness of CD8+ central memory T cells. *Immunity.* 2014; 41:116. [PubMed: 25035956]
92. Loupy A, Lefaucheur C, Vernerey D, et al. Complement-binding anti-HLA antibodies and kidney-allograft survival. *N Engl J Med.* 2013; 369:1215. [PubMed: 24066742]

93. Stegall MD, Diwan T, Raghavaiah S, et al. Terminal complement inhibition decreases antibody-mediated rejection in sensitized renal transplant recipients. *Am J Transplant*. 2011; 11:2405. [PubMed: 21942930]
94. Zhao Y, Li XC. Transplant tolerance: is it really free of concerns? *Trends in Immunology*. 2007; 28:376. [PubMed: 17662655]
95. Nabekura T, Lanier LL. Antigen-specific expansion and differentiation of natural killer cells by alloantigen stimulation. *J Exp Med*. 2014; 211:2455. [PubMed: 25366966]

Figure 1. Distinct fates of effector T cells

In response to antigen/TCR, costimulatory, and cytokine signals (known as signals 1, 2, and 3, respectively), naïve T cells are activated and clonally expanded to become effector T cells, which in turn mediate antigen clearance. Most effector T cells then undergo Bim-mediated apoptosis during the subsequent contraction phase. These short-lived effector T cells express high levels of KLRG1 and depend on Blimp-1 and Tbet for their terminal differentiation. Only a small percentage of effector T cells survive long term without antigen and develop into memory T cells. The generation of memory T cells is promoted by expression/activity of IL-7R, BCL-2, BCL-6, Eomes, FOXO1, and STAT3. When antigen is persistently present in the periphery, effector T cells progress through stages of dysfunction and become exhausted T cells expressing PD-1 and LAG-3. BATF and Blimp-1 have been shown to facilitate T cell exhaustion.