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Overcoming the memory barrier in tolerance induction: Molecular mimicry and functional heterogeneity among pathogen-specific T cell populations

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Abstract

Purpose of Review—This review highlights recent advances in our understanding of the frequency and nature of alloreactivity among memory T cell populations, and discusses recent successes in experimentally targeting these populations in order to prolong graft survival.

Recent Findings—Recent studies suggest that not only is alloreactivity present within peripheral T cell compartments of normal healthy individuals, but cross-reactivity between viral-specific T cells and alloepitopes may in fact be a very common occurrence. Furthermore, this cross-reactivity functions at the level of molecular mimicry of TCR recognition. Therapeutics that specifically target cell surface molecules or effector pathways used by memory T cells to mediate graft rejection will likely be required in order to attenuate the donor-reactive memory T cell response during transplantation.

Summary—A major challenge facing the field over the next decade is to define the heterogeneity that exists within memory T cell populations that impacts graft survival. Understanding the functional and phenotypic differences that modify the memory T cell barrier to tolerance induction might allow a strategy in which strength of immunosuppression could be tailored to fit the immunological history of a given transplant recipient in order to minimize non-immune toxicities, maximize protective immunity, and prolong graft survival.

Keywords

Transplant; costimulation; memory T cell; molecular mimicry

Introduction

T cell memory is a hallmark of adaptive immunity and plays a critical role in providing protective immunity to transplant recipients, who may lose the ability to mount *de novo* cellular immune responses while on immunosuppression. However, many studies over the last decades revealed the double-edged sword of memory T cells; that is, while providing protective immunity against the panoply of pathogens encountered on a daily basis, these cells may also pose a potent barrier to the attenuation of donor-specific immune responses

and the induction of tolerance or a tolerant-like state. Herein, we discuss recent advances in our understanding of the frequency and nature of alloreactivity among memory T cell populations, and advances in therapeutically targeting these populations in the hopes of prolonging graft survival following transplantation.

Evidence for the existence of alloreactive T cells among memory

Perhaps the first and most fundamental question when contemplating the potential barrier posed by alloreactive memory T cells is to consider the evidence that alloreactivity exists among memory T cells. The issue of whether alloreactive T cell precursor frequencies are higher, lower, or equivalent among memory T cell populations as compared to naive T cells has been debated for at least two decades [1,2]. Early experiments addressed the alloreactivity of cord blood-derived T cell populations, which would putatively contain little to no memory T cells [2]. During the last year however, this issue has been largely put to rest, mainly with the publication of a study by Lakkis and Metes, which analyzed the frequencies of alloreactive T cells in naive ($CD45RO^-CD62L^+$), central memory ($CD45RO^+CD62L^+$), effector memory ($CD45RO^+CD62L^-$), and terminal effector memory ($CD4RO^-CD62L^-$) compartments, and found roughly equal frequencies of alloreactivity among all four compartments [3]. Importantly, however, differences in the ways in which alloreactivity manifested in these different T cell populations were noted. For example, in the $CD8^+$ T cell compartment, naive T cells exhibited increased proliferation in response to allostimulation as compared to T_{EM} , while T_{EM} exhibited increased granzyme B/perforin expression as compared to naive T cells in response to alloantigen [3]. Thus, these data highlight that while the overall frequency of alloreactive T cells in memory vs. naive T cell compartments may be similar, the behavior of these alloreactive cells *in vivo* may be quite distinct, depending on their differentiation status.

Advances in our understanding in the generation of donor-reactive T cell memory

How do donor-reactive memory T cells arise in a previously untransplanted recipient? Existing ideas suggested that donor-reactive memory T cells can be generated via pregnancy, exposure to environmental pathogens, and frequent blood transfusions [4,5]. A recent report has conclusively demonstrated the latter phenomenon. Specifically, Zimring and colleagues found that immunity generated against transfused platelets, even across only minor histocompatibility antigens, was sufficient to induce rejection following a subsequent bone marrow transplantation in murine recipients [6]. These data indicate that patients receiving even leuko-reduced platelet products may be at an increased risk for memory T cell-mediated graft rejection following bone marrow transplantation; however further experiments examining the barriers posed by transfusion-derived minor antigens in experimental models of solid-organ transplantation are needed.

As mentioned above, donor-reactive memory T cells have also been purported to arise via exposure to environmental pathogens. Evidence for this exists from both human and mouse models. For example, studies by Burrows and colleagues demonstrated that T cells specific for EBV-EBNA3A restricted by HLA-B8 were cross-reactive with HLA-B44 (putatively

presenting a self-peptide) [7–9]. Earlier studies showed that CD4⁺ T cells-specific for tetanus toxoid in the context of HLA-DR3 were cross-reactive with HLA-DR4 [10], and a T cell specific for HSV-VP13/14 presented by A2 has been shown to also cross-react with HLA-B44 [11]. Thus, scattered throughout the literature are examples of specific donor-recipient pairs eliciting cross-reactivity between pathogen-specific and alloreactive T cell populations. But just how common is this phenomenon? This year, a new study by Heemskerk and colleagues suggests that it is in fact quite common, perhaps much more so than originally thought. In this elegant series of experiments, Amir et al. analyzed pathogen-specific T cell lines and clones against a panel of HLA-typed target cells, and found that fully 80% of T cell lines and 45% of pathogen-specific T cell clones exhibited alloreactivity [12]. For the pathogen-specific T cell clones, this cross-reactivity was usually confined to a single HLA molecule. Importantly, TCR gene transfer experiments demonstrated that the pathogen and allo-reactivity was conferred by a single TCR, thus confirming cross-reactivity at the molecular level [12]. These results have important implications for the field of transplantation, in that they indicate that the phenomenon of heterologous immunity is perhaps much more common than originally anticipated. Thus, the question becomes not just *whether* donor reactive memory T cells exist in a given recipient, but rather *to what degree* their frequency, phenotype, and functionality determines the relative barrier posed by donor-reactive memory T cell populations present in a given individual.

On the nature of T cell allorecognition via molecular mimicry

While the existence of heterologous immunity in transplantation has been recognized at the cellular level for more than a decade [13], the molecular mechanisms underlying this phenomenon are just beginning to be elucidated. Heterologous immunity as a broader term refers to the observation that one stimulus can lead to an immune response, either cell-mediated or humoral, that affects the response to a second, distinct stimulus [14]. For T cell response, one could conceive of at least two possible mechanisms that could contribute to this observation: bystander activation and molecular mimicry. Recently, important experimental evidence supporting the existence of molecular mimicry as an underlying cause of alloreactivity among heterologous immune responses has emerged. For instance, Allen and colleagues published a seminal study analyzing the molecular basis underlying the specificity of alloreactive T cells for peptide:MHC complexes, and found that alloreactive T cells have the ability to respond to multiple, distinct peptide epitopes that share no sequence homology [15]. Thus, this work demonstrated that alloreactive T cells are in fact “poly-specific” and may have the ability to recognize many unrelated peptide sequences [16]. These conclusions were extended by the findings of McCluskey and colleagues, who reported on the molecular mechanisms underlying the observed cross-reactivity of EBV-EBNA3A/HLA-B08 restricted TCR with HLA-B44 molecules [17]. The results of their analyses suggested that dual recognition of unrelated cognate and allogeneic peptide:MHC complexes by a single TCR involved binding modes that were almost identical. Interestingly, only following TCR ligation were the viral and allopeptides forced into this identical conformation, suggesting an induced-fit mechanism of TCR recognition in these instances [17,18]. These seminal studies have therefore definitively demonstrated that

heterologous immunity between pathogen-derived and transplant antigens can function at the level of molecular mimicry.

Cross-reactive allospecific memory T cell repertoire is likely to be more focused than the naïve alloreactive T cell repertoire

While alloreactivity is equally present in naive and memory T cell compartments, the spectrum of specificities within these repertoires is likely to be quite different. Due to the restricted TCR repertoire of pathogen-specific memory T cells [19,20], the alloreactive T cell repertoire among memory is likely to be confined to fewer discrete TCR clones. However, the frequency of any given clone is much higher within the memory pool as compared to the naive T cell repertoire. Thus, given T cell clones within the memory T cell pool are likely to be responding at higher frequency than given clones within the naive T cell pool. Because high T cell precursor frequency is known to constitute a barrier to long-term graft survival induced via costimulation blockade [21–23], this may be another method by which donor-reactive memory T cells constitute a barrier to tolerance induction.

Stimulation history profoundly impacts antigen-specific memory T cell quantity, quality, and recall requirements

In recent years, it has become increasingly apparent that a large degree of heterogeneity exists among memory T cells in mouse and man. For example, many groups now have characterized the existence of central (T_{CM}) vs effector (T_{EM}) memory T cells which express different homing receptors that allow them to traffic to the lymphoid organs or peripheral tissues, respectively [24]. These subsets are therefore thought to be responsible for the initiation of recall responses and the surveillance of peripheral tissue sites for invading pathogens, respectively. The functional phenotypes of memory cells can be influenced by a variety of different factors. Current thinking holds that the route of exposure, dose, replication rate, recurrence, and tropism of the infectious challenge may impact qualitative aspects of memory T cell development [25]. For example, there is evidence that repeated exposure to a given antigen results in not only increased quantity of antigen-specific memory T cells, but also altered quality in that the cells became increasingly T_{EM} -like [25]. Therefore, donor-reactive memory T cells that are cross-reactive to antigens presented by recurrent infections, such as the common cold, might be present at a higher precursor frequency and be more likely to exhibit a T_{EM} phenotype than donor-reactive memory T cells generated via exposure to a single infection. Are these cells differentially distributed within the body? Are they mobile or sessile? Do they have distinct homing molecules and recirculation patterns? Do they differ with regard to expression of costimulatory and adhesion molecules? Do they possess altered cytokine or cytolytic function? These questions remain to be answered.

Likewise, donor-reactive memory T cells generated following a latent viral infection, such as a herpesvirus infection, might also possess unique phenotypes and functional characteristics. This is likely due to the fact that, unlike acute infections (measles, influenza) where virus is cleared, latent or persistent infections such as EBV, CMV and the polyoma

viruses maintain reservoirs of virus within host cells that can periodically reactivate and cause recurrent clinical disease, resulting in populations of persistently activated, viral-specific memory T cells that possess a predominantly T_{EM} phenotype [26]. Due to this recurring antigen exposure and T_{EM} phenotype, these cells may exhibit altered trafficking patterns, obviate the need for initiation of a recall response in secondary lymphoid tissues [27], and demonstrate increased resistance to both tolerance-induction strategies [28–30] and common immunosuppressive agents [31].

Thus, understanding the relative barrier posed by different subsets of cells within these heterogeneous populations, particularly in terms of resistance to new costimulation blockade-based therapies for transplantation, remains an important goal.

Effector mechanisms used by memory T cells to mediate rejection

As discussed above, it is likely not just the presence of donor-reactive memory T cells, but their functional phenotypes, that likely determine the potency of the relative barrier they pose to transplantation tolerance induction. Evidence characterizing the varied subsets of memory T cells is mounting, and it is becoming increasingly clear that these cells may mediate their alloreactivity via different effector functions [3].

For example, effector memory T cells are poised in peripheral tissues, with the potential to rapidly respond and infiltrate allografts within 12–24 hours [32]. It was previously unknown as to whether these cells remain static residents of peripheral tissues or continuously recirculate throughout peripheral tissues, and a study published this year now suggests that they in fact recirculate to peripheral tissues and are not static [33]. This finding potentially explains the fact that memory T cells are able to infiltrate transplants so quickly, and also suggests that tissue-resident effector memory T cells could encounter monoclonal antibodies or other therapeutics in the bloodstream, and suggests that achieving high levels of therapeutics in peripheral tissues may not be necessary in order for these reagents to be effective against memory T cells which home there.

Memory T cells are also heterogeneous with regard to their cytokine producing capabilities. Studies of pathogen-specific immune responses and vaccine development first suggested that multi-cytokine producing cells might be more effective at providing protection from reinfection than single cytokine producers [34]. However, it was not known whether this was also true during memory T cell-mediated rejection of transplanted tissue. In 2009, Kirk and colleagues published a study examining the cytokine producing potential of different memory T cell subsets, and found that those T cells that formed the most potent barrier to long-term graft survival (CD2^{hi} T_{EM} cells) were also multi-cytokine producing effector cells [35]. In addition, these cells showed evidence of cytolytic function in that they expressed the cytolytic effector molecule granzyme and showed evidence of degranulation in vitro [35]. Thus, possessing a spectrum of effector functions, each of which could contribute to graft destruction, may determine the relative potency of a given population of memory T cells with regard to their ability to mediate graft rejection.

Potential Strategies for Attenuation of Donor-Reactive Memory T Cell Responses

While a few studies have pointed to an increased risk of rejection in calcineurin-inhibitor-treated recipients possessing an initial high frequency of donor-reactive memory T cells, it is likely that as a general rule calcineurin inhibitors may effectively inhibit the reactivation and effector function of memory T cells [31]. However, in order to improve the side-effect profile of immunosuppressive regimens, many investigators over the past 15 years have contributed to the development of belatacept [36], a second-generation CD28 blocker that has shown immense promise in recent clinical trials [37–39]. For example, in a Phase III multi-center clinical trial in renal transplantation, patients treated with belatacept exhibited reduced nephrotoxicity, reduced hyperlipidemia, and a decrease in cardiovascular events as compared to the control group, which received a standard cyclosporine-based immunosuppressive regimen [38]. While these results were certainly promising, this study also revealed that belatacept-treated patients receiving the moderate intensity dosing regimen exhibited a significant increase in both frequency and severity of acute rejection episodes [38]. From studies by many groups in murine models, it is clear that the pre-existence of donor-reactive memory T cells is a potent barrier to long-term graft survival induced by costimulation blockade [28,40]. Thus, it is interesting to speculate that the pre-existence of donor-reactive memory in some patients may be one risk factor contributing to higher incidence and severity of acute rejection following costimulation blockade-based therapy. Thus, as use of more selective immunosuppressive therapies such as the CD28 costimulation blockers are increased clinically, the need to simultaneously attenuate donor-reactive memory T cell responses by targeting memory cell-specific pathways becomes more pressing.

Over the last year, several such novel pathways have been suggested in the literature. First, as mentioned above, Kirk and colleagues observed the ability of CD2^{hi} T_{EM} cells to mediate costimulation blockade-resistant rejection, and targeted these cells using the CD2 blocker LFA-3-Ig (alefacept) in a non-human primate model of renal transplantation [35]. Blockade of CD2, when used as part of a regimen consisting also of CTLA-4 Ig (CD28 blockade) and sirolimus (mTOR blockade), resulted in renal allograft survival beyond the period of treatment (>90 days) in five out of eight rhesus macaques [35]. These results suggested that alefacept, which is currently FDA-approved for use in plaque psoriasis, may be an appropriate adjunct therapy for use in combination with CD28 blockers such as belatacept.

As discussed above, memory T cells are known to rapidly traffic into grafts and mediate rejection by exacting effector functions and recruiting in other inflammatory mediators. Thus, one potential strategy to limit their pathogenicity might be to block the ability of memory T cells to migrate into the transplanted tissue. Fairchild and colleagues demonstrated that treatment with anti-LFA-1 reduced the early infiltration of memory T cells into donor tissue [41], and we have shown that anti-LFA-1 mAb was effective at inhibiting donor-reactive memory T cell responses when combined with traditional costimulation blockade in murine models [42]. Translational studies in non-human primate models demonstrated that anti-LFA-1 used as an induction agent worked synergistically

with belatacept in inhibiting graft rejection in a model of islet transplantation (Badell and Larsen, manuscript in preparation). Furthermore, investigator-initiated exploratory phase II studies using the anti-LFA-1 mAb, efalizumab have suggested efficacy in clinical islet cell transplantation (Larsen, submitted and Peter Stock and Andrew Poselt (UCSF), personal communication). These data suggest that targeting LFA-1, and perhaps more broadly the adhesion pathways used by memory T cells to infiltrate grafted tissue, may be a potent method of attenuating memory T cell-mediated allograft rejection.

If donor-reactive memory T cells are able to infiltrate into the graft, what are the pathways necessary for their in situ effector function? Valujskikh and colleagues assessed the role of inducible costimulatory molecule (ICOS, CD278) in eliciting effector function from early graft-infiltrating memory T cells [43]. Their results revealed that while ICOS was not constitutively expressed on memory T cells, its expression was upregulated in situ following proliferation of memory T cells within the graft itself. Importantly, blockade of ICOS signaling via an anti-ICOS mAb resulted in a significant decrease in the early expression of IFN- γ , perforin, granzyme B, and FasL mRNA within the graft [43].

In addition, reagents which block proteasome degradation and thereby inhibit NFkB nuclear translocation are increasingly showing promise as inhibitors of donor-reactive memory T cell responses. For example, a recent in vitro study demonstrated the ability of bortezomib, one such proteasome inhibitor, to suppress activation of resistant memory T cells in vitro [44]. Importantly, use of bortezomib was shown to preserve regulatory T cell function while inhibiting the activation of donor-reactive memory T cells [44].

Conclusions

Studies published within the last year have definitively demonstrated that not only is alloreactivity present within peripheral T cell compartments of normal healthy individuals, but cross-reactivity between viral-specific T cells and alloepitopes may in fact be a very common occurrence. Thus, the challenge facing the field over the next decade will be to define the functional phenotypes that exist within memory T cell populations, as a result of the type and frequency of pathogen exposure, that results in a greater or lesser barrier to tolerance induction. For example, allo-crossreactive memory in HLA-B08/B44 donor-recipient pairs is related to a latent pathogen (EBV), while the allo-crossreactive memory found in HLA-DR3/DR4 combinations is related to a single or multi-acute exposure to a cleared antigen (tetanus toxoid). Because these memory T cell type responses generated against these distinct types of exposures may pose different barriers and thus require different therapies, knowing the number and quality of these types of cells for individual patients may be important. Ultimately, one might foresee a strategy in which strength and type of immunosuppression could be tailored to fit the immunological history of a given transplant recipient in order to minimize non-immune toxicities, maximize protective immunity, and prolong graft survival.

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