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MEMORY T CELLS AND THEIR EXHAUSTIVE DIFFERENTIATION IN ALLOGRAFT TOLERANCE AND REJECTION

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Abstract

Purpose of review—Memory T cells have emerged as a major threat to transplant survival; they are well equipped and well positioned to respond to antigens in an accelerated fashion. They participate in transplant rejection and resist interventions that usually contain naïve T cells. Thus, means to prevent memory T cells from attacking allotransplants is an important issue in transplantation.

Recent findings—Recent studies in other models suggest that effector T cells, which include both freshly activated T cells and memory T cells, can acquire “an exhausted phenotype” in that they progressively loss their effector activities. This response is highly regulated, antigen specific, and driven primarily by antigen persistence. This exhausted phenotype has not been carefully explored in transplant models, and its role in transplant survival remains largely unknown.

Summary—Studies of T cell exhaustion reveal additional facets of the fundamental mechanisms of transplant survival. T cell exhaustion may be an alternative pathway of preventing memory development. Future studies are needed to further improve our understanding of T cell exhaustion in transplantation.

Keywords

T cells; exhaustion; tolerance; antigen persistence

Introduction

T cells are the primary drivers of transplant rejection, thus finding ways to disarm alloreactive T cells is central to graft survival. The phenomenon of “T cell exhaustion” was first observed almost two decades ago and has since been actively investigated in a variety of animal models and in humans [1]. T cell exhaustion has been described for both CD8+ and CD4+ T cell subsets, and in some cases, the B cells; it is commonly defined as a gradual loss of T cell effector activities due to a prolonged or a persistent exposure to antigen

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Conflicts of interest

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stimulation. Specifically, exhausted T cells have been observed in viral, bacterial, and parasitic infections as well as in many tumor models. The exact mechanisms governing T cell exhaustion remain incompletely defined, but they may represent an alternative pathway of T cell differentiation, distinct from that of effector differentiation and memory generation. Although exhaustion has been described in multiple cell subsets, CD8⁺ T cells are by far the best-studied cell type. Recent studies have led to a much better appreciation of the phenotype and characteristics of exhausted T cells as well as various factors that contribute to the development and maintenance of T cell exhaustion. The purpose of this review is to highlight the latest findings in this area and to discuss possible implications of T cell exhaustion in transplant settings.

T cell exhaustion – an alternative differentiation pathway?

Exhausted T cells exhibit certain general features. First and foremost, otherwise competent effector T cells gradually and progressively lose their effector activities; this process is not an all or none response but rather a hierarchical event, and in the case of CD8⁺ effector cells, their proliferation capacity is often lost first; cells refuse to undergo further proliferation and respond poorly to cytokines that normally drive proliferation and self renewal of memory T cells. The exhausted T cells markedly downregulate CD122 and CD127 expression and respond poorly to IL-7 and IL-15 [2]. But cells at this stage may still be capable of executing some effector functions. This is often followed by the loss of cytolytic functions or the ability to degranulate cellular granules, and production of certain cytokines, especially TNF- α , is also impaired at this stage. A severe form of exhaustion is manifested by additional and complete loss of IFN- γ production. The final stage of exhaustion is the physical deletion of exhausted cells from the T cell repertoire. Despite the revelation of these events, how such events are coordinated in exhausted T cells is unclear. Second, exhausted T cells identify themselves by expression of cell surface receptors that usually transduce inhibitory signals, and such receptors include PD-1, LAG-3, 2B4, CD160, Tim-3, CTLA-4 and many other inhibitory receptors [3]. Some of these inhibitory receptors (e.g., PD-1) are critical to the exhausted phenotype. For example, blocking the PD-1 pathway can rescue T cells from exhaustion in models chronic viral infections, thus enhancing protective anti-viral immunity [4]. Similar findings are reported in humans [5, 6]. This led to the belief that T cell exhaustion is an active process, at least in part under the control of inhibitory receptors. Lastly, exhausted T cells are mechanistically distinct from anergic T cells, though both show similarly impaired effector activities. Anergic T cells are induced at the early stage of T cell priming, often the results of TCR stimulation in the absence of costimulatory signals. They express anergic factors such as Grail, Egr-1, Egr-2 that are not expressed in exhausted T cells [7].

Recently, genomic approaches have been used to further characterize the exhausted T cell phenotype, and studies in this area have uncovered additional insights concerning the transcriptional pathways governing the exhausted phenotype. Specifically, several transcriptional pathways have been identified and potentially play an important role in T cell exhaustion. For example, the transcriptional repressor Blimp-1 is highly up-regulated in exhausted CD8⁺ T cells and its levels of expression far exceed that in effector cells and memory T cells [8]. A close association between Blimp-1 and the expression of inhibitory receptors, including PD-1, CD160, LAG-3, has been observed. Genetic deletion of blimp-1 abrogated the expression of such inhibitory receptors, and consequently, restored the normal pattern of memory T cell development [8]. These findings suggest that Blimp-1 plays an important role in T cell exhaustion. The fact that Blimp-1 is also expressed by many other cell types suggests that its role is unlikely confined to exhausted T cells only. For example, Blimp-1 can be expressed by APCs and is functionally involved in the tolerogenic features of DCs [9]. Given the central role of PD-1 in T cell exhaustion, efforts to define genes that

are induced by PD-1 ligation in exhausted T cells identified BATF as a key transcription factor downstream of the PD-1 receptor in exhausted cells ^[10]. BATF is an AP-1 family transcription factor; it partners with c-jun to prevent AP-1 transcription ^[11]. In fact, BATF is highly expressed in exhausted CD8+ T cells and siRNA mediated knockdown of BATF can prevent T cell exhaustion during chronic HIV infections ^[10], establishing a critical role for BATF in T cell exhaustion. Although the fine details of how these transcription factors mediate the exhausted phenotype are largely unknown, the identifications of such transcription pathways in the control of T cell exhaustion provides compelling arguments that T cell exhaustion is an actively acquired phenotype and that T cell exhaustion may represent an alternative fate of T cell memory.

The exact roadmap to exhaustion is unclear, but several known factors favor the emergence of exhausted T cells. Prolonged antigen stimulation or antigen persistence in the immune system is a key contributing factor; this can be in the form of chronic, latent infections or in the form of cancer progression. In addition, an inhibitory process, both intrinsic and extrinsic, is also required for effector T cells to transit to an exhausted phenotype. The expression of multiple inhibitory receptors on the cell surface (mentioned above) provides an intrinsic mechanism for T cell exhaustion, 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 the extrinsic mechanisms permitting the development of exhausted T cells. In certain models, lack of help from CD4+ T cells or disruption of lymphoid structures also favors T cell exhaustion ^[1]. In a broad sense, all of these factors are well represented in transplant settings, and whether T effector cells could be pushed to exhaustion in favor of graft survival deserves more attention. Another unique feature to transplantation is the use of immunosuppressive drugs, which purposely create an immunosuppressive milieu in transplant recipients ^[12]. The contribution of immunosuppression to the induction or the inhibition of the exhausted T cell phenotype remains poorly studied.

Is T cell exhaustion relevant to transplantation?

As mentioned above, immune responses to transplanted organs resemble those elicited against chronic infections and tumors, especially considering the high antigenic load and antigen persistence. Intriguingly, as compared to other models, very few studies directly address the extent of T cell exhaustion in transplant models. A closer look at the published data revealed a multiplicity of terms used to describe T cell hypo-responsiveness or unresponsiveness in transplantation. It remains to be determined whether previously reported “exhaustive differentiation”, “adaptation”, “acquired tolerance”, “anergy”, or “ignorance” of alloreactive T cells in various transplantation models possess any features of “exhaustion” or whether they are induced and maintained by completely different mechanisms.

Analysis of T cell dysfunction during immune responses to persistent pathogens, tumors, and autoantigens suggests that the conditions leading to T cell exhaustion often occur in solid organ transplantation. One example of such a situation is long term allograft survival achieved by immunosuppressive drugs, costimulatory blockade, pre-emptive donor antigen administration or combination of these approaches. Unless the entire repertoire of donor-reactive T cells is depleted by aggressive depletion, T cells are exposed to persistent graft antigens for prolonged periods of time. Furthermore, as donor APCs disappear over time, the alloantigens are predominantly presented by host APCs or non-hematopoietic donor cells in the periphery, a condition favoring the potential deterioration of T cell functionality in the long run, as predicted based on the exhaustion theory outlined above. Donor-specific unresponsiveness in transplant settings may bear characteristic features of T cell exhaustion,

such as requirements for inhibitory receptor signaling, regulatory T cells, and regulatory cytokines IL-10 and TGF- β [13–15].

Another situation that may facilitate exhaustion of donor-reactive T cells is when donor and recipient have few antigenic differences such as transplantation across single minor histocompatibility antigens (mHA) mismatch. Indeed, studies of syngeneic C57BL/6 male to female or bm12 to wild type C57BL/6 heart transplantation demonstrated that anti-donor T cell responses are induced by heart transplants, and prominent lymphocyte infiltration into the grafts is observed early after transplantation. However, the anti-donor responses are not sustained and the grafts are not rejected acutely but instead develop signs of chronic rejection [16, 17]. The time frame needed to establish this type of pathology and the progressive loss of T cell function may be closely related, a notion that warrants further investigation.

Transplantation of a large amount of donor tissue can also induce hyper-stimulation of T cells resulting in T cell dysfunction and eventual apoptosis. Thus, liver allografts have been shown to cause early overstimulation of donor-reactive CD8 T cells, as a much higher proportion of cells undergo intensive cell division when compared to kidney or heart allografts [18]. While some of these hyper-activated T cells were gradually lost, the residual cells exhibited hypo-responsiveness to donor alloantigens despite the fact that such hypo-responsive cells were capable of rejecting skin allografts after adoptive transferring into a secondary host. These features are more characteristic of exhausted rather than anergic T cells.

Competition among different T cell clones appears to be a factor limiting the persistence of chronically activated autoreactive T cells [19]. As a result, the combination of antigen-driven and homeostatic T cell expansion in lymphopenic recipients is a potent driver of T cell exhaustion, analogous to the initial robust responses during chronic viral infections. Studies by Noval Rivas et al. investigated the functions of TCR transgenic CD4 T cells persistently stimulated by mHA after adoptive transfer into lymphopenic host [20]. Chronically stimulated CD4 T cells developed a long-lasting state of unresponsiveness demonstrating high activation threshold and inability to proliferate or produce effector cytokines upon re-challenge. However, this state was reversible as blocking PD1/PD-L1 pathway led to recovery of T cell function and enabled seemingly exhausted T cells to induce severe GVHD. These findings may be highly relevant to clinical transplantation as lymphocyte ablation is a common form of induction therapy in transplant recipients. Indeed, the recent analysis of T cell reconstitution and functions in rabbit anti-thymocyte globulin -induced renal transplant patients showed that the repopulating memory CD8⁺ T cell subset has impaired responses to IL-2, as shown by the decreased STAT-5 phosphorylation [21]. Investigation of the fate of donor T cells after bone marrow transplantation provides important insights into the mechanisms underlying exhaustion of alloreactive T cells. Flutter and colleagues have reported that antigen expression by non-hematopoietic host cells is critical for driving TGF- β -dependent exhaustion of CD8 T cells in a model of delayed donor lymphocyte infusion following allogeneic BM transplantation [22]. The exhausted CD8 T cells demonstrated minimal proliferative capacity, impaired IFN- γ production and low responsiveness to cytokines IL-2, IL-7 and IL-15. The functions of exhausted CD8 T cells were partially restored by PD-1/PD-L1 blockade, however, in contrast to the findings by Noval Rivas et al [20], this was not sufficient to cause GVHD. Those are circumstantial evidence supporting the possible role of T cell exhaustion in transplant settings.

An interesting and clinically relevant situation is the chronic viral infections and virus reactivation in immunosuppressed transplant patients. This problem shifts focus from donor-reactive to virus specific T cells and to the ability of transplant recipients to control

transplant rejection and life-threatening infections. In an attempt to address this issue, Macedo et al. have demonstrated that in thoracic transplant patients with high EBV load, EBV-specific CD8 T cells display characteristic features of exhausted phenotype, namely PD-1+CD127- and low IFN- γ production [23]. Similar findings on CD8 T cells from CMV-infected transplant patients were observed also. While infection control is important in these settings, it is unclear how reversal of viral-specific exhausted T cell populations may affect survival of organ transplants.

Can T cell exhaustion be harnessed to facilitate transplant survival?

Despite the paucity of studies that directly evaluate alloreactive T cell exhaustion in transplant models, there are accumulating data in the literature highlighting the expression of typical T cell exhaustion markers in allograft rejection. Among several cell surface molecules commonly associated with T cell exhaustion, PD-1/PD-L1 is the best studied pathway with well-established inhibitory functions. We have previously mentioned that blocking PD-1/PD-L1 interactions restored effector functions of exhausted alloreactive CD4 and CD8 T cells as a result of adoptive transfer or bone marrow transplantation [20, 22]. In addition, the lack of PD-L1 on donor cells or administration of anti-PD-L1 antibodies is known to accelerate allograft rejection or abrogate the graft-prolonging effects of CTLA-4Ig and anti-CD154 in various murine transplantation models [24–26].

Another molecule with known T cell inhibitory functions in the context of transplantation is TIM-3 (T cell immunoglobulin domain, mucin domain-3). Initially, TIM-3/galectin 9 pathway was described as a negative regulator of Th1 responses, in particular IFN- γ production [27]. Studies in mouse transplant models showed that the prevention of TIM-3 signaling with blocking monoclonal antibodies accelerated allograft rejection and the onset of acute GVHD [28]. Furthermore, blocking TIM-3 interfered with the establishment of long term allograft survival induced by costimulatory blockade [29]. On the other hand, treatment of recipient mice with TIM-3 ligand galectin-9 dampened alloresponses leading to prolonged survival of MHC-mismatched heart and skin allografts and amelioration of GVHD [30, 31]. The role of LAG-3 in transplant rejection and acceptance appears to be more complex, possibly due to the broad expression pattern of this molecule. LAG-3 is expressed by functional effector T cells and by exhausted T cells, but it is also found on regulatory T cells [32]. This may account for the findings that antibody blockade of LAG-3 prolonged heart allograft survival in rats when administered early after transplantation but abrogated the beneficial effects of donor specific transfusion in the same model [33]. Conversely, LAG-3 contributed to CD8 T cell tolerance induced by donor bone marrow transplantation and anti-CD154 antibody [34]. We propose that T cell exhaustion may be a fundamental mechanism of memory prevention in transplant models, and this phenotype may contribute significantly to transplant survival.

Concluding remarks

Despite recent progress in characterizing T cell exhaustion in other models, the potential implications of T cell exhaustion in transplant rejection and acceptance remain to be clearly determined. The existing data suggest that mechanisms closely resembling T cell exhaustion may contribute to the efficacy of different graft prolonging therapies. The conditions favoring recipient T cell differentiation toward an exhausted state include surviving allografts (persistence of alloantigens), minor histocompatibility mismatched transplants, and the reconstitution of T cell repertoire after lymphoablation. The findings in these models need to be re-evaluated in the context of our recent understanding on the development, maintenance, and reversal of T cell exhaustion. In addition, the contribution of T cell exhaustion to prolonged allograft survival should be clarified by further experimentation.

An important question is whether T cell exhaustion can be eventually harnessed to prevent graft tissue damage. The analysis of existing data presents two major problems with this approach. First, the loss of T cell functions in the form of exhaustion is hierarchical and gradual. Therefore, acute allograft rejection or irreversible tissue damage may occur prior to the establishment of exhaustion. Second, in situations where T cell hypo-responsiveness was described, donor-specific tolerance has not been achieved and the ongoing immune response eventually resulted in the development of chronic graft injury. Despite the hurdles, future studies of donor-specific T cell exhaustion should improve our understanding of this process in organ transplantation and facilitate its application to benefit transplant survival.

KEY POINTS

- Memory T cells are key players in rejection as well as tolerance resistance.
- T cell exhaustion is an alternative pathway of effector differentiation to memory T cells.
- Exhausted T cells are induced and maintained by both intrinsic and extrinsic molecular mechanisms.
- Antigen persistence and expression of inhibitory receptors by effector cells are key determinants of T cell exhaustion.
- Exhausted T cells can have profound impacts on immune outcomes in other models and their roles in transplant models are not clear.

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