Published in final edited form as:

Transplantation. 2009 May 15; 87(9 Suppl): S74–S77. doi:10.1097/TP.0b013e3181a2b83b.

Memory T cells: How might they disrupt the induction of tolerance?

Nick D. Jones

Transplantation Research Immunology Group, Nuffield Department of Surgery, University of Oxford, John Radcliffe Hospital, Oxford, United Kingdom. OX3 9DU

Abstract

Clinical and experimental evidence suggests that alloreactive memory T cells may be part of the normal T cell repertoire and that such cells are detrimental to the survival of foreign organ allografts induced either by the administration of conventional immunosuppression or experimental tolerance-inducing therapies. The potential mechanisms by which alloreactive memory T cell may form a barrier to the induction of tolerance will be discussed.

Keywords

alloreactive memory T cells; immunosuppression; organ transplantation; tolerance

Introduction

Current therapies to prevent the rejection of foreign organs in clinical transplantation, whilst relatively successful, are associated with a number of debilitating side-effects related to the long-term use of certain drugs and their non-specific mode of action. Therefore, one of the major goals in transplantation is the design of therapies to induce transplantation tolerance that can be administered in the short-term with long-term effects, and which only target immune responses to the transplanted organ. Ideally the induced tolerance is self-perpetuating by inducing a regulatory network that may afford the graft protection against further immunological insults.

Certainly the induction of tolerance to allografts has been demonstrated to be readily achievable in small animal models of transplantation but the successful application of such strategies to large animal models and clinical transplantation has proved challenging. One potential explanation that continues to gain credence is that the relative difficulty to induce tolerance may relate to the presence of alloreactive memory T cells. Indeed, Lombardi and Lechler first demonstrated in individuals who had not been previously exposed to alloantigens that allo-reactivity was contained within the memory as well as the naïve T cell compartment (1). This data was confirmed and extended by Heeger and colleagues who found that non-sensitized patients harboured significant numbers of alloreactive interferon (IFN) γ -secreting memory T cells prior to transplantation and that the precursor frequency of such cells correlated with the risk of rejection episodes following renal transplantation (2). Such alloreactive memory T cells are likely to have been generated by cross-reactivity to

Address correspondence to Dr. Nick D. Jones, Nuffield Department of Surgery, John Radcliffe Hospital, Oxford OX3 9DU, U.K.
Telephone: 00441865 221305 Fax: 00441865 768876 E-mail address: nicholas.jones@nds.ox.ac.uk.
N.D.J. is a Kidney Research U.K. Senior Research Fellow.

The author has no conflict of interest.

environmental pathogens or by homeostatic proliferation following a period of lymphopenia. Indeed, where alloreactive memory T cells have been generated in experimental models either by priming with donor alloantigen (3), following viral infection (4) or following homeostatic proliferation (5) then the presence of alloreactive memory T cells has been shown to preclude the induction of tolerance.

In summary, there is now convincing evidence to suggest that alloreactive memory T cells form part of the normal peripheral T cell pool where they may form a barrier to the induction of tolerance to allografts.

How Do Memory T Cells Disrupt The Induction Of Tolerance?

Whilst it is clear that memory T cells may interfere with the induction of tolerance the cause of this phenomenon remains unknown, although it is likely to be attributable to differential activation requirements, rapid induction of effector function and the microenvironment in which T cells encounter alloantigen.

It is well established that memory T cells are able to respond to lower concentrations of antigen and have less of a requirement for costimulation than naïve T cells. Therefore, memory T cells may be able to respond to alloantigen despite blockade of costimulatory pathways such as the CD28-CD80/86 and CD40-CD154 pathways (6). However, it is worth noting that although memory T cells may be re-activated in the absence of certain costimulatory molecules this does not necessarily mean that under these conditions the memory T cell response is unaffected, only that the response is sufficient to perturb tolerance induction. In addition, memory T cells may be able to utilise other costimulatory pathways such as the OX40-OX40L (7), 4-1BB-4-1BBL (7, 8), ICOS-ICOSL (9) and CD27-CD70 (10) pathways, which may substitute for defective CD28 and CD40 signaling.

Following antigen recognition, naïve T cells have little effector function and undergo extensive division before acquiring the ability to produce cytokines, activate other immune cells and differentiate into effector cells and thus kill targets. In contrast, memory T cells acquire effector function rapidly upon reactivation and are capable of immediate effector function that can precede cell division and clonal expansion (11, 12). Therefore, memory T cells may be able to provide “help” for naïve T cells and B cells by secreting lymphokines or providing accessory molecules that may circumvent the tolerance-inducing effect of costimulatory molecule blockade (Fig. 1). Although evidence in this regard is scant, Chen et al. demonstrated that the presence of alloreactive memory CD4⁺ T cells prevented tolerance induction by donor-specific transfusion and anti-CD154 mAb which was shown to be in part due to the provision of “help” for both CTL and humoral immunity (13).

Finally, it is well established that regulatory T cells (Treg) are a critical component for successful tolerance induction by costimulatory molecule blockade. Just where, when and how Treg function to induce and maintain tolerance remains an area of intense investigation. However, although it has been demonstrated that the lymphoid homing receptor CD62L is absolutely critical for regulation (14), we and others have shown that with time the location of regulation extends to the graft itself (15, 16). Memory T cells have been shown to possess different homing properties to naïve T cells by for example being able to traffic through non-lymphoid tissues as well as through lymphoid tissues (17). Furthermore, unlike naïve T cells, memory T cells can reject allografts in the complete absence of lymphoid tissue (18). Therefore, taken together these data may imply that memory T cells circumvent regulation in the short term by trafficking to the graft and consequently bypassing conventional activation in the lymphoid tissues (Fig. 1).

We have conducted a series of experiments to compare and contrast the response of naïve and memory T cells to an allograft in vivo to attempt to dissect inherent differences between these responses which may shed light on how memory T cells may provide a barrier to the induction of tolerance. Studies such as these are frequently hampered by the fact that as alloreactive memory T cells have received some form of prior activation (not necessarily in the form of an alloantigen), they may exist at a different precursor frequency and be clonally distinct (with different specificity and affinity) from alloreactive T cells present in the naïve repertoire. Therefore observed differences in the response of naïve and memory T cells may relate to these confounding factors rather than to a fundamental difference between naïve and memory T cell subsets. To eradicate these problems we have developed a model whereby the response of naïve and memory CD8⁺ T cells bearing a single transgenic TCR (derived from BM3RAG mice, which confers reactivity to the major histocompatibility complex class I alloantigen, H2K^b) can be visualized following transplantation of an H2K^b skin allograft. We have previously shown that memory BM3 T cells that have been generated from naïve T cells following prior exposure to either a skin allograft or an infusion of donor H2K^b splenocytes have a memory phenotype (CD44⁺) and produce vast amounts of IFN γ within 24 hours of reactivation with alloantigens in vitro (19). Importantly, the adoptive transfer of 1×10^5 memory BM3 T cells into syngeneic RAG^{-/-} recipients facilitated rejection of a subsequent H2K^b skin graft more rapidly than 1×10^5 naïve BM3 T cells (19).

Based on the literature we proposed that rapid skin graft rejection by memory compared to naïve T cells may be the result of memory T cells (a) undergoing more rapid clonal expansion following alloantigen recognition, (b) demonstrating an increased ability to traffic to the graft or (c) developing increased or earlier effector function.

However, when the response of naïve and memory BM3 T cells to a skin allograft was compared, we found that both subsets proliferated in an identical fashion following activation in the draining lymph nodes (as judged by loss of CFSE; manuscript in preparation). Furthermore, effector T cells derived from either memory or naïve T cells trafficked to the graft with the same kinetics (between 5 and 10 days post transplantation) and in the same numbers induced a similar pro-inflammatory gene expression profile; these studies used real-time PCR for the expression of a number of pro-inflammatory cytokines (e.g. IFN γ), effectors (e.g. perforin, granzyme B) and chemokines (e.g. CXCL9 and 10, CCL5). However, analyses of grafts taken 10 days post transplantation revealed that grafts transplanted to animals that had received memory but not naïve T cells harbored a significant GR-1⁺ (polymorphonuclear cells (PMN)) infiltrate. Interestingly, a similar finding has been reported by Fairchild and colleagues (20).

To determine whether the recruitment of PMN to the graft was the underlying cause of the rapid skin allograft rejection seen with memory T cells, further cohorts of RAG^{-/-} mice received adoptive transfer of either naïve or memory T cells with and without administration of a depleting anti-GR-1 mAb (kindly provided by Prof. Fairchild). We found that when GR-1⁺ cells were depleted, naïve and memory BM3 T cells rejected allografts with an identical kinetic, thus confirming the role of PMN in enhanced allograft rejection.

Finally, we have previously demonstrated that regulatory T cells were unable to prevent rejection induced by either CD4⁺ and CD8⁺ memory T cells whilst being completely effective at controlling naïve CD4⁺ or CD8⁺ T cell-mediated rejection (21). Therefore, we asked whether regulatory T cells were able to prevent rejection mediated by memory BM3 T cells if the rejection was attenuated by GR-1⁺ cell depletion. We found that in the absence of PMN, regulatory T cells derived from mice that had received a tolerance-inducing therapy of anti-CD4 mAb combined with a donor leukocyte infusion significantly attenuated

rejection elicited by BM3 Tm with approximately 60% of the grafts surviving for over 100 days (manuscript in preparation).

Taken together these data suggest that an additional way to prevent the disruption of tolerance by memory T cells is through the transient depletion of additional effector mechanisms utilized by memory T cells to elicit graft rejection. Furthermore, we would suggest that the transient attenuation of memory responses may allow the establishment of regulatory mechanisms that are able to control further T cell responses. Indeed, it has already been demonstrated that the transient depletion of PMN can enhance the effectiveness of costimulatory molecule blockade even in the face of a memory CD8 T cell response (22).

Unresolved issues

Although it is clear that alloreactive memory T cells can negatively influence the induction of tolerance, a number of questions related to memory T cell responses to allografts that remain unanswered.

For example, memory T cells can be split not only into CD4⁺ and CD8⁺ T cells but also can be further divided into central and effector memory subsets (that have different trafficking and functional properties). Which of these subsets confer the greatest resistance to tolerance induction is still unknown although there is evidence that suggests that all memory T cell subsets may be detrimental to tolerance induction. It is also worth noting that when memory T cells are generated during the course of an infection or following immunization, these cells mount a dominant T_h1, T_h2 or perhaps a T_h17 response according to the conditions of the primary response when the cells re-encounter the priming antigen. In contrast, alloreactive memory T cells that are present prior to transplantation might have originated from a number of distinct responses to a number of different infections/immunizations. Although most studies have focused on T_h1 type memory T cells, it is possible that memory T cells mount multiple classes of response to an allograft. How these different memory subtypes interact, which class of response is dominant (if any), how these responses influence the subsequent alloresponse of naïve T cells and whether the same or distinct costimulatory pathways are utilized by different memory T cell subsets is unknown. The answers to these questions will be important for the future individualization of immunosuppression according to a patient's immune history and attempts to induce tolerance via costimulatory molecule blockade in clinical transplantation.

In conclusion, clearly, our understanding of the way in which memory T cells respond to allografts, even in the presence of immunosuppression or despite the blockade of certain costimulatory molecules, remains incomplete. However, the available evidence suggests that memory T cell responses to allografts may require distinct costimulatory molecules and that these responses are susceptible, under the right conditions, to suppression by regulatory T cells. Therefore, these data maintain the promise of novel therapies that can induce tolerance to foreign organ transplants in the clinic, even in the face of a pool of pre-formed alloreactive memory T cells.

Acknowledgments

This work was supported by The Wellcome Trust and Kidney Research U.K.

References

1. Lombardi G, Sidhu S, Daly M, Batchelor JR, Makgoba W, Lechler RI. Are primary alloresponses truly primary? *Int Immunol*. 1990; 2:9. [PubMed: 1708274]

2. Heeger PS, Greenspan NS, Kuhlenschmidt S, et al. Pretransplant frequency of donor-specific, IFN- γ -producing lymphocytes is a manifestation of immunologic memory and correlates with the risk of posttransplant rejection episodes. *J Immunol.* 1999; 163:2267. [PubMed: 10438971]
3. Valujskikh A, Pantenburg B, Heeger PS. Primed allospecific T cells prevent the effects of costimulatory blockade on prolonged cardiac allograft survival in mice. *Am J Transplant.* 2002; 2:501. [PubMed: 12118893]
4. 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]
5. Wu Z, Bensinger SJ, Zhang J, et al. Homeostatic proliferation is a barrier to transplantation tolerance. *Nat Med.* 2004; 10:87. [PubMed: 14647496]
6. London CA, Perez VL, abbas AK. Functional characteristics and survival requirements of memory CD4⁺ T lymphocytes in vivo. *J. Immunol.* 1999; 162:766. [PubMed: 9916697]
7. Dawicki W, Bertram EM, Sharpe AH, Watts TH. 4-1BB and OX40 act independently to facilitate robust CD8 and CD4 recall responses. *J Immunol.* 2004; 173:5944. [PubMed: 15528328]
8. Bertram EM, Dawicki W, Sedgmen B, Bramson JL, Lynch DH, Watts TH. A switch in costimulation from CD28 to 4-1BB during primary versus secondary CD8 T cell response to influenza in vivo. *J Immunol.* 2004; 172:981. [PubMed: 14707071]
9. Khayyamian S, Hutloff A, Buchner K, et al. ICOS-ligand, expressed on human endothelial cells, costimulates Th1 and Th2 cytokine secretion by memory CD4⁺ T cells. *Proc Natl Acad Sci U S A.* 2002; 99:6198. [PubMed: 11983910]
10. Yamada A, Salama AD, Sho M, et al. CD70 Signaling Is Critical for CD28-Independent CD8⁺ T Cell-Mediated Alloimmune Responses In Vivo. *J Immunol.* 2005; 174:1357. [PubMed: 15661893]
11. Barber DL, Wherry EJ, Ahmed R. Cutting edge: rapid in vivo killing by memory CD8 T cells. *J Immunol.* 2003; 171:27. [PubMed: 12816979]
12. Kambayashi T, Assarsson E, Lukacher AE, Ljunggren HG, Jensen PE. Memory CD8⁺ T cells provide an early source of IFN- γ . *J Immunol.* 2003; 170:2399. [PubMed: 12594263]
13. 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]
14. Ochando JC, Yopp AC, Yang Y, et al. Lymph node occupancy is required for the peripheral development of alloantigen-specific Foxp3⁺ regulatory T cells. *J Immunol.* 2005; 174:6993. [PubMed: 15905542]
15. Graca L, Cobbold SP, Waldmann H. Identification of regulatory T cells in tolerated allografts. *J Exp Med.* 2002; 195:1641. [PubMed: 12070291]
16. Carvalho-Gaspar M, Jones ND, Luo S, Martin L, Brook MO, Wood KJ. Location and time-dependent control of rejection by regulatory T cells culminates in a failure to generate memory T cells. *J Immunol.* 2008; 180:6640. [PubMed: 18453583]
17. Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature.* 1999; 401:708. [PubMed: 10537110]
18. Chalasani G, Dai Z, Konieczny BT, 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]
19. Jones ND, Carvalho-Gaspar M, Luo S, Brook MO, Martin L, Wood KJ. Effector and Memory CD8⁺ T Cells Can Be Generated in Response to Alloantigen Independently of CD4⁺ T Cell Help. *J Immunol.* 2006; 176:2316. [PubMed: 16455988]
20. El-Sawy T, Miura M, Fairchild R. Early T cell response to allografts occurring prior to alloantigen priming up-regulates innate-mediated inflammation and graft necrosis. *Am J Pathol.* 2004; 165:147. [PubMed: 15215170]
21. 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]
22. El-Sawy T, Belperio JA, Strieter RM, Remick DG, Fairchild RL. Inhibition of polymorphonuclear leukocyte-mediated graft damage synergizes with short-term costimulatory blockade to prevent cardiac allograft rejection. *Circulation.* 2005; 112:320. [PubMed: 15998678]

Figure.

Potential mechanisms contributing to the disruption of tolerance induction by memory T cells

Memory T cells may respond to alloantigens despite blockade of certain costimulatory molecules. Memory T cells may form a barrier to tolerance induction themselves by eliciting rapid graft rejection following the activation of other immune effector cells such as polymorphonuclear cells (PMN) (A). Furthermore, memory T cell responses have been shown to be more resistant to suppression by regulatory T cells (Treg; B), allowing such responses to be initiated. In addition to directly contributing to rejection, memory T cells may provide help for B cells to produce alloantibodies and naïve T cells to respond to an allograft in the absence of costimulation via the secretion of growth factors such as interleukin (IL)-2 and IL-4 (C and D). It has also been suggested that memory T cells may further activate antigen-presenting cells (APC) bearing alloantigens in a process termed “licensing”, which may result in an enhanced ability to activate naïve T cells (particularly CD8⁺ T cells) due to the further upregulation of the major histocompatibility complex, alternate costimulatory molecules and secreted lymphokines (E).