Homeostatic T cell proliferation as a barrier to T cell tolerance

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The maintenance of T cell numbers in the periphery is mediated by distinct homeostatic mechanisms that ensure the proper representation of naïve and memory T cells. Homeostatic proliferation refers to the process by which T cells in lymphopenic hosts divide in the absence of cognate antigen to reconstitute the peripheral lymphoid compartment. During this process T cells acquire effector-memory like properties, including the ability to respond to low doses of antigen in the absence of CD28 costimulation. Furthermore, this capacity is retained long after proliferation has ceased. Accumulating data implicates homeostatic proliferation in autoimmune diseases and transplant rejection, and suggests that it may represent a barrier to tolerance in protocols that use T cell depletion. Implementing combination therapies that aim to promote the development and expansion of regulatory T cell populations while specifically targeting alloresponsive T cells may be the soundest approach to attaining allograft tolerance in the aftermath of T cell depletion and homeostatic proliferation.

Keywords: homeostasis; proliferation; tolerance; memory; costimulatory; T cell

1. HOMEOSTATIC PROLIFERATION

The peripheral T cell pool is composed of a large and heterogeneous repertoire of naïve and memory T cells capable of recognizing both foreign- and self-antigens. This pool of lymphocytes is tightly regulated by homeostatic mechanisms that serve to control the numbers of T lymphocytes in circulation and the types (i.e. CD4 versus CD8, naïve versus memory). This is important to ensure the organism has continued diversity of naïve T cells able to respond to random antigenic challenges while preserving immunological memory to microbial pathogens formerly encountered.

Recent evidence suggest that the set point for homeostatic equilibrium involves an external mechanism of quorum sensing, which the T cell ‘interprets’ as a measure of available or free ‘space’. Indeed, T cells seem to be under severe pressure to fill this space as evidence by their robust expansion in the absence of antigen soon after adoptive transfer into T cell-deficient syngeneic recipients, a term referred to as homeostatic or lymphopenia-induced proliferation (Surh & Sprent 2000). Homeostatic proliferation occurs primarily within the T cell areas in the spleen (periarteriolar lymphocyte sheaths) and the lymph node (paracortex) (Dummer *et al.* 2001) and is dependent on many factors. In general, the degree of lymphopenia is critical in determining the extent of proliferation (Gudmundsdottir & Turka 2001). For example, the co-transfer of large numbers of bystander T cells but not B cells has been reported to limit the expansion of CFSE labelled donor T cells in irradiated

hosts (Ernst *et al.* 1999). The level of suppression was found to be directly proportional to the dose of co-injected bystander T cells (Dummer *et al.* 2001).

The strength of interaction between the T cell receptor (TCR) and self-peptide major histocompatibility complex (MHC) also seems to regulate the homeostatic behaviour of T cells. Early work suggested that the level of CD5 expression was a good predictor of proliferative potential as its expression was believed to correlate with high TCR avidity for MHC (Smith *et al.* 2001; Azzam *et al.* 1998). Select TCR transgenic T cells that expressed high levels of CD5 were found to undergo a more robust expansion in lymphopenic hosts than those TCR transgenic T cells that expressed low levels of CD5 (Ge *et al.* 2004). The notion that CD5 predicts expansion potential was puzzling in particular because this molecule is a known negative regulator of TCR signalling. A more careful analysis has revealed that CD5 and the level of TCR expression cooperatively regulate the avidity for self-peptide MHC to influence the homeostatic capacity of a T cell. The T cell clones with the greatest potential for expansion were those that expressed high levels of TCR and whose TCR had a high avidity for self-peptide MHC as evidenced by CD5 expression. In this scenario high TCR expression could mitigate the inhibitory affect of CD5. In contrast, T cells that expressed high levels of CD5 but low levels of TCR could not overcome the negative signalling imparted by CD5 expression and thus were unable to proliferate well (Kassiotis *et al.* 2003). Consistent with this view that overall TCR signal strength determines the degree of homeostatic proliferation, T cells that lack CD4 or CD8 undergo little to no expansion in lymphopenic recipients (Ge *et al.* 2001; Wang *et al.* 2001).

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One contribution of 16 to a Theme Issue ‘Immunoregulation: harnessing T cell biology for therapeutic benefit’.

Figure 1. Requirements for homeostatic proliferation. (a) Naïve T cells migrating to lymph nodes enter from the peripheral blood and roll along the endothelium until CCR7 is engaged by CCL21 or CCL19 (not shown) presented on the surface of the high endothelial venules (HEV). Extravasation through the HEVs occurs and T cells proceed to migrate to the T cell zones where the TCR and IL-7 receptor become engaged by self-peptide/MHC expressing dendritic cells and by IL-7 produced locally by stromal cells. (b) Memory CD4 and CD8 positive T cells can proliferate in the lymph node (not shown) or in non-lymphoid tissues independent of TCR engagement.

(a) T cell requirements for homeostatic proliferation

Many studies have examined the specific requirements of naïve and memory T cells as they undergo homeostatic proliferation (figure 1). Reconstitution experiments have shown that naïve CD4⁺ and CD8⁺ T cells require contact with two ligands, primarily the cytokine IL-7 (a member of a family of cytokines that utilize the IL-2 receptor common gamma (γ -) chain to signal) and self-MHC-peptide complexes to undergo

optimum expansion in lymphopenic host (Murali-Krishna *et al.* 1999). Moreover, these same signals facilitate their prolonged survival *in vivo* as well. The relevant source of IL-7 is unknown, but recent data would suggest that a radiation-resistant non-bone marrow-derived stromal cell is most likely involved (Gimble *et al.* 1989; Schluns *et al.* 2000). CCR7 mediated trafficking of T cells into secondary lymph nodes has also been implicated in regulating homeostatic proliferation. Interestingly, naïve CD4⁺

lymphocytes have an additional requirement for homeostatic expansion: they fail to undergo any significant degree of proliferation when transferred into irradiation-depleted plt/plt mice which lacked expression of the CCR7 ligands CCL21 and CCL19 (Ploix *et al.* 2001). Tissue-specific overexpression of CCL21 in the same mice overcame the defect and, intriguingly, resulted in the homeostatic proliferation of CD4+ T cells even in non-irradiated (non-lymphopenic) recipients. CD4+ T cells then appear to be strongly dependent on CCR7 ligand expression to support their homeostatic proliferation, while CD8+ T cells can function independently of CCR7-mediated signals through mechanisms that remain undefined.

In the case of memory cells, Tan *et al.* found that IL-15 and IL-7 jointly controlled the homeostatic proliferation of memory CD8+ T cells, but were unable to show evidence for the involvement for either of these cytokines in memory CD4+ T cells (Tan *et al.* 2002). In fact memory CD4+ T cells remain an enigma as no cytokine or molecule to date has been assigned a prominent role in augmenting their homeostatic proliferation *in vivo*. A confounding question that still remains unanswered is how lymphopenia regulates sensitivity to the availability of soluble factors that drive homeostatic proliferation. Is it competition for shared resources that is the rate limiting step or does the 'lymphopenic state' actually enhance expression of these factors? Support for the former hypothesis is provided by findings in humans in which the levels of IL-7 were found to be elevated after allogeneic bone-marrow transplantation (Bolotin *et al.* 1999), cytotoxic chemotherapy treatment (Fry *et al.* 2001), and at late stages of HIV infection (Napolitano *et al.* 2001): all conditions in which the T cell population is severely depleted and the consumption of IL-7 would presumably be limited. As many laboratories attempt to answer these and other questions, homeostatic proliferation will likely continue to be an intense area of investigation.

With regard to MHC dependency, several studies have demonstrated that the survival and homeostatic proliferation of both CD4+ and CD8+ memory T cells is equivalent upon transfer into MHC deficient- and MHC-replete hosts (Murali-Krishna *et al.* 1999; Gudmundsdottir & Turka 2001; Tan *et al.* 2002). These data, which are in stark contrast to observations made with naïve T cells, strongly argue that contact with MHC ligands is not essential and perhaps likely explains why 1–3% of memory T cells in the periphery are cycling at any given point in time (Tough & Sprent 1994; Bruno *et al.* 1996). Nevertheless, some TCR signalling may still be necessary for maintenance of normal functional responses as memory CD4+ T cells deprived of MHC class II show defects in activation upon antigenic re-encounter (Kassiotis *et al.* 2002).

(b) Consequences of homeostatic proliferation

The key hallmark of homeostatic proliferation is that it induces naïve T cells to express conventional memory T cell markers and to differentiate into a memory-like state (Cho *et al.* 2000; Goldrath *et al.* 2000, 2004). In particular, L-selectin (CD62L) which is preferentially expressed on naïve T cells as it is necessary for entry

into lymph nodes, is progressively lost as cells undergo homeostatic proliferation. Concomitant with the loss in CD62L is the upregulation of CD44, a molecule whose expression is required for adhesion and entrance into peripheral tissue. Functionally, these cells behave similarly to memory cells as their requirement for CD28 costimulation is diminished, thus lowering their activation threshold (Gudmundsdottir & Turka 2001). Furthermore, the rapidity and magnitude of effector responses following activation, such as cytokine production, CTL activity and proliferative capacity and kinetics, are also greatly enhanced (Cho *et al.* 2000; Goldrath *et al.* 2000). Interestingly, one distinctive characteristic of cells as they undergo homeostatic proliferation is that acute activation markers such as CD69 and CD25 do not appear to be upregulated, thus allowing them to be phenotypically distinguished from antigen-activated T cells (Murali-Krishna & Ahmed 2000; Kieper *et al.* 2001).

2. HOMEOSTATIC PROLIFERATION AS A BARRIER... WHEN MEMORY IS PROBLEMATIC

(a) The association with autoimmunity

While homeostatic proliferation is important to maintain T cells numbers in the periphery, this highly regulated process may be hazardous under select circumstances to the host. The danger arises as a result of the non-specific expansion of all T lymphocytes during homeostatic proliferation, including those that are auto-reactive. Under normal conditions, usually, auto-reactive T cells that escape negative selection in the thymus are controlled by tightly regulated, multi-layered mechanisms that work in concert to mediate tolerance in the periphery (Walker & Abbas 2002). However, events occurring as a consequence of homeostatic proliferation can break this tolerance. For example, Powrie *et al.* observed that the adoptive transfer of naïve T cells (CD45RB^{hi}) into lymphopenic SCID or RAG^{−/−} mice is followed by the development of a progressive and chronic inflammatory bowel disease about four weeks later (Singh *et al.* 2001). The co-transfer of CD45RB^{lo} cells or CD4+CD25+ regulatory T cells (Tregs) not only prevents the induction of disease but also reverses established pathology (Liu *et al.* 2003; Mottet *et al.* 2003).

Initially it was believed that the regulatory T cell population was providing protection exclusively by the direct negative regulation of self-reactive T cells. Stockinger and colleagues have shown that this may not be the entire story. In their studies, the co-transfer of AND TCR-transgenic T cells of known TCR specificity unrelated to antigens present in the gut was able to completely prevent the development of colitis (Barthlott *et al.* 2003). The observed protective effect was not due to the acquisition of a regulatory phenotype by AND T cells, but instead was dependent on their ability to expand which indirectly inhibited the homeostatic proliferation of the pathogenic cells within the CD45RB^{hi} population. As added proof, the authors performed the same experiment in H-2^b hosts, where the homeostatic proliferative capacity of AND T cells is known to be limited. In this model, AND T cells failed to protect recipients from autoimmune disease and this

correlated with the robust expansion of the cotransferred naïve T cells. Thus, these data suggest that homeostatic proliferation can induce cells with self-reactive TCR specificities to cause overt clinical autoimmune disease.

(b) *Resistance to tolerance induction*

Many modalities of treatment for transplant recipients use T cell depletion in conjunction with immunosuppression as a strategy to promote allograft acceptance. In this situation depletion is rarely complete, with roughly 10–15% of cells remaining. Our laboratory has recently shown that homeostatic proliferation readily occurs in this setting as modelled using anti-CD4 and anti-CD8 depletion in mice (Wu *et al.* 2004). Interestingly, we have found that the residual T cells undergoing homeostatic proliferation are resistant to costimulatory blockade, a manoeuvre that normally produces long-term graft acceptance. Furthermore, the T cells that underwent homeostatic proliferation could dominantly transfer tolerance resistance to naïve mice receiving cardiac allografts. We hypothesize that resistance to tolerance was directly linked to the development of memory cells as a consequence of homeostatic proliferation. If correct, the lack of efficacy of CD28 costimulatory blockade in our model is consistent with the fact that effective activation of memory T cells can be CD28-independent (Croft *et al.* 1994; Swain *et al.* 1996).

3. HOW ALLOREACTIVE MEMORY DEVELOPS

Accumulating evidence suggest that immunological memory is a potential barrier to achieving long-term allograft survival (Baid *et al.* 2001). The fact that 40–50% of human peripheral T cells have a memory phenotype is suggested to be a contributing factor for the consistent failure of tolerance strategies to work in humans, despite proven success in rodent models (McFarland *et al.* 2000). Indeed, Heeger *et al.* found that freshly isolated normal human PBLs from patients pre-transplant exhibited memory-like alloreactivity characterized by the rapid production of interferon- γ to donor antigen (Heeger *et al.* 1999). The pre-existing anti-donor response seemed to be independent of the degree of HLA mismatch and was shown to correlate with post-transplant acute rejection in a cohort of patients examined. Since T cell memory can affect the outcome of graft acceptance, how allospecific memory cells are generated *in vivo* remains an important question.

Host sensitization through blood transfusions, pregnancy, or past failed grafts is one obvious means. The subsequent development of alloreactive memory T cells poised to mount anti-graft responses following antigenic re-exposure has been well documented (Kupiec-Weglinski 1996). T cells that express dual TCRs, which are a significant portion of the human peripheral T cell pool (up to 30%), can potentially be involved in mediating memory allogeneic responses as well (Padovan *et al.* 1993, 1995). In the situation where the second TCR is alloreactive, the activation through unrelated antigens of the first TCR can 'create' an allospecific memory T cell without prior exposure to

the alloantigen. Accruing evidence from both clinical and experimental studies indicate that heterologous immunity may be a significant yet unappreciated component of tolerance resistance. Heterologous immunity refers to the theory that a microbial infection (i.e. viral) can induce anti-viral specific T cells that display cross reactivity to alloantigens without prior sensitization (Alexander-Miller *et al.* 1993; Adams *et al.* 2003). This type of molecular mimicry has been documented in humans in which HLA-B8-restricted Epstein-Barr virus specific T cells have the known ability to alloreact to HLA-B14, -44, and -35 expressing donor cells (Burrows *et al.* 1994). The possible importance of heterologous immunity to transplantation tolerance was demonstrated in several studies in which animals challenged with virus during the period of tolerance induction therapy almost categorically failed to become tolerized using a regimen that was successful in naïve uninfected recipients (Welsh *et al.* 2000; Williams *et al.* 2001, 2002; Forman *et al.* 2002). In an elegant study, Adams *et al.* showed that a history of multiple viral infections rendered animals resistant to tolerance induction by a non-myeloablative mixed chimerism approach (Adams *et al.* 2003). Importantly, this effect was long-lived and did not require continuous viral exposure. In a related vein, work from our laboratory indicates that homeostatic proliferation can also generate alloreactive memory T cells and that the resistance to tolerance persists even after repopulation has ceased (Wu *et al.* 2004).

4. OVERCOMING THE BARRIER... REGULATORY T CELLS AS PROTECTORS

The best defined population of immunoregulatory cells are a naturally occurring subset of CD4⁺ T lymphocytes that constitutively express the IL-2 receptor- α subunit (CD25) (Sakaguchi *et al.* 1995). Originally identified by Sakaguchi and colleagues where they were found to constitute 5–10% of CD4⁺ peripheral T cells and were shown to be critical for the control of autoimmunity, Tregs have been implicated in regulating anti-tumour immunity and in modulating responses to infectious diseases as well (Shimizu *et al.* 1999; Woo *et al.* 2001, 2002; Aseffa *et al.* 2002; Xu *et al.* 2003). Once activated, CD4⁺CD25⁺ Tregs non-specifically suppress proliferation of either CD4 or CD8⁺ T cells through mechanisms that are not entirely clear but are known to require cell to cell contact and which involve limiting IL-2 production from responding cells (Thornton & Shevach 2000).

Regulatory T cells have long been implicated in transplant tolerance. Early work in the field found that the therapeutic administration of combinations of anti-CD4 and anti-CD8 mAbs at the time of transplantation induced a robust form of peripheral tolerance (Qin *et al.* 1993). Later, modification of these tolerance therapies using donor specific transfusion in combination with non-depleting anti-CD4 mAbs, CD154, or CTLA-4-Ig were shown to be as effective in inducing tolerance in rodents (Pearson *et al.* 1994; Parker *et al.* 1995; Graca *et al.* 2000). Tolerance impart was actively being maintained by a population of CD4⁺ T lymphocytes that contained regulatory activity as

removal of CD4+, but not CD8+ T cells led to rejection in thymectomized animals in which long-term skin allograft survival had been achieved by CTLA4-Ig treatment (Markees *et al.* 1998). Previous work has also shown that tolerance could be dominantly transferred to naïve recipients and was specific as only third party but not same-donor skin grafts were rapidly rejected (Hara *et al.* 2001; Graca *et al.* 2002a). Moreover regulatory T cells observed colonizing the tolerated allograft could exit the graft and repopulate the peripheral immune systems of immunodeficient recipients when the graft was retransplanted (Graca *et al.* 2002a). These recipients, when adoptively transferred with naïve T cells and challenged with a fresh skin graft, were able to resist rejection without further treatment. Thus regulatory T cells found within tolerated grafts when retransplanted could inhibit alloreactive T cells and dominantly establish tolerance to new skin grafts.

(a) Ineffectiveness of Tregs in depletion model of tolerance induction

There is widespread use of T cell depletion as a means to promote engraftment and induce tolerance. However, we have found that homeostatic proliferation that occurs as a consequence of depletion can promote the development of memory cells which resist tolerance. Given the potential importance of regulatory T cells in tolerance, an important question is whether or not they can regulate or prevent the development of alloreactive memory T cells. Here are a number of issues that are relevant.

First, there is little available information regarding how different populations of T cells, naïve, memory, and regulatory, may be differentially susceptible to depletion. Two reports in abstract form suggest that memory T cells may be relatively spared by depletion with Campath (Pearl *et al.* 2004a,b), but no information is currently available regarding regulatory T cells. Second, and related to this, even after depletion, the kinetics of reconstitution of regulatory T cells may vary from that of non-regulatory cells. Third, an important question is whether or not regulatory cells can inhibit the responses of memory T cells. One study has reported that they are able to block the responses of CD8+ memory T cells, but at present there are no data on the effect of regulatory T cells on CD4 memory responses (Dai *et al.* 2004). Fourth, the efficacy of Tregs in suppressing T cell responses *in vitro* is dose-dependent; high level suppression can only be attained at relatively high ratios of Tregs to effector cells (Kuniyasu *et al.* 2000). Fifth, efficacy is also dependent on stimulation of the Treg via the TCR, as activation is required for suppressor activity. Consistent with this, several reports have demonstrated that the transfer of many more naïve than tolerized CD4+CD25+ Tregs are needed to suppress rejection mediated by naïve T cells in skin or islet transplant models (Graca *et al.* 2002a,b; Davies *et al.* 1999), suggesting that transplantation may have led to activation of Tregs. Alternatively, however, transplantation in these settings might instead be leading to expansion of the Treg population. Of course, these two possibilities are not mutually exclusive and both may play a role.

(b) Enhancing the outgrowth of Tregs over alloreactive T cells

It would be an obvious benefit to promote the 'outgrowth' of Tregs compared to non-regulatory T cells in the aftermath of T cell depletion. However, this has been hampered by the failure to identify specific stimuli that favour their expansion. In the early literature, the inability of Tregs to proliferate *in vitro* following TCR ligation prompted many to ask whether these cells could homeostatically proliferate at all. Gavin *et al.* were first to demonstrate that CD4+CD25+ Tregs were quite capable of homeostatically expanding in lymphopenic environments despite their hyporesponsiveness to strong antigenic stimuli *in vitro* and *in vivo* (Gavin *et al.* 2002). The expansion profiles of Tregs were similar to naïve CD4+CD25- T cells in kinetics and dependence on MHC class II expression. Recent reports would also suggest that the selecting self-peptide is critical for the lymphopenia induced peripheral expansion of Tregs. 6.5 TCR transgenic CD4+CD25+ T cells derived from TS1 X HA28 mice were unable to homeostatically proliferate in irradiated host that lacked the selecting self-peptide while TCR transgenic CD25-CD4+ T cells showed no similar defect (Cozzo *et al.* 2003).

Of the known costimulatory molecules CD28 and OX40 have shown the greatest potential in regulating Treg expansion and homeostasis *in vivo*. In the presence of CD28, CFSE labelled CD4+CD25+ but not CD25- T cells were shown to readily expand under steady-state conditions in normal mice who display a full lymphoid compartment (Tang *et al.* 2003). This can be explained by the finding that tissue-specific Tregs preferentially divide in the draining lymph nodes that express the target self-antigen (Fisson *et al.* 2003). Interestingly, when B7 specific antibodies were used to block CD28 mediated signalling, CD4+CD25+ regulatory T cells were no longer able to proliferate and displayed a decreased ability to survive long-term. Along these same lines, CD28 deficient NOD mice were also found to have a dramatic reduction in the numbers of CD4+CD25+ Tregs in their spleens and lymph nodes (Salomon *et al.* 2000). Taken together, these results were instrumental in demonstrating that CD28 mediated signals in Tregs function to support their survival and promote their self-renewal *in vivo*. In contrast to CD28 deficient mice, OX40-/- mice express normal numbers of Tregs in the periphery (Tang *et al.* 2003). Despite this lackluster phenotype, recent data is suggestive that OX40 mediated signalling can play a positive role in driving the homeostasis of Tregs in the periphery (Takeda *et al.* 2004). Tregs were shown to expand poorly when adoptively transferred into OX40 ligand (OX-40L)/Rag double knockout mice. Remarkably, the transgenic overexpression of OX40L in Rag deficient recipients led to a profound expansion of Tregs that was far superior to the proliferative response observed in RAG-/- mice alone. Thus, OX40-OX40L interactions can potentiate the homeostatic proliferative capacity of Tregs.

The common γ -chain signalling cytokines (IL-2, -7, and -15) serve an integral role that supports the development, survival, and homeostatic proliferation

of T lymphocytes. IL-2 has been the most studied. CD4+CD25+ Tregs are almost completely absent in the thymus and periphery of mice that lacked IL-2 or other components of the IL-2 receptor (IL-2R β -/- mice) (Kneitz *et al.* 1995; Papiernik *et al.* 1998; Malek *et al.* 2002). Furthermore, Tregs transferred into IL-2-/- mice failed to successfully engraft in the absence of IL-2. These data indicate that IL-2 is important both for the development and maintenance of Tregs. IL-2R signals activate both the JAK-STAT and PI-3K signalling pathways. It appears that the former is key in Treg development, as there is a 5–10 fold increase in thymic (and peripheral) Treg numbers in mice that express a constitutively active version of the STAT5b (Burchill *et al.* 2003). STAT5b is also activated via the IL-7 and IL-15 receptors, but to date there is little published information regarding the role of either of these pathways in Treg development, maintenance or homeostatic expansion. CD4+CD25+ regulatory T cells do express the complete IL-7 receptor, albeit at reduced levels compared to CD25-CD4+ T cells (Cozzo *et al.* 2003). While the expression of IL-15 receptor on Tregs has not specifically been reported, human studies have shown that IL-15 can synergize with TCR-activated pathways to successfully expand CD4+CD25+ T cells *in vitro* (Koenen *et al.* 2003), and our own studies have shown that IL-15 can promote murine Treg survival (Bensinger *et al.* 2004).

5. REMOVING THE BARRIER BY SPECIFICALLY TARGETING ALLOREACTIVE T CELLS

Enhancing the pool of regulatory T cells post-depletion is ideal, but the beneficial effects of these treatments would still have to overcome the deleterious effects of homeostatically expanding alloreactive T cells that are present in the periphery. A recent report by Terry Stroms' group has demonstrated that actively proliferating T cells can be selectively targeted by the combined treatments of rapamycin with lytic IL-2-Fc and IL-15-Fc fusion proteins (Zheng *et al.* 2003). Indeed, the study demonstrated that administration of the combined therapy served to inhibit alloantigen triggered CD4+ and CD8+ T cell proliferation *in vivo* and could accelerate the initiation of apoptosis among activated T cells to achieve tolerance to skin and heart allografts even in recipients such as NOD mice that have a well-documented generalized resistance to tolerance induction (Pearson *et al.* 2003). Interestingly, CD4+CD25+ regulatory T cells whose survival was not adversely affected by these treatments were required to ensure indefinite allograft survival, since CD25 depleted skin allograft recipients rapidly rejected their grafts once treatment was discontinued.

6. CAVEATS: PROMOTING TREG OUTGROWTH MAY ALSO ENHANCE THE ALLOSPECIFIC MEMORY RESPONSE

Controlling the allospecific memory response is essential if the ultimate goal is to achieve long-term graft survival. Implementing therapies to enlarge the pool of regulatory T cells post-depletion should be examined closely as these treatments should not come at the expense of also potentially enhancing the activity

of the alloreactive T cell population. For example, tolerogenic/immature DCs expressing low levels of MHC class II, CD40, and CD80/86 and a limited ability to produce inflammatory cytokines such as IL-12 in comparison to matured DCs have been used therapeutically to demonstrate tolerance in murine models of allograft rejection (Hirano *et al.* 2000; Lu & Thomson 2002; Min *et al.* 2003). Indeed experimental evidence has shown that immature DCs cannot only induce the expansion of CD4+CD25+ T cells but can also promote the development of other regulatory T cell populations (i.e. Tr1, Th3) *in vivo* and *in vitro* (Steinman *et al.* 2003). Nevertheless, immunization with donor specific tolerogenic DCs could potentially exacerbate the alloresponse as memory T cells would be expected to be quite immune to the tolerogenic effects of immature DCs due to their lowered threshold for activation. Engagement of OX-40 was also proposed as a method to augment the homeostatic expansion of Tregs *in vivo*. This too is cause for concern as recent data has shown that OX40 signalling can potentiate the extent of T cell expansion in the primary T cell response to positively influence their ability to become long-lived memory T cells (Gramaglia *et al.* 2000). Importantly, OX40-positive T cells have been visualized *in situ* at the site of inflammation during graft-versus-host disease (Tittle *et al.* 1997) and OX40 agonistic antibody can break an existing state of tolerance to restore normal functionality to CD4 T cells (Bansal-Pakala *et al.* 2001). On the basis of these data then, antagonistic reagents to OX40 instead may be more useful therapeutically to limit rejection resulting from chronic inflammatory responses mediated in part by memory T cells. Given that Tregs do not have distinct molecules that can be targeted to solely augment their expansion *in vivo*, therapies that aim directly at removing or decreasing the survival of the alloreactive memory T cell currently hold more promise. As an example, recent work has shown that under normal T cell-sufficient conditions, homeostasis of CD8+ memory phenotype is controlled by IL-15 (Kennedy *et al.* 2000; Ku *et al.* 2000; Becker *et al.* 2002). Neutralizing IL-15 may limit the survival of alloresponsive memory CD8+ T cells and thus prove to be beneficial in limiting the extent of graft destruction.

7. CONCLUSION

Depletion as a method to induce immunosuppression is a powerful tool that serves to remove the pool of alloreactive CD4+ and CD8+ T cells in the periphery. Unfortunately, the homeostatic mechanisms that are essential in ensuring diversity within the T cell repertoire can undermine the beneficial effects that depletion affords. The residual cells that homeostatically expand represent a novel population of alloreactive T helper and cytotoxic T cells that express characteristically 'memory-like' responses and, more importantly, memory-like refractoriness to tolerance induction by costimulatory molecule blockade. Although all the factors that regulate homeostatic proliferation of naïve and memory, CD4+ and CD8+ T cells are not yet identified, available

knowledge does offer hints as to which pathways can be targeted to limit the expansion of alloreactive T cells.

Creating an environment in which the graft is well tolerated would also require the development of T cells with suppressor or regulatory properties functioning to continuously educate new alloreactive T cells being exported from the thymus as well as those that have escaped deletion. The homeostatic proliferative capacity of regulatory T cells as well as the factors that drive this process have just begun to be addressed.

In sum, homeostatic proliferation and the memory T cells that arise as a result of this process continue to present an obstacle to achieving long-term allograft survival. Understanding the mechanisms that regulate this process (to restrict their expansion while enlarging the regulatory T cell population) represents a potent means by which the immune response can be therapeutically manipulated to favour tolerance instead of rejection.

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