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Sinks, suppressors and antigen presenters: how lymphodepletion enhances T cell-mediated tumor immunotherapy

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Lymphodepletion followed by adoptive cell transfer (ACT) of autologous, tumor-reactive T cells boosts antitumor immunotherapeutic activity in mouse and in humans. In the most recent clinical trials, lymphodepletion together with ACT has an objective response rate of 50% in patients with solid metastatic tumors. The mechanisms underlying this recent advance in cancer immunotherapy are beginning to be elucidated and include: the elimination of cellular cytokine 'sinks' for homeostatic γ_C -cytokines, such as interleukin-7 (IL-7), IL-15 and possibly IL-21, which activate and expand tumor-reactive T cells; the impairment of CD4⁺CD25⁺ regulatory T (Treg) cells that suppress tumor-reactive T cells; and the induction of tumor apoptosis and necrosis in conjunction with antigen-presenting cell activation. Knowledge of these factors could be exploited therapeutically to improve the *in vivo* function of adoptively transferred, tumor-reactive T cells for the treatment of cancer.

Introduction

Adoptive cell transfer (ACT) of large numbers of autologous tumor-reactive T cells into a tumor-bearing host represents a promising therapy for the treatment of metastatic cancer in humans [1]. This exciting therapy uses the rapid *ex vivo* expansion of tumor-infiltrating or -reactive lymphocytes (TILs), which are subsequently transferred in conjunction with the administration of a high-dose of a stimulatory cytokine, in particular interleukin-2 (IL-2). Although other forms of immunotherapy, such as tumor-antigen (Ag) vaccination or the administration of immune-stimulating cytokines alone, are capable of raising tumor-reactive T cells *in vivo*, they do not reliably induce the regression of large established solid tumors (reviewed in Ref. [2]). ACT is capable of mediating

tumor regression [3–6], however, these effects are even more pronounced in the absence of host lymphocytes [7,8]. This approach has resulted in the most consistent and dramatic clinical responses observed in the treatment of metastatic cancer [7] (Figure 1a).

These clinical responses are associated with autoimmune manifestations in sites that express shared melanocyte or melanoma Ags, such as the skin, in the form of vitiligo. With lymphodepletion, we have also observed melanocyte destruction at immune privileged sites, such as the eye (Figure 1b,c). Although skin-depigmentation has been previously associated with immunotherapies, inflammation of the anterior segment of the eye has not been previously observed, and might be indicative of a more potent activating stimulus. Fortunately, these eye manifestations are treatable with local steroids, which do not detract from the antitumor immune destruction.

The specific mechanisms that contribute to this enhanced state of immunity remain poorly understood. Recent insights in two rapidly expanding fields, the cytokine-mediated homeostasis of mature lymphocytes by γ_C -cytokines, such as IL-7, IL-15 and IL-21, and the control of autoreactive T cells by CD4⁺CD25⁺ regulatory T (Treg) cells, provide the foundation for what might be occurring after lymphodepletion. The removal of lymphocytes that compete for homeostatic cytokines or suppress tumor-reactive T cells might contribute to the enhancement and subsequent tumor destruction by the adoptively transferred T cells. The less described role of antigen-presenting cell (APC) activation by lymphodepletion might also have a role in T-cell activation. Knowledge of these factors presents the potential for therapeutic exploitation in the treatment of metastatic cancer in humans.

A link between lymphodepletion and augmented immune function

It has long been observed that transfer of small numbers of T cells into lymphopenic hosts results in T-cell

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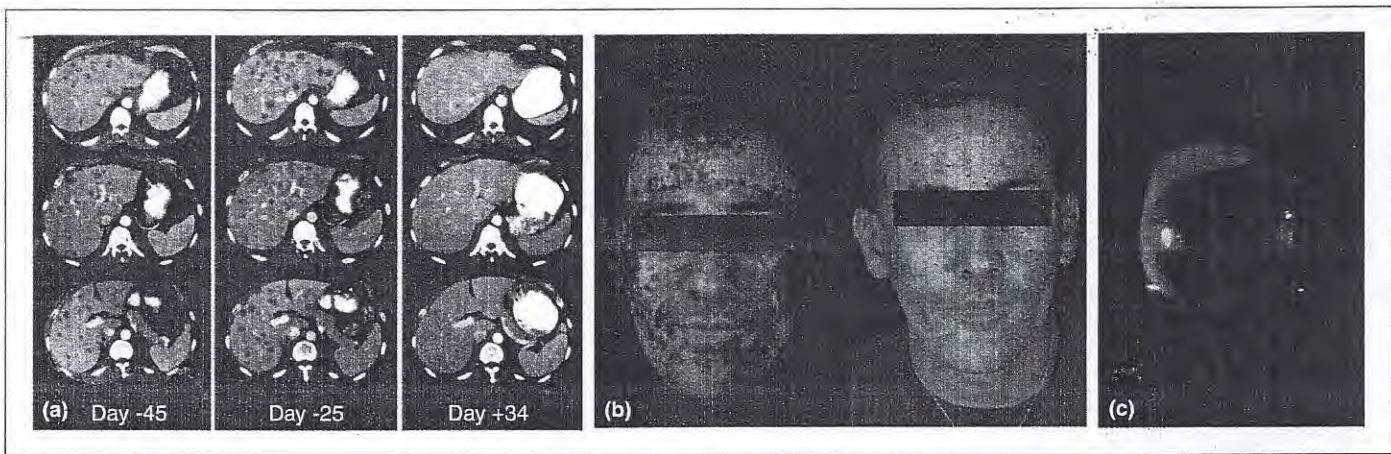


Figure 1. Potentiation of immune response to non-mutated shared self-antigens and tumor antigens by lymphodepletion. Following adoptive cell transfer therapy with tumor-infiltrating lymphocytes (TILs), in the setting of chemotherapy-mediated lymphoablation: (a) Computed tomography (CT) scan of liver metastases in a melanoma patient. Metastatic lesions progressed rapidly before the treatment (from day -45 to day -25) then regressed dramatically after adoptive T-cell transfer (day +34). (b) Extensive destruction of normal melanocytes (vitiligo) in a patient who experienced a remarkable clinical response. (c) Anterior uveitis (inflammation of the eye) in a patient who exhibited >99% tumor reduction. Photographs courtesy of S.A. Rosenberg.

expansion, a process described as homeostatic proliferation [9–16]. As the T cells proliferate, they assume an Ag-experienced or memory phenotype, which is indicated by upregulation of CD44, Ly6C and CD122 (IL-2–IL-15R β) [12,15]. This T-cell expansion and acquisition of a memory phenotype is also associated with enhanced effector functions, determined by *ex vivo* analysis of interferon- γ (IFN- γ) release and cytotoxicity [12,14,15]. In a recent autoimmunity study, King *et al.* showed that the inherent lymphopenia in non-obese diabetic (NOD) mice and increased expression of IL-21 drives the homeostatic expansion of autoreactive cells, precipitating self-tissue destruction [17]. Furthermore, adoptive transfer of naive lymphocytic choriomeningitis virus (LCMV)-specific CD8⁺ T-cell receptor (TCR) transgenic splenocytes into LCMV-infected lymphodepleted, but not replete, hosts rapidly reduced viral titers to below detection limits [13]. Analogous results have also been observed in another model, based on the 2C T-cell receptor (TCR) transgenic model. 2C TCR transgenic T cells are specific for an L^d-restricted α -ketoglutarate dehydrogenase epitope or the SIYRYGL peptide in the context MHC I H2-K^b. These cells expand homeostatically in *Rag*^{-/-} mice and specifically treat tumors, at a comparable level to the T cells derived from Ag-specific vaccination [15]. Dummer *et al.* observed that adoptive transfer of a polyclonal population of T cells into a *Rag*^{-/-} or sublethally irradiated mouse specifically inhibits tumor growth [18].

It is interesting to note that in these models, lymphopenic-induced activation and pathogen or tumor clearance are generated in the absence of Ag-specific vaccination. Ag-specific CD8⁺ T cells are required for pathogen or tumor clearance, and the need for subsequent immunization might be negated [19,20]. These model Ags have given us great insight into homeostatic proliferation but focus primarily on Ags that are of a foreign origin. Perhaps using a self- or tumor-antigen might provide a setting that is more analogous to the human condition. A recently described tumor model, Pmel-1, which uses a transgenic CD8⁺ T-cell specific for both the native and altered peptide ligand of the melanocyte and melanoma

differentiation molecule gp100, might enable us to better model unmutated self-antigens in the clinical setting [5,21].

Evidence for the presence of homeostatic cellular cytokine 'sinks'

The proliferation of adoptively transferred T cells in lymphopenic hosts can be reduced in a dose-dependent manner, either by increasing the total number of Ag-specific cells transfused or by co-transferring an 'irrelevant' population of T cells [9,15,16,22]. Host CD8⁺ T cells have a dominant role in modulating both donor CD8⁺ and CD4⁺ T-cell expansion in lymphopenic hosts [16]. In the same study, host CD4⁺ cells inhibited donor CD4⁺, and to a lesser extent CD8⁺ T-cell proliferation.

The ability of the host to inhibit the proliferation of adoptively transferred T cells could be the result of physical contact between T cells or competition between T cells for self-MHC-peptide complexes and supportive homeostatic cytokines (Figure 2). Naive T-cell proliferation is a result of self-MHC-peptide interactions in a lymphopenic environment (reviewed in Ref. [23]), whereas memory T cells proliferate independently of these interactions [12,16]. This is suggestive of Ag-independent activation, a notion that has recently received a great deal of attention. Some studies have focused on the role of host-derived IL-7 and IL-15, and more recently IL-21, γ C cytokines known to stimulate and induce the proliferation of mature T cells [24–26].

In transgenic mice that overexpress IL-7 or IL-15, there is a substantial increase in the absolute number of T cells [27,28]. This increase is driven largely by the expansion of CD8⁺ CD44^{high} memory T cells. Exogenous administration of IL-15 or induction of host expression of IL-15 in wildtype mice by reagents, such as poly I:C, lipopolysaccharide (LPS) and type I-IFNs, causes a selective increase in the proliferation of CD8⁺ CD44^{high} T cells *in vivo* [29–31]. Exogenous administration of IL-7 to mice enhances T-cell number and function [32,33] and, in both intact and immunodeficient non-human primates, causes a considerable yet reversible increase in the circulating levels of naive and Ag-experienced CD4⁺ and CD8⁺

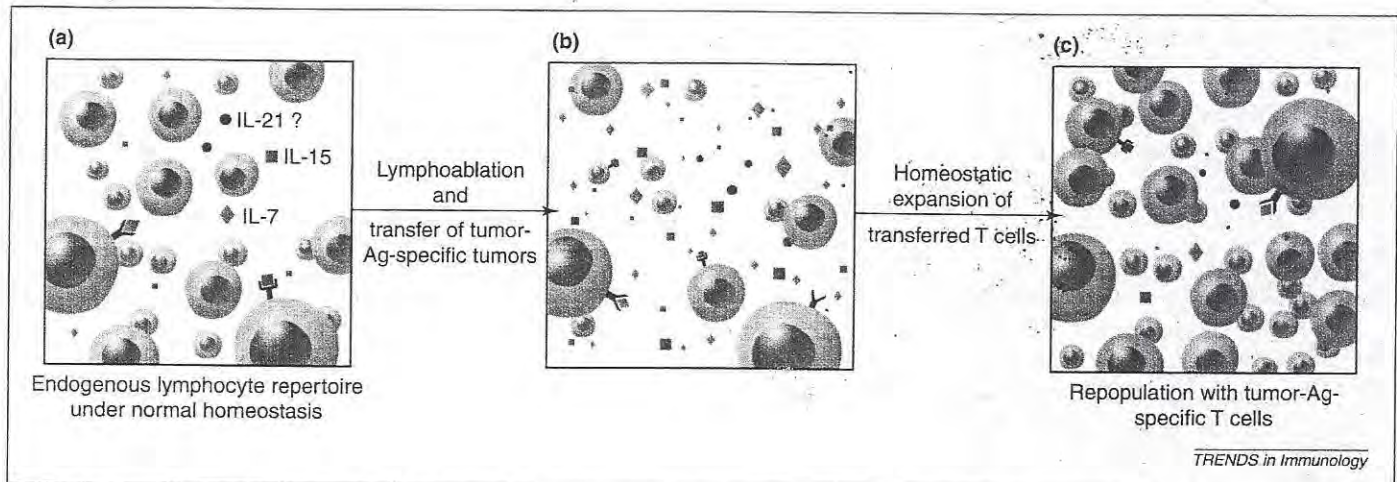


Figure 2. Removal of consumptive cytokine 'sink' by lymphoablation. (a) Under normal homeostasis, a smaller quantity of cytokines [IL-7 (yellow), IL-15 (green) and possibly IL-21 (red)] are available owing to consumption by the endogenous lymphocyte population. (b) After lymphodepletion preconditioning by chemotherapy or irradiation, the consumptive cellular cytokine 'sinks' are removed, enabling the adoptively transferred T cells a less competitive environment and greater access to IL-7, IL-15 and IL-21. (c) This is followed by preferential homeostatic expansion and re-population of the peripheral lymphoid compartments with the adoptively transferred, tumor-reactive T cells.

T cells [34]. Although similar studies have yet to be conducted in humans, there is evidence to support an inverse correlation between serum levels of IL-7 and the severity of lymphopenia caused by conditions, such as AIDS and cytotoxic drug therapy (reviewed in Ref. [35]). Currently, there are no data available that evaluate the *in vivo* effects of IL-15 in primates or humans.

Studies that examine mice deficient in IL-7 or IL-15 have demonstrated that these cytokines have a supportive role in the survival and/or proliferation of adoptively transferred T cells in lymphodepleted hosts [36–40]. The absence of IL-7 inhibits naïve CD4⁺ and CD8⁺ T-cell homeostatic proliferation and survival in a lymphopenic environment. The proliferation of donor cells in these IL-7^{-/-} hosts is rescued by the administration of exogenous IL-7 [36,41]. IL-7 has a significant role in naïve T-cell homeostatic proliferation but has little impact on memory T-cell expansion and survival in a lymphopenic host [37,38]. In contrast to IL-7, IL-15 does not contribute to naïve T-cell homeostasis but does have a pivotal role in memory CD8⁺ T-cell proliferation and durability [38]. The homeostatic expansion of memory CD8⁺ T cells does not rely solely on IL-7, however, these cells do constitutively express high levels of both IL-7R α (CD127) and the components of the IL-15–IL-2 heterodimeric complex, IL-2–IL-15R β (CD122) and γ_c (CD132), and thus might be sensitive to IL-7 [30,38,42].

In summary, it is clear that cytokines can have a profound impact in regulating the homeostasis of T cells in

Box 1. Unresolved questions regarding the use of γ_c cytokines

- Does the absence of endogenous IL-15 impair the early or late *in vivo* proliferation, functionality, and maintenance of adoptively transferred tumor-specific T cells, and thus their ability to mediate tumor destruction?
- What influence does host-derived IL-7 alone, or in combination with IL-15, have on tumor-reactive T cells and their function?
- What is the role of IL-21 in homeostatic proliferation, and does exogenous administration improve the ability of adoptive transferred tumor-specific T cells to kill tumor?

both the normal and lymphopenic settings. The expression of IL-7 and IL-15 receptors on memory T cells might indicate that the administration of these cytokines could enhance both the survival and proliferation of adoptively transferred, tumor-reactive T cells *in vivo*. In a recently described murine tumor model [5], it appears that the long-term tumor treatment efficacy of adoptively transferred, tumor-specific CD8⁺ T cells is compromised in whole-body irradiated IL-15^{-/-} but not wildtype hosts [43]. Endogenously produced IL-15 might contribute to a sustained antitumor treatment response by adoptively transferred CD8⁺ T cells in a lymphopenic setting. It is likely that non-tumor-specific T cells and other immune cells, such as natural killer (NK) cells, consume IL-15 in the wildtype host. Thus, there could be competition for homeostatic cytokines in the replete host. It is also important to mention the newly discovered cytokine IL-21, which shares homology with IL-2 and IL-15 and binds their common γ_c receptor [44,45]. This cytokine might augment the function of adoptively transferred tumor-specific CD8⁺ T cells [17,46] and is the subject of ongoing investigations. Many unresolved questions regarding the use of γ_c receptor cytokines remain to be answered (Box 1).

Role of regulatory cells in antitumor immunity

Naturally occurring and induced CD4⁺CD25⁺ Treg cells can potently suppress immune responses to self-Ags and foreign Ags in both humans and mice. The phenotype and function of Treg cells have been reviewed in detail elsewhere [47,48]. There are some features of Treg cells that might be of particular interest to tumor immunologists. CD4⁺CD25⁺ Treg cells express high levels of cell-surface molecules typically associated with activation; these include CD25 (IL-2R α), glucocorticoid-induced tumor necrosis factor (TNF)-receptor (GITR) and cytotoxic T lymphocyte-associated Ag-4 (CTLA-4). In addition, they also express a unique intracellular protein, *Foxp3*. Treg cells are completely absent in mice that are deficient in the genes encoding IL-2, IL-2R α , IL-2R β and *Foxp3*, suggesting a crucial role for both IL-2 signaling and *Foxp3*

expression in Treg-cell ontogeny and survival [49–51]. The role of Treg cells in maintaining immunological tolerance to self-Ags has been illuminated, in part, through adoptive transfer experiments. A spectrum of tissue-specific autoimmune diseases, including thyroiditis, oophoritis, gastritis and inflammatory bowel disease, occurs when immunodeficient mice are transfused with T cells depleted of CD4⁺CD25⁺ cells [52,53]. The concomitant transfer of Treg cells abrogates development of these autoimmune diseases. In some models, transfer of Treg cells after the onset of disease can even be curative [54]. The close correlation between autoimmunity and tumor immunity [5,7,55–59] suggests that Treg cells have a crucial role in T-cell tolerance to tumor [60–62].

Although the significance of Treg cells in human cancers has only recently begun to be explored, many of the findings in mice could be recapitulated in humans [61,63,64]. Wang *et al.* recently reported on the isolation of CD4⁺CD25⁺ TIL clones derived from a melanoma patient [65]. These clones displayed many of the phenotypic and functional properties associated with naturally occurring Treg cells and were antigen-specific. Another report has shown that Treg cells present in the circulation of patients vaccinated against melanoma Ags can suppress the proliferation of a polyclonal population of CD4⁺CD25⁻ T cells [66], however, the Ag-specificity of these cells was not determined. Treg cells capable of suppressing the *in vitro* function of tumor-reactive T cells in humans has also been found in other tumors besides melanoma [67,68]. In one instance, tumor deposits taken from patients with lung cancer reportedly contained large numbers of CD4⁺CD25⁺ Treg cells capable of suppressing the proliferation of autologous TILs [68]. In contrast to these murine studies, no conclusive data link the *in vivo* function of tumor-reactive Treg cells and the progression of cancer in humans, although some recent evidence suggests that Treg tumor infiltration might be associated with survival of cancer patients [69]. However, removal of these regulatory elements (Box 2), perhaps in conjunction with cytokine sinks, could reverse or prevent the tolerance of adoptively transferred tumor-reactive T cells (Figure 3).

Effects of lymphodepleting radiation and chemotherapy on APC function

Lymphodepletion before ACT uses total body irradiation (TBI) or cytotoxic drugs. Although these modalities were initially intended to deplete the lymphoid compartment of recipients, they can also facilitate the presentation of tumor antigens by triggering tumor cell death and antigen release. Subsequently, these antigens can be taken up and presented by APCs to enhance the activation of the adoptively transferred tumor-reactive cells [70].

Indirect evidence indicates that there might be other beneficial effects of lymphodepletion in our current ACT model. Irradiation and/or chemotherapy leads to activation of host cells, resulting in the release of pro-inflammatory cytokines, such as TNF- α , IL-1 and IL-4 [71–74], and upregulation of co-stimulatory molecules, such as CD80 [75]. In a recent study, activation markers I-A^b (class II) and CD86 were upregulated on splenic DCs as early as 6 h after TBI. Furthermore, *ex vivo* IL-12

Box 2. Eradication of Tregs

Although Food and Drug Administration-approved drugs are available that can selectively deplete CD25-expressing cells in humans, including humanized anti-Tac (anti-CD25) and ONTAK™ (IL-2 conjugated to diphtheria toxin), it is currently unknown what effect these reagents might have on Treg cells as well as on activated tumor-reactive T cells.

Until a unique cell surface lineage marker capable of discriminating between Treg and activated T cells can be discovered, non-specific lymphodepletion might be the only practical approach to removing Treg cells from patients for the purpose of augmenting their *in vivo* immune reactivity to a tumor.

production was significantly higher from DCs isolated 6 h after irradiation than DCs from non-irradiated mice [76]. Consistent with this finding, serum levels of IL-12 were also increased in these mice. Elimination of competitive T cells through lymphodepletion might improve donor T-cell access to, and activation by, antigen-bearing APCs [77], although other workers have found that competition might not play an influential role [78].

In addition to enhanced APC function and availability, the preconditioning regimen can damage the integrity of mucosal barriers through radiation-induced apoptosis of the cells lining these organs. Damage inflicted on the intestinal tract might permit the translocation of bacterial products, such as LPS, into the systemic circulation [79]. LPS activates T cells *in vivo* [31] and might enhance the antitumor response. Thus, proinflammatory cytokines and microbial products provide crucial 'danger signals' for the activation and maturation of DCs, thus enhancing T cell-mediated tumor treatment.

Although initially beneficial, lymphodepletion can depress the absolute number of host APCs. As reported in several murine studies, the total number of monocytes, macrophages and DCs are only slightly reduced at 6 and 24 h but are significantly reduced 5 days after TBI [71,76]. This could be detrimental in the case of the current tumor therapy model, which requires an active vaccination for successful tumor therapy [5]. Interestingly, late clearance did not hinder the activation of the donor T cells [76]. This corroborates with several reports that demonstrate antigenic stimulation, before APC clearance, is all that is required to initiate activation and proliferation of T cells [80,81].

Therapeutic implications and future directions

It is clear that lymphodepletion before adoptive transfer of tumor-reactive T cells into animals and humans with cancer augments *in vivo* function of the transferred cells and the therapeutic outcome. Increased access to the homeostatic cytokines, such as IL-7 and IL-15, through elimination of cytokine sinks, eradication of the suppressive influence of Treg cells and enhancement of APC activation and availability appear to be the underlying mechanisms involved in this paradigm (Figure 4). The mechanisms are complex, however, elucidation through the use of targeted cytokine administration, add-back of sink or suppressor elements and selective deletion or addition of APCs might explain how these mechanisms interact.

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