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CD4⁺CD25⁺ T Regulatory Cells, Immunotherapy of Cancer, and Interleukin-2

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Abstract

Summary: CD4⁺CD25⁺ T regulatory (T_{reg}) cells control immunologic tolerance to self-antigens and play a role in suppressing antitumor immune responses, but the mechanism of suppression in vivo remains uncertain. Recently, signaling through the high-affinity interleukin-2 (IL-2) receptor has been shown to be critical for T_{reg} cell differentiation and survival in vivo. Mice deficient in IL-2 or its receptor (CD25 or CD122) or deficient in downstream signaling molecules, including JAK-3 and STAT-5, do not develop a stable population of T_{reg} cells and subsequently acquire lymphoproliferative disease and autoimmunity. In vitro, IL-2 is required to expand T_{reg} cells and to induce their suppressive characteristics. Conversely, IL-2-based regimens can activate cellular antitumor immunity and are the mainstay of immunotherapies directed against melanoma and kidney cancers. Given the seemingly disparate effects of IL-2, the authors discuss the possibility that IL-2 may not be the optimal T-cell growth factor in vivo, but rather an inducer of self-tolerance. The authors propose that other γ_c -signaling cytokines, including IL-15, may be alternative choices for the immunotherapy of cancer.

Keywords

interleukin-2; T regulatory cell; T helper cell; immunotherapy; melanoma; autoimmunity; IL-2 receptor; interleukin-15

Historically, interleukin-2 (IL-2) was known as a “T-cell growth factor.” IL-2 was originally isolated from T-cell culture supernatants and was shown to expand and support the growth of T cells in vitro.¹ The role of IL-2 as a growth factor stemmed initially from in vitro work, but its role in vivo remains largely unknown. Paradoxically, mice engineered to lack the *Il2* or *Il2ra* gene or a single human patient with a truncation mutation of the IL-2R α chain² developed fatal lymphoproliferative disease. It was argued that T cells proliferated because of lack of apoptosis-induced cell death. However, it is now accepted that the autoimmunity seen in these genotypes is not cell autonomous but is due to a deficiency in naturally occurring T regulatory (T_{reg}) cells.³

It is well established that naturally occurring T_{reg} cells control immunologic tolerance to self-antigens and represent 5% to 10% of the CD4⁺ T cells in rodents and 5% to 15% in humans.^{4–9} T_{reg} cells constitutively express high levels of the cell surface molecules CD25 (IL-2R α), GITR (glucocorticoid-induced TNF receptor), and CTLA-4. They also express a unique intracellular protein, *Foxp3*, which acts as a master regulator gene for inducing a T_{reg} phenotype and lineage. T_{reg} cells are either abnormally regulated or nonexistent in mice with genotypes

that are *Il2*^{-/-}, *Il2ra*^{-/-}, *Il2rb*^{-/-}, *stat5*^{-/-}, *jak3*^{-/-} and *foxp3*^{-/-}, which all develop some form of fatal lymphoproliferative disease, suggesting a critical role for IL-2 signaling and Foxp3 in the development of self-tolerance mediated through T_{reg} cells.¹⁰⁻¹³ Interestingly, IL-2 signaling is not related to the expression of Foxp3 in T_{reg} cells,¹⁴ but Foxp3 can negatively regulate the *Il2* gene by blocking an NFAT (nuclear factor of activated T cells) binding site in the IL-2 promoter.^{15,16}

CURRENT THINKING ABOUT T_{REG} CELLS AND IL-2

Compared with the decades-old notion that IL-2 is a growth factor for effector T cells, the role of IL-2 in maintaining T_{reg} cells in vivo is a relatively new concept.¹⁷ Recent findings have helped define a critical role for IL-2 in the development of tolerance rather than immunity.¹⁸ IL-2^{-/-} and IL-2Rα^{-/-} mice succumb not to lymphopenia but to lymphoproliferation and autoimmunity (Fig. 1A, B, lower-left inset), and injection of IL-2 at birth into IL-2^{-/-} mice protects them from disease.³ Autoimmunity in IL-2^{-/-} mice is mediated by the adaptive immune response, because it can be eliminated when IL-2^{-/-} mice are crossed with RAG-1^{-/-} mice.¹⁹ In addition, we have recently generated transgenic mice that express IL-2 constitutively. These mice are surprisingly healthy, with no signs of an overreactive immune system (personal communication, C. Hinrichs).

Adoptive transfer of T_{reg} cells into mice that are incapable of producing the IL-2 cytokine (IL-2^{-/-} mice) leads to unrecoverable levels of T_{reg} cells.³ However, transfer of T_{reg} cells into mice incapable of expressing the IL-2 receptor α- or β-subunits (IL-2Rα^{-/-} or IL-2Rβ^{-/-} mice) results in survival of T_{reg} cells and suppression of autoimmune disease.¹⁰ This is not surprising, as the main source of IL-2 comes from CD4⁺ T cells, although dendritic cells have been reported to express IL-2 transiently.²⁰ Therefore, IL-2 supplied by CD4⁺ T cells supports the engraftment and maintenance of T_{reg} cells in the periphery.

T_{reg} cells are capable of arising from an IL-2^{-/-} genotype if they are supplied with an endogenous source of IL-2. A 50/50 mixed-bone marrow chimera of IL-2^{-/-} and CD25^{-/-} T-cell-depleted bone marrow transferred into lethally irradiated hosts leads to a stable engraftment of a CD4⁺CD25⁺ T_{reg} population that suppresses the autoimmune disease that arose if either cell type were grafted separately.³ Subsequently, CD4⁺ T regulatory cells, which arise from an IL-2^{-/-} lineage, suppress both the IL-2^{-/-} and CD25^{-/-} T cells that alone caused fatal autoimmune disease (see Fig 1C), showing that lymphoproliferation is controlled through a cell non-autonomous pathway. However, a recent paper challenges this idea.²¹ Transgenic expression of CTLA-4 on CD4⁺CD25⁻ T cells in IL-2^{-/-} mice can also rescue the mice from fatal lymphoproliferation but does not restore normal T_{reg} cell numbers or prevent inflammatory bowel disease.²¹ In mice that do not overexpress CTLA-4 as the result of a transgene, IL-2 signaling is required to maintain CTLA-4 expression on conventional T cells.²² Thus, IL-2^{-/-} mice may manifest autoimmunity that may in part result from the loss of CTLA-4 expression and not solely from lack of T_{reg} cells. Therefore, in IL-2^{-/-} mice, autoimmunity may be induced through loss of T_{reg} cells and the loss of the ability of helper T cells (that start off as CD25⁻) to express CTLA-4.

IL-2 signaling through the heterotrimeric IL-2Rαβγ_c complex is critical for T_{reg} function in vivo. In a model of experimental autoimmune encephalitis (EAE), adoptive transfer of whole CD4⁺ T cells from IL-2^{-/-} mice, but not CD4⁺ T cells from CD25^{-/-} mice, into EAE susceptible recipients could prevent autoimmune disease.¹³ This interesting experiment showed that a population of regulatory cells was present in IL-2^{-/-} mice but not CD25^{-/-} mice, even though neither genotype expresses a stable population of T_{reg} cells (see Fig. 1A, B). The author suggested that T_{reg} precursors or recent thymic emigrants existed in the circulation of CD4⁺ T cells from IL-2^{-/-} mice but not in CD25^{-/-} mice because signaling through the IL-2R in the

thymus is required for T_{reg} cell development (see Fig. 1B). Signaling through the IL-2R on T_{reg} cells results in a unique signaling pattern that increases their survival rather than expanding their numbers.^{23,24} This is not surprising in light of the fact that T_{reg} cells are the only cells that constitutively express the high-affinity IL-2R trimeric complex.

IL-2 is also critical for activation of the suppressor function of T_{reg} cells in vitro, but addition of significant amounts of IL-2 to cultures can mask their suppressive function. That said, T_{reg} cells could still inhibit IL-2 production at the mRNA level in responder T cells, even in the presence of IL-2.²⁵ Furthermore, T_{reg} cells may compete with T_{helper} cells for IL-2 (Fig. 2A, B). This is a reasonable idea because the trimeric form of the IL-2R receptor has a 100-fold higher affinity for IL-2 than the dimeric form (IL-2Rβγ) (see Fig. 2C), despite clear evidence that both receptors signal exclusively through the dimeric IL-2Rβγ form. Thus, the IL-2Rα chain controls the sensitivity of how T cells respond to IL-2.

This basic physical property was confirmed in a recent study that showed that secretion of IL-2 from the responder T cell (T_{responder}) led to an upregulation of IL-2Rα expression on T_{reg} cells and a lowering of IL-2R on responder T cells.²⁶ Inhibition of IL-2 from binding to T_{reg} cells showed that there was a downregulation of IL-2R, confirming not only that IL-2 upregulates its own receptor,²⁷ but also that IL-2 from T_{responders} helps regulate IL-2R on T_{reg} cells. We (manuscript in preparation) and others have confirmed that in vivo CD4⁺CD25⁻ T cells secrete IL-2, which regulates and maintains CD25⁺ expression on T_{reg} cells.¹⁴ Thus, CD4⁺CD25⁻ T cells could regulate T_{reg} cells through a yin/yang type relationship, with one depending on the other for immunologic balance (Fig. 3).

Some authors have suggested that the sole mechanism of suppression was competition for IL-2. This is an attractive idea, but the true test of this hypothesis would be to test whether T_{reg} cells could suppress IL-2^{-/-} responders or whether CD25^{-/-} T cells are still capable of helping CD8⁺ T-cell responses in vivo. Evidence from our laboratory suggests that T-helper cells lacking the high-affinity IL-2Rα chain (CD25^{-/-} T_{helper} cells) are just as efficient as normal WT T_{helper} cells in vivo at triggering antitumor CD8⁺ T cells to mediate the destruction of large, established tumors (manuscript in preparation). Therefore, suppression of T_{responder} cells is likely to be complex, involving IL-2 sequestration from CD8⁺ T cells, suppression of IL-2 production by CD4⁺ T cells, and direct cell–cell contact mediated suppression, which is not fully characterized at this time. IL-2 administration can trigger CD8⁺ antitumor T-cell responses in a subset of patients, which can be associated with long-term complete response. However, it seems feasible that IL-2 might also transiently mask suppression and support T_{reg} cell expansion in vivo in some or even most patients in the setting of immunotherapy for metastatic cancer. This needs to be further evaluated in patients receiving IL-2 therapy for cancer treatment.

HISTORY OF T_{REG} CELLS AND IMMUNOTHERAPY OF CANCER

The literature described the existence of a cellular barrier to the immunotherapy of cancer almost 30 years ago.^{28,29} This “barrier” reportedly could be overcome by γ-irradiation or by administration of a lymphodepleting drug called cyclophosphamide to tumor-bearing animals.^{30–32} Cyclophosphamide was shown to eliminate a population of L3T4⁺ T cells (now known as CD4⁺ T cells) and could enhance immunotherapy to cyclophosphamide-resistant lymphomas.^{29,33} Later it was determined that a population of radiosensitive T cells hindered immunotherapy of mice expressing established, immunogenic tumors. Through adoptive transfer experiments into T-cell-deficient tumor-bearing hosts, it was shown that splenocytes from tumor-bearing mice, before 9 days after tumor challenge, could cause the regression of established tumors, but splenocytes from tumor-bearing mice adoptively transferred after 9 days of tumor challenge abrogated the antitumor effects.^{32,34} These experiments showed that

either a suppressive phenotype was being “programmed” into the effector cells or a population of cells was being induced and adoptively transferred with the effector cells, which suppressed their function. Although the specific cells were never isolated, T-cell-mediated suppression of adoptive immunotherapy was shown to be specific for the tumor that induced them.³⁴ Eventually, the field of suppressor T cells faded due to the lack of reliable cell surface markers to study and isolate them.

Now, the emergence of a naturally occurring CD4⁺CD25⁺ T suppressor or regulatory cell (T_{reg}) as well as other regulatory cell populations (Tr1, Th3, CD45RB^{low}, NK T, GR-1⁺/CD11b⁺, and plasmacytoid dendritic cells)^{8,35,36} has rekindled the idea that regulatory T and dendritic cell populations could be inhibiting an effective antitumor immune response. Understanding how these populations function and suppress immune responses is paramount for understanding how to treat established tumors expressing self-antigens in an environment of persisting self-antigen.

IL-2 AND IMMUNOTHERAPY OF CANCER

Once IL-2 was discovered and cloned, it was quickly used in vitro to expand cells isolated from the peripheral blood of mice and humans, termed lymphokine-activated killer cells (LAK cells) that were administered in combination with IL-2 in vivo in the immunotherapy of cancer.³⁷ These experiments led to the development of IL-2-based regimens that are still used today, with some modifications, in melanoma^{38,39} and renal cell carcinoma.⁴⁰ In the past decade, therapy with IL-2 has been augmented with adoptive transfer of T cells isolated from the tumor. Cells isolated from tumor are called tumor-infiltrating lymphocytes (TILs)⁴¹ and are mostly CD8⁺ T cells that have reactivity against tumor-associated antigens ex vivo.³⁹ When TILs were transferred back into patients receiving IL-2 at the same time, responses were twice those seen with IL-2 alone and more than 10- to 100-fold better than LAK cells.⁴² Recently, this paradigm has been combined with vaccination^{43,44} in the treatment of established tumors in mice, and this therapy was significantly enhanced with sublethal total body irradiation or in CD4⁺ T-cell-deficient mice.^{44,45}

The immune-enhancing effects of IL-2 in vivo are thought to be due to its activating and expanding potential for T cells, as shown in vitro.⁴⁶ However, in vivo, the role of IL-2 is still largely unknown and may not always involve T-cell expansion.⁴⁷ As stated above, exogenous IL-2 can enhance immunotherapy of established tumors in mice and humans^{43,48} as well as help mediate expansion of transferred lymphocytes to a viral vaccine.⁴⁹ These examples offer insight into the role of IL-2 in vivo. In the case of melanoma, high doses of IL-2 are required, although “low-dose” IL-2 is effective in kidney cancer. It has been shown in vitro that IL-2 masks suppression,²⁵ and therefore the ephemeral antitumor response induced by IL-2 may be caused by the reversal of the suppressive state induced by T_{reg} cells (Fig. 4). It is interesting that patients who initially respond to IL-2 treatment or are pretreated with IL-2 become refractory to subsequent treatments with IL-2 (personal communication, S.A. Rosenberg, NCI). Therefore, conditions that remove T_{reg} cells or block their use of IL-2 may offer temporary relief from T_{reg}-mediated suppression when IL-2 is given alone or with adoptive transfer or vaccination.⁵⁰

T_{REG} CELLS, AUTOIMMUNITY, AND TUMOR IMMUNITY

The removal of T_{reg} cells from normal mice or transfer of T_{helper} cells into lymphopenic hosts can cause autoimmune diseases that are tissue-specific, including thyroiditis, oophoritis, gastritis, and inflammatory bowel disease.^{5,51,52} Adoptive transfer of T_{reg} cells prevents the development of disease and in some models can cure disease after its initiation.⁵³ Organ-specific autoimmunity can be enhanced by vaccination or through the provision of inflammatory signals when T_{reg} cells are absent.⁵² Depletion of T_{reg} cells with an antibody to

CD25 can also enhance tumor protection (ie, tumor given after treatment is initiated) to tumor-associated antigens that are expressed as self-antigens.^{54–56}

Autoimmunity and tumor immunity may be two sides of the same immune phenomenon, and indeed can accompany one another.^{38,43,57,58} Therefore, T_{reg} cells may play a critical role in the induction of tolerance to self-antigens, including those expressed by tumors. Because most tumor antigens are normal, nonmutated self-antigens,⁴⁸ it is conceivable that most tumors overexpressing self-antigens could induce tumor/self-specific T_{reg} cells that suppress an effective antitumor response.^{29,59,60} Strong evidence for this was found when depletion of CD25⁺ T cells and/or blocking CTLA-4 or GITR in vivo with antibodies could increase reactivity to known self-antigens, and enhance tumor rejection in mice.^{56,61–64} Although autoimmune destruction of self-antigen expressing tissues was readily seen, these reports did not always consider the effects that the antibodies have on CD4⁺ effector T cells.⁶⁵ These therapies revealed that it was possible to immunize against self-antigens. Most intriguing was the uncovering of enhanced immunity to several shared, tumor-rejection antigens.⁶³ Other costimulatory or “danger” signals, like signaling through Toll-like receptors (TLRs), may lead to a tipping of the balance in favor of an immune response against cancer antigens by reversing T_{reg} cell-mediated immunosuppression.^{55,66} Destructive autoimmunity beyond tumor treatment can occur^{58,67} but has been generally controllable in our hands.

Recently we published a tumor model where we described the necessary components required to treat large, vascularized, established melanoma. This highly aggressive mouse melanoma is amenable to treatment only by adoptive cell transfer of tumor/self-reactive CD8⁺ T cells (pmel-1), vaccination with a viral vaccine (either fowlpox or vaccinia virus) encoding a modified tumor/self-antigen, gp100, and exogenous administration of one or multiple γ_c -signaling cytokines: IL-2, IL-4, IL-7, IL-9, IL-15, or IL-21^{43,44} (and unpublished results). Transfer of 10⁷ pmel-1 T cells into tumor-bearing C57BL/6 mice led to complete cures. Transfer of 10⁶ pmel-1 T cells led to transient tumor regression, but this therapy was significantly enhanced in either sublethally irradiated or CD4⁺ T cell-deficient hosts.⁴⁵ Furthermore, adoptive cell transfer of naturally occurring CD4⁺ T cells depleted of CD4⁺CD25⁺ T_{reg} cells into tumor-bearing CD4⁺ T cell-deficient mice augmented and maintained the treatment of established tumors when co-administered with pmel-1 T cells and vaccination without the need for exogenous cytokine support.⁴⁵ These results suggested that CD4⁺ T cells were providing the essential cytokines or signals required to maintain stable antitumor immunity to a self-antigen in an environment of persisting self-antigen, but required the simultaneous absence of T_{reg} cells to be effective. Interestingly, T_{helper} cells derived from CD25^{-/-} mice, but not IL-2^{-/-} mice, generated antitumor immunity and autoimmunity in tumor-bearing CD4⁺ T cell-deficient mice⁴⁵ (manuscript in preparation). These results indicate a critical role for naturally occurring T_{reg} cells in depressing adoptive immunotherapy of established tumors but also establish a role for IL-2 in vivo for breaking tolerance to tumor/self-antigens: again, a yin/yang type relationship. Since IL-2 is also required to maintain T_{reg} cells, it is possible that autoreactive CD4⁺ T cells secrete IL-2, which maintains T_{reg} cells in the periphery. This circumstance, when T_{reg} cells are absent, leads to progressive autoimmunity (see Fig. 3).

Further evidence that self-antigen expressing tumors can activate T_{reg} cells was found in a murine tumor model using hemagglutinin (HA)-specific T_{reg} cells. T_{reg} cells from HA-TCR transgenic mice expressing HA as a self-antigen were isolated and transferred into a tumor-bearing host, whose tumor also expressed the HA antigen. The authors found that tumor-specific T_{reg} cells expressing an HA-TCR could upon adoptive transfer with HA-specific T_{helper} cells prevent tumor immunity.⁶⁸ What is intriguing about this finding is that T_{reg} cells with known specificity had been isolated. Although artificially generated in mice that expressed the HA antigen as a self-antigen, which forced the development of T_{reg} cells with the same

antigen-specific TCR, these studies, as well as other experiments by the same group and others, ^{69,70} showed that self-antigens induce the development of T_{reg} cells and tolerance to self-tissues. ⁷¹ Although mouse experiments using highly artificial systems can lead to spurious results, it is quite feasible that tumors overexpressing self-antigens may also induce T_{reg} cells with specificity for tumor antigens. However, whether T_{reg} cells use similar TCRs as effector CD4⁺ T cells is debatable. ⁷⁰

The importance of T_{reg} cells in human cancers has been revealed only in the past few years. ^{72–77} Recently a CD4⁺CD25⁺ TIL was discovered that was isolated from a patient's melanoma. ⁷⁵ This T_{reg} TIL clone suppressed other tumor-reactive TILs as well as a polyclonal CD4⁺ T-cell population in a dose-dependent fashion and displayed all the properties of naturally occurring CD4⁺CD25⁺ T_{reg} cells. The finding that the TIL recognized a tumor/self-antigen, LAGE-1, confirmed that CD4⁺CD25⁺ T cells recognize self-antigens and that tumors may induce their activation. More recently, T_{reg} cells were isolated from the ascites of patients with ovarian cancer. These cells, which were CD4⁺CD25⁺CCR4⁺GITR^{high}CTLA-4^{high}Foxp3⁺ cells, also suppressed other T cells and were reportedly recruited to tumor sites by the chemokine CCL22, which was predominantly expressed by ovarian tumors. ⁷³ It is thus possible that tumors may attract T_{reg} cells into their microenvironment to foster immune privilege. From our laboratory, studies showed that CD4⁺CD25⁺ T cells exist in the circulation of melanoma patients undergoing tumor/self-antigen immunization, ⁷² although their Foxp3 expression levels have not yet been determined.

In an earlier study, tumor deposits from lung cancer patients were reported to contain CD4⁺CD25⁺ T_{reg} cells that actively suppressed autologous TILs but not allogeneic TILs. ⁷⁸ This is a surprising finding, because in vitro it is known that naturally occurring CD4⁺CD25⁺ T_{reg} cells can suppress in a nonspecific fashion. ⁷⁹ Whether suppression is antigen-specific is debatable, but recently an in vivo model showed that antigen-specific T_{reg} cells suppressed only other T cells bearing the same antigen-specific TCR in vivo. ⁸⁰ This apparent antigen specificity may help explain how T_{reg} cells can discern self from non-self, allowing a productive antiviral response but keeping in check reactivity to self-tissues, and unfortunately to tumors.

MECHANISMS OF SUPPRESSION IN VIVO

In the tumor-bearing host, tumor-associated antigens (TAAs) could be taken up by tolerogenic dendritic cells present in the tumor microenvironment, such as GR1⁺/CD11b⁺, plasmacytoid (pDC), or IL-10-induced DCs. ^{36,81–85} pDCs have recently been shown to induce tolerance to an inhaled non-self-antigen. ³⁵ The allergen was taken up by pDCs, which presented the antigen to allergen-specific T cells that subsequently became T regulatory. This event may mimic activation of tumor-specific T_{reg} cells. These putative tumor-specific T_{reg} cells may then circulate throughout the body and respond to self-antigens shed by tumors in the tumor-draining lymph nodes. Subsequently, after activation, they may reside in the tumor-draining lymph node or infiltrate the tumor bed and suppress any precursor antitumor effector CD8⁺ T lymphocytes from destroying their self-target, either through cell–cell contact by inhibiting T-cell help and activation (eg, IL-2 deprivation or other mechanisms) or through release of immunosuppressive cytokines, such as IL-10 or TGF-β (see Fig. 4A).

Cytokines play an important role in T_{reg} cells' immunosuppressive function. The immunosuppressive state during Hodgkin lymphoma has been recently ascribed to T_{reg} cells (both naturally occurring CD4⁺CD25⁺ T_{reg} cells and induced Tr1 cells), which secrete IL-10. ⁷⁶ IL-10 plays a pivotal role in the immunosuppression of cancer and may be also induced by certain vaccinations to tumors. ^{83,85,86} Besides IL-10, TGF-β secreted by most melanomas ⁸⁷ could also be playing a critical role, as one of the suppressive mechanisms by

T_{reg} has been ascribed to this cytokine.^{88,89} Naive T_{helper} cells may develop a T_{reg}-like phenotype (Tr1) when exposed to TGF- β .^{90,91} This process, called infectious tolerance, may be one mechanism the immune system uses to expand T_{reg} cells and cause general immunosuppression.

Evidence that naturally occurring T_{reg} cells mediate suppression of CD8⁺ T cells was found in vitro.⁹² Although direct evidence in vivo has not been described in the literature, the level of T_{reg} cells inversely correlated with CD8⁺CD69^{high}CD44^{high} T cells (memory T cells) in vivo.⁹³ In another study, T_{reg} cells suppressed the expansion of co-transferred CD4⁺ T_{helper} cells, and this directly correlated with the ratio between the two populations.³ Evidence from our laboratory suggests that T_{reg} cells in vivo may be suppressing T-cell help of tumor-reactive T cells, thus preventing CD8⁺ T cells from being properly activated.⁴⁵

Tumor-specific TILs are expanded using anti-CD3 stimulation and high-dose IL-2 and then transferred into patients receiving exogenous IL-2.³⁸ A caveat to using this approach is that T_{reg} cells are known to undergo reversal of their suppressive state and expand with high-dose IL-2. T_{reg} cells also require IL-2 signaling for their function and development¹³ and most importantly induction of their suppressive characteristics.⁹⁴ Because it is likely that IL-2 therapy may be expanding T_{reg} cells in vivo (see Fig. 4B), the enhanced effects seen in patients rendered lymphopenic by nonmyeloablative conditioning³⁹ could be due to the preferential use of IL-2 by effector T cells in the absence of endogenous CD4⁺CD25⁺ T_{reg} cells (see Fig. 4C). Unpublished data from our laboratory using IL-2-overexpressing transgenic mice show an increase in CD4⁺CD25⁺ T cells. Since deprivation of IL-2 represents an attractive mechanism of suppression²⁶ (see Fig. 2), it would seem wise not to fuel the fire with exogenously provided IL-2 in patients with cancer.

METHODS TO CIRCUMVENT T_{REG} CELL-MEDIATED SUPPRESSION IN VIVO

So, how do we modify IL-2 therapy without compromising patients receiving immunotherapy? The identification of mutated self-antigens in human melanomas and other cancers^{48,95} may offer new strategies to circumvent this self-antigen-induced T_{reg} suppression. CD4⁺ T_{helper} cells could be generated to mutated self-antigens, which then could be used in adoptive immunotherapy protocols following nonmyeloablative conditioning along with tumor-reactive CD8⁺ TILs⁹⁶ alone or together with naturally occurring T_{helper} cells, which could be substituted if T cells against mutated antigens were unavailable.⁴⁵

Another approach already implemented in our laboratory and clinics is nonmyeloablative conditioning prior to adoptive immunotherapy in patients with advanced tumors. This represents an attractive modality for removing inhibitory T_{reg} cell subpopulations and consumptive cytokine sinks.^{38,39,97} Although there are selective drugs approved for depleting CD25⁺ T cells in humans, namely humanized anti-Tac⁹⁸ (anti-CD25) and ONTAK⁹⁹ (IL-2 conjugated to a diphtheria toxin), it is unknown what effect these treatments have on the T_{reg} population as well as activated tumor-reactive precursors. Depletion of CD25⁺-containing cells in mice can hinder adoptive cell transfer of activated antitumor CD8⁺CD25⁺ CTLs as well as any tumor-reactive CD4⁺ T cells, such as an activated T_{helper} cell that becomes CD4⁺CD25⁺.⁵⁶ Therefore, until a unique marker can be used to discriminate T_{reg} from activated T cells, total lymphodepletion may be the only practical method to remove T_{reg} cells (see Fig. 4C). This therapy, used in combination with cytokines, T helper cells, or complete ablation following stem cell transplantation, could theoretically augment tumor immunotherapy.⁴⁵

Recently, exogenous IL-15 was shown to replace exogenous IL-2 therapy during the treatment of established, nonmanipulated poorly immunogenic tumors.⁴⁴ Unpublished data from our

group show that any γ_c -signaling cytokine can help tumor-reactive CD8⁺ T cells treat established tumors, including IL-4, IL-7, IL-9, and IL-21. These findings are highly significant because most of these cytokines (IL-7, IL-9, IL-15, and possibly IL-21) do not affect T_{reg} growth and suppressive function in vitro⁹⁴ and have positive effects on the maintenance of CD8⁺ T cells and antitumor effects in vivo.^{100,101} Therefore, it is highly likely that TILs could be selectively expanded without generating or supporting CD4⁺CD25⁺ T_{reg} cells in vitro and in vivo by using a different γ_c -signaling cytokine during immunotherapy of cancer.

An alternative approach, which may go against conventional thinking, is to use anti-IL-2 therapy to decrease T_{reg} cell activity, since there are no antibodies that uniquely target T_{reg} cells, along with a cytokine growth factor that does not affect T_{reg} cells. The use of anti-IL-2 therapy has been shown to induce autoimmunity in mice (personal communication, S. Sakaguchi). Since IL-2 may be a major growth factor for T_{reg} cells, removing it and replacing it with a suitable factor that activates tumor-reactive T cells in combination with a lymphodepleting regimen may augment immunotherapy. We are studying this approach.

THE FUTURE OF IMMUNOTHERAPY: IL-2'S LAST STAND?

The main question now facing basic immunologists is the role of IL-2 in vivo. Is IL-2 required for expansion of CD8⁺ T cells, for CD4⁺ T cells, for T_{reg} cells, or for all of the above? What is the relationship between IL-2 and T_{reg} cells and suppression in vivo? It is fascinating that the role of IL-2 in vivo has not been elucidated,^{17,102,103} although its role as a T-cell growth factor has been quite diminished. Recent work by D'Souza et al showed that IL-2 in vivo is critical for sustaining the expansion of CD8⁺ T cells during activation,¹⁰⁴ but other groups have found no role for expansion at all.⁴⁷ Data from our laboratory suggest that IL-2 from T_{helper} cells is critical for the maintenance of tumor/self-reactive CD8⁺ T cells in an environment of persisting self-antigen.⁴⁵ However, help of CD8⁺ T cells could also involve other γ_c -signaling cytokines as well as other events that happen downstream from IL-2 signaling. We believe that IL-2 secreted by CD4⁺ T cells may be important in initiating autoimmunity, which is mediated through autoreactive cytotoxic CD8⁺ T cells. However, how it is maintained is being investigated.

Recently, in a diabetes mouse model, CD40L^{-/-} T_{helper} cells were shown to be inefficient in causing disease, but provision of anti-CD40 antibody could rescue this effect when CD40L^{-/-} T_{helper} cells were simultaneously present, but not when used alone.¹⁰⁵ These data show that other mediators are contributing to help autoreactive T cells as well, which may include (but are not limited to) IL-15 from a licensed APC, IL-21, and other signals and/or molecules such as 41BB, OX40, or CD28-B7-1/B7-2.

In summary, although IL-2 has shown promise in the immunotherapy of cancer, its use in vivo may be hampered by its potential to expand T_{reg} cells. Other γ_c -signaling cytokines such as IL-7, IL-15, and IL-21 in combination with anti-IL-2 therapy may offer alternative choices for expanding and activating TILs in vivo. Possibilities for effective cancer immunotherapies are expanding as the knowledge of the immune system evolves. Now that cancer antigens have been identified, harnessing the immunostimulatory properties of the immune system and reversing the immunoregulatory mechanisms against tumors in an environment of persisting self-antigens is now the main focus.

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FIGURE 1.

Phenotype of IL-2^{-/-} mice, CD25^{-/-} mice, and mixed bone marrow chimeras. A, IL-2^{-/-} mice develop fatal lymphoproliferative disease as a result of a paucity of naturally occurring T_{reg} cells due to a lack of IL-2 from CD4⁺CD25⁻ T cells in the periphery. Adoptive transfer of T_{reg} cells into IL-2^{-/-} mice leads to unrecoverable levels of T_{reg}. B, CD25^{-/-} mice also develop fatal lymphoproliferative disease as a result of a paucity of naturally occurring T_{reg} cells in the periphery, which is due to the lack of IL-2 signaling either in the thymus or the periphery. However, adoptive transfer of T_{reg} cells into CD25^{-/-} mice leads to recoverable levels of T_{reg} cells, presumably because CD25^{-/-} T cells can still make IL-2. C, Two wrongs make a right: mixed bone marrow chimeras of IL-2^{-/-} and CD25^{-/-} bone marrow lead to a stable engraftment of CD4⁺CD25⁺ T_{reg} cells (which are IL-2^{-/-} in phenotype) in the periphery due to IL-2 being provided by CD25^{-/-} T_{helper} cells. CD25^{-/-} T cells destined to be T_{reg} cells die. Subsequently, IL-2^{-/-} T_{reg} cells suppress both IL-2^{-/-} and CD25^{-/-} T_{helper} cells through an as-yet-undefined mechanism.

FIGURE 2.

T_h cells provide IL-2 to T_{reg} cells in the periphery. A, T_{reg} cells suppress CD4⁺ and CD8⁺ T cells and dendritic cells in the periphery through undefined mechanisms. B, T_{reg} cells may compete for IL-2 as a growth factor. T_h cells provide IL-2 that has a 100-fold higher affinity for its trimeric receptor on T_{reg} cells than its dimeric form on T-helper cells. C, Affinities (k_d) of IL-2 receptor complexes for IL-2.

FIGURE 3.

IL-2 maintains self-tolerance through T_{reg} cells. A yin/yang type relationship exists between T_{helper} cells and T_{reg} cells in vivo. T_{helper} cells provide IL-2 that is critical for the survival and function of naturally occurring T_{reg} cells in vivo. T_{reg} cells suppress autoreactive or foreign reactive T_{helper} cells, depending on the situation. If either one does not exist, an imbalance ensues. Autoimmunity develops if T_{reg} cells are absent or T_{reg} cells lose IL-2R and eventually die without IL-2 support when T_{helper} cells are absent.

FIGURE 4.

Status of the immune system during normal homeostasis, IL-2 therapy, or nonmyeloablative conditioning. A, During normal homeostasis, CD4⁺CD25⁺ T_{reg} cells (red) suppress CD4⁺ T_{helper} cells (green), CD8⁺ T cells (blue), and dendritic cells (pale red). CCR4⁺ T_{reg} cells are recruited to the tumor by chemokines, such as CCL22, and may suppress tumor-reactive T cells through TGF-β, IL-10, or other undefined mechanisms such as IL-2 deprivation, cell–cell contact, and so forth. B, Exogenous IL-2 therapy may expand T_{reg} cells, although it helps initially in a few patients (some tumor destruction). C, Nonmyeloablative conditioning (lymphodepletion) with cyclophosphamide and fludarabine depletes all endogenous T cells, including T_{reg} cells, and allows transferred tumor-reactive T cells (TILs) to be activated and kill tumor. Access by tumor-reactive T cells to homeostatic cytokines such as IL-7 and IL-15 in addition to exogenous IL-2 is improved and enhances CTL activity against tumor.