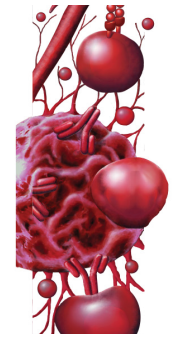
Editorial

Immunotherapy



Manipulating IL-2 and IL-2R in autoimmune diseases and transplantation

"...manipulating IL-2 and its receptor can dramatically shift the balance between Tregs and effector T cells and change the course of immune-mediated diseases."

Keywords: autoimmunity • CD25 • graft-versus-host disease • IL-2 • immunotherapy • regulatory T cell • transplantation

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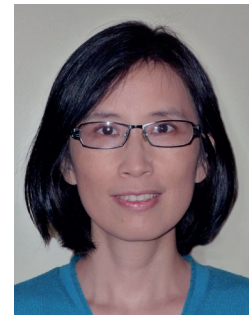

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An imbalance between Tregs and effector T cells (Teffs) is an underlying cause of many autoimmune diseases. IL-2 signaling is the crux of this delicate balance [1]. IL-2 was initially discovered as a growth factor for T cells and essential for Teff differentiation. The IL-2R consists of three chains, IL-2Rα (CD25), IL-2Rβ and a common γ chain shared with other cytokines. The αβγ trimer forms the high affinity IL-2R whereas the βγ dimer forms the intermediate-affinity IL-2R. The βγ dimer is broadly expressed on T cells and NK cells constitutively and CD25 is induced upon activation. Surprisingly, knocking out IL-2 or CD25 in mice led to systemic lymphoproliferation and early lethality from autoimmune attacks, demonstrating that the indispensible function of IL-2 and IL-2R is the maintenance of immune tolerance, not immune activation. This phenomenon is explained by the later discoveries that IL-2 promotes activation-induced cell death of Teffs and is essential for the survival and function of Tregs. One of the hallmarks of Tregs is their constitutive expression of CD25 and high sensitivity to IL-2. Moreover, Tregs are unable to make IL-2 and completely dependent on exogenous source of IL-2 from activated conventional T cells and dendritic cells. Thus manipulating IL-2 and its receptor can dramatically shift the balance between Tregs and Teffs and change the course of immune-mediated diseases.

IL-2 binding to its receptor brings together the β and γ chains and the associated Janus-activated kinases, Jak1 and Jak3, leading to their activation by transphosphorylation. These Jaks then initiate three streams of signaling that include PI3K, STAT5, and MAPK [2]. The PI3K pathway activates a downstream kinase Akt that is a hub for coordinating anabolic activities and cellular growth, whereas STAT5 and MAPK mainly mediate transcriptional activation and cellular differentiation. CD25 does not have signal transducing functions, but its binding to IL-2 alters the conformation of IL-2 such that affinity of IL-2 to the βγ dimers increases 100-fold, allowing cells to respond to much lower concentration of IL-2.

IL-2 signaling is quantitatively and qualitatively distinct between Tregs and other T cells. First, Tregs are highly sensitive to IL-2 due to their constitutive expression of CD25. Second, Tregs express high level of PTEN that antagonize the PI3K–Akt pathway; thus they preferentially signal through STAT5 [3]. Loss of PTEN or defect in PI3K signaling each leads to impaired Treg function, demonstrating that this pathway needs to be tightly controlled to ensure Treg stability and proper function [4,5]. Third, IL-2 signaling in Teffs, but not Tregs, is constrained intracellularly by inhibitory phosphorylation of IL-2Rβ, Jak3 and STAT5, making Teffs intrinsically less responsive to IL-2 even when they express CD25 [6,7]. Last, CD25 is cleaved from the cell surface after T-cell activation, limiting the duration of high-affinity IL-2 signaling. This effect is more pronounced on Teffs than on Tregs. The released soluble CD25 (sCD25) has low affinity for IL-2 and can serve to

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sequester IL-2 or potentiate IL-2 signaling [8,9]. The impact of sCD25 is likely to be stronger on $CD25^-$ T cells than on Tregs. Overall, IL-2 signaling is under tight temporal and cell-specific regulations to permit controlled immune activation and to function as a negative feedback to prevent immunopathology.

IL-2 was approved by the US FDA as an immunotherapy for metastatic renal cell carcinoma in 1992 and the therapy has been evaluated in various cancers. High-dose of IL-2 was needed to elicit a clinical response, but also resulted in severe dose-limiting toxicities [10]. The realization that IL-2 function at low dose to support Treg stability and function provides new therapeutic perspectives for the use of IL-2 in autoimmunity [11]. In a healthy immune system, activated $CD4^+$ T cells are the main source of IL-2. In chronic autoimmune and inflammatory diseases, differentiated Th1 and Th17 Teffs have low capacity to produce IL-2. The scarcity of IL-2 is further exacerbated by competition from activated $CD8^+$ T cells and NK cells and accumulation of sCD25, all contributing to Treg imbalance and disease progression. In graft-versus-host disease (GVHD) after hematopoietic stem cell transplantation, lymphopenia leads to IL-2 deficiency and an excess of IL-7. Expression of IL-7 on Tregs is much lower than that on naive and memory T cells. Thus, this cytokine imbalance contributes to Treg and Teff imbalance and development of GVHD [12]. Low-dose IL-2 therapy in these settings can potentially break the vicious cycle and restore immune homeostasis. The high abundance of Teffs in these settings also makes the use of IL-2 risky of inciting disease flare. Indeed, in a mouse model of Type 1 diabetes, low-dose IL-2 therapy is effective at inducing long-term disease remission, whereas high-dose IL-2 precipitates disease onset in a few days in prediabetic animals [13]. Safely translating this therapy to humans that are heterogeneous in their immune composition would require careful dose selection and the use of sensitive biomarkers to monitor disease and immune status.

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Several clinical reports have been published on the use of low-dose IL-2 in GVHD, HCV vaculitis and Type 1 diabetes [14–16]. The therapy showed promising efficacy in alleviating symptoms of chronic GVHD and vasculitis without toxicity associated with high-dose IL-2. Low-dose IL-2 therapy selectively increased Tregs with minimal effects on other T cells in GVHD patients [12] and increased basal STAT5 phosphorylation and CD25, GITR, CTLA-4 expression on Tregs in patients with Type 1 diabetes [17]. A recent study quantitatively measured IL-2 responsiveness of various T cell subsets and NK cells. The study found no difference between healthy subjects and patients with Type 1 diabetes in that Tregs were most responsive to IL-2 followed by $CD56^+$ NK cells, memory $CD8^+$ T cells, memory $CD4^+$ T cells and naive T cells [7]. Innate lymphocyte ILC2 constitutively express CD25 and can respond to IL-2 by secreting IL-5 and drive eosinophil expansion [18]. Thus monitoring NK and eosinophil responses is helpful in determining the selectivity of a particular regimen for Tregs. In this regard, a trial using a combination of low-dose IL-2 and rapamycin in patients recently diagnosed with Type 1 diabetes found increased Tregs, NK cells, eosinophils, but not $CD8^+$ T cells. Alarmingly, all patients experienced significant and transient decline of beta cell function measured by c-peptide response to a mixed-meal challenge [19]. Higher dose of IL-2 or combination with rapamycin used in this trial may be responsible for this decline. This experience highlights the challenge in using IL-2 therapeutically and the importance of sensitive and objective biomarkers in monitoring trial safety and efficacy.

Considering the promising efficacy of low-dose IL-2 therapy in autoimmune diseases, it is rather paradoxical that anti-CD25 therapies are found effective at stalling progression of multiple sclerosis and autoimmune uveitis. Anti-CD25 antibodies were originally developed with the intention to prevent immune activation by blocking IL-2 signaling. Randomized controlled trials and over two decades of clinical experiences show that two forms of anti-CD25 antibodies, daclizumab and basiliximab, are effective at reducing acute transplant rejection [20,21]. The therapy leads to significant loss of $FOXP3^+$ Tregs, and the lack of detrimental effect on allografts may be explained by the deletion of $FOXP3^-CD25^+$ Teffs in these patients [22]. In multiple sclerosis, daclizumab was found to inhibit activation of antigen-specific T cells with limited effect on non-specific T cells. This highly selective effect was found to involve a novel mechanism of IL-2 transpresentation by activated DCs to T cells during cognate interactions [23]. Activated DCs express IL-2 and CD25. These cells capture IL-2 on their surface via CD25 and present the complex to T cells that are being stimulated by antigens, enabling high affinity IL-2 signaling in antigen-specific T cells. DC transpresentation is inhibited by declizumab. The mechanism of antigen-specific deletion by anti-CD25 suggests that this therapy is likely to be most effective during disease flare when Teffs and DCs are activated to express CD25. These studies together show that anti-CD25 therapy can

delete CD25-expressing activated effectors nonspecifically and CD25-negative Teffs in an antigen-specific manner.

Analysis of multiple sclerosis patients treated with daclizumab showed increased serum IL-2 and a significant expansion of $CD56^{bright}$ NK cells similar to that seen in patients treated with IL-2. Daclizumab therapy inhibited IL-2 signaling in all T cells; but IL-2 signaling in $CD56^{bright}$ NK cells and their *in vivo* proliferation and cytotoxicity actually increased after the therapy [24]. Increased NK-cell activation is also observed in mice after Treg depletion, suggesting that Tregs normally limit IL-2 bioavailability by consumption and inhibition of production [25,26]. Deletion of Tregs and blockade of CD25 leads to an excess IL-2 that drives NK-cell activation. More importantly, the expansion of $CD56^{bright}$ NK cells was found to be associated with protective response to the therapy likely because NK-mediated killing of activated T cells [27]. Thus, in patients with multiple sclerosis, $CD56^{bright}$ NK cells function as a 'backup' for Tregs after anti-CD25 therapy explaining the lack to negative impact of Treg deletion. It remains to be determined if this backup system operates similarly in other disease settings. In a mouse model of Type 1 diabetes where NK cells are deficient, Treg deletion leads to increase in islet killing, which was associated with the activation of $CD8^+$ Teffs in the inflamed islets [28]. Taken together, effects of anti-CD25 therapy will likely vary depend on the disease context. The aforementioned mouse studies and results from human trials provide ideas on assessing immune regulatory responses in future trials.

In conclusion, interventions directed at the IL-2 and IL-2R have shown promise in promoting immune tolerance in autoimmune diseases and transplantation. Manipulating this axis also has potential application in cancer immunotherapy. Sensitive biomarkers of responsiveness, efficacy and toxicity are much needed to gain mechanistic insights and to personalize this treatment for specific disease and specific patients. Approaches to achieve cell-specific delivery of IL-2 [29,30] and combinational therapies may help to maximize efficacy and minimize toxicity.

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