Low-dose IL-2 as a therapeutic agent for tolerance induction

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KEYWORDS: IL-2 ■ tolerance ■ T regulatory cells ■ Type 1 diabetes

Thomas R Malek

Author for correspondence: Department of Microbiology & Immunology Miller School of Medicine, University of Miami, PO Box 016960, Miami, FL 33101, USA and The Diabetes Research Institute, Miller School of Medicine, University of Miami, PO Box 016960, Miami, FL 33101, USA
tmalek@med.miami.edu

Alberto Pugliese

Department of Medicine, Division of Endocrinology, Diabetes & Metabolism & Department of Microbiology & Immunology, Miller School of Medicine, University of Miami, PO Box 016960, Miami, FL 33101, USA and The Diabetes Research Institute, Miller School of Medicine, University of Miami, PO Box 016960, Miami, FL 33101, USA

IL-2 is produced primarily by recently activated naive and memory T cells after antigen stimulation of the T-cell receptor. The duration of IL-2 secretion is short lived as production of this cytokine is under stringent transcriptional and post-transcription regulation. IL-2 mediates its biological activity by binding to a high affinity receptor consisting of three subunits, IL-2Rα (CD25), IL-2Rβ (CD122) and γc (CD132). Expression of the high affinity IL-2R is also under stringent transcriptional regulation that is positively linked to T-cell receptor and IL-2 stimulation, leading to IL-2R expression mainly on recently activated T effector (Teff) cells and Tregs. Under physiological levels of IL-2, T-cell immunity is enhanced by increasing expansion and effector activity of antigen-specific T cells and by promoting memory cell development. IL-2 also promotes T-cell tolerance by providing essential signals for thymic development and peripheral homeostasis of Tregs. More detailed information concerning the immunobiology of IL-2 and its receptor has recently been reviewed [1].

"An important consideration with IL-2-mediated tolerance induction is that the dose of IL-2 used for immunotherapy is critical."

Given the dual role of IL-2 in tolerance and immunity, the application of IL-2 to modulate the immune system in therapeutic situations by design affects both Teff cells and Tregs. Up until very recently, almost all clinical trials using IL-2 aimed at boosting Teff-cell function taking advantage of the immune-stimulating activity of IL-2. This bias is historically grounded in the early work on IL-2 that showed it was a powerful T-cell growth factor *in vitro*. A short time after cloning IL-2, exciting preclinical work demonstrated that tumors readily regressed in mice that received IL-2 therapy and/or IL-2 expanded lymphocytes [2]. This work was followed by some initial success in patients suffering from renal cell carcinoma and melanoma [3]. However, as many more patients underwent IL-2 therapy, it became apparent that the response rate is low [4]. Nevertheless, IL-2 is still sometimes used as a therapy for renal cell carcinoma and melanoma cancers owing to their poor prognosis and lack of more efficacious treatment. IL-2 was also heavily tested to boost immunity in HIV/AIDS patients, but this approach was proven to be ineffective [5].

There are two fundamental reasons that are likely to account for these poor outcomes when using IL-2 to boost immunity. First, sufficient antigen-specific T cells may not be mobilized by IL-2 therapy because the expression of the high affinity IL-2R does not persist on antigen-specific T cells. IL-2 has a very short half-life in the circulation (~30 min) after infusion *in vivo*. Trials were designed to avoid this problem by infusion of IL-2 at levels as high as possible, but avoiding life-threatening nonspecific toxicities, particularly vascular leak syndrome. This type of IL-2 therapy not only stimulates antigen-specific T cells expressing the high-affinity IL-2R but also NK and naive $CD4^+$ T cells expressing the intermediate affinity IL-2R (i.e., cells expressing only IL-2Rβ and γc). These latter cells substantially outnumber the antigen-specific compartment and may limit induction of the most efficacious immune responses. Second, IL-2 also enhances the Treg compartment that may further limit the capacity to stimulate a desirable immune response [6,7]. Indeed, increased Tregs within a tumor are associated with ineffective immunotherapy [8].

Only in the last 7 years or so has IL-2 been accepted as an essential cytokine for Tregs. Therefore, failure to promote immunity by IL-2

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immunotherapy has only been recently considered as a direct effect of immune inhibition by Tregs stimulated by IL-2. Perhaps of greater interest, the requirement for IL-2 by Tregs raises the possibility that IL-2 may serve as a biologic to promote T-cell tolerance. A number of recent preclinical studies support this notion. Autoimmune-mediated Type 1 diabetes (T1D) was prevented and new-onset T1D was reversed in nonobese diabetic (NOD) mice by IL-2 immunotherapy [9,10]. This effect is achieved by using either IL-2 or agonist IL-2–anti-IL-2 complexes, the latter targeting the high-affinity IL-2R and extending the *in vivo* half-life of IL-2 [6,11]. Inducible adeno-associated virus-mediated expression of IL-2 was also shown to prevent diabetes in NOD mice [12]. The protective effects in these studies were associated with increased Treg numbers and several key molecules associated with Treg function and survival. The same application of IL-2–anti-IL-2 complexes also rendered mice resistant to experimental autoimmune encephalomyelitis, and when combined with rapamycin, ongoing disease was suppressed [13]. In addition, the use of IL-2–anti-IL-2 complexes in mouse models prevented the rejection of full MHC-mismatched mice pancreatic islet grafts, suppressed experimental myasthenia gravis and inhibited allergic airway disease [13–15]. Tolerance in each of the latter situations was also associated with the expansion of Tregs.

> *"...the protection from severe autoimmunity afforded by minimal signaling to Tregs demonstrates the remarkable sensitivity to low IL-2-dependent STAT5 activation by key genes in Tregs that powerfully support Treg function and immune tolerance."*

An important consideration with IL-2-mediated tolerance induction is that the dose of IL-2 used for immunotherapy is critical. In studies of T1D in NOD mice, a relatively low dose of IL-2 favored tolerance, whereas a higher dose promoted Teff cell activity and exacerbated T1D [10]. This observation indicates that IL-2 therapy can favor tolerance over autoimmunity by carefully controlling the level of IL-2. Our recent work on IL-2R signal transduction provides a mechanistic explanation for these preclinical therapeutic successes, whereby IL-2 suppresses autoimmune disease and tissue rejection responses. By developing mouse models whose T lymphocytes express tyrosine→phenylalanine mutations within the signaling domains of IL-2Rβ, IL-2R signaling, including STAT5 activation, was severely impaired, but not abolished [16]. Even the most severe tyrosine→phenylalanine mutations, which induce weak transient STAT5 activation, but not IL-2-dependent MAPK and PI3K/Akt signaling, readily supported Treg thymic maturation and peripheral homeostasis. By contrast, IL-2-driven T-cell growth factor activity and Teff function remained severely impaired. Thus, weak IL-2R signaling promotes natural Treg development and homeostasis, such as normal numbers and proportion of mature Foxp3^{hi} cells, and this weak signaling does not result in the severe autoimmune disease that is associated with the complete absence of IL-2R signaling. It is important to point out that Tregs with weak IL-2R signaling still harbor important intrinsic defects that may weaken their function. For example, C57BL/6 mice with impaired IL-2R signaling were long-lived, but eventually later in life exhibited inflammatory infiltrates in various tissues, particularly the salivary gland, lungs, liver, small intestine and occasionally the pancreas. Thus, unlike the lethal autoimmunity in the complete absence of IL-2R signaling, weak IL-2R signaling represents a risk for autoimmunity. Nevertheless, the protection from severe autoimmunity afforded by minimal signaling to Tregs demonstrates the remarkable sensitivity to low IL-2-dependent STAT5 activation by key genes in Tregs that powerfully support Treg function and immune tolerance.

These findings raise the possibility that IL-2 may represent a reasonable therapeutic approach to promote T-cell tolerance by boosting Treg numbers and function. In clinical trials for autoimmune diseases such as T1D, an immune-stimulatory therapy to induce tolerance is preferable to current approaches where immunosuppression is achieved through broad-acting drugs. Both chronic and short-term immune interventions with a variety of immunosuppressive agents and antigen-specific therapies have been largely ineffective in achieving long-lasting preservation of residual insulin secretion following the diagnosis of T1D [17]. Thus, while these treatments may have perturbed the autoimmune process, self-tolerance was not restored. A total of three main lines of evidence provide a strong rationale to use IL-2 to boost Treg function and improve IL-2R signaling in patients with T1D:

- Genetic variants of the *IL2RA* gene have been linked to T1D susceptibility, as well as to other autoimmune diseases [18];
- Functional defects have been associated with Tregs from patients with T1D [19];

- Patients with T1D have been reported to exhibit defective IL-2 production or IL-2R signaling [20–22], which may impair IL-2R signaling and Treg function, respectively.

Importantly, we propose that a clinical trial based on the administration of IL-2 should involve a 'low dose' that selectively stimulates Treg function but has no or minimal effect on Teff cells, so the dominant outcome is improved regulation. This reasoning is based on the preclinical studies demonstrating that low-dose IL-2 suppresses, while a higher dose worsens T1D [10]. Similarly, as reported at the American Diabetes Association Annual Meeting, a clinical trial in new-onset T1D patients showed that treatment with rapamycin and a relatively high-dose of IL-2 (4.5×10^6 IU/day subcutaneously for three times/week for 4 weeks) resulted in greater loss of insulin secretion at 3 months and overall was considered to worsen pancreatic β-cell function [23]. While this IL-2 therapy restored defective IL-2 responsiveness and expanded Tregs, there was also enhanced IL-2 responsiveness by Teff and NK cells and an increased frequency of NK cells and eosinophils. The effects on Teff cells are likely explained by high doses of IL-2 inducing a rapamycin-resistant STAT5 signaling enhancement in Teff cells. Based on the preclinical data previously mentioned [9,10,12–15], and our own studies of IL-2 signaling in Tregs and Teff cells where the former are highly reactive to low IL-2R signaling [16], we believe that a lower dose of IL-2 therapy is required to safely promote Treg function and to effectively reduce the severity of autoimmune disease.

Linking tolerance over immunity to a low dose of IL-2 makes teleological sense with respect to proper regulation and function of the immune system. Such a mechanism helps to ensure that antiself immune responses are not easily engaged and that key thresholds must be passed before an immune response is initiated. Thus, a key component to translating this preclinical work is to define a dose of IL-2 that is highly selective for human Tregs while avoiding or minimizing stimulatory effects on Teff cell responses. This dose of IL-2 is unlikely to have been used in past studies, as 'low dose' IL-2 often represented a relatively high dose to stimulate an immune response but avoid nonspecific toxicities that was a major complication in the early use of IL-2 in the clinical setting. To this end, we are currently evaluating the effect of various doses of IL-2 on key functional and molecular parameters in human Treg and Teff cells. Our goals are to identify a suitable dose range and biomarkers that characterize a specific IL-2-dependent response by Tregs. This information will be useful to design clinical trials for T1D or other autoimmune diseases with the aid of well-defined immunological biomarkers of response. As of now an IL-2 clinical trial in new-onset T1D has been recently launched in France [101], aiming at improving Treg activity and preserving insulin secretion. This study will compare placebo with 0.33, 1 and 3 million IU IL-2 administered subcutaneously for 5 days. We eagerly await the results of this study.

If this or similar trials can provide evidence for efficacy and safety of low-dose IL-2, combination therapies with autoantigens (e.g., insulin and glutamic acid decarboxylase) could be envisioned with the objective to boost antigen-specific Tregs, correcting plausible deficiencies in the Treg repertoire, which may depend on genetic factors controlling thymic expression of self-antigens and thymic selection [24]. The potential to induce antigen-specific Tregs may possibly address the earliest pathogenic mechanism in autoimmunity and may take us a step closer to restoring self-tolerance.

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