# Low-dose Interleukin-2 in the Treatment of Autoimmune Disease

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## Abstract

CD4+ regulatory T cells (Tregs) act to maintain peripheral immune tolerance. Decreased numbers or defective function of Tregs has been implicated in the pathogenesis of various autoimmune diseases. Interleukin-2 (IL-2) at high doses is approved by the US Food and Drug Administration (FDA) as an immune stimulant to induce anti-tumor cytotoxicity. However, at physiologic doses, IL-2 is necessary for the expansion and function of Tregs. Treatment with low-dose IL-2 can selectively enhance Treg function while avoiding the activation of effector T cells and ameliorate immune inflammation. Administration of low doses of IL-2 to patients suffering from chronic graft versus host disease (cGvHD) or chronic hepatitis C-mediated vasculitis resulted in significant clinical benefit, which was linked to improved Treg cell function. Preclinical studies suggest that low-dose IL-2 may offer benefit in other autoimmune diseases including systemic lupus erythematosus and type 1 diabetes. Ongoing preclinical and clinical studies indicate a wider potential role for low-dose IL-2 based Treg therapeutics in human autoimmune diseases.

## Keywords

Autoimmune disease, interleukin-2, systemic lupus erythematosus, graft versus host disease, vasculitis, type-1 diabetes

**Disclosure**: John Koreth, MBBS, DPhil, has received research funding from Prometheus. Jerome Ritz, MD, has received research funding from Prometheus. Michelle Rosenzwajg, MD, PhD, is an inventor of a patent application claiming low-dose interleukin-2 (IL-2) in autoimmune and inflammatory diseases, owned by her academic institutions and licensed to ILTOO Pharma in which she holds shares. David Klatzmann, MD, PhD, is an inventor of a patent application claiming low-dose IL-2 in autoimmune and inflammatory diseases, owned by his academic institutions and licensed to ILTOO Pharma in which he holds shares. George C Tsokos, MD, Alberto Pugliese, MD, and Thomas R Malek, PhD, have no conflicts of interest to declare.

**Acknowledgments**: Editorial assistance was provided by Katrina Mountfort, PhD, at Touch Medical Media, London, UK and funded by Prometheus.

**Received**: October 22, 2014 **Accepted**: November 6, 2014 **Citation**: *Oncology & Hematology Review,* 2014;10(2):157–63

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**Support**: The publication of this article was supported by Prometheus. The views and opinions expressed are those of the authors and do not necessarily reflect those of Prometheus.

Autoimmune diseases comprise more than 80 chronic conditions that collectively affect approximately 5 to 8 % of the US population and are a leading cause of death in young and middle-aged women.[1] Moreover, the incidence and prevalence of autoimmune diseases are rising. The age of onset of autoimmune diseases varies widely but many start during childhood[2] and, being chronic and debilitating in nature, require long-term therapy and invoke considerable medical costs, long-term impaired quality of life, and constitute a significant burden to families and society. Type 1 diabetes (T1D), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and inflammatory bowel disease (IBD) account for the majority of the patients with autoimmune diseases. Understanding the dysregulated immune response underlying autoimmune diseases will help the development of disease-specific therapeutics. In this short review we will present an overview of the pathophysiology of autoimmune diseases in the context of regulatory T cell (Treg) dysfunction with a focus on the emerging role for interleukin-2 (IL-2) based Treg therapeutics in restoring immune regulation and mitigating organ damage.

## Pathophysiology of Autoimmune Disease—The Role of Tregs

Autoimmune diseases are characterized by a breakdown of mechanisms that allow the immune system to distinguish between self and nonself and

maintain immunologic self-tolerance. Organ damage and the ensuing clinical manifestations may result from the action of autoantibodies, self-reactive effector T cell (Teff) responses, secreted cytokines, or other elements that participate in the autoimmune response.[3] In recent years, there has been increasing interest in the role of Tregs, which are important in the maintenance of peripheral immune tolerance.[4] Tregs can suppress immune-mediated inflammation through a number of complementary mechanisms that may involve cell–cell contact and the release of regulatory cytokines that directly limit the responses of effector immune cells.

Several subtypes of Tregs exist, the most well studied being $CD4^+$ cells that express high-level CD25 and the transcription factor forkhead box P3 (FOXP3), which is a critical determinant of Treg cell phenotype and function. Treg deficiency or dysfunction is associated with autoimmune disease. For example, in experimental animal studies, genetic or pharmacologic depletion of Tregs causes autoimmunity.[5,6]

Mutations of the *FOXP3* gene in humans result in impaired Treg function and are associated with the 'immunodysregulation polyendocrinopathy enteropathy X-linked' (IPEX) syndrome, a rare disorder characterized by fulminant multi-organ autoimmunity.[7] In clinical studies, decreased levels of circulating $CD25^+CD4^+$ T cells have been reported in patients with autoimmune disease, including vasculitis, rheumatic disease, juvenile idiopathic arthritis, and Kawasaki disease,[8–12] and are associated with high disease activity or poor prognosis.[8,10,12] Graft versus host disease (GvHD), which is a manifestation of allo-immunity following hematopoietic stem cell transplantation (HSCT), has also been associated with Treg cell deficiency.

These data have led to the hypothesis that augmentation of Tregs may be a useful therapeutic strategy in autoimmune disease. Treg augmentation has resulted in clinical improvements in numerous animal models of autoimmune diseases.[13] Furthermore, the administration of *in vitro* expanded $CD4^+CD25^{high}CD127$-Tregs has been found to be safe and may help to preserve β-cell function in children with T1D.[14,15] Targeting Treg cell enhancement at the cellular and molecular levels may therefore be an attractive therapeutic strategy. Cytokines that can modulate and boost Treg-mediated suppression of immune responses may play an important role in the control of autoimmunity. This article will focus on the ability of IL-2 to augment the numbers and function of $CD4^+$ Tregs.

## The Role of IL-2 in the Pathophysiology of Autoimmune Disease

IL-2 was originally discovered as a growth factor for activated T cells *in vitro*. Physiologically, however, IL-2 is essential to maintain the peripheral homeostasis of $CD4^+CD25^{high}Foxp3^+$ Tregs. IL-2 and IL-2 receptor (IL-2R) knockout mice have preserved cytotoxic immune responses, but exhibit autoimmunity. In a study in which the genes for IL-2 were inactivated by gene targeting, the knockout mice died early due to lymphoproliferative syndrome accompanied by severe autoimmunity.[16] Neutralization of circulating IL-2 by anti-IL-2 monoclonal antibody has also been found to result in a number of autoimmune diseases, including gastritis, thyroiditis, sialadenitis, and severe neuropathy.[17] Autoimmunity associated with impaired IL-2 or IL-2R signaling is due to a failure in the development and homeostasis of Tregs.[18] Importantly, a low level of IL-2 receptor signaling is sufficient to provide these key functions to Tregs, while not supporting IL-2-dependent T effector activity. These latter observations provided some of the initial rationale for low-dose IL-2 therapy for autoimmunity.[19]

$CD4^+$ Tregs do not produce IL-2 but their survival and function depend on IL-2 uptake via the constitutively expressed high affinity heterotrimeric IL-2 receptor complex (CD25, CD122, and CD132).[20,21] By contrast, conventional $CD4^+$ and $CD8^+$ T cells constitutively express a moderate affinity IL-2 receptor complex (CD122 and CD132) and do not express CD25; they do not express the high affinity IL-2R complex until they have been activated through their antigen (Ag) receptor. When conventional $CD4^+$ and $CD8^+$ T cells are activated, IL-2 also promotes Th1 and Th2 development while it actively opposes the development of Th17 and Tfh cells.[22,23] This dual role for IL-2 in tolerance and immunity suggests that it may have pleotropic effects in the context of autoimmune diseases. As IL-2 is vital for amplifying T-cell-mediated effector immune responses, including T-cell memory, it could function to enhance autoreactive T cells at higher doses. However, the capacity of IL-2 at low doses to support Tregs indicates that it also critically serves as a negative regulator of autoimmunity.[6,16] Low-dose IL-2 is defined operationally, i.e. IL-2 doses that preferentially enhance the proliferation, activation, and survival of Treg (versus conventional $CD4^+$ T cells [Tcon]). Practically, the doses used are suitable for outpatient administration. IL-2 is also well known to be important for activation-induced cell death (AICD), which controls the expansion of immune effector cells.[24,25] In autoimmune disease e.g. SLE, AICD is decreased and this defect has been considered responsible for the poor elimination of self-reactive T cells.[26] Therefore, IL-2 not only promotes effector immune responses but also acts to eliminate activated T cells.

Defects in the IL-2 pathway or reduced IL-2 availability are seen in some autoimmune conditions including SLE[27] and T1D,[28–30] whereas in others IL-2 deficits are associated with disease progression; these include RA[31] and IBD.[32] Studies in SLE patients have noted dysregulation of host immune responses, including decreased $CD4^+$ Treg function and/or numbers, decreased AICD and compromised cytotoxic cell responses.[27,33–36] The molecular mechanisms responsible for the decreased IL-2 production involve an imbalance between the transcription factors cyclic adenosine monophosphate (cAMP) response element-binding protein (CRE)-binding protein (CREB) and its modulator (cAMP response element modulator-α [CREMα][25,37,38] T cells from patients with SLE express increased amounts of calcium/calmodulin-dependent protein kinase IV (CaMK4) in the nucleus, which phosphorylates CREM to bind to the IL-2 promoter.[35] At the same time, T cells express increased amounts of serine/threonine-specific protein phosphatase 2A (PP-2A), which dephosphorylates phosphorylated cAMP response element binding protein (pCREB), tipping the balance of CREB/CREM toward CREM and suppression of IL-2 promoter activity (see *Figure 1*).[33,39] Recent studies have indicated that estrogen enhances the expression of CREMα, resulting in IL-2 suppression in human T lymphocytes.[40] Altered immune function leads to increased rates of infection and tissue inflammation in patients with SLE.[41]

The mechanisms by which low-dose IL-2 treatment can re-establish immune tolerance have recently been partly elucidated in a study of patients with chronic GvHD, a common complication of allogeneic HSCT arising due to donor effector immune responses to allogeneic (donor/recipient polymorphic) and autologous (donor/recipient nonpolymorphic) Ags. Chronic GvHD is characterized clinically by multisystem immune inflammation and fibrosis. Immunologically, there is a relative failure of

Treg compared with Teff reconstitution after allogeneic HSCT in adults, arising due to reduced CD4+ Treg thymogenesis, with a compensatory peripheral Treg proliferation that ultimately cannot be sustained owing to increased apoptosis and impaired survival of the circulating CD4+ memory Treg. Additionally chronic GvHD patients have a documented imbalance in the activation of CD4+ Treg versus Teff cell activation as measured by phosphorylation of signal transducer and activator of transcription 5 (pStat5) that acts downstream of IL-2 receptor activation on immune cells.[20] In GvHD, low-dose IL-2 therapy increased pStat5 activation in Tregs and decreased production of phosphorylated pStat5 in conventional CD4+ T cells. However, this was not a study of patients with T1D[42] (Alberto Pugliese, Tom Malek, Michelle Rosenzwajg, and David Klatzmann, unpublished data). Treatment with low doses of IL-2 also resulted in restoration of Treg homeostasis, including increased peripheral proliferation, increased thymic neogenesis, and enhanced resistance to apoptosis.[43]

To summarize, at high doses, IL-2 activates immune Teffs; but at lower doses, IL-2 induces Tregs and also natural killer (NK) cell numbers and function to a lesser extent and in a dose-dependent manner.[18] However, it is not known whether the therapeutic effects of low-dose IL-2 are solely attributable to the expansion of Tregs; in an animal model of experimental autoimmune encephalomyelitis (EAE), the IL-2 induced expansion of NK cells contributed to disease protection.[44] Overall, these data provide a strong rationale for the therapeutic use of low-dose IL-2 in the treatment of autoimmune inflammation.

## Therapeutic Use of IL-2 in Autoimmune Disease

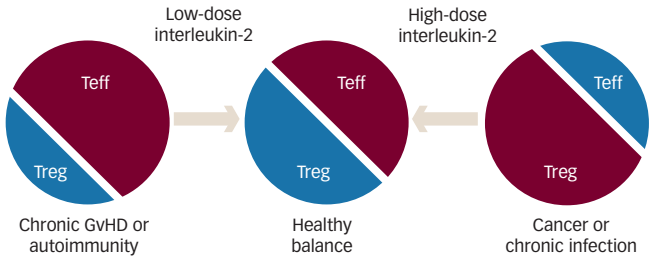
High doses of IL-2 induce proliferation of cytotoxic CD4+ Teff cells, and this led to the use at high doses (100–150 x $10^6$ IU/$m^2$/day) to boost cytotoxic immune responses in patients with advanced cancers. Treatment with high-dose IL-2 led to a durable and complete tumor regression in ~8 % of patients with renal carcinoma and melanoma, leading to its approval by the US Food and Drug administration (FDA) to treat these malignancies. However, administration of high-dose IL-2 was associated with significant toxicity including the development of the vascular or capillary leak syndrome (VLS/CLS).[45] At lower doses, IL-2 has been used to boost immune responses in patients with advanced HIV and induced a significant rise in the CD4+ T-cell count compared with patients treated with antiretroviral therapy alone.[46]

Lowering the dose to reduce toxicity greatly reduces therapeutic efficacy as an immune stimulant. The limited efficacy of high doses of IL-2 in the cancer and HIV setting may also be explained by the unintended, concomitant activation of Tregs, which can suppress the effector responses that the therapy aimed to stimulate.[47,48] IL-2 administration increases CD4+ Tregs in cancer patients.[48,49] Such increases, while detrimental to IL-2-mediated anticancer therapy, may be advantageous in autoimmune diseases. At high doses, IL-2 activates immune Teffs; however, at low doses, IL-2 induces Tregs and NK cell numbers and function.[18] These findings and the high sensitivity of Tregs for IL-2 support the therapeutic use of low-dose IL-2 for autoimmune disease.[50]

### Preclinical Studies

Mouse studies cannot help in determining appropriate dosing of IL-2 as Treg activation by IL-2 in mice require doses in the range of 25–50 x $10^3$ U/day, which correspond to high doses in humans. IL-2 has been found to have a proliferative effect on Tregs in low doses (0.5–1 × $10^6$ IU/$m^2$/day) in nonhuman primates.[51] Administration of IL-2 to lupus-prone mice protected against end-organ damage and suppressed inflammation by limiting the production of IL-17-producing double-negative T cells and by expanding Tregs.[52] Interestingly, in this study it was demonstrated that IL-2 acts on CD8+ cells and augments their ability to kill $CD3^+CD4^-CD8^-$ (double negative) T cells,[52] which are expanded in SLE patients and lupus-prone mice and contribute to the immunopathogenesis of the disease.[36] IL-2 administered to mice with EAE, prior to disease onset resulted in significant improvement in the clinical manifestations of EAE as well as expansion of Tregs while preventing the induction of T helper 17 (Th17) cells.[53,54] However, the efficacy of low-dose IL-2 in these studies is dependent on disease stage; administration of IL-2 after the onset of clinical symptoms failed to suppress disease despite the induction of Tregs.

### Figure 1: Role of IL-2 in Suppression of Tregs in Autoimmune Disease




*GvHD = graft versus host disease; Teff = effector T cell; Treg = regulatory T cell. Adapted from Bluestone.[39]*

In the nonobese diabetic (NOD) mouse model of T1D, low-dose IL-2 protects against disease development[55] and reverses established T1D.[56] Adeno-associated virus (AAV)-based expression of IL-2 selectively in pancreatic beta cells of NOD mice expanded islet-resident Foxp3(+)Tregs, resulting in effective long-term suppression of T1D.[57] Administration of NOD mice with a vector able to deliver IL-2 in an inducible manner resulted in enhanced Treg function and disease improvement.[58] In addition, there is initial evidence that IL-2 may promote beta cell replication.[59,60]

### Clinical Studies

Studies reported in 1992 and 1994 administered low-dose IL-2 after autologous or T-cell depleted allogeneic stem cell transplantation. Patients received low-dose IL-2 by continuous intravenous infusion for a 90-day period beginning approximately 3 months after transplant. Treatment was well tolerated and the major immunologic effect observed at that time was a marked increase in the number of circulating NK cells.[61,62] Subsequent examination of cryopreserved blood samples obtained from patients on these studies demonstrated that $CD4^+CD25^+FOXP3^+$ cells were also expanded during low-dose IL-2 therapy.[63] Due to the high toxicity of high-dose IL-2, many groups attempted to lower IL-2 doses in the fields of cancer and chronic infection. For example, a phase I study found that administration of low-dose IL-2 (0.9 × $10^6$ IU/$m^2$/day for 8 weeks) is safe and relatively well tolerated in patients with HIV infection or HIV and cancer.[64]

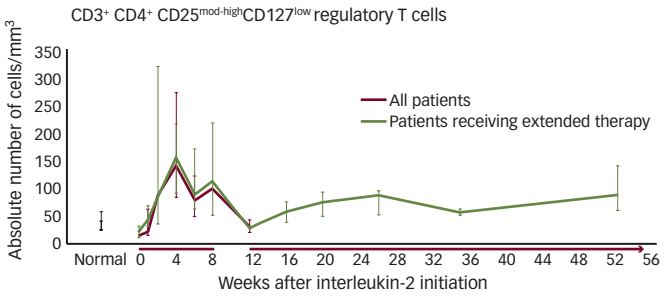
Subsequent clinical studies investigating the use of low-dose IL-2 in autoimmune diseases are summarized in *Table 1*. Two successful clinical

## Table 1: Clinical Studies of the Use of IL-2 in Autoimmune Disease

| Condition | Trial Description | Efficacy Outcomes | Safety Outcomes | Reference |
|---|---|---|---|---|
| Hepatitis C-induced vasculitis | n=10, 9 weeks | Tregs from 3.6 % to 11.8 % (p=0.004). Clinical improvement of vasculitis in 80 % | No AEs related to treatment >grade 1, no significant changes in granulocytes, red cells, or liver enzymes | [65] |
| Chronic GvHD | n=29, 8 weeks | $CD4^+$ Tregs at 4 weeks were more than 8x higher than at baseline (p<0.001), without any change in the conventional T cells | No leukopenia, neutropenia, thrombocytopenia, or hepatic dysfunction | [66] |
| Alopecia areata | Prospective pilot study, n=5 | 4 patients attained considerable hair regrowth, as well as an increase in Tregs | No serious AEs | [67] |
| T1D | Phase I/II, n=24 | Increased production of Tregs at all doses | No serious AEs, AEs were dose-dependent | [42] |

*AE = adverse effects; EAE = experimental autoimmune encephalomyelitis; GvHD = graft versus host disease; T1D = type 1 diabetes; Tregs = regulatory T cells.*

## Figure 2: Immunologic Effects of IL-2 in Patients with Graft versus Host Disease




trials of low-dose IL-2 in the treatment of auto and allo-immune diseases have been reported. Patients (n=10) with hepatitis C-induced vasculitis that was refractory to standard antiviral therapy, rituximab or both, were given low-dose IL-2 ($1.5 \times 10^6$ IU once a day for 5 days followed by $3 \times 10^6$ IU for 5 days for 3 cycles on weeks 3, 6, and 9).[65] Percentages of Tregs constantly and significantly increased at each course. By week 9, there was a significant increase in the proportion of $CD4^+$ Tregs, from 3.6 % at baseline to 11.8 % (p=0.004). The selective expansion of Tregs was associated with marked clinical improvement, and the treatment did not induce Teff activation or vasculitis flare, further demonstrating the selective effect of low-dose IL-2 on Tregs and no Teffs *in vivo*. The treatment also did not increase HCV viremia. There were no significant changes in circulating levels of granulocytes, red cells, or liver enzymes and no adverse events (AEs) related to the treatment higher than grade 1. A clinical improvement of vasculitis was seen in eight of 10 patients.

Another study investigated the use of IL-2 in patients with chronic GvHD (cGvHD). Patients (n=29) with cGvHD refractory to glucocorticoids were given daily low-dose IL-2 treatment (either $0.3 \times 10^6$, $1 \times 10^6$, or $3 \times 10^6$ IU/$m^2$/day) for 8 weeks.[66] The highest dose, however, resulted in unacceptable grade 2 AEs, including fever, malaise, and arthralgia. Thus, the maximum tolerated dose was $1 \times 10^6$ IU/$m^2$/day. Low-dose IL-2 treatment had an acceptable toxicity profile and did not induce significant leukopenia, neutropenia, thrombocytopenia, or hepatic dysfunction. Of the 23 patients who could be evaluated for a clinical response, 12 had a partial response involving multiple sites and 11 had stable disease. No patient had cGvHD progression. Immunologically, the peak median number of $CD4^+$ Tregs at 4 weeks was more than eight times higher than at baseline (p<0.001), without any change in the $CD4^+$ Teff cells. The NK cell count also rose above baseline. Additionally, daily low-dose IL-2 resulted in restoration of various parameters of Treg homeostasis, including peripheral proliferation, thymic neogenesis, and enhanced resistance to apoptosis.[43] Low-dose IL-2 therapy also preferentially increased pStat5 activation status of Tregs, to resolve the $CD4^+$ Treg:Teff pStat5 activation imbalance noted in cGvHD patients. Extended low-dose IL-2 therapy led to sustained clinical and immunologic responses (see *Figure 2*), enabling tapering of glucocorticoids by an average of 60 %. However, despite Treg-cell expansion, clinical benefit was not seen in all patients. Clinical response may be influenced by numerous factors, including patient characteristics, underlying disease, concomitant immunosuppressive medications, and the extent of tissue injury. Treg-cell activity may also vary from one patient to another. Clinical response rate was higher in patients with an above median Treg/Teff ratio at study entry. Further studies are required to confirm these findings.

In T1D, in a recent phase I/II study, 24 adult patients with T1D were randomized to three IL-2 dosing regimens. This was the first randomized placebo-controlled, double-blind clinical study of low-dose IL-2 in an autoimmune disease. IL-2 was well tolerated at all doses, with no serious AEs. Nonserious AEs, the most common of which were injection-site reaction and influenza-like syndrome, were slightly more frequent in the higher-dose group. Treatment with IL-2 increased in the proportion of Tregs at all doses compared with placebo (see *Figure 3*).[42] While the study was not powered to detect significant improvements of insulin secretion, no impairment was observed. At the $1 \times 10^6$ U/day dose, there were no elevations of Teffs or of NK cells, suggesting that this dose may be both efficacious and safe. Thus, there is no evidence so far that low-dose IL-2 may worsen beta cell function as a result of direct effects on beta cells or activation of autoreactive effector cells.

Alopecia areata is an autoimmune disease associated with infiltration of $CD4^+$ and $CD8^+$ T cells around the hair follicles. In a prospective pilot study, five patients with alopecia areata were treated with low-dose IL-2. Of these, four patients attained considerable hair regrowth, as well as an increase in Tregs and no serious AEs. Histologic examination revealed a reduction of infiltrating $CD8^+$ T cells paralleled by an increase of FOXP3 Tregs.[67] A phase I/II study in patients with severe and resistant alopecia areata is currently recruiting.

Further clinical studies are in progress, including the phase II Induction of Tregs by low-dose IL-2 in Autoimmune and Inflammatory Diseases (TRANSREG) study that will assess the safety and biological efficacy of low-dose IL-2 as a Treg inducer in 11 autoimmune and auto-inflammatory

diseases: RA, ankylosing spondylitis, SLE, psoriasis, Behcet's disease, Wegener's granulomatosis, Takayasu's disease, Crohn's disease, ulcerative colitis, autoimmune hepatitis, and sclerosing cholangitis.[68]

A 9-week, single-center, nonrandomized, single-dose, open label, adaptive dose-finding trial, the Adaptive study of IL-2 dose on Tregs in T1D (DILT1D), is ongoing.[69] The ultra-low-dose IL-2 to fight T1D trial (DIABIL-2), a double-blind, randomized, European phase IIb clinical trial, is also in progress.[70]

In addition, ultra-low-dose IL-2 (0.1 or 0.2 × $10^6$ IU/$m^2$/day) was administered to 12 healthy volunteers with minimal AEs, mainly grade 1–2 injection site reactions. The levels of $CD4^+$ Treg and CD56bright NK significantly increased at 7 days.[71] Low-dose IL-2 may affect the function of additional immune cells including innate lymphoid cells 2 (ILC2), which express CD25.[72] Finally, a recent case study has reported that low-dose IL-2-therapy induced a rapid, strong, and sustained reduction of disease activity as well a substantial and selective expansion of the Treg population in a SLE patient with increased disease activity.[73] Further cases of SLE improvements after low-dose IL-2 have also been recently reported.[74,75]

## Practical Aspects of Administration of Low-dose IL-2

Patients with autoimmune diseases represent a heterogeneous group and it may be difficult to predict an optimal IL-2 dosage *a priori* and this may also vary by underlying condition. Thus, caution must be exercised in translating IL-2-based therapies to the clinic. Factors to consider include the definition of an appropriately low dose of IL-2 that is efficacious and safe in a specific autoimmune disorder, also the consideration of the frequency of administration and the cumulative dosing as a function of treatment duration. The potential effects of concomitant therapies must also be considered, if given in combination. Future trials should further evaluate individual patient's responses versus dosing and frequency of administration, and aim at developing biomarkers of IL-2 response that could be used to personalize doses, especially in the context of combinatorial therapies. For instance, a study of low-dose IL-2 to enhance $CD4^+$ T cell counts in patients with HIV found that daily dosing was associated with fewer adverse effects than intermittent dosing.[76] Furthermore, IL-2 has a very short half-life in human serum (5–7 minutes).[77] Subcutaneous or intraperitoneal injections can prolong the half-life.[78] In addition, coupling IL-2 to IL-2 monoclonal antibodies prolongs its half-life, leading to a 40-fold higher *in vivo* biological activity compared with soluble IL-2, and may offer a promising approach to the treatment of autoimmune disease.[79] The efficacy of this approach has been demonstrated in experimental models of the autoimmune disease myasthenia gravis.[80] Attempts to couple IL-2 to larger proteins, such as albumin and unrelated antibodies to create IL-2-IgG fusion proteins, have had limited success.[20]

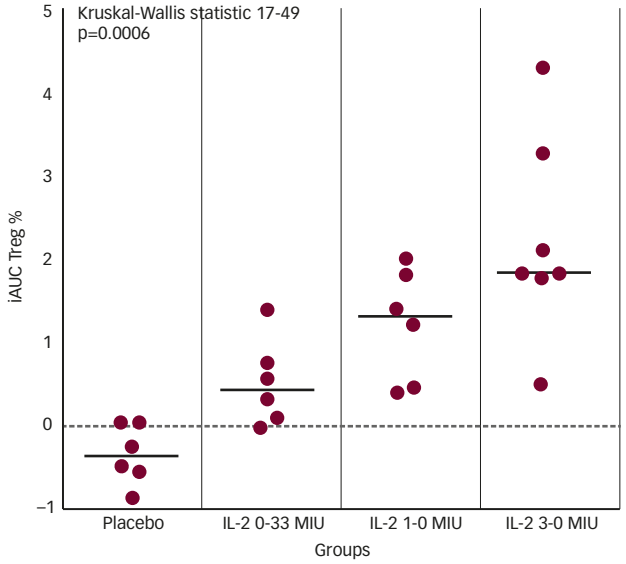
## Use of Low-dose IL-2 in Conjunction with Other Therapies

Another approach to the treatment of autoimmune disease with low-dose IL-2 is in combination with immunosuppressive drugs that are selectively sparing of Treg. In T1D patients, IL-2 was first tested in combination with rapamycin, a drug that blocks cell-cycle progression and cytokine signal transduction through inhibition of the mammalian target of rapamycin (mTOR), another downstream IL-2 signaling component. Rapamycin increases the production of Tregs and also enhances their suppressive ability.[81,82] The combination of IL-2 and Rapamycin has been shown to promote induction, survival, and expansion of functional Treg from $CD4^+CD25^-$ cells.[83] Moreover, combined therapy with IL-2 and rapamycin prevents T1D in animal models.[84] However, a phase I clinical trial involving combination therapy with IL-2 and rapamycin reported a temporary worsening of C-peptide production. In this study, nine adult patients were treated with rapamycin for 3 months.[85] During the first month, patients were also treated with 12 injections of IL-2, given at a dose of 4.5 × $10^6$ IU, 3 times a week. Analysis of the 3-month data for seven patients revealed that they all had experienced a transitory but significant reduction in stimulated C-peptide responses. It is important to consider that IL-2 was given only during the first month, and yet the impaired secretory was observed at 3 months, while patients were still treated with rapamycin but were no longer treated with IL-2. Since patients were not evaluated earlier during the IL-2 course, the actual effects of this combination could not be directly evaluated. Insulin secretion improved after rapamycin was discontinued. This should not be surprising, since rapamycin is known to have a negative impact on beta cell function, survival, and peripheral insulin resistance; rapamycin inhibits the mTOR pathways, causing beta cell toxicity, reduced beta cell size, mass, proliferation, impaired insulin secretion, increased apoptosis, autophagy, and peripheral insulin resistance.[85–87] Thus, at least in the T1D setting, the effects of rapamycin on pancreatic beta cell function may overcome any possible synergistic effect on Tregs when given with low-dose IL-2.

Adoptive cell therapy involving the combination of IL-2 and infusion of Tregs is also the subject of clinical investigations, although research is at an early stage. This approach was shown to expand Tregs *in vivo* following allogeneic HSCT.[88] Translation of these promising data to clinical practice will require a robust method of generating large pure populations of Ag-specific Tregs; an alternative is the use of *ex vivo* expanded Tregs[89] or freshly isolated donor Tregs.[90]

Further clinical development of low-dose IL-2 in autoimmune diseases will likely require long-term administration of IL-2. A recent preclinical study showed that chronic low-dose IL-2 treatment does not suppress

### Figure 3: Increase in Tregs Induced by IL-2 in Humans with Type 1 Diabetes[42]




*Each circle represents one treated patient. Adapted from Hartemann et al.[42] MUI = million international units; iAUC = incremental area under the curve.*

beneficial effector immune responses, for example against infectious agents or in response to vaccination, at doses that prevent autoimmune diabetes.[91] Another recent study found that low-dose IL-2 for GvHD prophylaxis after allogeneic HSCT reduced the frequency of infections and mediated the expansion of Tregs without diminishing its antiviral and anti-leukemic activity.[92]

## Summary and Concluding Remarks

Autoimmune disease is chronic and debilitating, and there is an unmet need for safe and effective novel treatments. Autoimmunity is often characterized by dysregulation of CD4+ Tregs, which play a central role in immune tolerance and prevention of aberrant immune responses. Recent studies have improved our understanding of the role of IL-2 in restoring CD4+ Treg homeostasis and support a rationale for its use at low doses to induce Tregs and treat autoimmune diseases. Low doses of IL-2 in soluble form or coupled to anti-IL2 mAbs can also enhance Tregs, while avoiding stimulation of Teffs. A growing number of early phase clinical trials have provided initial safety and efficacy data in various autoimmune diseases but there remains a need for larger, long-term confirmatory studies. Nonetheless, based on extensive preclinical data and early clinical results, low-dose IL-2-based Treg therapy offers a promising avenue for the treatment of immune inflammation. Data from ongoing clinical trials are eagerly awaited. ■


1. AAARD, Autoimmune statistics. Available at: http://www.aarda.org/autoimmune-information/autoimmune-statistics/ (accessed August 4, 2014).
2. Amador-Patarroyo MJ, Rodriguez-Rodriguez A, Montoya-Ortiz G, How does age at onset influence the outcome of autoimmune diseases?, *Autoimmune Dis*, 2012;2012:251730.
3. Bluestone JA, Mechanisms of tolerance, *Immunol Rev*, 2011;241:5–19.
4. Belkaid Y, Tarbell K, Regulatory T cells in the control of host-microorganism interactions (*), *Annu Rev Immunol*, 2009;27:551–89.
5. Wing K, Sakaguchi S, Regulatory T cells exert checks and balances on self tolerance and autoimmunity, *Nat Immunol*, 2010;11:7–13.
6. Geng X, Zhang R, Yang G, et al., Interleukin-2 and autoimmune disease occurrence and therapy, *Eur Rev Med Pharmacol Sci*, 2012;16:1462–7.
7. d'Hennezel E, Ben-Shoshan M, Ochs HD, et al., FOXP3 forkhead domain mutation and regulatory T cells in the IPEX syndrome, *N Engl J Med*, 2009;361:1710–3.
8. de Kleer IM, Wedderburn LR, Taams LS, et al., CD4+CD25bright regulatory T cells actively regulate inflammation in the joints of patients with the remitting form of juvenile idiopathic arthritis, *J Immunol*, 2004;172:6435–43.
9. Cao D, van Vollenhoven R, Klareskog L, et al., CD25brightCD4+ regulatory T cells are enriched in inflamed joints of patients with chronic rheumatic disease, *Arthritis Res Ther*, 2004;6:R335–46.
10. Boyer O, Saadoun D, Abriol J, et al., CD4+CD25+ regulatory T-cell deficiency in patients with hepatitis C-mixed cryoglobulinemia vasculitis, *Blood*, 2004;103:3428–30.
11. Kuhn A, Beissert S, Krammer PH, CD4(+)CD25 (+) regulatory T cells in human lupus erythematosus, *Arch Dermatol Res*, 2009;301:71–81.
12. Furuno K, Yuge T, Kusuhara K, et al., CD25+CD4+ regulatory T cells in patients with Kawasaki disease, *J Pediatr*, 2004;145:385–90.
13. Brusko TM, Putnam AL, Bluestone JA, Human regulatory T cells: role in autoimmune disease and therapeutic opportunities, *Immunol Rev*, 2008;223:371–90.
14. Marek-Trzonkowska N, Mysliwiec M, Dobyszuk A, et al., Therapy of type 1 diabetes with CD4(+)CD25(high)CD127-regulatory T cells prolongs survival of pancreatic islets – results of one year follow-up, *Clin Immunol*, 2014;153:23–30.
15. Marek-Trzonkowska N, Mysliwiec M, Dobyszuk A, et al., Administration of CD4+CD25highCD127- regulatory T cells preserves beta-cell function in type 1 diabetes in children, *Diabetes Care*, 2012;35:1817–20.
16. Malek TR, The main function of IL-2 is to promote the development of T regulatory cells, *J Leukoc Biol*, 2003;74:961–5.
17. Setoguchi R, Hori S, Takahashi T, et al., Homeostatic maintenance of natural Foxp3(+) CD25(+) CD4(+) regulatory T cells by interleukin (IL)-2 and induction of autoimmune disease by IL-2 neutralization, *J Exp Med*, 2005;201:723–35.
18. Malek TR, Bayer AL, Tolerance, not immunity, crucially depends on IL-2, *Nat Rev Immunol*, 2004;4:665–74.
19. Yu A, Zhu L, Altman NH, et al., A low interleukin-2 receptor signaling threshold supports the development and homeostasis of T regulatory cells, *Immunity*, 2009;30:204–17.
20. Shevach EM, Application of IL-2 therapy to target T regulatory cell function, *Trends Immunol*, 2012;33:626–32.
21. Baecher-Allan C, Wolf E, Hafler DA, Functional analysis of highly defined, FACS-isolated populations of human regulatory CD4+ CD25+ T cells, *Clin Immunol*, 2005;115:10–8.
22. Laurence A, Tato CM, Davidson TS, et al., Interleukin-2 signaling via STAT5 constrains T helper 17 cell generation, *Immunity*, 2007;26:371–81.
23. Ballesteros-Tato A, Leon B, Graf BA, et al., Interleukin-2 inhibits germinal center formation by limiting T follicular helper cell differentiation, *Immunity*, 2012;36:847–56.
24. Richter GH, Mollweide A, Hanewinkel K, et al., CD25 blockade protects T cells from activation-induced cell death (AICD) via maintenance of TOSO expression, *Scand J Immunol*, 2009;70:206–15.
25. Boyman O, Sprent J, The role of interleukin-2 during homeostasis and activation of the immune system, *Nat Rev Immunol*, 2012;12:180–90.
26. Kovacs B, Vassilopoulos D, Vogelgesang SA, et al., Defective CD3-mediated cell death in activated T cells from patients with systemic lupus erythematosus: role of decreased intracellular TNF-alpha, *Clin Immunol Immunopathol*, 1996;81:293–302.
27. Lieberman LA, Tsokos GC, The IL-2 defect in systemic lupus erythematosus disease has an expansive effect on host immunity, *J Biomed Biotechnol*, 2010;2010:740619.
28. Zier KS, Leo MM, Spielman RS, et al., Decreased synthesis of interleukin-2 (IL-2) in insulin-dependent diabetes mellitus, *Diabetes*, 1984;33:552–5.
29. Roncarolo MG, Zoppo M, Bacchetta R, et al., Interleukin-2 production and interleukin-2 receptor expression in children with newly diagnosed diabetes, *Clin Immunol Immunopathol*, 1988;49:53–62.
30. Giordano C, Panto F, Caruso C, et al., Interleukin 2 and soluble interleukin 2-receptor secretion defect *in vitro* in newly diagnosed type I diabetic patients, *Diabetes*, 1989;38:310–5.
31. Kitas GD, Salmon M, Farr M, et al., Deficient interleukin 2 production in rheumatoid arthritis: association with active disease and systemic complications, *Clin Exp Immunol*, 1988;73:242–9.
32. Sadlack B, Merz H, Schorle H, et al., Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene, *Cell*, 1993;75:253–61.
33. Tsokos GC, Thai TH, Interleukin-2 in systemic autoimmunity hits the micro way, *Arthritis Rheum*, 2012;64:3494–7.
34. Konya C, Paz Z, Tsokos GC, The role of T cells in systemic lupus erythematosus: an update, *Curr Opin Rheumatol*, 2014;26:493–501.
35. Koga T, Ichinose K, Mizui M, et al., Calcium/calmodulin-dependent protein kinase IV suppresses IL-2 production and regulatory T cell activity in lupus, *J Immunol*, 2012;189:3490–6.
36. Tsokos GC, Systemic lupus erythematosus, *N Engl J Med*, 2011;365:2110–21.
37. Solomou EE, Juang YT, Gourley MF, et al., Molecular basis of deficient IL-2 production in T cells from patients with systemic lupus erythematosus, *J Immunol*, 2001;166:4216–22.
38. Hedrich CM, Crispin JC, Rauen T, et al., cAMP response element modulator alpha controls IL2 and IL17A expression during CD4 lineage commitment and subset distribution in lupus, *Proc Natl Acad Sci U S A*, 2012;109:16606–11.
39. Bluestone JA, The yin and yang of interleukin-2-mediated immunotherapy, *N Engl J Med*, 2011;365:2129–31.
40. Moulton VR, Holcomb DR, Zajdel MC, et al., Estrogen upregulates cyclic AMP response element modulator alpha expression and downregulates interleukin-2 production by human T lymphocytes, *Mol Med*, 2012;18:370–8.
41. Iliopoulos AG, Tsokos GC, Immunopathogenesis and spectrum of infections in systemic lupus erythematosus, *Semin Arthritis Rheum*, 1996;25:318–36.
42. Hartemann A, Bensimon, G, Payan CA, et al., Low-dose interleukin 2 in patients with type 1 diabetes: a phase 1/2 randomised, double-blind, placebo-controlled trial, *Lancet Diabetes Endocrinol*, 2013;1:295–305.
43. Matsuoka K, Koreth J, Kim HT, et al., Low-dose interleukin-2 therapy restores regulatory T cell homeostasis in patients with chronic graft-versus-host disease, *Sci Transl Med*, 2013;5:179ra43.
44. Hao J, Campagnolo D, Liu R, et al., Interleukin-2/interleukin-2 antibody therapy induces target organ natural killer cells that inhibit central nervous system inflammation, *Ann Neurol*, 2011;69:721–34.
45. Dutcher J, Atkins MB, Margolin K, et al., Kidney cancer: the Cytokine Working Group experience (1986–2001): part II. Management of IL-2 toxicity and studies with other cytokines, *Med Oncol*, 2001;18:209–19.
46. Group I-ES, Committee SS, Abrams D, et al., Interleukin-2 therapy in patients with HIV infection, *N Engl J Med*, 2009;361:1548–59.
47. de Vries IJ, Castelli C, Huygens C, et al., Frequency of circulating Tregs with demethylated FOXP3 intron 1 in melanoma patients receiving tumor vaccines and potentially Treg-depleting agents, *Clin Cancer Res*, 2011;17:841–8.
48. Ahmadzadeh M, Rosenberg SA, IL-2 administration increases CD4+ CD25(hi) Foxp3+ regulatory T cells in cancer patients, *Blood*, 2006;107:2409–14.
49. Boyman O, Kovar M, Rubinstein MP, et al., Selective stimulation of T cell subsets with antibody-cytokine immune complexes, *Science*, 2006;311:1924–7.
50. d'Hennezel E, Kornete M, Piccirillo CA, IL-2 as a therapeutic target for the restoration of Foxp3+ regulatory T cell function in organ-specific autoimmunity: implications in pathophysiology and translation to human disease, *J Transl Med*, 2010;8:113.
51. Aoyama A, Klarin D, Yamada Y, et al., Low-dose IL-2 for *in vivo* expansion of CD4+ and CD8+ regulatory T cells in nonhuman primates, *Am J Transplant*, 2012;12:2532–7.
52. Mizui M, Koga T, Lieberman LA, et al., IL-2 Protects lupus-prone mice from multiple end-organ damage by limiting CD4-CD8- IL-17-producing T cells, *J Immunol*, 2014;193:2168–77.
53. Rouse M, Nagarkatti M, Nagarkatti PS, The role of IL-2 in the activation and expansion of regulatory T-cells and the development of experimental autoimmune encephalomyelitis, *Immunobiology*, 2013;218:674–82.
54. Webster KE, Walters S, Kohler RE, et al., *In vivo* expansion of T reg cells with IL-2-mAb complexes: induction of resistance to EAE and long-term acceptance of islet allografts without immunosuppression, *J Exp Med*, 2009;206:751–60.
55. Tang Q, Adams JY, Penaranda C, et al., Central role of defective interleukin-2 production in the triggering of islet autoimmune destruction, *Immunity*, 2008;28:687–97.
56. Grinberg-Bleyer Y, Baeyens A, You S, et al., IL-2 reverses established type 1 diabetes in NOD mice by a local effect on pancreatic regulatory T cells, *J Exp Med*, 2010;207:1871–8.
57. Johnson MC, Garland AL, Nicolson SC, et al., Beta-cell-specific IL-2 therapy increases islet Foxp3+Treg and suppresses type 1 diabetes in NOD mice, *Diabetes*, 2013;62:3775–84.
58. Goudy KS, Johnson MC, Garland A, et al., Inducible adeno-associated virus-mediated IL-2 gene therapy prevents autoimmune diabetes, *J Immunol*, 2011;186:3779–86.
59. Diaz-de-Durana Y, Lau J, Knee D, et al., IL-2 Immunotherapy reveals potential for innate beta cell regeneration in the non-obese diabetic mouse model of autoimmune diabetes, *PLoS One*, 2013;8:e78483.
60. Dirice E, Kahraman S, Jiang W, et al., Soluble factors secreted by T cells promote beta-cell proliferation, *Diabetes*, 2014;63:188–202.
61. Soiffer RJ, Murray C, Cochran K, et al., Clinical and immunologic effects of prolonged infusion of low-dose recombinant interleukin-2 after autologous and T-cell-depleted allogeneic bone marrow transplantation, *Blood*, 1992;79:517–26.
62. Soiffer RJ, Murray C, Gonin R, et al., Effect of low-dose interleukin-2 on disease relapse after T-cell-depleted allogeneic bone marrow transplantation, *Blood*, 1994;84:964–71.
63. Zorn E, Nelson EA, Mohseni M, et al., IL-2 regulates FOXP3 expression in human CD4+CD25+ regulatory T cells through a STAT-dependent mechanism and induces the expansion of these cells *in vivo*, *Blood*, 2006;108:1571–9.
64. Shah MH, Freud AG, Benson DM, Jr, et al., A phase I study of ultra low dose interleukin-2 and stem cell factor in patients with HIV infection or HIV and cancer, *Clin Cancer Res*, 2006;12:3993–6.
65. Saadoun D, Rosenzwajg M, Joly F, et al., Regulatory T-cell responses to low-dose interleukin-2 in HCV-induced vasculitis, *N Engl J Med*, 2011;365:2067–77.
66. Koreth J, Matsuoka K, Kim HT, et al., Interleukin-2 and regulatory T cells in graft-versus-host disease, *N Engl J Med*, 2011;365:2055–66.
67. Castela E, Le Duff F, Butori C, et al., Effects of low-dose recombinant interleukin 2 to promote T-regulatory cells in alopecia areata, *JAMA Dermatol*, 2014;150:748–51.
68. Induction of regulatory T Cells by low-dose Il2 in autoimmune and Inflammatory Diseases (TRANSREG) Available at: http://clinicaltrials.gov/show/NCT01988506 (accessed August 5, 2014).
69. Waldron-Lynch F, Kareclas P, Irons K, et al., Rationale and study design of the Adaptive study of IL-2 dose on regulatory T cells in type 1 diabetes (DILT1D): a non-randomised, open label, adaptive dose finding trial, *BMJ Open*, 2014;4:e005559.
70. Ultra-low dose Interleukin-2 to fight T1D trial (DIABL-2). Available at: http://www.diabil-2.eu/ (accessed October 6, 2014).
71. Ito S, Bollard CM, Carlsten M, et al., Ultra-low dose interleukin-2 promotes immune-modulating function of regulatory T cells and natural killer cells in healthy volunteers, *Mol Ther*, 2014;22:1388–95.
72. Klein Wolterink RG, Kleinjan A, van Nimwegen M, et al., Pulmonary innate lymphoid cells are major producers of IL-5 and IL-13 in murine models of allergic asthma, *Eur J Immunol*, 2012;42:1106–16.

73. Humrich JY, von Spree C, Rose A, et al., Induction of remission by low-dose IL-2-therapy in one SLE patient with increased disease activity refractory to standard therapies: A case report, *Ann Rhem Dis*, 2014;73:A46.
74. Klatzmann D, Immunoregulation without immunosuppression: the promise of low-dose IL2. Presented at the FOCiS meeting, June 25–28, 2014, Chicago, Illinois.
75. Yu D, Low-dose interleukin-2 in active systemic lupus erythematosus, presented at the FOCiS meeting, June 25–28 2014, Chicago, Illinois.
76. Lalezari JP, Beal JA, Ruane PJ, et al., Low-dose daily subcutaneous interleukin-2 in combination with highly active antiretroviral therapy in HIV+ patients: a randomized controlled trial, *HIV Clin Trials*, 2000;1:1–15.
77. Lotze MT, Frana LW, Sharrow SO, et al., *In vivo* administration of purified human interleukin 2. I. Half-life and immunologic effects of the Jurkat cell line-derived interleukin 2, *J Immunol*, 1985;134:157–66.
78. Piscitelli SC, Wells MJ, Metcalf JA, et al., Pharmacokinetics and pharmacodynamics of subcutaneous interleukin-2 in HIV-infected patients, *Pharmacotherapy*, 1996;16:754–9.
79. Letourneau S, van Leeuwen EM, Krieg C, et al., IL-2/anti-IL-2 antibody complexes show strong biological activity by avoiding interaction with IL-2 receptor alpha subunit CD25, *Proc Natl Acad Sci U S A*, 2010;107:2171–6.
80. Liu R, Zhou Q, La Cava A, et al., Expansion of regulatory T cells via IL-2/anti-IL-2 mAb complexes suppresses experimental myasthenia, *Eur J Immunol*, 2010;40:1577–89.
81. Monti P, Scirpoli M, Maffi P, et al., Rapamycin monotherapy in patients with type 1 diabetes modifies CD4+CD25+FOXP3+ regulatory T-cells, *Diabetes*, 2008;57:2341–7.
82. Kopf H, de la Rosa GM, Howard OM, et al., Rapamycin inhibits differentiation of Th17 cells and promotes generation of FoxP3+ T regulatory cells, *Int Immunopharmacol*, 2007;7:1819–24.
83. Long SA, Buckner JH, Combination of rapamycin and IL-2 increases de novo induction of human CD4(+)CD25(+)FOXP3(+) T cells, *J Autoimmun*, 2008;30:293–302.
84. Rabinovitch A, Suarez-Pinzon WL, Shapiro AM, et al., Combination therapy with sirolimus and interleukin-2 prevents spontaneous and recurrent autoimmune diabetes in NOD mice, *Diabetes*, 2002;51:638–45.
85. Long SA, Rieck M, Sanda S, et al., Rapamycin/IL-2 combination therapy in patients with type 1 diabetes augments Tregs yet transiently impairs beta-cell function, *Diabetes*, 2012;61:2340–8.
86. Baeyens A, Perol L, Fourcade G, et al., Limitations of IL-2 and rapamycin in immunotherapy of type 1 diabetes, *Diabetes*, 2013;62:3120–31.
87. Barlow AD, Nicholson ML, Herbert TP, Evidence for rapamycin toxicity in pancreatic beta-cells and a review of the underlying molecular mechanisms, *Diabetes*, 2013;62:2674–82.
88. Zorn E, Mohseni M, Kim H, et al., Combined CD4+ donor lymphocyte infusion and low-dose recombinant IL-2 expand FOXP3+ regulatory T cells following allogeneic hematopoietic stem cell transplantation, *Biol Blood Marrow Transplant*, 2009;15:382–8.
89. Lapierre P, Beland K, Yang R, et al., Adoptive transfer of *ex vivo* expanded regulatory T cells in an autoimmune hepatitis murine model restores peripheral tolerance, *Hepatology*, 2013;57:217–27.
90. Riley JL, June CH, Blazar BR, Human T regulatory cell therapy: take a billion or so and call me in the morning, *Immunity*, 2009;30:656–65.
91. Churlaud G, Jimenez V, Ruberte J, et al., Sustained stimulation and expansion of Tregs by IL2 control autoimmunity without impairing immune responses to infection, vaccination and cancer, *Clin Immunol*, 2014;151:114–26.
92. Kennedy-Nasser AA, Ku S, Castillo-Caro P, et al., Ultra low-dose IL-2 for GVHD prophylaxis after allogeneic hematopoietic stem cell transplantation mediates expansion of regulatory T cells without diminishing antiviral and antileukemic activity, *Clin Cancer Res*, 2014;20:2215–25.