The Journal of Immunology

Low-Dose IL-2 Therapy in Transplantation, Autoimmunity, and Inflammatory Diseases

Maryam Tahvildari and Reza Dana

J Immunol 2019; 203:2749-2755; ;
doi: 10.4049/jimmunol.1900733
http://www.jimmunol.org/content/203/11/2749

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Translating Immunology

The Journal of Immunology

Low-Dose IL-2 Therapy in Transplantation, Autoimmunity, and Inflammatory Diseases

Maryam Tahvildari*,† and Reza Dana†

Regulatory T cells (Tregs) play a central role in the induction and maintenance of immune homeostasis and self-tolerance. Tregs constantly express the high-affinity receptor to IL-2. IL-2 is a pleiotropic cytokine and a key survival factor for Tregs. It maintains Tregs' suppressive function by promoting Foxp3 expression and subsequent production of immunoregulatory cytokines. Administration of low-dose IL-2 is shown to be a promising approach to prevent allograft rejection and to treat autoimmune and inflammatory conditions in experimental models. The combination of IL-2 with its mAb (JES6-1) has also been shown to increase the $t_{1/2}$ of IL-2 and further enhance Treg frequencies and function. Low-dose IL-2 therapy has been used in several clinical trials to treat conditions such as hepatitis C vasculitis, graft-versus-host disease, type 1 diabetes, and systemic lupus erythematosus. In this paper, we summarize our findings on low-dose IL-2 treatment in corneal allografting and review recent studies focusing on the use of low-dose IL-2 in transplantation, autoimmunity, and other inflammatory conditions. We also discuss potential areas of further investigation with the aim to optimize current low-dose IL-2 regimens. *The Journal of Immunology*, 2019, 203: 2749–2755.

Regulatory T cells (Tregs) are a subpopulation of T cells that mediate immune suppression in an Ag-specific manner in an array of inflammatory responses that include immune responses to self-antigens (autoimmunity), foreign Ags (pathogens or alloantigens), and tumors. Tregs, therefore, play a central role in the maintenance of immune homeostasis and self-tolerance. They are characterized by expression of CD4, CD25, and the transcription factor Foxp3 (1). Foxp3 is a family of transcriptional regulators, the protein product of which, scurfin, is essential for normal immune homeostasis. Foxp3 is shown to be absent in scurfy mice and in patients with immune dysregulation, polyendocrinopathy, enteropathy, and X-linked syndrome. Mutations in the Foxp3 gene have also been associated with other autoimmune diseases such as type 1 diabetes (T1D), allergies, and inflammatory bowel disease in humans (2–5). Foxp3 plays an essential role in development and differentiation of Tregs in the periphery, as well as maintaining Treg suppressive function (6). $CD4^+CD25^-$ T cells, NK cells, and $CD8^+$ CTLs only express the β- and the γ-chains of the IL-2R and have lower affinity to bind to IL-2 in the microenvironment. Therefore, low concentrations of IL-2 selectively activate Tregs, whereas high doses expand Tregs, effector T cells (Teffs), NK cells, and CTLs (7, 8). Multiple studies have shown that Treg deficiency leads to development of autoimmunity both in humans and mice, and defects in Treg function have been identified in numerous inflammatory conditions, including systemic lupus erythematosus (SLE), T1D, and chronic kidney disease (9, 10). In vivo expansion of $CD4^+CD25^+Foxp3^+$ Tregs using low-dose IL-2 has shown promising results in controlling inflammation and inducing immune tolerance in both autoimmunity and transplantation. This approach bypasses the major obstacle in previous approaches to adoptively transfer Tregs, as Tregs have relatively low frequencies in lymphoid tissues, requiring in vitro expansion prior to adoptive transfer. Prolonged in vitro expansion of Tregs itself is shown to lead to loss of Foxp3 expression and decreased suppressive function (11).

IL-2 is a pleiotropic cytokine that was originally discovered in the 1970s as a T cell growth factor. However, further studies in the 1990s showed that mice deficient in the genes encoding for IL-2, IL-2Rα, or IL-2Rβ lacked Tregs and developed severe autoimmunity (9, 12). IL-2 is a key survival factor for Tregs in the periphery that is required for their functional competence and stability (9, 13). Administration of s.c. low-dose IL-2 is shown to be promising in treating autoimmune conditions such as chronic refractory graft-versus-host disease (GVHD), hepatitis C virus–induced vasculitis, and T1D (14–16). Since then, a number of phase I/II clinical trials have focused on determining the optimal dose and frequency of administration of low-dose IL-2 in patients with T1D and SLE and have reported promising results (16–18). Table I summarizes the initial human studies on low-dose IL-2 therapies in autoimmune diseases (15–17, 19–23).

*Kresge Eye Institute, Wayne State University, Detroit, MI 48201; and †Schepens Eye Research Institute, Massachusetts Eye and Ear Infirmary, Department of Ophthalmology, Harvard Medical School, Boston, MA 02114

ORCID: 0000-0002-5732-5100 (R.D.).

Received for publication July 3, 2019. Accepted for publication October 6, 2019.

This work was supported by the National Institutes of Health (Project R01EY012963).

Address correspondence and reprint requests to Dr. Reza Dana, Schepens Eye Research Institute, Massachusetts Eye and Ear Infirmary, Department of Ophthalmology, Harvard Medical School, 20 Staniford Street, Boston, MA 02114. E-mail address: Reza_Dana@meei.harvard.edu

Abbreviations used in this article: CNI, calcineurin inhibitor; EAE, experimental autoimmune encephalitis; GVHD, graft-versus-host disease; HCV, hepatitis C virus; IRI, ischemia reperfusion injury; rhIL-2, recombinant human IL-2; SLE, systemic lupus erythematosus; T1D, type 1 diabetes; Teff, effector T cell; TRALI, transfusion-related acute lung injury; Treg, regulatory T cell.

www.jimmunol.org/cgi/doi/10.4049/jimmunol.1900733

Table I. Summary of the findings of the initial studies on the use of low-dose IL-2 in the treatment of autoimmune conditions in humans

Year	Author	Clinical Setting	Route, Dosage, Frequency of IL-2 Administration	Biological Effect	Clinical Outcome	Side Effects
2011	Saadoun et al. (19)	HCV-induced vasculitits	s.c., 1 million IU/d for 5 d, followed by three 5-d courses of 3 million IU/d at weeks 3, 6, and 9 (52.5 million IU cumulative dose)	Increased $CD4^+CD25^{hi}Foxp3^+$ Tregs No Teff activation Decreased inflammatory and oxidative stress mediators No increased HCV viremia	No vasculitis flare Decline in cryoglobulinemia in 9 of 10 patients Improvement of vasculitis in 8 of 10 patients First time to show low-dose IL-2 could be used as immunoregulatory drug for treatment of autoimmune disease	No serious adverse events
2011	Koreth et al. (15)	Refractory GVHD	s.c., 0.3, 1, or 3 million IU/m^2 of body surface area daily for 8 wk, followed by a 4-wk hiatus (extended treatment if response observed)	Increased $CD4^+Foxp3^+$ Tregs with a peak median value at 4 wk (more than eight times the baseline value) No effect on Tcon Treg:Tcon ratio increased to five times the baseline value, declined when treatment stopped	Of the 23 patients, 12 had major responses involving multiple sites with alleviation of the manifestations of chronic GVHD	Maximum tolerated dose: 1 million IU/m^2 Highest dose induced severe constitutional symptoms
2013	Hartemann et al. (16)	Insulin-dependent T1D	s.c., 0.33, 1, or 3 million IU/d for a 5-d course, followed for 60 d	A dose-dependent increase in Tregs (significant at all doses)	IL-2 did not induce deleterious changes in glucose metabolism variables	Tolerated at all doses with no serious adverse effects Dose-dependent Most common: injection-site reaction and influenza-like syndrome
2014	Castela et al. (20)	Alopecia areata	s.c., 1.5 million IU/d during 5 d, followed by three 5-d courses of 3 million IU/d at weeks 3, 6, and 9	Notable increase in Treg count in four of five patients at the end of the treatment compared with baseline	Partial regrowth achieved in four of five patients	No serious adverse event was reported
2015	Rosenzwajg et al. (21)	T1D	s.c., 0.33, 1, or 3 million IU daily for 5 d A dose-finding trial to define safety and immunological responses	Expands and activates Tregs at 0.33 and 1 million IU/d without effects on Teffs or NK cells Greater expansion of Tregs at the dosage of 3 million IU/d but with NK cell expansion, cytokine/chemokine increase A clear shift of the peripheral blood immune environment toward a regulatory milieu	Help delay or prevent the onset of the full-blown disease Decrease the frequency and severity of disease	No serious adverse events More frequent mild-to-moderate side effects with higher dosage (3 million IU/d)
2015	Humrich et al. (22)	SLE A 36-y-old female with SLE and high disease activity	s.c., 1.5 or 3.0 million IU of IL-2 daily for 5 consecutive d, four treatment cycles separated by washout periods of 9–16 d and followed by a 9-wk follow-up period	Remarkable $CD25^+Foxp3^+CD127^{lo}$ Treg expansion Decreased serum levels of anti-dsDNA Abs Very slight and transient decreases in the levels of total Ig Intact Treg suppressive function on days 57 and 83 by in vitro suppression assays Marginal effects on other cell subsets	Rapid and robust reduction of disease activity No organ manifestations during the treatment cycles Disease activity remained low First evidence for clinical efficacy of s.c. low-dose IL-2 in SLE A successful biological treatment strategy	Well-tolerated Mild and transient adverse effects: erythema at the injection site, increased day and night sweats, and one episode of fever

(*Table continues*)

Table I. (*Continued*)

Year	Author	Clinical Setting	Route, Dosage, Frequency of IL-2 Administration	Biological Effect	Clinical Outcome	Side Effects
2016	Todd et al. (23)	T1D	40 participants with T1D	Optimal IL-2 doses that increased Treg frequencies by 10 and 20% were defined	Not looked at	Single dose: small self-limiting injection-site reaction
			Optimal doses of aldesleukin to induce 10 (minimal) and 20% (maximal) increases in Tregs were 0.101 and 0.497 million IU/m^2	Desensitization of Tregs can occur in some patients receiving daily doses of Aldesleukin of 1.0 million IU/m^2 or more Following treatment, Tregs had a decreased sensitivity to IL-2 that returned to baseline on day 3 after treatment		Two episodes of rhinitis possibly related to drug administration Transient lymphopenia Transient eosinophilia
2016	Spee-Mayer et al. (17)	Refractory SLE	s.c., 1.5 million IU daily for 5 consecutive d	Lack of IL-2 production by CD4$^+$ T cells was reversed by stimulation with low doses of IL-2 Selectively expanded Tregs in vivo CD56^{hi} NK cells (with immunoregulatory properties) showed a strong response to in vitro and in vivo stimulations with IL-2	Not looked at	Not looked at

Tcon, conventional T cell.

Using a high-risk model of corneal transplantation, our group has previously shown that low-dose IL-2 treatment can increase Treg frequencies and function with minimal expansion of CD4$^+$IFN-y$^+$ Th cells (Teffs) and significantly improve allograft survival in mice (24).

Despite these promising results, efficacy of low-dose IL-2 therapy has been limited because of the short $t_{1/2}$ of IL-2 (12). To overcome this obstacle, an IL-2–specific mAb has been discovered (JES6-1A12, known as JES6-1) that increases IL-2 $t_{1/2}$ while focusing the activity of IL-2 on CD25$^+$ cells, thus minimizing its effect on CD25$^-$ cells, which will in turn prevent the potential side effects of high-dose IL-2. These side effects stem from increased capillary permeabilization resulting in vascular leak syndrome, which leads to hypotension, pulmonary edema, liver congestion leading to hepatocyte damage, and renal failure (25). In experimental models, low doses of IL-2 have been combined with anti–IL-2 JES6-1 mAb. JES6-1 is known to bind to an IL-2 site that is crucial for interaction with CD122 (IL-2Rβ) but is less crucial for binding to CD25 (IL-2Rα); this is as opposed to another IL-2 mAB, S4B6, which binds to an IL-2 site that partly occludes binding to CD25 but does not impede binding to CD122. Therefore, treatment with IL-2/S4B6 Ab complexes might be clinically useful for tumor immunotherapy and for expanding T cell numbers after bone marrow transplantation. In contrast, the selective expansion of Tregs by IL-2/JES6-1 complexes (IL-2c) would be useful for treating autoimmune disease (7). IL-2c is shown to reduce the severity of allergen-induced inflammation in the lung by expanding Tregs in vivo in a mouse model of allergic airway disease (26). It has also shown to increase the survival of skin and islet cell allografts and effectively diminish inflammation in an experimental model of autoimmune encephalitis (27, 28).

In this paper, we review recent studies focusing on the use of low-dose IL-2 in transplantation, autoimmunity, and other inflammatory conditions. We also discuss potential areas of further investigation with the aim to optimize currently used treatment regimens for low-dose IL-2.

Low-dose IL-2 therapy in transplantation

The first studies to use low-dose IL-2 in transplantation were performed in experimental models of pancreatic islet cell grafting, in which i.p. injections of IL-2c were given (28). Authors showed that the maximal Treg expansion could be achieved in the spleen on day 3 after three daily injections of IL-2 (1 μg) mixed with 5 μg of mAb. With this regimen, the frequencies of the CD25$^+$Foxp3$^+$ Treg population increased from 10.3 to 57.4% among CD4$^+$ spleen cells. Authors further showed that pretreating mice with IL-2c with the above regimen rendered them resistant to induction of experimental autoimmune encephalitis (EAE) and induced tolerance to fully MHC-incompatible pancreatic islet cells in the absence of immunosuppression, leading to the majority of grafts being accepted indefinitely (28). Effects of IL-2c treatment have also been investigated in various allogeneic combinations in skin grafting; specifically, IL-2c has been shown to expand Tregs, inhibit Th1 alloreactivitiy, and increase survival in a mouse model of a single MHC class II disparity (29). In a mouse model of corneal transplantation, we have shown that low-dose IL-2 therapy significantly improves graft survival. We demonstrated that injection of IL-2 alone (1-μg daily i.p. injections)

starting 3 d prior to transplantation until 1 wk after grafting, followed by twice weekly injections up to 6 wk posttransplantation, increases Treg frequencies and improves their immunosuppressive function and long-term graft survival (24). This was the first study showing that the use of low-dose IL-2 alone could induce transplant survival. Previous reports in corneal transplantation reported superiority of the use of IL-2 with rapamycin (compared with IL-2 alone) in corneal allograft survival (30). We believe that frequent injections of IL-2 alone is required for sustained expansion of Tregs and to prevent graft rejection in our model. In addition, starting IL-2 treatment prior to grafting would expand the Treg population prior to allosensitization and prevent graft rejection.

As mentioned above, low-dose IL-2 therapy has been used in combination with interventions that block Teff responses. In a murine model of skin grafting, it is shown that IL-2, when added to rapamycin, increases Tregs and decreases Teff activation in grafted mice and significantly delays skin rejection, an effect that was not observed using one of the two molecules injected alone (27), possibly because of concomitant expansion of Tregs and Teffs with IL-2 and simultaneous inhibition of Treg and Teff proliferation with rapamycin.

Low-dose IL-2 therapy has also been used to induce the expansion of Tregs as an adjunct to previously established strategies that inhibit Th1 activation in autoimmunity and transplantation. As an example, IL-2 has been added to calcineurin inhibitors (CNIs) such as tacrolimus or cyclosporine A. CNIs block the TCR-induced translocation of NFAT into the nucleus, thereby blocking Teff function and IL-2 transcription (31). Therefore, these agents have been shown to limit the availability of IL-2 as a growth factor for Tregs, resulting in a decrease in Treg numbers, as shown in liver and kidney transplant patients (32). In addition, experimental studies in skin allografts have shown that Tregs collected from tacrolimus-treated mice were less efficient in suppressing Teff proliferation. However, the addition of IL-2c to tacrolimus therapy rescued the Treg phenotype, normalized Treg suppressive properties, restored the survival and suppressive properties of Tregs exposed to CNIs, and improved allograft survival in murine skin transplantation (32).

Another strategy to inhibit Teff response is to block the costimulatory mechanisms using CTLA4-Ig; this approach was shown to be superior to cyclosporine in improving renal function in kidney-transplanted patients (33), which raised the possibility that the combination of treatments aiming at inhibiting effector function while expanding Treg numbers or enhancing their function may represent a valuable strategy to achieve immune tolerance. Therefore, in a study by Charbonnier et al., the effect of using CTLA4-Ig and an IL-2–induced Treg expansion on allograft survival was studied. In contrast to the original speculations, the authors demonstrated that CTLA4-Ig prevents graft acceptance induced by exogenous IL-2 therapy through inhibition of Treg homeostasis and suppressive capacities. Therefore, inhibition of Treg function should be taken into account when designing tolerance protocols based on costimulatory blockade (34).

Low-dose IL-2 therapy in autoimmune diseases

One of the earliest clinical reports of low-dose IL-2 has been in the treatment of patients with refractory GVHD following hematopoietic stem cell transplantation (15, 35). Since this report, low-dose IL-2 has been widely used as a modulator of Treg homeostasis in treatment of various autoimmune diseases (36). Different regimens have been suggested in clinical trials to test the efficacy and safety of low-dose IL-2 in the treatment and prevention of GVHD. Early studies showed successful use of s.c. IL-2, with a maximum tolerable dose of 1 million IU/m^2 of body surface area daily for 8 wk, which could be repeated after a 4-wk hiatus. Alleviation of the manifestations of chronic GVHD was observed in a substantial proportion of these patients; out of 23 patients, 12 had major responses involving multiple sites (15). Further studies proposed similar regimens for the prevention of GVHD [e.g., s.c. injections of low-dose IL-2 (1 million IU/m^2) daily for 14 d, followed by a 14-d hiatus (37), or 0.1–0.2 million IU/m^2 three times per week for days 0–90 (38)]. These findings suggested that the prophylactic administration of low-dose IL-2 could effectively enhance early Treg expansion and suppress acute and chronic GVHD (35, 36). In patients with hepatitis C virus (HCV)-induced vasculitis, administration of 1.5–3 $\times$ 10^6 IU/d IL-2 for a total of 10 d was found to exert significant clinical improvement in the majority of patients, with a reduction in cryoglobulinemia in 9 of 10 patients and improvement of vasculitis in 8 of 10 patients (19).

Low-dose IL-2 administration has also shown efficacy in treating patients with T1D. Tregs from T1D patients are shown to be dysfunctional and have a relative deficiency in IL-2 production and IL-2 signaling (39). Accordingly, exogenous IL-2 is thought to restore impaired Treg function associated with defects in the IL-2/IL-2R signaling (40–42). The first dose-defining trial of low-dose IL-2 in T1D was published by Hartemann et al. (16) in 2013, aiming to determine the lowest active dose of IL-2 that could safely expand and activate Tregs in patients with established T1D. This study showed that daily s.c. injection of 0.33–1 million IU/d IL-2 for 5 consecutive d effectively expanded Tregs in a dose-dependent manner with minimal effects on Teffs or NK cells. At higher dosages (3 million IU/d), despite more pronounced and lasting expansion of Tregs, NK cell expansion and more frequent mild-to-moderate side effects were observed. The authors, therefore, established a dosage range of 0.33–1 million IU/d by which Tregs could safely and specifically be expanded in T1D. Based on the results of this study, an efficacy trial has been initiated in patients with new-onset T1D (ClinicalTrial.gov identifier NCT01862120).

Low-dose IL-2 treatment has also been used to restore Treg function in patients with SLE. Impaired IL-2 production by T cells from SLE patients was first described in the 1980s (long before the discovery of Tregs) (43). More recently, several studies have shown that lack of IL-2 production by $CD4^+$ T cells of these patients accounts for the loss of CD25 expression in Tregs, which could be selectively reversed by stimulation with low doses of IL-2 (17, 18, 43). In addition, data from mouse models have suggested that IL-2 deficiency in SLE is acquired and develops as a result of displacement of IL-2–producing T cells by chronically activated Teffs as well as memory T cells, which are known to lose their ability to express IL-2 (17, 18). In April 2013, the first patient with active SLE was treated off-label with recombinant human IL-2 (rhIL-2); a rapid and robust reduction of disease activity was observed, which was in parallel with a remarkable expansion

of the Treg population (43). This finding was in accordance with the published study in patients with hepatitis C–associated vasculitis (19, 21). Subsequently, in April 2014, the same group performed a combined phase I/IIa trial addressing the safety, tolerability, clinical efficacy, and immunological responses of a repetitive and cyclic s.c. application of low-dose IL-2 in patients with active and refractory SLE (PRO-IMMUN). The regimen consists of four treatment cycles, each consisting of daily s.c. injections of rhIL-2 (aldesleukin) at single doses of 0.75, 1.5, and 3.0 million IU on 5 consecutive d, separated by washout periods of 9–16 d (43). Similarly, in a case series of five patients with refractory SLE, it has been shown that daily s.c. injections of 1.5 million IU of rhIL-2 for 5 consecutive d selectively corrected Treg functional defects in vivo (17).

In another study, 38 patients with SLE received three cycles of rhIL-2 administered s.c. at a dosage of 1 million IU every other day for 2 wk, followed by a 2-wk break in treatment. Treatment with low-dose rhIL-2 selectively enhanced Tregs and decreased numbers of follicular Th cells and Th17 cells but not Th1 or Th2 cells; this was accompanied by marked reductions of disease activity in all patients with SLE. This study provided further evidence that treatment with low-dose IL-2 is capable of altering the Teff-to-Treg balance and improving clinical outcomes in patients with SLE (27).

Currently, multiple phase I/II clinical trials are ongoing to use low-dose IL-2 in the treatment of patients with 11 different autoimmune conditions, including rheumatoid arthritis, ankylosing spondylitis, SLE, and several forms of vasculitis. The most commonly used regimen is to s.c. inject 1 million IU/d IL-2 for 5 d and then once every 2 wk for 6 mo. In all of these conditions, Tregs were successfully expanded without any effect on Teffs, which indicates a potential therapeutic use for low-dose IL-2 across the spectrum of autoimmune diseases (44). Future randomized trials of low-dose IL-2 in the treatment of these conditions are required to determine the efficacy of low-dose IL-2 in their treatment and its potential corticosteroid-sparing effects in these patients.

Use of IL-2/anti–IL-2 complexes to increase the $t_{1/2}$ of IL-2 has been studied in different animal models of autoimmunity similar to transplantation models. Webster et al. showed that in a model of multiple sclerosis, EAE, pretreating the mice for 3 d with IL-2–JES6-1 mAb led only to mild neurologic symptoms of EAE. To examine the effect of therapeutic administration of IL-2c on EAE progression, after EAE induction, mice were treated with IL-2c, rapamycin, or both for 3 d, starting on day 2 after priming (i.e., during the early stages of the immune response). With this regimen, injecting either rapamycin or IL-2c alone delayed the onset of clinical symptoms; however, all of the mice eventually developed severe disease. In contrast, the combined treatment of rapamycin and IL-2c resulted in a marked reduction in disease severity, indicating a stronger therapeutic effect (28).

In a recent study by Izquierdo et al. (45) using NOD mice, treatment with IL-2c was associated with expansion of both polyclonal and Ag-specific Foxp3$^+$ Tregs. IL-2c therapy also expanded Ag-specific Foxp3$^-$ IL-10–producing T cells that persisted over prolonged periods of time, leading to complete prevention of diabetes and minimal islet infiltration. In a mouse model of lupus nephritis, IL-2c significantly attenuated glomerular and tubular injury, vasculitis scores, and renal deposition of IgG and complement component 3 (C3). Disease activity markers such as high levels of anti-dsDNA Abs and Ig levels and low levels of complement were improved in sera of IL-2c–treated mice (46). IL-2c therapy also decreased renal expression of TNF-α and IL-6 and the frequencies of IFN-γ$^+$ IL-17–producing CD4$^+$ T cells in the kidneys and spleen. Importantly, when compared with combination therapy of steroid and mycophenolate mofetil, IL-2c therapy showed comparable or superior outcomes and protected lupus-prone mice against lupus nephritis by expanding Tregs (46). In addition, IL-2c therapy has been shown to be effective in a mouse model of rheumatoid arthritis in which three consecutive daily i.p. injections of IL-2c resulted in Treg expansion and inhibited synovial cell proliferation and IL-17, IL-6, and TNF-α levels. It also reduced the frequencies of IFN-γ$^+$ IL-17–producing cells and expanded IL-10–producing Tregs in the spleen (47). Despite successes of IL-2c therapy in experimental models, the use of human mAbs against IL-2 is under investigation, and further studies are needed to determine the safety and efficacy of this approach in humans.

Low-dose IL-2 therapy in other inflammatory conditions

Treg deficiency has been shown in multiple inflammatory scenarios. It has been shown that patients with chronic kidney disease have significantly lower frequencies of peripheral Tregs than those of healthy volunteers, and IL-2 can selectively expand Tregs and upregulate Foxp3 expression in these patients. It has also been demonstrated that STAT5 activation is required for IL-2–induced expansion of Tregs and expression of Foxp3 mRNA in chronic kidney disease patients, supporting findings of clinical Treg impairment in glomerular diseases and the rationale for low-dose IL-2 therapy in these patients (48). Similarly, in patients with ischemic heart disease and acute coronary syndrome, low-dose IL-2 therapy is being investigated through a phase I/II randomized double-blind controlled trial. In this ongoing clinical trial, patients will be randomized to receive s.c. doses of either IL-2 (aldesleukin; dose range 0.3–3 million IU) or placebo once daily for 5 consecutive d. Five different dose levels will be studied, and doses will be determined based on the initial responses. This study is looking at the safety and tolerability of aldesleukin and also aims to determine the dose that increases Treg levels by 75% (49).

In animal models, effect of IL-2c therapy have been studied in various inflammatory conditions. In a murine model of renal ischemia reperfusion injury (IRI), three daily doses of IL-2c from 5 d before induction of injury successfully expanded Tregs and decreased inflammatory cells and cytokine levels as well as apoptosis in the renal tissues (50). Interestingly, IL-2c administered after the development of IRI also enhanced Treg frequencies, resulting in improved tubular cell proliferation and renal function and reduced renal fibrosis. More recently, administration of IL-2c before induction of myocardial IRIs induced Treg expansion in the heart and decreased tissue infiltration of inflammatory cells and apoptosis as well as frequencies of Th1 and Th17 cells and expression of inflammatory cytokines, resulting in improved myocardial function (49). In an experimental model of transient ischemic stroke, IL-2c therapy was shown to induce Treg expansion as well as promote expression of CD39 and CD73 by Tregs, which correlates with their immunosuppressive function (51). Also, in a mouse model of food allergy,

IL-2c therapy combined with sublingual immunotherapy reversed IgE-mediated allergy, reduced IL-5 secretion by spleen cells, and increased expression of IL-10 and TGF-β in the lamina propria of buccal and duodenal mucosa (52). In a murine model of sclerosing cholangitis, expansion of intrahepatic Tregs with IL-2c downregulated hepatic expression of osteopontin (a profibrogenic cytokine) and TNF-α, reduced frequencies of intrahepatic $CD8^+$ lymphocytes, and diminished biliary injury and fibrosis. In addition, treatment with IL-2c upregulated hepatic expression of CD39 in the Tregs. Hepatic $CD8^+$ T lymphocytes drive biliary injury and fibrosis in murine sclerosing cholangitis. Their proliferation is controlled by hepatic Tregs through the purinergic pathway, which is responsive to IL-2c, suggesting Treg-directed low-dose IL-2 as a potential therapy for sclerosing cholangitis (53). Finally, in transfusion-related acute lung injury (TRALI), daily i.p. injection of recombinant murine IL-2 (1 μg/kg) or IL-2c, which comprised a mixture of IL-2 and anti–IL-2 at a 1:10 ratio (i.e., 1 mg of recombinant murine IL-2 and 10 mg of mouse IL-2 Ab), for 5 consecutive d before induction of the TRALI prevented the onset of edema and reduced pulmonary protein levels and proinflammatory factors inhibiting polymorphonuclear neutrophil aggregation in the lungs (54). This study revealed that progression of disease in TRALI is associated with altered Th17 and Treg responses and that the addition of exogenous IL-2 and IL-2c could potentially prevent TRALI.

Future directions

Low-dose IL-2 treatment has been offered as a promising tool in restoring immune quiescence in transplantation, autoimmunity, and various inflammatory disorders. Multiple clinical trials are ongoing using IL-2 in T1D, SLE, and ischemic heart disease. Dose-finding trials have been performed in patients with T1D to optimize the dose and frequency of administration of IL-2 to maximize its effects on Tregs without expansion of Teffs or NK cells (16). One existing challenge is the short $t_{1/2}$ of IL-2 when used alone, which necessitates repeated injections. This obstacle has been overcome in experimental models by combining IL-2 with JES-6 mAb, which has resulted in a significant enhancement in Treg expansion and Foxp3 expression with minimal effects on the Teff population (28, 29, 45–47, 50–55). These results warrant the need to translate that knowledge to humans. Recently, a novel anti-human IL-2 Ab has been identified that inhibits the Teff responses to IL-2 without blocking the Treg pSTAT5 pathway (56). This is the first strong evidence for a human anti–IL-2 Ab that can be used therapeutically to specifically target human Tregs and induce tolerance.

In a recent study, a pharmacologically superior and Treg-selective human IL-2 has been engineered that preferentially binds and activates cells expressing high levels of the IL-2Rαβγ, and has the potential to be used for the treatment of autoimmunity and other immune-based disorders. This approach was explored previously by increasing IL-2 affinity to the α-chain (57). Another approach is to decrease IL-2 affinity to the β-chain to reduce the ability of IL-2 to activate IL-2R present on $CD4^+$ and $CD8^+$ Teffs and NK cells, which predominately signals through the intermediate affinity form of the receptor (IL-2Rβγ). This IL-2 mutein is coupled to an effector-silent human IgG1 to enhance its pharmacologic $t_{1/2}$ and enhance its avidity to Treg high-affinity IL-2Rαβγ. This new IL-2 molecule has been highly Treg-selective both in vitro in a human whole blood pSTAT5 assay and in vivo in monkeys (58). Its administration in vivo activated and expanded $CD4^+$ and $CD8^+CD25^+Foxp3^+$ Tregs. Such enhanced and selective Treg responses have the potential to restore 3the immune homeostasis that is perturbed in most autoimmune diseases (58).

These novel therapeutic approaches with more selective effects on different subsets of immune cells can serve as a strong potential tool in the induction of immune quiescence in transplantation, autoimmunity, and a variety of inflammatory disorders and decrease the dependence on generalized immunosuppressive medications such as corticosteroids and cytotoxic agents.

Disclosures

The authors have no financial conflicts of interest.

References

1. Sakaguchi, S., N. Sakaguchi, M. Asano, M. Itoh, and M. Toda. 2011. Pillars article: immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor α-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J. Immunol. 1995. *J. Immunol.* 186: 3808–3821.
2. Brunkow, M. E., E. W. Jeffery, K. A. Hjerrild, B. Paeper, L. B. Clark, S. A. Yasayko, J. E. Wilkinson, D. Galas, S. F. Ziegler, and F. Ramsdell. 2001. Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. *Nat. Genet.* 27: 68–73.
3. Setoguchi, R., S. Hori, T. Takahashi, and S. Sakaguchi. 2005. 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.* 201: 723–735.
4. Gambineri, E., T. R. Torgerson, and H. D. Ochs. 2003. Immune dysregulation, polyendocrinopathy, enteropathy, and X-linked inheritance (IPEX), a syndrome of systemic autoimmunity caused by mutations of FOXP3, a critical regulator of T-cell homeostasis. *Curr. Opin. Rheumatol.* 15: 430–435.
5. Bennett, C. L., J. Christie, F. Ramsdell, M. E. Brunkow, P. J. Ferguson, L. Whitesell, T. E. Kelly, F. T. Saulsbury, P. F. Chance, and H. D. Ochs. 2001. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat. Genet.* 27: 20–21.
6. Fontenot, J. D., M. A. Gavin, and A. Y. Rudensky. 2003. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat. Immunol.* 4: 330–336.
7. Boyman, O., M. Kovar, M. P. Rubinstein, C. D. Surh, and J. Sprent. 2006. Selective stimulation of T cell subsets with antibody-cytokine immune complexes. *Science* 311: 1924–1927.
8. Caligiuri, M. A., C. Murray, M. J. Robertson, E. Wang, K. Cochran, C. Cameron, P. Schow, M. E. Ross, T. R. Klumpp, R. J. Soiffer, et al. 1993. Selective modulation of human natural killer cells in vivo after prolonged infusion of low dose recombinant interleukin 2. *J. Clin. Invest.* 91: 123–132.
9. Klatzmann, D., and A. K. Abbas. 2015. The promise of low-dose interleukin-2 therapy for autoimmune and inflammatory diseases. *Nat. Rev. Immunol.* 15: 283–294.
10. Tang, Q., J. A. Bluestone, and S. M. Kang. 2012. CD4(+)Foxp3(+) regulatory T cell therapy in transplantation. *J. Mol. Cell Biol.* 4: 11–21.
11. Wang, X., L. Lu, and S. Jiang. 2011. Regulatory T cells: customizing for the clinic. *Sci. Transl. Med.* 3: 83ps19.
12. Boyman, O., and J. Sprent. 2012. The role of interleukin-2 during homeostasis and activation of the immune system. *Nat. Rev. Immunol.* 12: 180–190.
13. Gratz, I. K., M. D. Rosenblum, and A. K. Abbas. 2013. The life of regulatory T cells. *Ann. N. Y. Acad. Sci.* 1283: 8–12.
14. Tomova, R., K. Antonov, A. Ivanova, J. J. Jacobs, J. W. Koten, W. Den Otter, and Z. Krastev. 2009. Low-dose IL-2 therapy reduces HCV RNA and HBV DNA: case report. *Anticancer Res.* 29: 5241–5244.
15. Koreth, J., K. Matsuoka, H. T. Kim, S. M. McDonough, B. Bindra, E. P. Alyea, III, P. Armand, C. Cutler, V. T. Ho, N. S. Treister, et al. 2011. Interleukin-2 and regulatory T cells in graft-versus-host disease. *N. Engl. J. Med.* 365: 2055–2066.
16. Hartemann, A., G. Bensimon, C. A. Payan, S. Jacqueminet, O. Bourron, N. Nicolas, M. Fonfrede, M. Rosenzwajg, C. Bernard, and D. Klatzmann. 2013. Low-dose interleukin 2 in patients with type 1 diabetes: a phase 1/2 randomised, double-blind, placebo-controlled trial. *Lancet Diabetes Endocrinol.* 1: 295–305.
17. von Spee-Mayer, C., E. Siegert, D. Abdirama, A. Rose, A. Klaus, T. Alexander, P. Enghard, B. Sawitzki, F. Hiepe, A. Radbruch, et al. 2016. Low-dose interleukin-2 selectively corrects regulatory T cell defects in patients with systemic lupus erythematosus. *Ann. Rheum. Dis.* 75: 1407–1415.
18. He, J., X. Zhang, Y. Wei, X. Sun, Y. Chen, J. Deng, Y. Jin, Y. Gan, X. Hu, R. Jia, et al. 2016. Low-dose interleukin-2 treatment selectively modulates CD4(+) T cell subsets in patients with systemic lupus erythematosus. *Nat. Med.* 22: 991–993.

19. Saadoun, D., M. Rosenzwajg, F. Joly, A. Six, F. Carrat, V. Thibault, D. Sene, P. Cacoub, and D. Klatzmann. 2011. Regulatory T-cell responses to low-dose interleukin-2 in HCV-induced vasculitis. *N. Engl. J. Med.* 365: 2067–2077.
20. Castela, E., F. Le Duff, C. Butori, M. Ticchioni, P. Hofman, P. Bahadoran, J. P. Lacour, and T. Passeron. 2014. Effects of low-dose recombinant interleukin 2 to promote T-regulatory cells in alopecia areata. *JAMA Dermatol.* 150: 748–751.
21. Rosenzwajg, M., G. Churlaud, R. Mallone, A. Six, N. Dérian, W. Chaara, R. Lorenzon, S. A. Long, J. H. Buckner, G. Afonso, et al. 2015. Low-dose interleukin-2 fosters a dose-dependent regulatory T cell tuned milieu in T1D patients. *J. Autoimmun.* 58: 48–58.
22. Humrich, J. Y., C. von Spee-Mayer, E. Siegert, T. Alexander, F. Hiepe, A. Radbruch, G. R. Burmester, and G. Riemekasten. 2015. Rapid induction of clinical remission by low-dose interleukin-2 in a patient with refractory SLE. *Ann. Rheum. Dis.* 74: 791–792.
23. Todd, J. A., M. Evangelou, A. J. Cutler, M. L. Pekalski, N. M. Walker, H. E. Stevens, L. Porter, D. J. Smyth, D. B. Rainbow, R. C. Ferreira, et al. 2016. Regulatory T cell responses in participants with type 1 diabetes after a single dose of interleukin-2: a non-randomised, open label, adaptive dose-finding trial. *PLoS Med.* 13: e1002139.
24. Tahvildari, M., M. Omoto, Y. Chen, P. Emami-Naeini, T. Inomata, T. H. Dohlman, A. E. Kaye, S. K. Chauhan, and R. Dana. 2016. In vivo expansion of regulatory T cells by low-dose interleukin-2 treatment increases allograft survival in corneal transplantation. *Transplantation* 100: 525–532.
25. McDermott, D. F., and M. B. Atkins. 2004. Application of IL-2 and other cytokines in renal cancer. *Expert Opin. Biol. Ther.* 4: 455–468.
26. Wilson, M. S., J. T. Pesce, T. R. Ramalingam, R. W. Thompson, A. Cheever, and T. A. Wynn. 2008. Suppression of murine allergic airway disease by IL-2:anti-IL-2 monoclonal antibody-induced regulatory T cells. *J. Immunol.* 181: 6942–6954.
27. Pilon, C. B., S. Petillon, S. Naserian, G. H. Martin, C. Badoual, P. Lang, D. Azoulay, E. Piaggio, P. Grimbert, and J. L. Cohen. 2014. Administration of low doses of IL-2 combined to rapamycin promotes allogeneic skin graft survival in mice. *Am. J. Transplant.* 14: 2874–2882.
28. Webster, K. E., S. Walters, R. E. Kohler, T. Mrkvan, O. Boyman, C. D. Surh, S. T. Grey, and J. Sprent. 2009. 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.* 206: 751–760.
29. Vokaer, B., L. M. Charbonnier, P. H. Lemaître, and A. Le Moine. 2012. Impact of interleukin-2-expanded regulatory T cells in various allogeneic combinations on mouse skin graft survival. *Transplant. Proc.* 44: 2840–2844.
30. Wang, X., W. Wang, J. Xu, and Q. Le. 2013. Effect of rapamycin and interleukin-2 on regulatory CD4+CD25+Foxp3+ T cells in mice after allogenic corneal transplantation. *Transplant. Proc.* 45: 528–537.
31. Hermann-Kleiter, N., and G. Baier. 2010. NFAT pulls the strings during CD4+ T helper cell effector functions. *Blood* 115: 2989–2997.
32. Whitehouse, G., E. Gray, S. Mastoridis, E. Merritt, E. Kodela, J. H. M. Yang, R. Danger, M. Mairal, S. Christakoudi, J. J. Lozano, et al. 2017. IL-2 therapy restores regulatory T-cell dysfunction induced by calcineurin inhibitors. *Proc. Natl. Acad. Sci. USA* 114: 7083–7088.
33. Vincenti, F., C. Larsen, A. Durrbach, T. Wekerle, B. Nashan, G. Blancho, P. Lang, J. Grinyo, P. F. Halloran, K. Solez, et al; Belatacept Study Group. 2005. Costimulation blockade with belatacept in renal transplantation. *N. Engl. J. Med.* 353: 770–781.
34. Charbonnier, L. M., B. Vokaer, P. H. Lemaître, K. A. Field, O. Leo, and A. Le Moine. 2012. CTLA4-Ig restores rejection of MHC class-II mismatched allografts by disabling IL-2-expanded regulatory T cells. *Am. J. Transplant.* 12: 2313–2321.
35. Matsuoka, K., J. Koreth, H. T. Kim, G. Bascug, S. McDonough, Y. Kawano, K. Murase, C. Cutler, V. T. Ho, E. P. Alyea, et al. 2013. Low-dose interleukin-2 therapy restores regulatory T cell homeostasis in patients with chronic graft-versus-host disease. *Sci. Transl. Med.* 5: 179ra43.
36. Matsuoka, K. I. 2018. Low-dose interleukin-2 as a modulator of Treg homeostasis after HSCT: current understanding and future perspectives. *Int. J. Hematol.* 107: 130–137.
37. Zhao, X. Y., X. S. Zhao, Y. T. Wang, Y. H. Chen, L. P. Xu, X. H. Zhang, W. Han, H. Chen, Y. Wang, C. H. Yan, et al. 2016. Prophylactic use of low-dose interleukin-2 and the clinical outcomes of hematopoietic stem cell transplantation: a randomized study. *OncoImmunology* 5: e1250992.
38. Betts, B. C., J. Pidala, J. Kim, A. Mishra, T. Nishihori, L. Perez, J. L. Ochoa-Bayona, F. Khimani, K. Walton, R. Bookout, et al. 2017. IL-2 promotes early Treg reconstitution after allogeneic hematopoietic cell transplantation. *Haematologica* 102: 948–957.
39. Rosenzwajg, M., G. Churlaud, A. Hartemann, and D. Klatzmann. 2014. Interleukin 2 in the pathogenesis and therapy of type 1 diabetes. *Curr. Diab. Rep.* 14: 553.
40. Concannon, P., S. S. Rich, and G. T. Nepom. 2009. Genetics of type 1A diabetes. *N. Engl. J. Med.* 360: 1646–1654.
41. Wicker, L. S., J. Clark, H. I. Fraser, V. E. Garner, A. Gonzalez-Munoz, B. Healy, S. Howlett, K. Hunter, D. Rainbow, R. L. Rosa, et al. 2005. Type 1 diabetes genes and pathways shared by humans and NOD mice. *J. Autoimmun.* 25(Suppl): 29–33.
42. Dwyer, C. J., N. C. Ward, A. Pugliese, and T. R. Malek. 2016. Promoting immune regulation in type 1 diabetes using low-dose interleukin-2. *Curr. Diab. Rep.* 16: 46.
43. Humrich, J. Y., and G. Riemekasten. 2016. Restoring regulation - IL-2 therapy in systemic lupus erythematosus. *Expert Rev. Clin. Immunol.* 12: 1153–1160.
44. Collison, J. 2019. Low-dose IL-2 therapy for autoimmune diseases. *Nat. Rev. Rheumatol.* 15: 2.
45. Izquierdo, C., A. Z. Ortiz, M. Presa, S. Malo, A. Montoya, N. Garabatos, C. Mora, J. Verdaguer, and T. Stratmann. 2018. Treatment of T1D via optimized expansion of antigen-specific Tregs induced by IL-2/anti-IL-2 monoclonal antibody complexes and peptide/MHC tetramers. *Sci. Rep.* 8: 8106.
46. Yan, J. J., J. G. Lee, J. Y. Jang, T. Y. Koo, C. Ahn, and J. Yang. 2017. IL-2/anti-IL-2 complexes ameliorate lupus nephritis by expansion of $CD4^+CD25^+Foxp3^+$ regulatory T cells. *Kidney Int.* 91: 603–615.
47. Yokoyama, Y., T. Iwasaki, S. Kitano, A. Satake, S. Nomura, T. Furukawa, K. Matsui, and H. Sano. 2018. IL-2-anti-IL-2 monoclonal antibody immune complexes inhibit collagen-induced arthritis by augmenting regulatory T cell functions. *J. Immunol.* 201: 1899–1906.
48. Li, Y., X. Liu, W. Wang, S. Wang, J. Zhang, S. Jiang, Y. Wang, L. Li, J. Li, Y. Zhang, and H. Huang. 2018. Low-dose IL-2 expands $CD4^+$ regulatory T cells with a suppressive function in vitro via the STAT5-dependent pathway in patients with chronic kidney diseases. *Ren. Fail.* 40: 280–288.
49. Xiao, J., K. Yu, M. Li, C. Xiong, Y. Wei, and Q. Zeng. 2017. The IL-2/anti-IL-2 complex attenuates cardiac ischaemia-reperfusion injury through expansion of regulatory T cells. *Cell. Physiol. Biochem.* 44: 1810–1827.
50. Kim, M. G., T. Y. Koo, J. J. Yan, E. Lee, K. H. Han, J. C. Jeong, H. Ro, B. S. Kim, S. K. Jo, K. H. Oh, et al. 2013. IL-2/anti-IL-2 complex attenuates renal ischemia-reperfusion injury through expansion of regulatory T cells. *J. Am. Soc. Nephrol.* 24: 1529–1536.
51. Zhang, H., Y. Xia, Q. Ye, F. Yu, W. Zhu, P. Li, Z. Wei, Y. Yang, Y. Shi, A. W. Thomson, et al. 2018. *In vivo* expansion of regulatory T cells with IL-2/IL-2 antibody complex protects against transient ischemic stroke. *J. Neurosci.* 38: 10168–10179.
52. Smaldini, P. L., F. Trejo, J. L. Cohen, E. Piaggio, and G. H. Docena. 2018. Systemic IL-2/anti-IL-2Ab complex combined with sublingual immunotherapy suppresses experimental food allergy in mice through induction of mucosal regulatory T cells. *Allergy* 73: 885–895.
53. Taylor, A. E., A. N. Carey, R. Kudira, C. S. Lages, T. Shi, S. Lam, R. Karns, J. Simmons, K. Shanmukhappa, M. Almanan, et al. 2018. Interleukin 2 promotes hepatic regulatory T cell responses and protects from biliary fibrosis in murine sclerosing cholangitis. *Hepatology* 68: 1905–1921.
54. He, R., L. Li, Y. Kong, L. Tian, X. Tian, P. Fang, M. Bian, and Z. Liu. 2019. Preventing murine transfusion-related acute lung injury by expansion of $CD4^+$ $CD25^+$ $FoxP3^+$ Tregs using IL-2/anti-IL-2 complexes. *Transfusion* 59: 534–544.
55. Zhao, T. X., M. Kostapanos, C. Griffiths, E. L. Arbon, A. Hubsch, F. Kaloyirou, J. Helmy, S. P. Hoole, J. H. F. Rudd, G. Wood, et al. 2018. Low-dose interleukin-2 in patients with stable ischaemic heart disease and acute coronary syndromes (LILACS): protocol and study rationale for a randomised, double-blind, placebo-controlled, phase I/II clinical trial. *BMJ Open* 8: e022452.
56. Trotta, E., P. H. Bessette, S. L. Silveria, L. K. Ely, K. M. Jude, D. T. Le, C. R. Holst, A. Coyle, M. Potempa, L. L. Lanier, et al. 2018. A human anti-IL-2 antibody that potentiates regulatory T cells by a structure-based mechanism. *Nat. Med.* 24: 1005–1014.
57. Rao, B. M., I. Driver, D. A. Lauffenburger, and K. D. Wittrup. 2005. High-affinity CD25-binding IL-2 mutants potently stimulate persistent T cell growth. *Biochemistry* 44: 10696–10701.
58. Peterson, L. B., C. J. M. Bell, S. K. Howlett, M. L. Pekalski, K. Brady, H. Hinton, D. Sauter, J. A. Todd, P. Umana, O. Ast, et al. 2018. A long-lived IL-2 mutein that selectively activates and expands regulatory T cells as a therapy for autoimmune disease. *J. Autoimmun.* 95: 1–14.