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Low dose Interleukin-2 Therapy in Transplantation, Autoimmunity and Inflammatory Diseases

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Abstract

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 interleukin-2 (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 monoclonal antibody (JES6–1) has also been shown to increase the half-life 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, Type1 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.

Keywords

Regulatory T cells; low-dose IL-2; autoimmunity; transplantation; immune tolerance

Introduction

Regulatory T cells (Tregs) are a subpopulation of T cells that mediate immune suppression in an antigen-specific manner in an array of inflammatory responses that include immune responses to self-antigens (autoimmunity), foreign antigens (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, forkhead box protein 3 (Foxp3) (1). Foxp3 is a family of transcriptional regulators, the protein product of which, scurfin, is essential for normal immune homeostasis. Foxp3 is

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shown to be absent in scurfy mice and in patients with immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome. Mutations in Foxp3 gene has 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, natural killer cells (NKs) and $CD8^+$ cytotoxic T lymphocytes (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, Teffs, NKs 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 interleukin-2 (IL-2) have 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 1970s as a T cell growth factor. However, further studies in the 1990s showed that mice deficient in the gene encoding for IL-2, IL-2Rα or IL-2Rß lacked Tregs and developed severe autoimmunity (9, 12). IL-2 is a key survival factor for Tregs in the periphery, which is required for their functional competence and stability (9, 13). Administration of subcutaneous 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, which have reported promising results (16–18). Table-1 summarizes the initial human studies on low-dose IL-2 therapies in autoimmune diseases (15–17, 19–23).

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 $CD4^+IFNy^+$T helper cells (effector T cells or Teffs), and significantly improve allograft survival in mice (24).

Despite these promising results, efficacy of low-dose IL-2 therapy have been limited due to the short half-life of IL-2 (12). In order to overcome this obstacle, an IL-2 specific monoclonal antibody (mAb) has been discovered (JES6–1A12, known as JES6–1) that increases IL-2 half-life 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-2Rß), but is less crucial for binding to CD25 (IL-2Rα); this is as opposed to other 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 antibody complexes might be clinically useful for tumor immunotherapy and for expanding T cell numbers after bone marrow transplantation. On the other hand, the selective expansion of Tregs by IL-2/JES6–1 complexes (referred to as IL-2c in the rest of the text) 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 intraperitoneal 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 (1μg) mixed with 5μg of mAb. With this regimen, the frequencies of $CD25^{+}Foxp3^{+}$ 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 EAE, and induced tolerance to fully major histocompatibility complex (MHC)–incompatible pancreatic islet cells in the absence of immunosuppression, leading to the majority of grafts being accepted indefinitely (28). Effect of IL-2c treatment has 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 of daily intraperitoneal injections) starting 3 days prior to transplantation until 1 week after grafting followed by twice weekly injections up to 6 weeks post-transplantation increases Treg frequencies, 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 to 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 more effective in preventing 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 mic and significantly delays skin rejection, an effect that was not observed using one of the two molecules injected alone (27), possibly due to concomitant expansion of Tregs and Teff 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 strategy 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 T-cell receptor (TCR)-induced translocation of nuclear factor of activated T cells (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 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 effector T-cell proliferation. However, the addition of IL-2c to tacrolimus therapy rescued the Treg phenotype and 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 co-stimulatory 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 Tregs 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 contrary to the original speculations, 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 regulatory T cell 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 (HSCT) (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 subcutaneous IL-2 with maximum tolerable dose of 1 million IU/m^2 of body surface area daily for 8 weeks, which could be repeated after a 4-week hiatus. Amelioration 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. subcutaneous injections of low-dose IL-2 (1 million IU/m^2) daily for 14 days followed by a 14-day hiatus (37), or 0.1–0.2 million IU/m^2 3 times per week for days 0 to 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 HCV-induced vasculitis, administration of 1.5–3 million IU/day of IL-2 for a total of 10 days 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 type 1 diabetes. 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. in 2013, aiming to determine the lowest active dose of IL-2 that could safely expand and activate Tregs in patients with established T1D (16). This study showed that daily subcutaneous injection of 0.33–1 million IU/day IL-2 for 5 consecutive days effectively expanded Treg cells in a dose-dependent manner with minimal effects on Teffs or NK cells. At higher doses (3 million IU/day), 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 dose range of 0.33–1 million IU/day, by which Treg cells 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).

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 effector 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, same group performed a combined Phase I/IIa trial addressing the safety, tolerability, clinical efficacy, and immunological responses of a repetitive and cyclic, subcutaneously applied low-dose IL-2 in patients with active and refractory SLE (PRO-IMMUN). The regimen consists of four treatment cycles, each consisting of daily subcutaneous injections of rhIL-2 (aldesleukin) at single doses of 0.75, 1.5, and 3.0 million IU on 5 consecutive days separated by washout periods of 9–16 days (43). Similarly, in a case series of five patients with refractory SLE, it has been shown that daily subcutaneous injections of 1.5 million IU rhIL-2 for five consecutive days selectively corrected Treg functional defects in vivo (17).

In another study, 38 patients with SLE received three cycles of rhIL-2 administered subcutaneously at a dose of 1 million IU every other day for 2 weeks, followed by a 2-week break in treatment. Treatment with low-dose recombinant human IL-2 selectively enhanced Tregs and decreased numbers of follicular helper T cells and Th17 cells, but not Th1 or T helper 2 (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 effector to regulatory T cell 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 subcutaneously inject 1 million IU/day IL-2 for 5 days and then once every 2 weeks for 6 months. In all of these conditions, Tregs were successfully expanded without any effect on Teff, 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 half-life 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 (MS), experimental autoimmune encephalitis (EAE), pretreating the mice for 3 days with IL2c led only to mild neurological symptoms of EAE. In order 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 days, 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. Whereas, 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 Izquerdo et al. using non-obese diabetic (NOD) mice, treatment with Il-2c was associated with expansion of both polyclonal and antigen-specific $Foxp3^+$ Tregs. IL-2c therapy also expanded antigen-specific $Foxp3^-$ IL-10 producing T cells that persisted over prolonged periods of time, leading to complete prevention of diabetes and minimal islet infiltration (45). 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 antibodies and immunoglobulin 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 effective in a mouse model of rheumatoid arthritis (RA), where 3 consecutive daily intraperitoneal 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 monoclonal antibodies 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 (CKD) have significantly lower frequencies of peripheral Tregs than that 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 regulatory T cells and expression of Foxp3 mRNA in CKD 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 randomised to receive subcutaneous doses of either IL-2 (aldesleukin; dose range 0.3–3 million IU) or placebo once daily, for five consecutive days. 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 an murine model of renal ischemia reperfusion injury (IRI), 3 daily doses of IL-2c from 5 days before induction of injury successfully expanded Tregs, 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, 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 intraperitoneal 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 antibody), for 5 consecutive days before induction of the TRALI prevented the onset of edema, reduced pulmonary protein levels, and pro-inflammatory 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 type-1 diabetes, systemic lupus erythematosus, and ischemic heart disease. Dose-finding trials have been performed in patients with type-1 diabetes to optimize the dose and frequency of administration of IL-2 to maximize its effects on Tregs without expansion of effector T cells or natural killer cells (16). One existing challenge is the short half-life 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 effector T cell 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 antibody has been identified, which inhibits the effector T cell responses to IL-2 without blocking Treg pSTAT5 pathway (56). This is the first strong evidence for a human anti-IL-2 antibody 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, which preferentially binds and activates cells expressing high levels of the IL-2Rαβγ receptor, 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 alpha chain (57). Another approach is to decrease IL-2 affinity to the beta chain to reduce the ability of IL-2 to activate IL-2 receptors present on $CD4^+$ and $CD8^+$ effector T cells 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 half-life and enhance its avidity to Treg high-affinity IL-2Rαβγ receptors. 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 the 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.

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Table-1-

Summary of the findings of the initial studies on the use of low-dose IL-2 in the treatment of autoimmune conditions in humans. HCV= hepatitis C virus, SC= subcutaneous, Treg= regulatory T cell, Teff= effector T cell, GVHD= graft-versus-host-disease, Tcon= conventional T cell, T1D= type 1 diabetes, SLE= systemic lupus erythematosus, NK= natural killer cell.

Year	Author	Clinical setting	Route, dose, frequency of IL2 administration	Biological effect	Clinical outcome	Side effects
2011	Saadoun et al. (19)	HCV-induced vasculitits	SC, 1 million IU per day for 5 days, followed by three 5-day courses of 3 million IU per day 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	SC, 0.3, 1 or 3 million IU per square meter of body-surface area, daily for 8 weeks, followed by a 4-week hiatus (extended treatment if response observed)	• Increased $CD4^{+}Foxp3^{+}$Tregs with a peak median value at 4 weeks (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 amelioration of the manifestations of chronic GVHD	• Maximum tolerated dose: 1 million IU per square meter • Highest dose induced severe constitutional symptoms
2013	Hartemann et al. (16)	Insulin-dependent T1D	SC, 0.33, 1 or 3 million IU per day, for a 5-day course, followed for 60 days	A dose-dependent increase in Tregs (significant at all doses)	IL2 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	SC, 1.5 million IU/d for 5 days, followed by three 5-day courses of 3 million IU/d at weeks 3, 6, and 9	Notable increase in Treg cell count in 4 of 5 patients at the end of the treatment compared with baseline	Partial regrowth achieved in 4 of 5 patients	No serious adverse event was reported
2015	Rosenzwajg et al. (21)	T1D	SC, 0.33, 1 or 3 million IU, daily for 5 days • A dose finding trial to define safety and immunological responses	• Expands and activates Tregs at 0.33 and 1 million IU/day without effects on Teffs or NK • Greater expansion of Tregs at the dose of 3 million IU/day but with NK expansion, cytokine/chemokine increase • A clear shift of the peripheral blood immune environment towards 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 dose (3 million IU/day)
2015	Humrich et al. (22)	SLE • A 36-year-old female with SLE and high disease activity	SC, 1.5 or 3 million or 3.0 million IU of IL-2, daily for five consecutive days, 4 treatment cycles, separated by washout periods of 9–16 days and followed by a 9-week follow-up period	• Remarkable $CD25^{+}Foxp3^{+}CD127^{lo}$ Treg expansion • Decreased serum levels of anti-dsDNA-antibodies • 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 subcutaneous low-dose IL-2 in SLE	• Well-tolerated • Mild and transient adverse effects: erythema at the injection site, increased day and night sweats, and one episode of fever

Year	Author	Clinical setting	Route, dose, frequency of IL2 administration	Biological effect	Clinical outcome	Side effects
					• A successful biological treatment strategy	
2016	Todd et al. (23)	T1D	• 40 participants with T1D • Optimal doses of aldesleukin to induce 10% (minimal) and 20% (maximal) increases in Tregs were 0.101 and 0.497 million IU/m^2	• Optimal IL-2 dose that increased Treg frequencies raised by 10% and 20% were defined • Desensitisation of Tregs can occur in some patients receiving daily doses of • Aldesleukin of 1.0 million IU/m2 or more • Following treatment Tregs had a decreased sensitivity to IL-2 that returned to baseline on day 3 after treatment	Not looked at	• Single dose: small self-limiting injection site reaction • Two episodes of rhinitis possibly related to drug administration • Transient lymphopenia • Transient eosinophilia
2016	Spee-Mayer et al. (17)	Refractory SLE	SC, 1.5 million IU, daily for five consecutive days	• 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