

# Promoting Immune Regulation in Type 1 Diabetes Using Low-Dose Interleukin-2

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**Abstract** Dysregulation of the immune system contributes to the breakdown of immune regulation, leading to autoimmune diseases, such as type 1 diabetes (T1D). Current therapies for T1D include daily insulin, due to pancreatic  $\beta$ -cell destruction to maintain blood glucose levels, suppressive immunotherapy to decrease the symptoms associated with autoimmunity, and islet transplantation. Genetic risks for T1D have been linked to IL-2 and IL-2R signaling pathways that lead to the breakdown of self-tolerance mechanisms, primarily through altered regulatory T cell (Treg) function and homeostasis. In attempt to correct such deficits, therapeutic administration of IL-2 at low doses has gained attention due to the capacity to boost Tregs without the unwanted stimulation of effector T cells. Preclinical and clinical studies utilizing low-dose IL-2 have shown promising results to expand Tregs due to their high selective sensitivity to respond to IL-2. These results suggest that low-dose IL-2 therapy represents a new class of immunotherapy for T1D by promoting immune regulation rather than

broadly suppressing unwanted and beneficial immune responses.

**Keywords** Type 1 diabetes · IL-2 · Tregs · Low-dose IL-2 therapy · IL-2 receptor · Tolerance

## Introduction

Type 1 diabetes (T1D) is an autoimmune disorder primarily mediated by the adaptive immune response against several islet cell autoantigens, which eventually leads to the destruction of pancreatic  $\beta$  cells and in turn severe insulin deficiency [1–3]. Individuals with this disorder must take insulin daily to maintain normal blood glucose levels. Decades of research have led to improved management of T1D but there is still no cure. In this review, we will discuss how the IL-2 receptor (IL-2R) may represent a therapeutic target for controlling islet autoimmunity and restoring self-tolerance in patients with T1D, which in turn would preserve a functional mass of pancreatic  $\beta$  cells. IL-2 not only causes proliferation of regulatory T cells (Tregs) but may compensate for a genetic defect associated with T1D, as single nucleotide polymorphisms (SNPs) in *IL2RA* represent a genetic risk [4]. A completed clinical trial has demonstrated the safety of administering IL-2 to T1D patients and defined a low dose range at which IL-2 increases Tregs without reactivating autoreactive T cells and in general effector T cells [5•]. Patients with recent onset T1D are now being enrolled in a low-dose IL-2 phase 2 clinical trial to test the efficacy of this therapy (NTC02411253). Before describing ongoing work concerning low-dose IL-2 in the clinic, we will first briefly discuss our current understanding of the function of IL-2 in the immune system and how altered activity of the IL-2 pathway contributes to T1D.

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## The Role of Interleukin-2 in Tregs

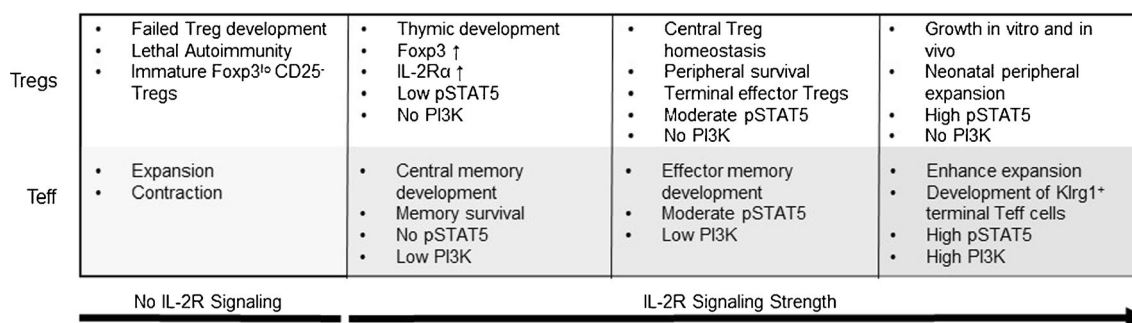
IL-2R signaling plays a non-redundant role for the development of CD4<sup>+</sup> Foxp3<sup>+</sup> Tregs and importantly contributes to Treg homeostasis [6–8]. IL-2<sup>-/-</sup>, IL-2Rα<sup>-/-</sup>, and IL-2Rβ<sup>-/-</sup> mice are characterized by severe systemic autoimmunity and lymphoproliferation that rapidly leads to the death of these mice [9–11]. Our laboratory showed nearly 14 years ago that the transfer of purified Tregs was sufficient to fully protect IL-2-sufficient IL-2Rβ<sup>-/-</sup> mice from disease [12]. This work and that of others directly demonstrated that impaired Tregs were the fundamental reason for lethal autoimmunity in these mice [13, 14]. In the complete absence of IL-2R signaling, mice harbor a low proportion of non-functional immature CD4<sup>+</sup> CD25<sup>-</sup> Foxp3<sup>lo</sup> cells [15]. During thymic development, IL-2 is a necessary second signal after TCR signaling that upregulates CD25 and Foxp3 in developing Tregs by a STAT5-mediated mechanism, leading to functional Tregs [16–19]. Under the appropriate conditions, conventional peripheral T cells can also develop into suppressive Tregs. These induced or peripheral Tregs, similar to thymic-derived Tregs, also require IL-2 for development in addition to retinoic acid and TGFβ to upregulate Foxp3 [20–22]. This pathway may also counteract RORγt expression and production of IL-17 to reinforce Treg suppressive function [22].

In the periphery, IL-2 regulates a number of activities in Tregs. During the neonatal period, blockade of IL-2 severely affects the initial peripheral amplification of Tregs [23], whereas interfering with IL-2R signaling in adult mice lowers the numbers of Tregs, but many Tregs are still detected [24, 25]. IL-2 mediates Treg homeostasis primarily by STAT5 activation of cell cycle progression and promoting expression of cell survival molecules such as Bcl-2 and Mcl-1 [26, 27]. Recent work suggests that IL-2 is most critical for the homeostasis of central Tregs, those Tregs that are CCR7<sup>+</sup> and CD62L<sup>hi</sup> and primarily reside in secondary lymphoid tissues [28•]. The homeostasis of activated or effector Tregs, e.g., CCR7<sup>-</sup>, CD62L<sup>lo</sup>, ICOS<sup>hi</sup> Tregs, is much more dependent on TCR signaling [29, 30]. However, the development of activated, terminally differentiated Tregs, characterized by

expression of Klrp1 and high levels of several key Treg suppressive molecules, also depends on IL-2 [31•].

Another important property of IL-2 in the periphery is to reinforce the expression of Foxp3 [32]. This function is mediated by direct IL-2-dependent STAT5 binding to regulatory regions within the promoter and conserved noncoding sequence 2 (CNS2) of *Foxp3* [33]. Foxp3 directly represses key genes related to T effector (Teff) cells, e.g., IL-2 and IL-17, and upregulates the expression of several Treg suppressive molecules, e.g., CTLA-4, IL-10, and IL-35 [34, 35]. Moreover, Foxp3 and IL-2 are in a positive regulatory loop through direct effects of *Foxp3* and STAT5 to upregulate CD25 expression. Besides effects on *Foxp3*, IL-2 may directly regulate some aspects of the Treg suppressive program. For example, a mechanism by which Tregs suppress autoimmunity involves perforin/granzyme B cytotoxicity, and both are partially regulated by IL-2-dependent STAT5 activation [36–38]. Besides activating mechanisms to promote Treg suppressive function, the IL-2R pathway passively promotes suppression of autoreactive T cells due to the high level of CD25 expression and the high affinity IL-2R on Treg cells. This property promotes Tregs to preferentially consume IL-2 and sequester IL-2 from autoreactive T cells [39, 40].

A very important aspect of Treg immunobiology uncovered by our laboratory is that Treg development and homeostasis are effectively supported with low levels of IL-2R signaling whereas Teff responses require more extensive signaling [26, 41] (Fig. 1). This conclusion stems from studies of genetically engineered mice in which T cells expressed mutated IL-2Rβ cytoplasmic tails resulting in lowered IL-2-dependent pSTAT5 and PI3K activation. This study also established that a gradient of IL-2R signaling impacts Treg cells. Some fundamental Treg properties, such as regulation of Foxp3, CD25, and CTLA-4 expression, are supported by low IL-2R signaling whereas other functions such as development of Klrp1<sup>+</sup> effector Tregs and expression of some Treg functional molecules, e.g., IL-10 and granzyme B, require more extensive IL-2R signal transduction. Importantly, these findings provide strong conceptual support for using low levels of IL-2 to selectively stimulate Tregs.



**Fig. 1** Varied IL-2R signaling strength leads to distinct outcome in Treg and Teff cells

## The Role of IL-2 in Teff Cells

IL-2 is also an important cytokine for mounting an optimal immune response. Recent work, however, shows that substantial T cell expansion and contraction occurs even in the absence of IL-2 [42]. Nevertheless, IL-2 promotes more robust T cell growth in vivo and sensitizes cells for apoptosis, which also facilitates contraction of an immune response [43]. IL-2 provides critical signals for the development of several key Teff cell functions and promotes Th1 cells by positive regulation of IFN $\gamma$ , IL-12R $\beta$ 2 and T-bet [44, 45]. Th2 differentiation is promoted by IL-2 through positive regulation of IL-4 and IL-4R $\alpha$  via STAT5-mediated mechanisms [46, 47]. Moreover, IL-2 is critical for development of cytotoxic T lymphocytes (CTLs) through regulation of granzyme B and perforin [38] by promoting the development of terminally differentiated CTLs through strong IL-2 signaling [48–50]. In striking contrast, the development of Th17 and Tfh subsets is favored in the absence of IL-2 [51, 52]. IL-2R signaling constrains Th17 development by activating STAT5 that directly competes with STAT3 for binding to regulatory regions in *IL-17* [53]. Tfh development is also constrained by IL-2R signaling through STAT5-dependent activation of Blimp-1, which represses Bcl-6 that is necessary for Tfh development [51]. Thus, IL-2 therapy may promote tolerance not only through its effects of enhancing Tregs but also by lowering Th17 and Tfh activity.

IL-2 provides important signals for optimal T memory responses and acts during the primary response to promote a strong memory recall response [54]. The development of central memory CD8<sup>+</sup> T cells are readily supported by low IL-2R $\beta$  signaling whereas the development effector and effector/memory CD8<sup>+</sup> T cells require more intensive IL-2R signaling [41]. IL-2 also impacts the survival of CD4<sup>+</sup> T memory cells through the upregulation of IL-7R $\alpha$  [55, 56]. Overall, just like for Tregs, IL-2 critically impacts the Teff compartment. However, most of these activities in Teff cells depend on persistent and intense IL-2R signaling (Fig. 1). Thus, at a proper low dose, IL-2 is expected to primarily affect the Treg compartment.

## IL2/IL2RA as a Genetic Risk for T1D

The non-obese diabetic (NOD) mouse model has many properties that are similar to human disease, including key components of the genetic susceptibility. Among the insulin-dependent diabetes (Idd) risk loci identified in the NOD mouse, the Idd3 region, which encodes *IL2* and *IL21*, is a major contributor to diabetes susceptibility [57–59]. Lower IL-2 levels represent an important aspect by which Idd3 contributes to diabetes. In fact, further lowering IL-2 activity in female NOD mice by neutralization with anti-IL-2 accelerates the onset of diabetes [25]. Congenic NOD mice that contain the *Idd3* interval from diabetes-free C57BL/6 mice, i.e.,

NOD<sup>B6Idd3</sup> mice, are largely protected from diabetes and exhibit reduced insulinitis [60]. These protective effects are due to approximately twofold increased IL-2 production when compared to NOD mice [61] that leads to improved Treg function and reduced pro-inflammatory cytokine production [61–63].

The first and most prominent genetic risk factor for human T1D is localized in the HLA complex with odds ratio values close to 7. The HLA complex is a multigene risk locus in which the most important genes are polymorphic variants of HLA class I and class II antigen-presenting molecules [64]. Underscoring the complexity of T1D, more than 50 loci have now been identified as genetic risks for T1D development [65–67], most individually providing small contributions to risk (odds ratio below 1.5). Among these, the four non-HLA genetic polymorphisms with stronger association (odds ratios in the 1.5–2.5 range) have been mapped to *CTLA4* [68], *INS* [69], *PTPN22*, and *IL2RA* [4, 65]. Statistically, each of these loci individually represents a small risk toward developing T1D. Furthermore, individuals express distinct patterns of these risk loci. However, by targeting important pathways that regulate the immune system, the cumulative biological effects of disease-related genetic polymorphisms in conjunction with environmental factors may trigger T1D. So far, the precise biological contributions of these genetic risks have been difficult to precisely define.

Genome-wide association studies (GWAS) and tag-SNPs have identified key SNPs associated with T1D and sometimes have defined variation in transcription or protein function. Using tag-SNP technology, 54 SNPs were identified near the regulatory regions or exons of *IL2RA* [70], initially implicating *IL2RA* as a genetic risk for T1D. GWAS established two specific SNPs (ss52580101 and ss52580109) in the region containing intron 1 of *IL2RA* and the 5' upstream regions of *IL2RA* and *RBM17* that specifically correlate with T1D disease susceptibility [4]. However, these two SNPs are not associated with eight regions known to control *IL2RA* transcription [4]. Susceptible SNP ss52580101, however, has been linked to reduced serum concentrations of soluble IL-2R $\alpha$  in patients with T1D, consistent with individuals with fewer IL-2R $\alpha$ <sup>+</sup> T cells, i.e., Tregs or Teff cells. Indeed, individuals with the *IL2RA* T1D susceptible SNPs have reduced CD25 expression on Tregs and T memory cells and reduced IL-2-induced tyrosine phosphorylated STAT5 (pSTAT5) activity [71]. Overall, lower IL-2R signaling in Tregs leads to decreased Foxp3 expression and impaired Treg function [71, 72]. Thus, low-dose IL-2 therapy has the potential to selectively correct potential genetic defects associated with altered IL-2R signaling in Tregs.

## IL-2 in the Therapy of Autoimmunity

We still lack a robust therapy for islet autoimmunity, to prevent progression to diabetes symptoms or to preserve residual  $\beta$ -cell mass after diagnosis. Current treatments focus on targeting

symptoms or consequences of the disease, from daily injections of insulin, use of immunosuppressive drugs, strategies to deplete autoreactive T cells [73–76], islet transplantation to repair disease tissues [77, 78], and the use of biologics to suppress the inflammatory response [79–81]. There is much interest in providing [82, 83] or boosting Tregs in patients with T1D as this may induce immunoregulation by restoring tolerance and leave beneficial aspects of the immune system intact that protect one from infectious disease [65, 84]. Based on the studies discussed above, IL-2 represents a prime candidate to mediate these effects as it can directly increase Tregs. Accordingly, IL-2 may reestablish impaired immune regulation and may correct impaired Treg activities associated with the IL-2/IL-2R genetic risk in this disease.

IL-2 has been an approved for clinical use for over 20 years with the first immunotherapeutic approaches to boost an immune responses in patients with cancer and HIV/AIDS [85–88]. In early clinical trials of renal cancer and metastatic melanoma, IL-2 was administered at high doses in attempts to maintain efficacious circulatory levels of IL-2 to boost immune responses, which was believed essential due to the relatively short half-life of IL-2 [85–87]. At such high doses, IL-2 stimulates an immune response in some patients but leads to extreme toxicities. Moreover, the accompany increase in Tregs inhibit the capacity to boost immunity in patients with cancer and HIV/AIDS. [88, 89].

Several preclinical studies raised the possibility that much lower doses of IL-2 might selectively boost Tregs while avoiding effect on Teff or T autoreactive T cells. One such study, described above, showed that low levels of IL-2R signaling were effective for the development and homeostasis of Tregs, but this low signaling did not support Teff cells [26]. More directly, administering low levels of recombinant IL-2 or in the form of agonist IL-2/anti-IL-2 complexes [90] to NOD mice increased Tregs and prevented the development of diabetes [91]. This type of treatment was also effective in NOD mice with recent onset diabetes [92]. Pancreas-targeted adeno-associated virus expressing IL-2 also effectively controlled diabetes in NOD mice [93]. Low levels of IL-2 have also been shown to increase Tregs, prolong allogenic islet allografts, and suppress disease in mouse models of experimental autoimmune encephalomyelitis (EAE), systemic lupus erythematosus (SLE), and muscular dystrophy [94–97].

Given the promise of low-dose IL-2 therapy in these pre-clinical studies, the focus has now shifted to the utilization of IL-2 in phase I/II clinical trials for autoimmune diseases. The first reports of the use of low levels of IL-2 in patients with overactive immune responses were in chronic graft versus host disease (GvHD) and hepatitis c virus (HCV) induced vasculitis [98•, 99•]. Patients suffering with chronic GvHD received IL-2 s.c. at  $0.3 \times 10^6$ ,  $1 \times 10^6$ ,  $3 \times 10^6$  IU/m<sup>2</sup> of body surface area daily for 8 weeks. For HCV, IL-2 was administered s.c. at  $1.5 \times 10^6$  IU/day for 5 days followed by three

additional applications of IL-2 at  $3 \times 10^6$  IU/day for 5 days at 3-week intervals. These levels of IL-2 were substantially lower than those given to patients with cancer and HIV/AIDS, where the goal was to boost immunity. Evaluation of these patients revealed that low-dose IL-2 was safe, and the highest tolerated dose was  $1 \times 10^6$  IU/m<sup>2</sup> in the GvHD study. Importantly, both studies found no indication that self-reactive T cells were activated. These levels of IL-2 led to sustained Treg expansion with clinical improvement of disease manifestations in many patients. The one off-target effect was an increase in CD56<sup>bright</sup> NK cells that was more prominent at the higher doses of IL-2.

There have been several additional reports using low-dose IL-2 in a limited number of patients with alopecia areata, SLE, and acute GvHD [98•, 100, 101]. Dosing of IL-2 in these studies ranged from  $2 \times 10^5$  to  $3 \times 10^6$  IU s.c. per injection. The frequency of administering IL-2 varied in these studies, but generally several injections were administered closely spaced, e.g., daily or every other day, followed by a rest period and repeat administration of IL-2. Again, Treg levels increased in all patients, and clinical improvement was noted in approximately 80 % of the patients. The main adverse reaction was inflammation at the injection sites; importantly, grade 3 or 4 toxicities were not seen. When low-dose IL-2 was administered to patients undergoing allogeneic hematopoietic stem cells transplants, no patients developed grades 2–4 acute GvHD, whereas 12 % of the patients in the control group developed severe acute GvHD. Low-dose IL-2 in SLE led to amelioration of clinical symptoms and decrease in disease-associated autoantibodies. The results with alopecia areata were particularly impressive in that before IL-2 administration skin biopsies revealed numerous CD4<sup>+</sup> and CD8<sup>+</sup> T cells, with few Tregs. After IL-2 treatment, inflammatory infiltrates were markedly reduced, and Tregs were now readily detected as Foxp3-positive cells. Remarkably, significant hair regrowth was also noted. Thus, all these studies point to the potential benefit of low-dose IL-2 to inhibit pathogenic effects mediated by self-reactive T cells through improved immune regulation via enhanced Treg function.

### Low-Dose IL-2 Therapy in T1D

Substantial efforts are now under way to test low-dose IL-2 in patients with T1D. The rationale for these efforts, as discussed above, is that Tregs are impaired in T1D, and this therapy targets a known genetic risk in T1D. Thus, expanding Tregs may restore tolerance mechanisms and preserve this residual islet activity to benefit the health of these patients. A phase I/II dose-limiting study aimed at establishing an optimal IL-2 therapeutic dose has already been completed. Twenty-four participants were randomly assigned to placebo or IL-2 groups where they received  $0.33 \times 10^6$ ,  $1 \times 10^6$ , or  $3 \times 10^6$  IU/day for five consecutive days. The participants were monitored for

60 days [5••]. No participants exhibited severe adverse effects, but an injection-site reaction was often seen. A dose-dependent increase in Tregs was seen in all participants with minimal NK cell expansion, especially when using  $1 \times 10^6$  IU of IL-2 or lower. Importantly, the IL-2-treated participants did not exhibit any detrimental changes in glucose metabolism, supporting the safety of using these levels of IL-2 in participants with T1D.

More detailed examination of samples from this trial [102] showed that all participants with T1D treated with low-dose IL-2 trial upregulated CD25 and Foxp3 on Tregs but not on CD4<sup>+</sup> T effector memory (T<sub>EM</sub>) cells. This is particularly relevant for CD25 as this target is substantially upregulated by IL-2 in Tregs and T<sub>EM</sub>, although T<sub>EM</sub> depends on a higher amount of IL-2. Importantly, low-dose IL-2 selectively induces pSTAT5 signaling in Treg cells *ex vivo*. Proportions of Tregs increased and remained elevated in patients given IL-2 at 1 or  $3 \times 10^6$  IU at 60 days post-treatment, but these levels were lower than detected immediately after the end of IL-2 administration (this was a 5-day course). Further characterization showed that expanded Tregs were largely of a CD45RO<sup>+</sup> memory phenotype with some features that suggested heightened activation. Plasma proteomics of cytokines were consistent with a shift towards a more regulatory environment. Transcriptome analysis of PBMCs showed upregulation of genes associated with cell cycle and transcription and downregulation of B cell signatures. At only the highest dose of IL-2, a NK gene signature was detected. Although IL-2 was safe at all treatments, it appears that dosing at  $1 \times 10^6$  IU of IL-2 is the higher end where Tregs are most selectively targeted with negligible effects on NK cells. However, this trial was not powered to assess effects on insulin secretion, a question that will be addressed by a clinical trial in Europe that is enrolling children and adults with recent onset T1D (NCT02411253).

We also believe that low-dose IL-2 therapy could be beneficial in patients with established T1D, beyond the immediate post-diagnosis period, as long as there is still residual insulin secretion. In support of this belief, we note that levels of stimulated C-peptide at diagnosis are only partially reduced in many patients [103–105]. While C-peptide production declines after diagnosis, it persists in many patients for several years. In a 2-year follow-up of 191 newly diagnosed patients conducted by the type 1 diabetes TrialNet, 93 % of the patients had detectable C-peptide 2 years after diagnosis, with 88 and 66 % of patients maintaining a peak stimulated C-peptide  $\geq 0.2$  nmol/L at 1 year and 2 years after onset, respectively; this level is used as entry criteria by most clinical trials in recently diagnosed patients. Emerging pathology data indicate that  $\beta$ -cell loss at diagnosis may be much less severe than previously believed [106–108]. Pathology studies also show that islet autoimmunity may persist for many years after diagnosis [109–112]. Thus, if low-dose IL-2 could simply prevent further loss of insulin secretion and maintain a  $\geq 0.2$  nmol/L C-

peptide response, this would be clinically significant because at this level of C-peptide, there is an association with lower incidence of complications [113].

### Low-Dose IL-2: Immunoregulation without a Compromised Immune Response

One concern with administration of IL-2 to stimulate a Treg response is its potential to stimulate a Teff immune response. Excess IL-2 accelerates T1D in NOD mice [91]. In a T1D study using IL-2 in combination with rapamycin, nine participants were treated orally with 2–4-mg/day rapamycin for 3 months and  $4.5 \times 10^6$  IU IL-2 three times per week for 1 month [114]. The goal in this trial was to inhibit Teff cells with rapamycin and to sensitize Teff cells to apoptosis through the action of IL-2 [115, 116]. However, a decline in insulin C-peptide levels was noted in participants with T1D that received IL-2 and rapamycin. It should be noted that this is not considered a low-dose regimen and represents 4.5-fold-higher initial and ninefold higher cumulative dose of IL-2 during the same time frame than is currently being used in ongoing efficacy trial of new onset participants with T1D (NCT02411253). Tregs also increased after IL-2/rapamycin, but glucose metabolism worsened in these participants, raising concerns that these higher levels of IL-2 may have led to a net increase in the activity of autoreactive T cells. Whether this actually explains this adverse reaction remains unclear, as rapamycin is known to cause  $\beta$  cell toxicity, leading to reduced  $\beta$  cell size, mass, proliferation, impaired insulin secretion, increased apoptosis, autophagy, and peripheral insulin resistance [117–119]. Robust preclinical data about the combined use of IL-2 with rapamycin also show that rapamycin impairs  $\beta$  cell function in NOD mice [119]. The overall experience with low-dose IL-2, however, points to its safety, including the inability to promote Teff responses at the levels currently used.

The above concerns, nevertheless, make plain that a more detailed understanding is required concerning the selectivity of human Tregs to low-dose IL-2. We quantified the levels by which IL-2 selectively activated human Tregs at initial proximal signaling and down-stream gene activation [120••]. IL-2 optimally stimulated tyrosine phosphorylated STAT5 (pSTAT5) at approximately tenfold lower levels of IL-2 than CD45RO<sup>+</sup> CD4<sup>+</sup> memory T cells. With respect to gene activation, quantitative analysis of 12 of 388 IL-2-dependent genes in human Tregs indicated that 10/12 were highly upregulated at a 100-fold lower level of IL-2 when compared to CD4<sup>+</sup> T memory cells. These included Foxp3 and CD25 which work together to reinforce the Treg suppressive program in response to therapeutically administered or endogenous IL-2. Thus, a substantial window exists in which Tregs are available to selectively respond to IL-2 during low-dose IL-2 therapy. This was shown in both healthy subjects and patients with T1D. This increase in IL-2 sensitivity was due

to in part to higher levels of IL-2R $\alpha$  and  $\gamma$ c subunits on Tregs. Moreover, PP2A activity may be higher in Tregs due to increased levels of the PP2A inhibitor, SET. This may lead to lower serine/threonine phosphorylation of IL-2R and/or associated signaling molecules, which would promote IL-2R signal transduction [121].

Another concern with boosting Tregs with low-dose IL-2 is the increased Tregs might limit beneficial immune responses. Several studies suggest that this may not be a serious problem. First, administration of low-dose IL-2 to patients undergoing an allogeneic hematopoietic stem cell transplanted showed increases in Tregs and lower instances of acute GvHD [122]. Importantly, these patients did not experience diminished anti-tumor and anti-viral responses. Second, administering  $10^{10}$  viral genomes of a recombinant adeno-associated viral (AAV) vector containing the IL-2 cDNA to NOD mice leads to continuous IL-2 production at levels that increased Tregs and protected mice from diabetes [123]. Although Tregs increased, protective immunity was elicited against influenza infection, growth of transplanted or chemically induced tumors were not accelerated, and allogeneic pregnancy was normal with regard to number of offspring and the male/female ratio. Overall, low-dose IL-2 promotes immune tolerance through selective action on Tregs while not obviously impairing immunity.

### Concluding Remarks

Boosting Tregs in patients with autoimmunity has been a major clinical objective since CD4<sup>+</sup> Foxp3<sup>+</sup> Tregs were accepted as an important population of T cells that critically maintain peripheral tolerance to self. Based on our much better understanding of the immunobiology of IL-2, new clinical approaches have been devised to take advantage of the powerful

activity of IL-2 on Tregs. The current experience with low-dose IL-2 therapy is very promising (Table 1). So far, clinical trials have been completed where 99 participants with autoimmunity or overactive immune responses have been treated with low-dose IL-2. The experience is that this therapy is safe, Tregs increase, and depending upon the trial design, therapeutic benefit has often been noted (Table 1). Nevertheless, the jury is still out on whether the promise of low-dose IL-2 therapy as an entirely new approach to treat autoimmunity, including T1D, becomes a reality. Clearly, the next key step is larger-scale efficacy trials. The research teams of David Klatzmann, John Todd and Linda Wicker, and ours are actively involved in such trials for T1D, focusing on IL-2 as a monotherapy. Thus, it will be a relatively short time before we learn whether this approach benefits patients with T1D. A major concern that will be more definitely answered in these and other trials is related to the potential of IL-2 to reactivate autoreactive T cells and worsen disease. We have defined a therapeutic window where low levels of IL-2 preferentially stimulate Tregs. This finding and the current experience in patients with low-dose IL-2 suggest that such selectivity is achievable, especially a doses of IL-2 at  $<1 \times 10^6$  IU/injection for an adult patient.

Current results indicate that IL-2 must be frequently administered at low doses to maintain increases in Tregs. Another key point, therefore, is the extent that low-dose IL-2 therapy on its own can induce robust immune tolerance after a limited course of therapy or whether chronic administration may be required. There is also increasing consensus that combination therapies may be more effective in controlling autoimmunity and supporting  $\beta$ -cell function in T1D [124]. Low-dose IL-2 may be combined with other agents, as well as with the tolerogenic administration of autoantigens to drive the specificity of Tregs. It is known that Tregs specific for a given autoantigen are much more effective in controlling the

**Table 1** Summary of completed clinical trials using low-dose IL-2 therapy

Autoimmune disease	Participants
Chronic graft vs. host disease (GvHD)	23
Hepatitis C virus-induced vasculitis	10
GvHD after allogeneic hematopoietic stem cell transplant	16
Type 1 diabetes	24
Systemic lupus erythematosus	21
Alopecia areata	5
Major clinical outcomes	
Optimal dosing: $0.3\text{--}1 \times 10^6$ IU of IL-2 s.c.	
Frequency: Daily or 5 day induction and biweekly maintenance	
Therapy well tolerated, minor injection site reaction	
Treg expansion in PBMCs of most patients	
No reactivation of self-reactive T cells	
Main off target effect: Increase in CD56 <sup>hi</sup> NK cells	
Improvement of clinical outcomes in many patients	

relevant autoimmune responses than polyclonal Treg cells and in much lower numbers [125]. Thus, what may ultimately be critical in developing robust long-lasting tolerance is induction of autoantigen specific Tregs where low-dose IL-2 may help to increase their numbers. A clinical trial in patients with T1D, who by necessity immunize themselves with a key autoantigen, insulin [126], on a daily basis, presents a unique opportunity to test this concept and may be relevant to other autoimmune diseases. Lastly, IL-2 exhibits poor pharmacokinetic with very short-half life in the blood, approximately 30 min. Thus, new analogs of IL-2 may improve low-dose IL-2 therapy by extending its half-life and potency to boost Tregs, perhaps as novel fusion proteins, agonist IL-2/anti-IL2 complexes or superkines, the latter to enhance selectively toward Treg.

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#### Compliance with Ethical Standards

**Conflict of Interest** Connor J. Dwyer, Natasha C. Ward, Alberto Pugliese, and Thomas R. Malek declare that they have no conflict of interest.

**Human and Animal Rights and Informed Consent** This article does not contain any studies with human or animal subjects performed by any of the authors.

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- Of importance
- Of major importance

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