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Mechanisms of action of low-dose IL-2 restoration therapies in SLE

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Interleukin-2 (IL-2) shortage is a hallmark of Systemic Lupus Erythematosus (SLE). Importantly, clinical and preclinical studies demonstrate the potential clinical benefits of IL-2-based restoration therapies for the treatment of SLE. Here we discuss the immunological consequences of IL-2 deficiency in SLE patients and the mechanisms underlying the therapeutic effects of low-dose IL-2 regimens.

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Introduction

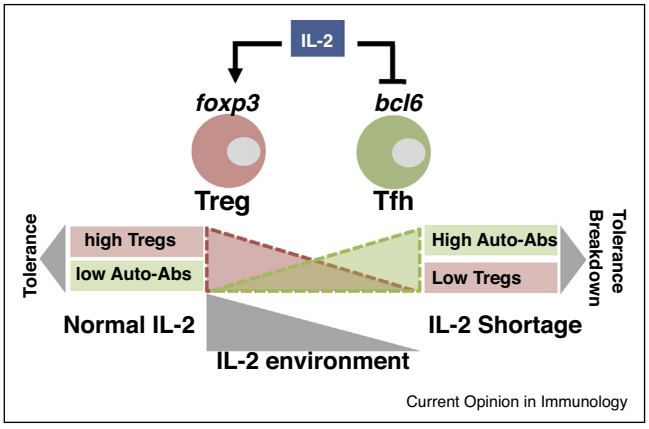
Systemic lupus erythematosus (SLE) is a multi-organ autoimmune disorder characterized by chronic immune activation, severe inflammation, and organ damage. Although multiple genetic and environmental factors synergize to promote immunopathology and tissue damage in SLE patients, cytokine deregulation plays a prominent role in tolerance breakdown by fueling innate and adaptive immune alterations that ultimately lead to disease progression [1]. Among the dysfunctional cytokine pathways observed in SLE patients, Interleukin-2 (IL-2) deficiency associates with SLE progression and immunopathology in both patients and spontaneous mouse models of lupus [2,3]. The first line of evidence suggesting a potential relationship between IL-2 scarcity and disease development is derived from early studies showing impaired IL-2 production in T cells from SLE patients [4] and lupus-prone mice [5]. Follow up studies, in which MRL/lpr mice were infected with an engineered vaccinia virus expressing the human IL-2 gene demonstrated that IL-2-treated mice survived longer, had decreased autoreactive antibody (Ab) titers, and showed reduced kidney damage [6•]. Altogether, these initial observations suggested a causative relationship between IL-2 shortage and disease development that can be corrected by IL-2 restoration. More recent studies, in which (NZBxNZW) F_1 [7] and MRL/Fas(lpr/lpr) [8] lupus-prone mice were treated with low-doses of rIL-2 confirmed these initial results, evidencing the therapeutic potential of low-dose IL-2 for the treatment of lupus.

The link between IL-2 deficiency and autoimmune disease development is not unique to lupus. For example, T cells obtained from rheumatoid arthritis (RA) patients produce less IL-2 compared with T cells from healthy donors [9]. Comparable defects have been observed in T cells from type I diabetes (T1D) patients [10] and non-obese diabetic (NOD) mice [11,12]. Similar to what was observed in lupus-prone mice [6•,7,8], treatment of NOD mice with low doses of IL-2 prevents the immune-mediated destruction of insulin-producing β cells, thereby precluding diabetes development [11–13]. Importantly, low-dose human-recombinant IL-2 (rIL-2 Aldesleukin) can be safely administered to humans and has potent immunosuppressive effects in patients with hepatitis C virus (HCV)-induced vasculitis [14] and chronic graft-versus-host disease (GVHD) [15]. Altogether, these clinical and preclinical data provide proof of concept for the current IL-2-based immunotherapies to treat SLE. In this regard, Humrich *et al.* first reported a case of a patient with active SLE that showed a significant reduction in disease activity and anti-dsDNA antibody titers after receiving four treatment cycles of low-dose rIL-2 ($1.3–3 \times 10^6$ IU) separated by 9–16 day resting periods [16••]. Similar clinical benefits were observed in a more recent study, in which thirty-eight SLE patients received three cycles of 1×10^6 units of rIL-2 subcutaneously every other day for 14 days followed by a two-week washout period [17••]. Additional clinical trials to further explore the potential benefits of low-dose IL-2 in SLE are now underway (NCT03312335, NCT01988506). Here, we will focus on how IL-2 shortage contributes to tolerance breakdown in SLE and review the potential immunological mechanisms underlying the therapeutic effects observed after IL-2 supplementation (Figure 1).

IL-2R and signaling

IL-2 is a member of the common γ-chain family of cytokines that can signal through two different conformations of the IL-2 receptor (IL-2R) [18,19]. The high-affinity IL-2R is a heterotrimer formed by the combination of the α chain (CD25), the β chain (CD122), and

Figure 1



Deregulated IL-2 production impacts both Treg and Tfh cell populations.
It is well established that Tregs lose their competitive fitness and immunosuppressive capacity in a low IL-2 environment. The same conditions, however, facilitate Tfh cell development. Thus aberrant expansion of self-reactive Tfh cells together with dysfunctional Treg populations due to IL-2 shortage synergize to promote tolerance breakdown in SLE patients.

the common γ chain (CD132). Although CD25 binds to IL-2 with low affinity and rapid on-off binding kinetics, the initial CD25/IL-2 interaction leads to a conformational change in IL-2 that stabilizes a complementary binding site to CD122, thereby increasing the binding affinity of IL-2 to CD122 [18]. In contrast, the intermediate-affinity IL-2R is a βγ heterodimeric complex that lacks CD25 [18,19]. Following IL-2 ligation, phosphorylation of Janus-Activated Kinase 1 (JAK1) and 3 (JAK3), which are associated with the cytoplasmic domains of the β and γ chains, initiates a cascade of signaling events that culminate with the activation of the MAPK and PI-3K kinase pathways, and the activation and translocation into the nucleus of the Signal Transducer and Activator of Transcription 5 (STAT5).

Naïve and memory T cells constitutively express the β and γ chains, and it is only after TCR activation that they transiently upregulate CD25 to form the trimetric high-affinity IL-2R. On the contrary, the majority of FoxP3-expressing $CD4^+$ regulatory T-cells (Tregs) express high levels of CD25, which allow them to better compete for the available IL-2 [20,21]. As a consequence, while large doses of rIL-2 activate both Treg and conventional T cells, low-IL-2 regimes preferentially target IL-2 to Tregs [22,23]. The differential expression of CD25 by different T cell subsets has important therapeutic implications for the function of IL-2. For example, whereas low-dose rIL-2 treatment selectively augments pancreatic Treg numbers and prevents diabetes development in NOD mice, high IL-2 dose enhances pathogenic effector T cell responses and accelerates the onset of disease [13]. In fact, based on its capacity to boost effector T cell responses when administered at high doses, IL-2 has been used as an antitumor agent [24]. In this regard, a variety of studies have shown that treatment with high doses of recombinant IL-2 enhances anti-tumor T cell responses *in vivo*, thereby promoting tumor regression [24]. As a consequence, in 1992, the U.S. Food and Drug Administration (FDA) approved the use of high-dose bolus of human recombinant IL-2 for the treatment of patients with metastatic renal cancer. In 1998, high-dose rIL-2 therapy was approved for patients with metastatic melanoma. Unfortunately, the overall efficacy of IL-2-based immunotherapies in cancer is lower than initially expected [24], most likely due to the simultaneous expansion of Tregs. Furthermore, when administered at high doses, IL-2 induces systemic toxicity, which compromises the clinical applicability of high-dose rIL-2-based immunotherapies [24,25]. Thus, the dose-dependent effects of IL-2 need to be taken into consideration when considering IL-2-based regimens for the treatment of SLE.

IL-2 shortage in SLE

It is well established that deficient IL-2 production from T cells is a hallmark of human and murine SLE. While the exact mechanisms underlying the IL-2 shortage in SLE patients are still unclear, changes in the expression of regulatory elements implicated in the control of the *Il2* locus, such as NF-κB, PP2A, NFAT, SRSF1, AP-1, CREB, and the microRNA (miR)-200a-3, have been associated with diminished IL-2 transcription in SLE patients [26–29]. In addition to intrinsic signaling defects, work from Dai *et al.* shows that IL-23, which is increased in the serum of SLE patients, limits IL-2 production [30], suggesting that alterations in the cytokine milieu of SLE patients may also impact the capacity of T cells produce IL-2. Supporting this conclusion, IL-23R deficiency restores IL-2 production in *lpr/lpr* mice, which correlates with decreased production of anti-dsDNA antibodies and nephritis in these animals [30,31].

Recent data from Tsokos' laboratory demonstrate that SLE patients also weakly respond to IL-2, even when IL-2 is administered exogenously [32•]. These findings indicate that in addition to low-IL-2 production, low IL-2 responsiveness could also contribute to deficient IL-2 signaling in SLE patients. Interestingly, studies done in the context of viral infections suggest that IL-6 negatively regulates IL-2R expression [33]. Given that IL-6 is increased in the serum of SLE patients [34], it is possible that high IL-6 signaling in SLE patients contributes to maintaining IL-2 hypo-responsiveness by inhibiting the expression of the IL-2R. Elevated levels of a circulating form of soluble CD25 observed in SLE patients could also preclude IL-2 responsiveness by competing with membrane-bound CD25 for IL-2 binding, thereby preventing IL-2/IL-2R interactions [35]. Thus, the combination of multiple and overlapping mechanisms are likely to be responsible for IL-2 deficiency and hyporesponsiveness in SLE patients.

IL-2 deficiency and Tregs

IL-2 is critical for the normal development and function of Tregs, an immunosuppressive population of $CD4^+$ T cells [36,37,38]. Mechanistically, binding of STAT5 to the *Foxp3* locus in response to IL-2 directly promotes FoxP3 expression, the master regulator of Treg differentiation [36,39]. In addition to supporting the initial upregulation of FoxP3, sustained IL-2 signaling is required for the long-term maintenance and the suppressive activity of Tregs in the periphery [37,38]. Evidencing the critical role of IL-2 in Treg homeostasis and immune tolerance, IL-2 and IL-2R-deficient mice develop life-threatening autoimmune pathology due to the lack of functional Tregs [40,41]. Considering these data, it is generally assumed that the primary mechanism by which IL-2 shortage contributes to the loss of tolerance and immunopathology in SLE patients is by disrupting Treg homeostasis [22,42,43]. Supporting this view, while no apparent differences in the immunosuppressive function of Treg are detected, Treg numbers gradually decline in lupus-prone mice as the disease progresses [7,44,45]. Importantly, the gradual decay in Treg numbers observed in these mice can be prevented by low-dose rIL-2 administration, which also results in improved disease [7]. Together, these data provide strong evidence for a causal link between IL-2 shortage, Treg disruption, and subsequent disease progression that can be ultimately corrected by IL-2 supplementation.

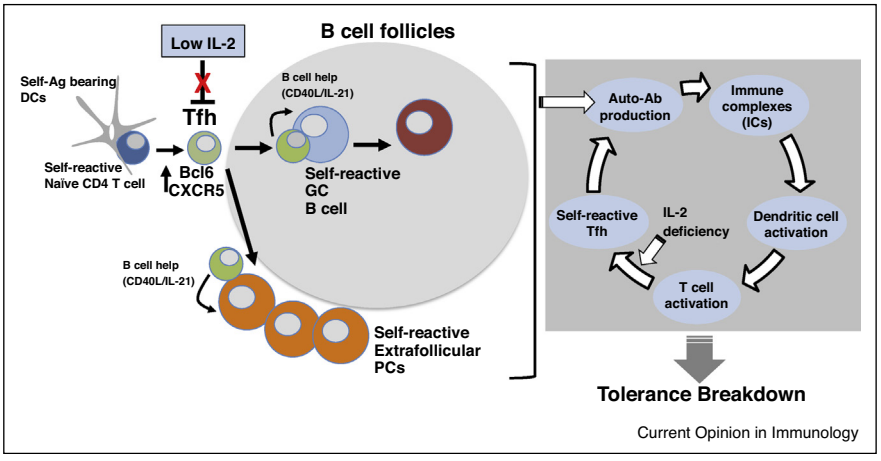
Despite the preclinical observations suggesting a link between Treg disturbances and disease activity in lupus-prone mice, conflicting data have been reported when analyzing Tregs in SLE patients [45]. Some studies suggest that SLE patients have lower numbers of Tregs compared to healthy controls [45]. Other studies show, however, average or even increased frequencies of Tregs [45,46]. Additionally, whereas Tregs from SLE patients with active disease do show changes in specific phenotypic markers, such as reduced CD25 and CD27 expression, or CCR6 upregulation, no alterations have been detected regarding their immunosuppressive function [42,46]. Thus, whether Tregs from SLE patients are dysfunctional still needs to be formally demonstrated. Importantly, however, three independent clinical studies in SLE suggest an association between augmented Treg numbers and decreased disease activity after low-dose IL-2 treatment [16••,17••,42]. The association between low-dose IL-2 administration, Treg expansion, and reduced immunopathology has been shown in other forms of autoimmune disorders, including type I diabetes (T1D) [11,47], HCV-induced vasculitis [14], alopecia areata [48], or GVHD [15]. Thus, these studies indicate a causal correlation between the IL-2-dependent expansion of Tregs and the clinical benefits observed following low-dose IL-2 treatment. Given the critical role of Tregs in immune tolerance is commonly accepted that low-dose IL-2 restoration therapy prevents immunopathology through promoting the expansion or immunosuppressive function of Tregs.

Low-dose IL-2 and Tfh cells

Autoreactive Abs secreted by autoreactive plasma cells (PCs) are primary drivers of the disease pathogenesis observed in SLE patients [49]. Autoreactive PCs can be derived from both the germinal center (GCs) or through the extrafollicular pathway, both of which require survival and co-stimulatory signals provided by $CD4^+$ T follicular helper (Tfh) cells [49,50]. In agreement with this, increased numbers of self-reactive Tfh cells correlate with elevated titers of autoreactive Abs and disease severity in mice and SLE patients [49,50]. Conversely, preclinical studies show that the blockade of Tfh cell activity prevents autoreactive-Ab production and disease progression in lupus-prone mice, indicating a direct correlation between Tfh cell expansion and Ab-mediated pathology [51]. Importantly, IL-2 signaling inhibits Bcl6 expression, the master regulator of Tfh cell differentiation [52,53]. As a consequence, Tfh cells fail to differentiate in the presence of high levels of IL-2 [21,54•]. Mechanistically, IL-2/STAT5 signaling intrinsically inhibits Tfh cell responses by inducing the expression of Blimp-1, the reciprocal antagonist of Bcl6, and by favoring the STAT5-dependent recruitment of transcriptional repressors to the *bcl6* locus [52,53]. Collectively, these studies indicate a critical role for IL-2 as an inhibitor of Tfh cell differentiation. Supporting this view, mice and human studies show that IL-2 deprivation facilitates Tfh cell development [21,52,53,54•,55]. Given the role of Tfh cells promoting Ab-mediated pathology [49,50,56], it is reasonable to speculate that one of the mechanisms, by which a low-IL-2 environment in SLE patients contributes to disease progression is by favoring the development of self-reactive Tfh cells (Figure 2).

Importantly, data from our laboratory demonstrate that Tfh cell responses can be exogenously manipulated by changing the physiological availability of IL-2 [21,54•,57,58]. In this regard, we have shown that low-dose rIL-2 treatment after influenza infection prevents the development of Tfh cells [54•], thereby preventing influenza-specific Ab responses. Notably, the capacity of IL-2 to prevent Tfh cell differentiation is independent of the presence of Tregs [21,54•]. A recent clinical trial also analyzed the effect of low-dose rIL-2 administration on Tfh cells from a cohort of 40 active SLE patients [17••]. In this work, He *et al.* demonstrate that, in addition to boosting Tregs, IL-2 treatment results in reduced frequencies of Tfh cells, which were associated with significantly decreased disease activity. Interestingly, in the same study, the authors treated ovalbumin-immunized mice with different doses of rIL-2 and found that, at the lowest dose (1×10^4 Units), IL-2 had no significant effects on Tregs but prevented Tfh and GC center formation. These data suggest that the minimum

Figure 2



Tfh cells promote self-reactive plasma cell responses.
Unwanted expansion of self-reactive Tfh cells in a low IL-2 environment helps the development of follicular and extrafollicular self-reactive plasma cells. Auto-Ab production by PCs favors the formation of self-Ag/IgG immune complexes that activate Fc-expressing cells, including dendritic cells, thereby initiating a positive feedback loop that ultimately leads to tolerance breakdown.

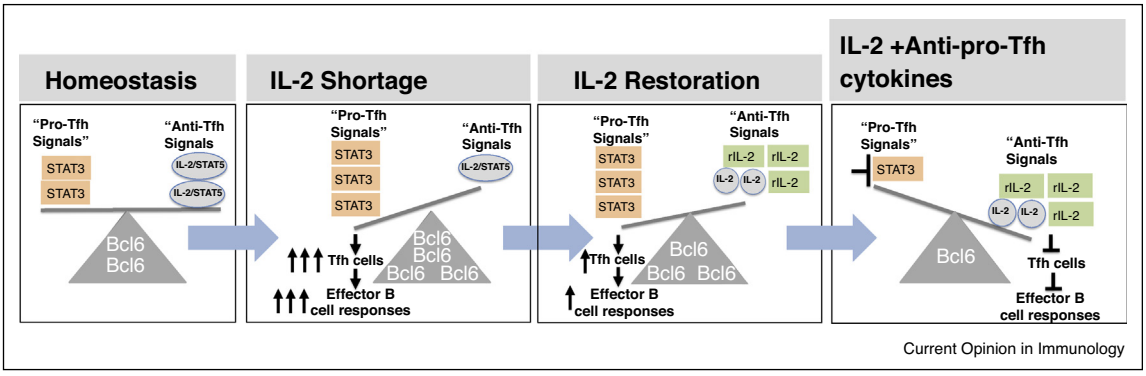
threshold of IL-2 signaling required for preventing Tfh cell responses is lower than the threshold needed for promoting Treg expansion. Collectively, these clinical and preclinical data support the notion that IL-2 shortage in SLE patients could lead to abnormal self-reactive Tfh cell expansion and the subsequent development of autoreactive PC responses, a cycle that can be interrupted by restoring IL-2 equilibrium after rIL-2 supplementation (Figure 2). Altogether, these results provide evidence for the potential of low-dose IL-2 regimens to suppress Tfh cell responses and Ab-mediated immunopathology. Thus, the consequences of IL-2 on Tfh cell homeostasis should be taken into consideration when designing and evaluating future low-dose rIL-2-based treatments.

Conclusions

The studies addressed above suggest a scenario, in which rIL-2 treatment contributes to preventing immunopathology by directly acting on both Tregs and Tfh cells. An important remaining question is whether these two populations need to be targeted simultaneously. For example, one could envision a scenario, in which targeting IL-2 to Tregs is required, but not sufficient for preventing immunopathology when the disease is already ongoing. Instead, when combined with Treg-mediated immunosuppression, concomitant Tfh cell depletion by IL-2 synergizes to induce full clinical benefits. Targeting the Tfh and Treg pathways simultaneously might, however, be challenging as preferential consumption of IL-2 by CD25-expressing Tregs might limit IL-2 availability, and thereby limiting the putative effect of low-IL-2 treatment on Tfh cells.

Interestingly, when IL-2 is complexed with the anti-IL-2 monoclonal Ab S4B6, this complex specifically targets CD122-expressing cells (IL-2/mAbCD122 complex), thus preventing the preferential consumption of IL-2 by $CD25^{+}$Tregs. In contrast, IL-2/JES6-1 complexes selectively target CD25-expressing Tregs (IL-2/mAbCD25 complex) [59,60]. Thus, one possibility to synchronously couple IL-2 to the Treg and Tfh cell pathways is combining the administration of low-dose IL-2/mAbCD122 and IL-2/mAbCD25 complexes. This approach has the potential of targeting Tfh (IL-2/mAbCD122) and Tregs (IL-2/mAbCD25) without the inherent competition for the available IL-2, thus promoting Tfh cell depletion while at the same time boosting Treg activity. One limitation of this strategy is the unwanted expansion of bystander CD122-expressing cells, such as T effector cells or NK cells. Tfh cells, however, express high levels of CD122 and respond to IL-2 with a lower threshold than conventional T cells [17••,57]. Thus, when administered at sufficiently low doses, the IL-2/mAbCD122 complex might preferentially target Tfh cells without boosting effector T cell responses. In a similar approach, IL-2 could also be selectively targeted to Tfh and Treg cells using synthetic IL-2 variants that have been shown to differentially target the intermediate and high-affinity IL-2R [61,62••]. For example, neoleukin-2/15, a recently developed mimetic IL-2 protein, binds to IL-2Rβγ with high affinity but lacks the binding site for CD25 [62••]. As a consequence, Tregs do not have a competitive advantage for neoleukin-2/15. Thus, it is possible that treatment with neoleukin-2/15 will more efficiently target Tfh cells than IL-2, even when IL-2 is administered at ultra-low doses, thereby preventing Ab-mediated pathology without inducing IL-2-mediated toxicity. Alternatively, blockade of STAT3-activating cytokine pathways that are known to induce

Figure 3



Targeting Tfh cells in SLE.
Tfh cell differentiation is fine-tuned by the relative levels of STAT3-activating cytokines and IL-2 signaling, which respectively promote and prevent Tfh cell differentiation. Therefore, it is likely that IL-2 shortage in combination with elevated levels of STAT3-activating cytokines promotes self-reactive Tfh cell differentiation and auto-Ab production in SLE patients. While low-dose rIL-2 treatment alone might help to prevent Tfh cell formation, it might not be sufficient to avoid self-reactive Tfh cell development completely. Importantly, blockade of STAT3-activating cytokines could render Tfh cells more sensitive to IL-2 therapy by lowering the threshold of IL-2 required to efficiently suppress Bcl6 expression, thus preventing Ab-mediated pathology even when IL-2 is administered at low doses.

Bcl6 expression, such as IL-6 or IL-21, may lower the threshold of IL-2 required to efficiently prevent Tfh cell responses, even in the presence of IL-2-consuming Tregs (Figure 3).

In summary, low-dose IL-2-based immunotherapies have shown promising results for the treatment of autoimmune manifestations, including SLE. The main limitations of the current IL-2-based therapies are their short half-life in serum and the risk of IL-2-dependent toxicity, particularly when considering administering it for extended periods of time. While the exact mechanisms by which low-IL-2 regimens restore immune tolerance and prevent disease progression are still unclear, changes in Treg and Tfh cell activity correlate with the therapeutic benefits of low-dose IL-2 treatments. Therefore, new therapeutic approaches aimed to more efficiently target IL-2 to these immune cell subsets will likely increase the effectiveness and clinical applicability of the current IL-2-based regimens.

Conflict of interest statement

Nothing declared.

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