International Immunopharmacology 72 (2019) 322–329

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Review

New therapeutic strategies based on IL-2 to modulate Treg cells for autoimmune diseases

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ARTICLE INFO

Keywords:
IL-2
Effector T cells
Regulatory T cells
Autoimmune diseases

ABSTRACT

Interleukin-2 (IL-2) is a multifunctional cytokine in immune regulation. It is essential for the differentiation, expansion and stability of $CD25^+Foxp3^+$ regulatory T (Treg) cells, which is an important factor in immune suppression and self-tolerance. Meanwhile, IL-2 also stimulate effector T (Teff) cells to promote immune responses. The opposite and diverse function of IL-2 impedes its application to boost Treg cell populations in autoimmune disease treatment. Thus, it became focus of the research to modulate IL-2 activities to enhance Treg cell functions selectively. Based on the characteristic properties of Treg cells such as constitutively expression of high affinity IL-2 receptors (IL-2Rs), multiple approaches, including IL-2/mAb complexes, IL-2 muteins and low-dose of IL-2 have emerged in recent years to selectively target Treg cells and treat autoimmunity. These therapeutic approaches have achieved favorable results in both clinical trials and experimental animal models, and provided engineering blueprints to develop novel strategies of IL-2 treatments for autoimmune diseases.

1. Introduction

Interleukin-2 (IL-2) was originally discovered as a T cell growth factor (TCGF) in 1976 [1] and was first cloned in 1983 [2]. As the first cytokine effectual in cancer immunotherapy, IL-2 signals stimulate different lymphocyte subsets, including T, B and natural killer (NK) cells, during their proliferation, differentiation and immune responses. Remarkably, abundant evidences demonstrate that IL-2 is essential for the immune homeostasis and balance of Treg and Teff cells in immune system, especially for cell number maintenance and functional activity of Treg cells. Thus, administration of IL-2 is considered as an effective method to boost Treg cell numbers and function to treat autoimmune diseases. However, the short half-life [3], IL-2-induced toxicity [4] and off-target effects on different cell populations limit the therapeutic

Abbreviations: IL-2, Interleukin-2; Treg cell, regulatory T cell; Teff cell, effector T cell; IL-2R, IL-2 receptor; TCGF, T cell growth factor; NK cell, natural killer cell; DCs, dendritic cells; TCR, T cell receptor; NFAT, nuclear factor of activated T cells; AP-1, activator protein-1; BLIMP-1, B lymphocyte-induced maturation protein 1; VLS, vascular leak syndrome; JAK, janus kinase; STAT, signal transducer and activator of transcription; RAS, rat sarcoma; MAPK, mitogen-activated protein kinase; PI3K, phosphoinositide 3-kinase; AKT, protein kinase B; SHC, SH2 domain-containing transforming protein; Foxp3, forkhead box P3; CTLA-4, cytotoxic T lymphocyte antigen-4; TGF-β, transforming growth factor-β; PTEN, phosphatase and tensin homologue; PD-1, programmed death-1; Mst, serine-threonine kinase; mAb, monoclonal antibody; NOD mice, nonobese diabetic mice; EAE, experimental autoimmune encephalomyelitis; EAMG, experimental autoimmune myasthenia gravis; CIA, collagen-induced arthritis; HSK, herpetic stromal keratitis; GVHD, chronic graft-*versus*-host disease; HSCT, hematopoietic stem cell transplantation; IRI, ischemia reperfusion injury; CDR, complementarity determining regions; DSS, dextran sodium sulfate; ATL, adult T cell leukemia; HTLV-I, human T cell lymphotropic virus-I; DTH, delayed-type hypersensitivity; SLE, systemic lupus erythematosus; FIH study, first-in-human study; T1D, Type 1 diabetes; CGI, Clinical Global Impression; AA, alopecia areata; ITP, immune thrombocytopenia; TNF, tumor-necrosis factor; ADA, anti-drug antibodies

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https://doi.org/10.1016/j.intimp.2019.03.064
Received 11 December 2018; Received in revised form 29 March 2019; Accepted 29 March 2019

application of IL-2 for cancer or autoimmune disorders. In recent years, other approaches like IL-2/mAb complexes, IL-2 muteins and low-dose-IL-2 emerged and revived IL-2 therapeutic strategies for autoimmune diseases. In this review, we discuss recent findings on the development of IL-2 therapeutics, focusing on the mechanisms how IL-2/mAb complexes, IL-2 muteins and low-dosage of IL-2 amplify the amount of Treg cells and maintain Treg cell functions.

2. IL-2 functions and its receptor system

IL-2 is a 15.5–16 kDa, four-α-helix-bundle type I cytokine with a hydrophobic core [5]. Although several immune cells have been shown to secrete IL-2, including $CD4^+$ T cells, $CD8^+$T cells, natural killer (NK) cells, activated dendritic cells (DCs) and mast cells [6], at resting conditions, the main production of IL-2 is from $CD4^+$ T helper cells [7]. After immune activation by T cell receptor (TCR) stimulation, IL-2 production by $CD4^+$ and $CD8^+$ T cells is rapidly increased, then supply to other immune cells regulated by IL-2 [8]. Various transcription factors such as nuclear factor of activated T cells (NFAT) [9] and activator protein 1 (AP-1) [10] activated by TCR signals can upregulate IL-2 expression. It's very interesting that Treg cells are unable to produce IL-2, even on activated conditions. TCR-induced IL-2 production by activated T cells is also regulated by several negative regulators, including transcription factor B lymphocyte-induced maturation protein 1 (BLIMP1) [11] and other immune checkpoint inhibitors [12]. Such mechanisms prevent overproduction of IL-2 which may result in immune disorders.

In the early time of study about IL-2 biological function, IL-2 was thought to be an essential cytokine to activate immune response because it promoted the differentiation of naive T cells into effector T cells. This view was challenged in 1993, when it was found that knockout of the gene encoding IL-2 in mice resulted in autoimmunity and lymphoproliferation rather than expected immune deficiency [13]. Subsequent studies demonstrated that knockout of the gene encoding IL-2Rα (also known as CD25) in mice also showed similar phenotypes [14]. Because of high level expression of CD25 on its cell surface, Treg cell was considered as the key factor in autoimmunity due to IL-2 signaling defects. This was confirmed by showing that in IL-2-deficient or IL-2R-deficient mice, Treg cells were absent as well, and the deficient mice which adopted Treg cells from normal mice could recover from autoimmunity [15]. Therefore, IL-2 has converse effects in immunoregulation. It can stimulate Teff cells and promote the immune responses to fight against cancer. Paradoxically, it can maintain Treg cells, suppress immune responses and be utilized in therapy for autoimmune diseases and rejection of transplanted organs. Moreover, it was observed that IL-2 was a key cytokine in T cells differentiation. It promoted Treg, T helper 1 (T_H1) [16] and T_H2 [17] cell differentiation but inhibited T_H17 [18] and T follicular helper (T_{FH}) [19] cell differentiation.

IL-2 exerts its function *via* binding to three classes of IL-2 receptors (IL-2Rs), monomeric, dimeric and trimeric IL-2Rs with different affinities. Monomeric IL-2R has low affinity for IL-2 (K_d $\sim 10^{-8}$ M) containing only IL-2Rα (also known as CD25). Due to lack of intracellular signal motif in CD25 peptide chain, interaction of IL-2 with monomeric IL-2R is unable to induce the signal transduction [20]. Conversely, IL-2 bind to dimeric IL-2R comprising IL-2Rβ (also known as CD122) and IL-2Rγ (known as common γ-chain, γ_c) with moderate affinity (K_d $\sim 10^{-9}$ M) and trimeric IL-2R comprising α, β and γ subunits with high affinity (K_d $\sim 10^{-11}$ M) [21]. Both IL-2Rβγ and IL-2Rαβγ receptors activate downstream signaling based on intracellular signal domains of IL-2Rβ and γ_c. For the IL-2Rαβγ receptor, the structural data of IL-2-receptor complex suggested that IL-2 initially binds to IL-2Rα and subsequently combine with IL-2Rβ and γ_c to form the quaternary complex. In addition, IL-2Rs binding sites and IL-2 key residues in interface were identified. Residues of IL-2 which primarily mediate receptor interactions contain R38, F42, Y45 for IL-2Rα, D20 and N88 for IL-2Rβ and Q126 for IL-2Rγ [22]. These interactions provide the basis for engineered IL-2 muteins to modulate their binding affinity for α, β and γ subunits. Following receptor binding, the IL-2-receptor complex is internalized, IL-2Rβ and γ_c are degraded while IL-2Rα returns to the cell membrane [23]. IL-2Rβγ receptors are expressed primarily on resting NK cells and naive $CD4^+$ or $CD8^+$ T cells. Upon TCR co-stimulation, naive cells express IL-2Rα transiently to respond to IL-2. Unlike effector T cells, Treg cells constitutively express IL-2Rα making this cell subset very sensitive to IL-2 [24]. In addition to lymphoid cells, non-immune cells, including endothelial cells can express IL-2Rαβγ receptors. [25]. IL-2 binding directly to endothelial cells in a CD25-dependent manner [26] will exert endothelial cell damage and result in vascular leak syndrome (VLS).

Signal transduction of IL-2 occurs *via* three principal signaling pathways including JAK-STAT, RAS-MAPK and PI3K-AKT pathways. IL-2-receptor interaction causes the assembly of cytoplasmic signal domains of receptor subunits and then activate JAK1 and JAK3 which associate with IL-2Rβ and γ_c respectively [27]. The activation of JAK1 and JAK3 phosphorylates tyrosine residues in IL-2Rβ. It was already identified that Tyr338, Tyr392 and Tyr510 in IL-2Rβ play crucial roles in signal transduction. Phosphorylated Tyr338 mediates recruitment of protein SHC and phosphorylates the downstream signal molecules, thus RAS-MAPK pathways is activated. Whereas phosphorylated Tyr392 and Tyr510 recruit STAT5 and cause their phosphorylation and nucleus translocation to control the target genes transcription [28]. Notably, there is a positive feedback loop provided by IL-2 that STAT5 binding to the *Cd25* gene locus makes T cells expressing more CD25 on cell surface to respond IL-2 more efficiently [7].

3. IL-2 and Treg cells

The early work identified a subset of $CD4^+$ T cells constitutively expressing high level of CD25 in 1995 [24]. These cells, known as regulatory T cells, are suggested as the major subset to suppress immune responses and maintain immunologic self-tolerance in immune system. Adoption of sufficient Treg cells rescued mice which suffered the autoimmune disease [15]. While high amounts of CD25 expression was unable to represent the most specific marker of Treg cells until the identification of transcription factor forkhead box P3 (Foxp3) [29–31]. The mutation in the *Foxp3* gene led to immune-mediated disorders such as diabetes, lymphadenopathy and cytokine storm *in vivo* [32]. These symptoms showed the correlation between the deficiency of Foxp3 in Treg cells and autoimmune diseases. On the basis of these discovery, subsequent experiments gave the additional evidence that Foxp3 had critical roles in differentiation and suppressor function of Treg cells. T cells-deficient mice only received Foxp3-sufficient precursor cells could generate Treg cells and recover from immune disorders [31]. Factitiously reduction of Foxp3 expression resulted in functional impairment of Treg cells [33]. Moreover, the normal immune response was not influenced by Foxp3 deficiency in effector T cells [34]. These observations showed that the expression of Foxp3 is required for the Treg cell suppressor function. Mechanisms of Treg cell-mediated suppression is now understood in considerable detail and substantial molecules are demonstrated to participate in suppression. Several cell-surface molecules were proposed to function as mediators to regulate Treg cell activities in immune tolerance, including cytotoxic T lymphocyte antigen-4 (CTLA-4) [35], CD25 [36], CD39 [37] and CD73 [38]. Other Treg cell-secreted immunosuppressive cytokines, such as TGF-β [39], IL-10 [40] and granzyme B [41], executed immunosuppression through dampening responds of effector T cells or contact-dependent cytotoxicity.

Differentiation of Treg cells occurs in the thymus relied on TCR and IL-2 signals. After reception of a TCR signal, a number of CD4 single-positive (SP) thymocytes are survived and instructed by cytokines to differentiate into thymus derived $Foxp3^+$ Treg cells [42]. Among the cytokines which promote the Treg cell differentiation, IL-2 is the most

essential factor. The evidence was given by experiments that in IL-2-deficient or IL-2Rα-deficient mice, the number and proportion of Treg cells decrease drastically, on the contrary, loss of IL-15 or IL-7 alone was unable to impair the Treg cell generation [43]. Moreover, injection of neutralizing IL-2 antibodies into normal mice reduced the number of Treg cells in the thymus and led to autoimmune disease symptoms [44]. In addition to the peripheral homeostasis of Treg cells, IL-2 has been shown to be important for increasing the suppressive activity of Treg cells [45]. As mentioned above, JAK-STAT5 is one of the IL-2-induction signaling pathway, and STAT5 binds to Foxp3 promoter to upregulate the expression of Foxp3 [46]. In this line, some experiments showed that STAT5-deficient mice were lack of $CD4^+CD25^+Foxp3^+$ T cells [47] and constitutively expression of STAT5 leads to recover and expansion of Treg cells [48]. These studies strongly support the indispensable role of IL-2 in Treg cell development. More remarkable, several pathways for inhibiting TCR and IL-2 signaling outputs involve phosphatase and tensin homologue (PTEN) [49], programmed death 1 (PD-1) [50] and CTLA-4 [51] in Treg cells. PI3K-AKT signaling inhibition by these negative regulators makes Treg cells more dependent on IL-2-induction-STAT5 signaling pathway than effector T cells. A new study identified Mst1-Mst2 as the amplifier of IL-2-STAT5 signaling in Treg cells [52], underscoring the requirement for IL-2 to maintain Treg cell populations and immune tolerance (Fig. 1).

Collectively, Treg cells make a large contribution in suppressing immune response and keeping immune homeostasis, meanwhile, IL-2 is the key factor to maintain expansion and function of Treg cells by regulating STAT5 signaling pathway and Foxp3 expression. Thus, IL-2 analogues such as IL-2/antibodies complex and IL-2 muteins are promising therapeutic strategies to treat autoimmune diseases.

4. New therapeutic strategies based on IL-2 analogues

4.1. IL-2/mAb complexes

Administration of IL-2 combined with its specific monoclonal antibody (mAb) can delay the degradation of IL-2 *in vivo*. More important, when coupled with IL-2, some IL-2/mAb complexes cause expansion of effector T cells showing the anti-cancer effectiveness, whereas, other IL-2/mAb complexes selectively maintain the biological function of Treg cells leading to immunosuppression.

4.1.1. IL-2/JES6–1 complex

JES6–1 and S4B6 are mouse monoclonal antibodies against mouse IL-2, but they have distinct functions *in vivo*. IL-2/S4B6 complexes preferentially stimulated T cells with high level expression of IL-2Rβγ receptors, especially $CD8^+$ T cells, causing the cells above 100-flod expansion *in vivo*. While short courses injections of IL-2/JES6–1 (anti-mouse IL-2 antibodies) complexes increased the number of $CD25^+$ $Foxp3^+$ Treg cells without significantly affected $CD8^+$ T cells and NK cells [53]. Lots of researches focused on IL-2/JES6–1 complexes have demonstrated promising results in the treatment of several autoimmune diseases and immunological rejection. Injection of IL-2/JES6–1 complexes in nonobese diabetic (NOD) mice promoted Treg cell survival and protected mice from developing diabetes [54]. A 1:10 ratio of IL-2: JES6–1 complexes was used to expand Treg cells and prevent murine allergic airway diseases including airway inflammation and airway hyperresponsiveness [55]. In experimental autoimmune encephalomyelitis (EAE) mice model, IL-2/JES6–1 complexes render the mice resistant to EVE, and combined with rapamycin, the complexes could also be used to treat ongoing disease [56]. In experimental autoimmune myasthenia gravis (EAMG) mice model, mice treated with IL-2/JES6–1 complexes of 1.5 μg of IL-2 with 50 μg of JES6–1 improved the symptoms of EAMG [57]. For arthritis, IL-2/JES6–1 complexes (1.5 μg/7.5 μg) were injected in collagen-induced arthritis (CIA) mice, and suppressed the induction of CIA and inflammatory responses [58]. In another similar experiment, 5 μg of JES6–1 with 1 μg of IL-2 effectively boosted a 1.6-fold expansion of $CD4^+Foxp3^+$ Treg cells in peripheral blood, and the level of IL-10 production was also increased [59]. Preventing development of virus-induction inflammation was suggested by a recently research that administration of the IL-2/JES1–6 complexes to mice, prior to corneal HSV-1 infection, significantly expanded Treg cells particularly and resulted in a marked reduction in the development of severe herpetic stromal keratitis (HSK) [60]. Additionally, IL-2/JES6–1 complexes show promising potency for the prevention of skin allograft rejection [61] and chronic graft-*versus*-host disease (GVHD) in allogeneic hematopoietic stem cell transplantation

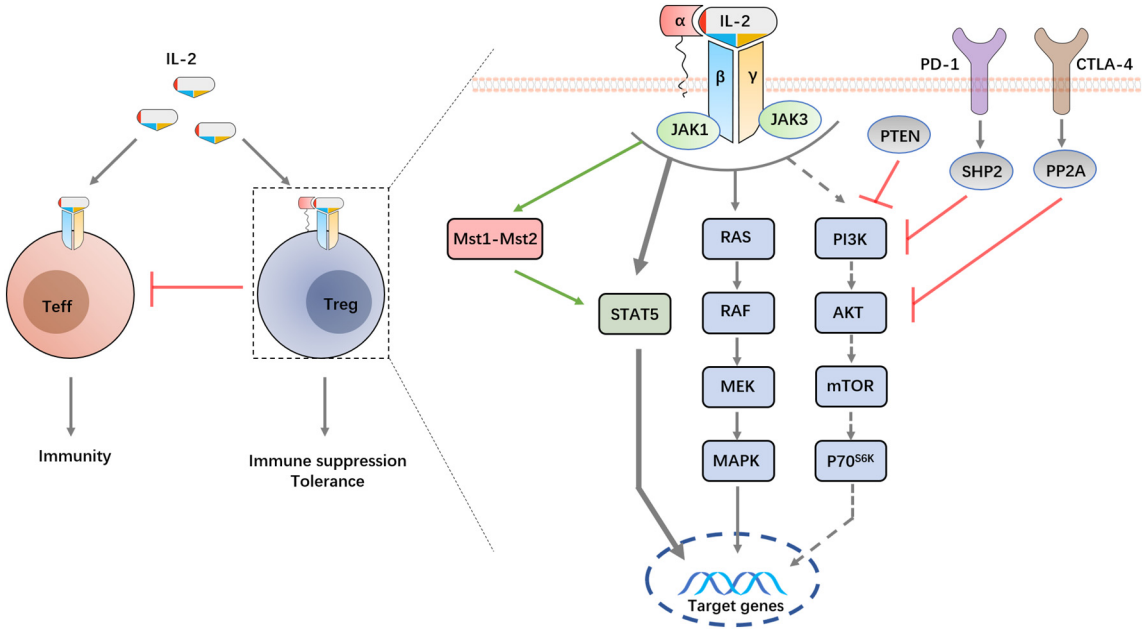

Fig. 1. IL-2-induced signal network in Treg cells.
IL-2 is a pleiotropic cytokine that activates both regulatory T cells and effector T cells through three signal pathways: JAK-STAT, RAS-MAPK and PI3K-AKT pathways. However, compare with Teff cells, major differences in Treg cells are that Mst1-Mst2 amplifies the STAT5 signaling while PTEN, PD-1 and CTLA-4 inhibit PI3K-AKT signaling. Those co-regulations make Treg cells more dependent on IL-2-induction-STAT5 pathway.

(HSCT) [62]. For some metabolic disorders or immunologic damage, such as renal ischemia reperfusion injury (IRI) [63] and atherosclerosis [64], administration of the complexes also improved the pathology with expansion of $CD25^+$ $Foxp3^+$ Treg cells.

The mechanisms that IL-2/JES6–1 complexes selectively promote expansion of Treg cells was suggested by crystallographic and biophysical data. Firstly, IL-2/JES6–1 complexes crystal structure demonstrated that there existed steric competition between JES6–1 and both β and γ subunits. IL-2 residues (Q22 and M23) which were originally essential to the IL-2/IL-2Rβ and IL-2/γ_c interactions now participated in hydrogen bonds formation with JES6–1. The binding between IL-2/JES6–1 complex and IL-2Rβγ receptor was inhibited by steric hindrance. Therefore, complexes were unable to target effector T cells which mainly expressed IL-2Rβγ receptors. However, compared with IL-2Rα-bound IL-2, JES6–1 binding distorted presumed receptor-bound states of IL-2 AB loop. That allosterically distortion potentially led to an exchange mechanism [65] that JES6–1 would be displaced by IL-2Rα when encountering sufficient IL-2Rα on Treg cell surface. After dissociation of JES6–1, the IL-2Rα-bound IL-2 was liberated to recruit IL-2Rβ and γ_c to form the functional signaling complexes ultimately. Additionally, this mechanism provided a positive transcriptional feedback loop that only cells with sufficient amounts of surface IL-2Rα like Treg cells had the capacity to displace JES6–1. IL-2 signaling-induced elevation of IL-2Rα expression in turn boosted the sensitivity of Treg cells to the immunocomplex [65–67] (Fig. 2).

4.1.2. IL-2/F5111.2 complex

Recently experimental data showed that administration of an anti-human IL-2 antibody F5111.2 resulted in similar effect as JES6–1 *in vivo*. F5111.2 was screened by a single-chain variable fragment phage display library and IL-2/F5111.2 complexes could expand Treg cell populations selectively. The crystal structure revealed that the IL-2Rβ-binding site of IL-2 was completely blocked by F5111.2. Light-chain CDR1 and CDR3 loops and the heavy-chain CDR2 and CDR3 loops interacting with IL-2 caused steric obstruction for IL-2Rβ. Meanwhile, the antibody generated steric perturbations on IL-2 A–B and B−C loops which propagated to the IL-2Rα-binding site [68]. These perturbations slightly decreased the IL-2 affinity for CD25 and gave a competitive advantage for Treg cells with high affinity IL-2Rs binding to IL-2/F5111.2 complexes. In addition, in NOD mice model, EAE model and xeno-GVHD model [69], administration of IL-2/F5111.2 complexes for several consecutive days revealed the tremendous potential in treatment for autoimmune diseases and immunological rejection. Consist with IL-2/JES6–1 complexes [65], the strong evidences suggested that IL-2/F5111.2 complexes induced substantial increase of Foxp3, CD25 and p-STAT5 signals without influence in Teff cell populations. Active efforts are underway to translate the application of the antibody F5111.2 into autoimmune diseases and promote this immunotherapy method forward into clinical trial [68].

4.1.3. IL-2/mAb fusion-JY3 IC

Although IL-2/mAbs complexes show gratifying results in immune disease treatment, the optimal cytokine/mAbs ratio and stability of complexes *in vivo* remain of concern [70]. Off-target effects release free IL-2 from complexes, leading to same behavior as the naked cytokine which activates both Treg and Teff cells unselectively. To overcome this trouble, Garcia and his colleagues used a flexible (Gly4Ser)2 linker to fuse the IL-2 and JES6–1 together. Indeed, the cytokine/mAb fusion (denoted the IC) avoided cytokine falling off, but lost its capacity to stimulate the IL-2Rα$^+$ cells. The intramolecular high affinity between IL-2 and JES6–1 in IC disrupted the exchange mechanism which exists in IL-2/JES6–1 complexes, resulting in cytokine activity abolishment. Through the exchange mechanism [65], there must be an optimal IL-2/mAb affinity to expand Treg cells specifically. Thus, they selected some residues at the cytokine/mAb interface to design JES6–1 Ab alanine mutants which reduced the cytokine/mAb affinity significantly [71]. Subsequently, they reformatted the mutants as the IC fusions. In C57BL/6 mice and NOD mice, experimental results indicated one IC

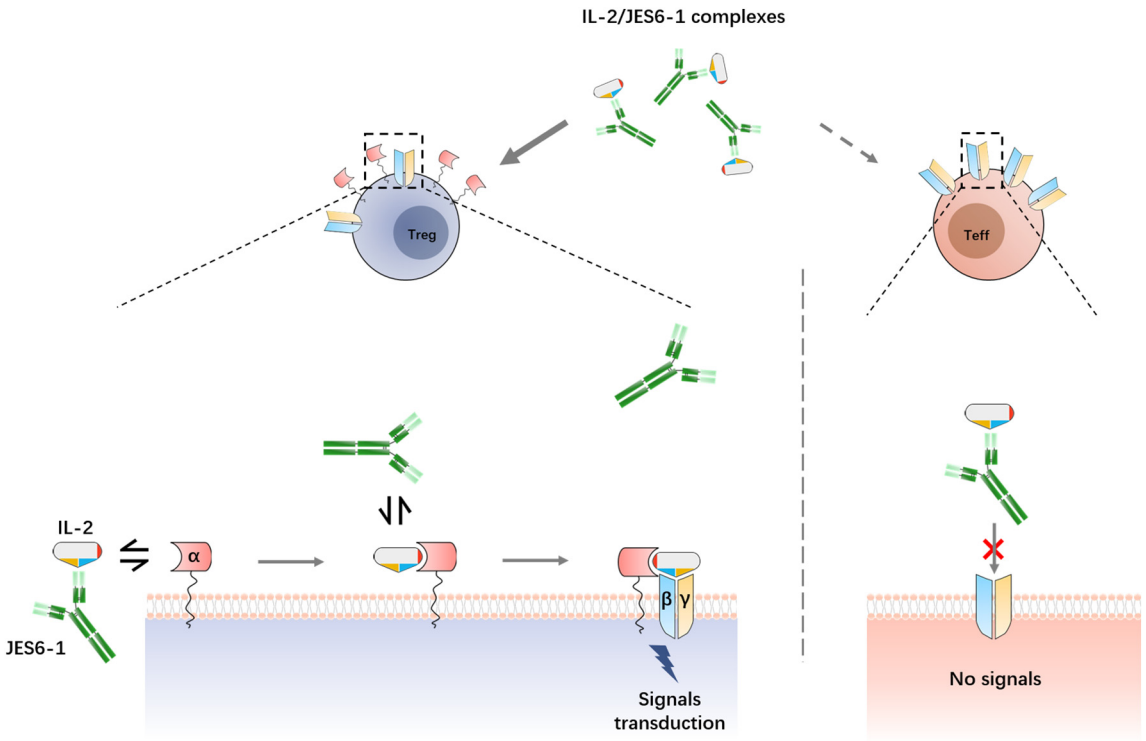

Fig. 2. Exchange mechanism of IL-2/JES6–1 complexes
The JES6–1 sterically blocked the IL-2/IL-2Rβ and IL-2/IL-2Rγ interactions and distorts IL-2Rα binding site. Sufficient IL-2Rα can displace the JES6–1 and release IL-2 to form the IL-2-receptors complexes. This mechanism induces a preferential activation of Treg cells.

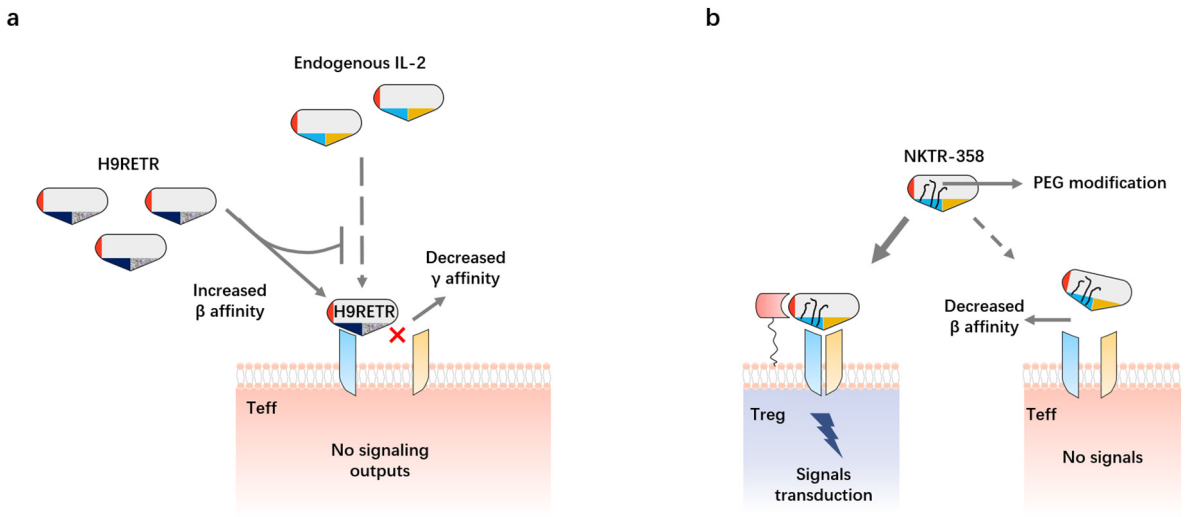

Fig. 3. Mechanism of IL-2 muteins.
(a) H9RETR has higher β binding affinity and lower γ binding affinity. It antagonizes endogenous IL-2 binding. Meanwhile, without IL-2Rγparticipation, H9RETR is unable to activate signaling pathway. This mutein act as an antagonist to suppress Teff cell activity. (b) Treg cells with high affinity receptors are more sensitive to NKTR-358 because PEG modification decreases β binding affinity.

mutant denoted JY3 IC, preferential expanded Treg cells *versus* Teff cells. Compared with the IL-2/JES6–1 complexes, the JY3 IC treatment increased the CD25 expression on Treg cells more intensely. Moreover, the toxicity caused by off-target effects in IL-2/JES6–1 complexes treatment was alleviated when used equivalent dose of the JY3 IC. In the adoptive T cell transfer model, studies suggested that JY3 IC had a greater ability to expand IL-2Rα$^+$ cell subsets relative to IL-2/JES6–1 complexes. In dextran sodium sulfate (DSS) colitis model, JY3 IC also achieved better therapeutic effect than IL-2/JES6–1 complexes [71]. Overall, the JY3 IC enhanced the stability of cytokine/mAb, meanwhile, it maintained the exchange mechanism which allowed the cytokine regulating immune cells. ICs showed therapeutic advantages to the mixed cytokine/mAb complexes in selectively Treg cell expansion and autoimmune disease treatment. This approach extends the application of cytokine-antibodies system as another novel immunotherapeutic strategy.

4.2. IL-2 muteins

Engineered IL-2 muteins are considered interesting tools to modulate IL-2-mediated immune responses by antagonizing endogenous IL-2. Focus on reducing endothelial damage and VLS [72], some IL-2 muteins, such as 'no-α mutein' [73], were generated with attenuated binding to IL-2Rα. Other IL-2 muteins with decreased affinity for IL-2Rβ were generated to disfavors contact with NK cells for decreasing IL-2-induced toxicity [74–76]. Although, these muteins did not achieve decreased toxicity in clinical trials, they showed significant selectivity for high affinity IL-2Rs and Treg cells. For instants, BAY 50–4798, an IL-2 mutein (N88R) exhibited a 2700-fold increase in affinity for IL-2Rαβγ relative to IL-2Rβγ [74]. It revived this kind of muteins in expansion Treg cells and autoimmunity treatments. In this line, another IL-2 mutein (N88D) with reduced binding to the IL-2Rβ was fused with IgG to selectively activates and expands Treg cells. In this study, treatment of cynomolgus monkeys or mice with single low doses of this mutein induced sustained preferential activation of Treg cells accompanied by demethylation of *FOXP3* and *CTLA4* genes which strongly predicted that Treg cells were functional and immunosuppressive [77]. The therapeutic abilities of this mutein in autoimmune disease models are anticipated.

Since decreased affinity for IL-2Rβ makes muteins Treg cell-biased, on the contrary, muteins with increased binding affinity for IL-2Rβ can bind with moderate affinity IL-2Rs on Teff cells irrespective of IL-2Rα. The agonistic IL-2 mutein H9 (known as IL-2 superkine) that functions independently of IL-2Rα was generated with 200-fold increased affinity for IL-2Rβ. This mutein resulted in efficient expansion of cytotoxic T cell populations to upregulate anti-tumor responses [78]. On the basis of H9 property, H9-RETR (L18R, Q22E, Q126T, and S130R) was engineered as a potent antagonist [79]. It retained the high binding affinity for IL-2Rβ but showed pronounced decreased affinity for γ_c. The results showed that little IL-2-induced STAT5 binding site could be induced by H9-RETR. Hence, H9-RETR were incapable of activating the signaling pathways (Fig. 3). When used *in vivo*, H9-RETR showed more effective in blocking IL-2-induced pSTAT5 signaling and suppressing action of T cells regardless of Teff or Treg cells than CD25 (Daclizumab) [80] and CD122 antibodies (Mikβ1) [81]. Because of the powerful and indiscriminate silencing effects on T cells, mice treated with H9-RETR for 10 days had longer survival than control groups in mismatched bone marrow transplantation mice model, which demonstrated that the mutein could inhibit lethal GVHD. In addition, the malignant and spontaneous proliferation of CD4$^+$ T cells was inhibited effectively by H9-RETR at 10 μg/ml in a patient with smoldering adult T cell leukemia (ATL) caused by human T cell lymphotropic virus-I (HTLV-I) [21,79].

Due to the enormous therapeutic potential, pharmaceutical companies flock to IL-2 muteins in autoimmune disease treatment [82]. NKTR-358, developed by Nektar Therapeutics, was a novel IL-2/polyethylene glycol (PEG) conjugate aiming to promote Treg cells selectively. Chemical modification by PEG in NKTR-358 attenuated affinity for the IL-2Rβ relative to native IL-2, thus, Treg cells with high affinity receptors were far more sensitive to NKTR-358 than Teff cells (Fig. 3). Experimental results in the mouse delayed-type hypersensitivity (DTH) model, mouse systemic lupus erythematosus (SLE) model and primate cutaneous hypersensitivity model demonstrated that NKTR-358 restored sustained preferential Treg cell functions [83]. Currently investigation of NKTR-358 in Phase 1 study were anticipated. Celgene also bought a company which developed a novel IL-2 mutein-Fc fusion protein called DEL106 [84]. AMG 592, another new IL-2 mutein in investigational studies was recently designed by Amgen. AMG 592 showed robust preference to Treg cell expansion relative to conventional effector T cells and NK cells *in vitro*. In first-in-human (FIH) study, it increased Treg: Tcon ratio approximately fourfold *via* baseline at the highest dose in healthy volunteers [85]. Further investigations of therapeutic mechanism of AMG 592-induced modulation of immune homeostasis are eagerly anticipated.

Table 1
Summary of new therapeutic strategies based on IL-2.

Category	Name	Technology	Stage	Disease treatment	References
Antibody	IL-2/JES6–1	IL-2/anti-mIL-2 antibody complexes	Animal experiments	T1D, allergic airway disease, EAE, EAMG, CIA, HSK, GVHD, HSCT, IRI, atherosclerosis	[54–64]
	IL-2/F5111.2	IL-2/anti-hIL-2 antibody complexes	Animal experiments	T1D, EAE, GVHD	[68]
	JY3 IC	IL-2/antibody fusion	Animal experiments	DSS-induced colitis	[71]
Muteins	H9RETR	IL-2 mutein	Animal experiments	GVHD, ATL	[79]
	NKTR-358	IL-2/PEG conjugate	Clinical trials	DTH, SLE, cutaneous hypersensitivity	[83]
	AMG 592	IL-2 mutein Fc fusion protein	Clinical trials	FIH study (healthy subjects)	[85]
Recombinant cytokine	Recombinant IL-2	Low-dose recombinant IL-2	Clinical trials	GVHD, T1D, hepatitis C virus-related autoimmunity, SLE, rheumatoid arthritis, ankylosing spondylitis, psoriasis, Behcet's disease, granulomatosis with polyangiitis, Takayasu's disease, Crohn's disease, ulcerative colitis, autoimmune hepatitis and sclerosing cholangitis, AA, ITP	[87–95]

4.3. Low-dose IL-2

Administration of low-dose IL-2 has got a wide range of applications in several clinical settings to restore Treg cell populations and treat autoimmune diseases. The underling mechanism is that Treg cells constitutively express high affinity IL-2Rs which is sensitive to low level IL-2 [86]. Low-dose IL-2 has shown promising results in the treatment of several autoimmune such as GVHD [87], Type 1 diabetes (T1D) [88], hepatitis C virus-related autoimmunity [89]. Of note, in a study by Humrich et al., one female patient with severe SLE refractory or intolerant to traditional therapies was recovered after receiving daily administrations of recombinant human IL-2 (aldesleukin) at single doses of 1.5 or 3.0 million IU for four treatment cycles. The data showed that low-dose IL-2 treatment improved skin eruptions and laboratory signs of myositis, decreased the levels of anti-dsDNA-antibodies and increased the population of Treg cells [90]. In the study of von Spee-Mayer et al., five patients with refractory SLE were treated daily with 1.5 million IU of human IL-2 for five consecutive days [91]. Both the case report and case series gave the first implications of the possible efficacy of low-dose IL-2 therapy in systemic autoimmune disease. Subsequently, He et al. completed the first clinical trial of low-dose IL-2 in SLE [92]. 38 patients with SLE were administered 1 million IU of human IL-2 every other day for 2 weeks, followed by a 2-week break. Their results demonstrated that administration of low-dose IL-2 to patients with active SLE could increase the population and function of Treg cells, decrease the (T_{FH} + T_H17) cell/Treg cell ratio and reduce SLE disease activity. This first open-labelled study of low-dose IL-2 therapy in SLE highlighted the detailed clinical efficacy of low-dose IL-2 treatment in SLE.

Recently, Rosenzwajg et al. assessed the biological and clinical effects of low-dose IL-2 in a single clinical trial treating 46 patients with 11 autoimmune diseases including rheumatoid arthritis, ankylosing spondylitis, systemic lupus erythematosus, psoriasis, Behcet's disease, granulomatosis with polyangiitis, Takayasu's disease, Crohn's disease, ulcerative colitis, autoimmune hepatitis and sclerosing cholangitis. All patients received 1 million IU/day of IL-2 from day 1 to day 5 and then every 2 weeks from day 15 to day 180. With this dose and treatment scheme used, low-dose IL-2 selectively activated and expanded Treg cells without impacting on Teff cells, and there was no serious adverse event induced by treatment. Additionally, Clinical Global Impression (CGI) scores and disease-specific score were improved at month 3 and continued to month 6. Likewise, arthralgia and chronic fatigue symptoms were improved significantly [93]. This comprehensive study demonstrated the clinical efficacy, safety and tolerance of low-dose IL-2 treatment in heterogeneous patients with autoimmune diseases. Some autoimmune disorders like alopecia areata (AA) [94] and immune thrombocytopenia (ITP) [95] were also demonstrated to be treated by Low-dose IL-2.

Although the results of low-dose IL-2 treatment are promising, there are some challenges need to be overcome. An appropriate dose or scheme of low-dose IL-2 administrated to various autoimmune diseases needs more abundant clinical data to support. In addition, the half-life of IL-2 is short. Finally, although the low-dose IL-2 can selectively activate Treg cells generally, it is unavoidable to affect Teff cells in a dose-dependent manner. Off-target effects and inflammation will limit the application of IL-2. Thus, it worth to explore other novel IL-2 related drugs such as IL-2/antibody complexes and IL-2 muteins which more selectively expand Treg cells than low-dose IL-2.

In addition to nascent low-dosage IL-2 therapy, anti-TNF and anti-IL-6 agents have already been a commercial success and treated millions of patients with autoimmune diseases such as rheumatoid arthritis, inflammatory bowel disease and Castleman disease [96,97]. Tumor-necrosis factor-α (TNF-α) and interleukin-6 (IL-6) are proinflammatory cytokines with multifunctional functions on inflammation and antitumor responses in immune system, and they can induce inflammation, autoimmunity and tissue degeneration. Anti-TNF biologics

include antibodies and “TNF receptor-Fc fusion proteins”. Infliximab, Adalimumab, and Golimumab are anti-TNF antibodies, Etanercept is a TNF receptor 2 and human IgG_1 Fc fusion protein. The drugs mentioned above treat autoimmune diseases by neutralizing the endogenous TNF and inhibiting its function *in vivo*. However, anti-TNF antibodies still have limitations including low rates of disease remission, the development of adverse effects and the formation of anti-drug antibodies (ADA) which is one of the causes of clinical nonresponse to treatment [98–100]. IL-6 is considered as a driver of autoimmunity and chronic inflammation because it promotes the differentiation of T_H17 cells which are major factors in inducing tissue damage in autoimmune and inflammatory disorders. Thus, IL-6R antibodies like Tocilizumab and Sarilumab prevent endogenous IL-6 receptors ligand binding, block its functions on targeted cells and inhibit its activity [97]. While clinical studies demonstrate that anti-IL-6 antibodies treatments increase the risk of bacterial infections. This major side effect is caused by attenuating host defense against infection due to blocking of IL-6 signaling [101]. Compared with anti-TNF or anti-IL-6R antibodies, IL-2 treats autoimmune diseases by expanding Treg cells and balancing Treg/Teff ratio. In addition, low-dose IL-2, IL-2/mAb complexes and IL-2 muteins can selectively target on Treg cells to induce immunosuppression and maintain immune tolerance with relatively mild efficacy and low toxicities. New therapeutic strategies based on IL-2 can applicated more broadly not only for autoimmune diseases treatments (Table 1), but also for rejection of transplanted organs.

5. Conclusions

The immunologic balance of Treg and Teff cells are important for the immune homeostasis and IL-2 is the key factor to maintaining balance. First thought to be a TCGF which was related with anti-tumor response, IL-2 was subsequently demonstrated by experimental results that it had diverse actions including its ability to drive the differentiation and expansion of Treg cells, as well as to control self-tolerance and inflammatory reaction. Revolving around the cytokine, low-dose IL-2, IL-2/mAb complexes and IL-2 muteins were three potential therapeutic methods to treat autoimmune diseases. Recently developments of these methods provided foundation for novel therapeutic strategies, which could be adapted to other cytokines. Although establishing more precise and efficient methods to achieve the most optimal ratio of Treg and Teff cells are challenges, further studies will show potency of this remarkable cytokine IL-2 and its analogues in immunotherapy.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (Grant No. 81273308 to JS and Grant No. 81771743 to RL) and China State Key Research (Grant 2016YFA0501404 to YZ).

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