Trends in Immunology

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Review

Interleukin-2: Biology, Design and Application

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Interleukin-2 (IL-2) exerts crucial functions during immune homeostasis via its effects on regulatory T (Treg) cells, and the optimizing and fine-tuning of effector lymphocyte responses. Thus, somewhat paradoxically, low doses of recombinant IL-2 have been used for Treg cell-based immunosuppressive strategies against immune pathologies, while high-dose IL-2 has shown some success in stimulating anti-tumor immune responses. Recent studies of the functional, biophysical and structural characteristics of IL-2 have led to the generation of IL-2 formulations, including IL-2/mAb complexes and IL-2 variants (muteins) that selectively enhance IL-2's immune stimulatory versus inhibitory properties. Here, we review these findings, placing new mechanistic insights into improved next-generation IL-2 formulations within the broader context of IL-2 biology. We conclude by integrating these findings into a framework for understanding IL-2-mediated selective immune modulation.

Introduction

Discovered, isolated, and cloned between 1976 and 1983, IL-2 was the first immunotherapy demonstrating clinical efficacy in metastatic cancer [1–5]. Despite these promising data, IL-2 immunotherapy has not been widely adopted for various reasons, including its difficult administration due to its short *in vivo* half-life ($T_{½}$), its toxic adverse effects when administered at high doses (as needed for antitumor immunotherapy), and its ability to stimulate both cytotoxic effector T cells and regulatory T (Treg) cells [6]. While activation of Treg cells is an unwanted effect in anticancer IL-2 immunotherapy, as Treg cells can dampen effector T cell responses against tumor antigens, the property of IL-2 – even at low doses – to stimulate Treg cells could be harnessed for the treatment of Treg cell-deficient autoimmune and chronic inflammatory disorders [7].

The past 10 years rekindled an interest in IL-2 immunotherapy, which came from structural and biophysical insights into the complex formed by IL-2 with its IL-2 receptor (IL-2R) subunits [8] and the finding that IL-2 could be modified to selectively stimulate either cytotoxic effector T cells or Treg cells [9]; these studies have led to the generation of IL-2 formulations with improved and selective immune stimulatory capacities [10]. Furthermore, clinical trials using low-dose IL-2 have demonstrated IL-2's potential in expanding Treg cells and modulating immune pathologies [11,12]. Four recent publications exemplify this development and have motivated this review, in that they provide structural insight into the selective IL-2-mediated modulation of immune responses using IL-2/monoclonal antibody (mAb) complexes [13], highlight the possibility of using IL-2 muteins to antagonize, rather than stimulate, IL-2R-induced signals [14], and report novel data on the clinical use of low-dose IL-2 therapy in organ-specific and systemic autoimmune disease [15,16].

To place these findings in context, we begin by providing an introduction into the biology of IL-2 and its receptors, followed by a discussion of IL-2/mAb complexes and IL-2 muteins, and end by

Trends

Interleukin-2 (IL-2) exerts immunosuppressive and immunostimulatory effects by activating regulatory T (Treg) versus cytotoxic effector cells.

These IL-2 effects hinge on different IL-2 receptor (IL-2R) expression patterns: $CD8^+$ T and natural killer cells carry high levels of dimeric IL-2Rs comprising IL-2Rβ (CD122) and IL-2Rγ (γ_c); Treg cells express high IL-2Rα (CD25) levels along with intermediate levels of CD122 and γ_c.

Selective IL-2 formulations, such as IL-2 complexes and IL-2 muteins, preferentially stimulate cells expressing high CD25 versus high CD122 levels, and recent studies extend these concepts to also include muteins that inhibit IL-2R-induced responses.

These data converge into a framework of IL-2-mediated selective immune modulation where CD25-biased IL-2 formulations primarily expand Treg cells and CD122-directed IL-2 formulations stimulate cytotoxic effector cells.

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summarizing the key concepts arising from these studies as well as indicating gaps in our current understanding.

The Biology of IL-2

IL-2 is a 15.5–16-kDa, four-α-helix-bundle cytokine (Figure 1) that exerts its actions via binding to various IL-2Rs, notably monomeric, dimeric, and trimeric IL-2Rs [6,17,18]. Monomeric IL-2Rs, comprising IL-2Rα (CD25), are usually cell membrane associated but also exist in soluble form and bind IL-2 with a low K_d of $\sim 10^{-8}$ M. Interaction of IL-2 with CD25 alone does not induce a signal [19]; hence, isolated membrane-bound or soluble CD25 molecules might serve as scavenger or decoy receptors for IL-2 [6,20]. Conversely, both dimeric and trimeric IL-2Rs lead to a downstream signal on binding to IL-2. Dimeric IL-2Rs comprise IL-2Rβ (CD122) and IL-2Rγ [better known as common γ-chain (γ_c) or CD132], whereas trimeric IL-2Rs comprise CD25, CD122, and γ_c (Figure 2). Of note, CD122 is also part of IL-15R, whereas γ_c is shared by IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 [18]. Considering only IL-2Rs with signaling capacity, dimeric IL-2Rs can be referred to as low-affinity (K_d $\sim 10^{-9}$ M) and trimeric IL-2Rs as high-affinity (K_d $\sim 10^{-11}$ M) IL-2Rs [19]. On a molecular level, a single trimeric IL-2R binds IL-2 with roughly 10–100-fold higher affinity than a single dimeric IL-2R, illustrating that one function of CD25 is to improve IL-2 binding to the dimeric IL-2R. Association of IL-2 with IL-2R causes internalization of the quaternary complex, on which IL-2, CD122, and γ_c become degraded in vesicles, whereas CD25 is recycled via endosomes to the cell surface [21]. Notably, internalization of IL-2 has been suggested to depend on the cytoplasmic part of γ_c, suggesting that γ_c-mediated signaling is involved [22].

On triggering of IL-2R, signal transduction occurs via three major pathways, involving: (i) Janus kinase (JAK)–signal transducer and activator of transcription (STAT); (ii) phosphoinositide 3-kinase (PI3K)–AKT; and (iii) mitogen-activated protein kinase (MAPK) [6,18] (see Figure 2 for more details). Moreover, IL-2 signaling activates the transcription factor B lymphocyte-induced maturation protein 1 (Blimp1), encoded by *Prdm1*, which serves as a negative feedback loop by repressing IL-2 production.

Several immune cells have been shown to secrete IL-2 when activated, including T cell receptor (TCR) $\alpha\beta^+$ and TCR$\gamma\delta^+$ T cells, natural killer (NK) cells, NKT cells, dendritic cells (DCs), and mast cells [6]. At resting conditions, $CD4^+$ helper T (Th) cells are the main source of the constant but low levels of IL-2. On immune activation, IL-2 production rapidly rises. Low IL-2 secretion by activated DCs has been suggested to provide an early IL-2 source [23], thereby supporting T cell stimulation. In parallel, activated T cells, including $CD4^+$ and $CD8^+$ T cells, start secreting large amounts of IL-2 for their own (autocrine) use and to stimulate in a paracrine fashion neighboring IL-$2R^+$ cells [6,17,18]. Interestingly, Treg cells are unable to produce IL-2, even on stimulation, unless they constitute peripherally derived ('induced') Treg cells redifferentiating to Th cells [24]. IL-2 production by activated T cells is transient and transcriptionally regulated, including silencing by Blimp1 and Aiolos (encoded by *Ikzf3*) [18]. Moreover, negative feedback appears to also exist on the cellular level involving IL-2-producing $CD4^+$ T and IL-2-consuming Treg cells [25]. Such regulation mechanisms might be key in preventing T cell overstimulation by persistent IL-2 signals in conjunction with repetitive TCR stimulation by antigen (including self- and tumor antigens), which can lead to T cell exhaustion or Fas (CD95)-mediated activation-induced cell death [26,27].

In terms of IL-2 responsiveness, *in vitro* activated human T cells have been reported to carry about 2000 high-affinity and 11 000 low-affinity IL-2Rs per cell [4,28]. Dimeric IL-2Rs are expressed at high levels on antigen-experienced (memory) $CD8^+$ T cells and NK cells, whereas low to intermediate levels of dimeric IL-2Rs are found on memory $CD4^+$ and naive T cells [6]. On TCR stimulation, T cells transiently upregulate CD25, thus expressing now trimeric IL-2Rs. In