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Interleukin-2 Immunotherapy for Advanced Cancer

İleri evre kanser tedavisinde interlökin-2 immünoterapisi

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Abstract

Interleukin-2 (IL-2) was the first approved immunotherapy to show efficacy in advanced cancer. 13–20% of patients with metastatic renal cell carcinoma and metastatic melanoma receiving high-dose IL-2 treatment showed objective clinical responses, some enduring for up to 20 years and more. However, the use of IL-2 immunotherapy was hampered by the short *in vivo* half-life of IL-2, dose-dependent toxicity and stimulation of immunosuppressive regulatory T cells. Recent efforts have explored the biology of IL-2 and its receptors to generate improved IL-2 formulations. Such IL-2 formulations provide targeted and potent stimulation of selected lymphocyte subsets, and they include IL-2/anti-IL-2 monoclonal antibody complexes (briefly, IL-2 complexes), IL-2 muteins, and versions of IL-2 bound to polyethylene glycol or other molecules. In this article, we review the use of IL-2 for cancer immunotherapy, and discuss the preclinical and translational aspects of IL-2 complexes and their potential for the treatment of advanced cancer.

Keywords: Interleukin-2 (IL-2), immunotherapy, advanced cancer, tumor immunology

Öz

Interlökin-2 (IL-2), ileri evre kanser vakalarında etkisi kanıtlanan ve tedavi için onaylanan ilk immünoterapi stratejisidir. Yüksek doz IL-2 tedavisi gören metastatik renal hücreli karsinom ve metastatik melanom hasarının %13–20'sinde tedaviye objektif yanıt alınmış ve hastaların bir bölümünde hastalıksız sağkalım 20 yılı aşmıştır. Ancak, IL-2 immünoterapisinin kullanımı, *in vivo* yarı-ömrünün kısa olması, doza bağlı toksisite ve immünosupresif düzenleyici T hücrelerinin stimülasyonu nedeniyle yaygınlaşmamıştır. IL-2 ve IL-2 reseptörlerindeki ilerleyen güncel çalışmalar sonucunda, geliştirilmiş IL-2 formülasyonları üretilmiştir. Bu formülasyonlar, IL-2/anti-IL-2 monoklonal antikor kompleksleri (kısaca, IL-2 kompleksleri), IL-2 muteinleri ve IL-2'nin polietilen glikol ve benzeri moleküllere bağlanmasını içermekte ve seçilmiş lenfosit alt-gruplarının selektif ve güçlü stimülasyonunu sağlamaktadır. Bu makalede, IL-2'nin kanser immünoterapisindeki rolü özetlenmekte ve IL-2 komplekslerinin klinik-öncesi çalışmaları ile kanser tedavisindeki potansiyeli tartışılmaktadır.

Anahtar Kelimeler: Interlökin-2 (IL-2), immünoterapi, ileri evre kanser, tümör immünolojisi

The Biology of IL-2

Interleukin-2 (IL-2) has been one of the most studied cytokines since its discovery as a T cell growth factor in 1976. IL-2 is a four α-helical bundle cytokine that belongs to the family of common gamma chain (g_c, also termed CD132) cytokines.[1–3] In resting conditions, IL-2 is produced at low levels predominantly by $CD4^+$ T helper (Th) cells.[2] Upon activation of the immune system, IL-2 concentrations can rise significantly due to increased secretion by $CD4^+$ Th cells, $CD8^+$ T cells, natural killer (NK) cells, NKT cells, dendritic cells, and mast cells.[4–6] IL-2 production by activated T cells results only in a transient accumulation of IL-2 because such production becomes transcriptionally repressed by negative feedback loops, among others by the action of B lymphocyte-induced maturation protein 1 (Blimp1).[3,7] Blimp1 is activated by IL-2, which in turn binds to the IL-2 promoter region to inhibit IL-2 transcription.[8] Antigen-experienced (i.e., memory) T cells express low levels of Blimp1 and are able to produce IL-2 when re-stimulated, while terminally-differentiated effector T cells upregulate Blimp1 expression upon prolonged antigen stimulation thus losing their ability of IL-2 production.[9,10]

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IL-2 exerts its actions on its target cells by binding to two different signaling IL-2 receptor (IL-2Rs) complexes, termed dimeric and trimeric IL-2Rs.[11] Dimeric IL-2Rs are comprised of the γ_c and IL-2Rβ chain (also called CD122), whereas trimeric IL-2Rs consist of the g_c, CD122, and IL-2Rα chain (also termed CD25). Although CD25 is dispensable for signaling, it enhances the binding affinity of IL-2 to the dimeric complex of CD122 and g_c by 10–100 fold.[12] Upon binding of IL-2 to its receptor, the IL-2-IL-2R complex is internalized and IL-2, CD122 and γ_c are degraded, whereas CD25 is recycled back to the cell membrane.[13] IL-2R signaling depends on the cytoplasmic tails of CD122 and γ_c and occurs via three major intracellular pathways, namely, (i) Janus kinase (JAK)-signal transducer and activator of transcription (STAT), (ii) phosphoinositide 3-kinase (PI3K)-AKT, and (iii) mitogen-activated protein kinase (MAPK) pathway.[11] Monomeric IL-2Rs, comprising only CD25, also exist in membrane-bound or soluble forms. Since CD25 is not involved in signaling these receptors are thought to function as scavengers for IL-2, reducing free IL-2 levels or trans-presenting bound IL-2 to effector T cells.[2,14]

Dimeric IL-2Rs are expressed by memory $CD8^+$ T and NK cells at high levels and by naïve $CD8^+$ T and memory $CD4^+$ T cells at intermediate levels.[11] Following T cell receptor (TCR) stimulation, $CD8^+$ T cells transiently upregulate CD25, thus expressing the high affinity trimeric receptor. In steady state, thymus-derived $CD4^+$ fork head box p3 $(Foxp3)^+$ T regulatory cells (T_{regs}) constitutively express high levels of CD25 and intermediate levels of CD122 and γ_c.[15,16] Type 2 innate lymphoid cells and B cells also express functional trimeric IL-2Rs and expand upon stimulation with IL-2.[17–20] Furthermore, the trimeric IL-2Rs are expressed by nonimmune cells, such as pulmonary endothelial cells, which have been reported to contribute to the toxic adverse effects seen with high-dose (HD) IL-2 immunotherapy.[21] The physiological role of IL-2R expression by endothelial cells is unknown, but we hypothesized that leakiness in endothelial cells upon triggering of their IL-2Rs by locally produced IL-2 could allow the transition of intravascular leukocytes to sites of inflammation and infection.[2,11] Monomeric CD25 is expressed by dendritic cells,[22,23] where it can trans-present bound IL-2 to $CD25^{low}$ T cells early during T cell activation.[24]

The low levels of IL-2 present at the steady state are crucial for the development and survival of $CD4^+$ $CD25^{high}$ T_{regs}.[25,26] Since T_{regs} do not produce IL-2, they depend on paracrine IL-2 production.[27] This dependency of T_{regs} is also well illustrated in mice lacking IL-2, CD25, or CD122, all of which suffer from systemic autoimmunity.[28–30] The adoptive transfer of wild-type T_{regs} is able to rescue the phenotype of $CD25^{-/-}$and $CD122^{-/-}$mice.[31,32]

The role of IL-2 for $CD8^+$ T cells is subtler and becomes evident during $CD8^+$ T cell responses. IL-2 signaling is required for efficient primary and secondary expansion of $CD8^+$ T cells, as well as the stimulation of resting memory T cells and NK cells.[33] Moreover, the persistence and intensity of IL-2 signaling plays a major role in the differentiation of recently-activated $CD8^+$ T cells during primary expansion. Cells that sustain high CD25 expression are exposed to strong IL-2 signals and, together with repetitive TCR stimulation, become effector cells that are short-lived due to exhaustion or activation-induced cell death. Cells that show only transient upregulation of CD25 following activation are subjected to less IL-2 signaling and differentiate into central memory $CD8^+$ T cells. These cells are long-lived and can home to secondary lymphoid organs owing to their expression of L-selectin (CD62L) and CCR7.[34–36]

IL-2 Immunotherapy

Due to its potent immune stimulatory effects on T and NK cells, IL-2 was investigated as an immunotherapy for advanced malignancies already shortly after its discovery. Initial studies in mice demonstrated the potential of IL-2 to enhance the endogenous anti-tumor immune response to achieve tumor regression.[37] Subsequently, IL-2 was tested in clinical trials for metastatic renal cell carcinoma and metastatic melanoma. Treatment regimens with HD IL-2 (600'000–720'000 international units per kg of body weight per infusion, every 8 hours for up to 14 cycles) led to objective clinical responses in 13–16% (with 4–7% complete responses) of metastatic renal cell carcinoma patients and in 14–20% (with 5–9% complete responses) of metastatic melanoma patients. Even though only some patients showed clinical responses to HD IL-2 treatment, the responses were remarkably durable, some lasting for 20 years and more.[38–41] Based on these results, the United States Food and Drug Administration approved HD IL-2 for the treatment of metastatic renal cell carcinoma and metastatic melanoma in 1992 and 1998, respectively.

IL-2 therapy in patients with advanced melanoma has also been tested in combination with various approaches, including peptide vaccination, immune checkpoint inhibitors (such as ipilimumab), interferon (IFN)-α, chemotherapy (cisplatin, vinblastine and dacarbazine), and adoptive cell transfer (ACT). Vaccination studies resulted in contradictory reports ranging from no effect to significant improvements, [40,42–44] while co-treated with ipilimumab, IFN-α and chemotherapy failed to show any additive effect.[45,46] Combination of IL-2 with ACT demonstrated the most promising results in the clinic.[37] The stimulatory effects of IL-2 on T cell growth and survival enabled the *ex vivo* culturing of T cells isolated from tumors. T cells infiltrating solid tumors are enriched in tumor-antigen specific T cells that can be used for ACT following IL-2-mediated *in vitro* expansion.[47] Moreover, co-administration of HD IL-2 with ACT was used to improve the proliferation and survival of transferred T cells in patients. As this therapeutic strategy developed, the combination of HD IL-2 and ACT following lymphodepletion was found to be the most effective treatment, resulting in an objective response rate of up to 72% in metastatic melanoma patients.[37] However, this approach is performed in a few centers thus benefitting only a limited number of patients.

Despite the above-mentioned benefits of IL-2 immunotherapy, the use of IL-2 for advanced cancer is hampered by several factors, such as the short half-life of IL-2, dose-dependent IL-2-related adverse effects, and the stimulation of immunosuppressive T_{regs} by IL-2.[11] The half-life of IL-2 *in vivo* is measured in minutes, as IL-2 is rapidly cleared by the renal system.[37] The half-life of IL-2 can be prolonged by different strategies, such as coupling IL-2 to large proteins.[48] Administration of HD IL-2 leads to endothelial cell damage resulting in vascular leak syndrome (VLS), which causes severe side effects in various organs, including pulmonary edema, renal failure, and liver cell damage.[49,50] The pathomechanism of IL-2-mediated toxicity has been proposed to hinge, firstly, on damage of endothelial cells by the direct interaction and toxic effect of IL-2 with trimeric IL-2Rs on them,[21,51] followed by the secretion of pro-inflammatory cytokines and vasoactive mediators by stimulated T cells and NK cells.[11,52,53] IL-2-mediated toxicity is dose-dependent, and decreasing the dose of IL-2 to 72'000 international units per kg of body weight causes significantly less adverse effects. However, such reduction in the dose of IL-2 is also accompanied with a significant loss of anti-tumor efficacy.[54] IL-2 is expected to stimulate effector T cells and NK cells only, once the high-affinity IL-2Rs of T_{regs} are saturated with IL-2, thus necessitating high IL-2 doses (Figure 1). $CD25^+$ immunosuppressive cells in turn curtail the anti-tumor activities of IL-2-stimulated $CD8^+$ T cells and NK cells, among others. In order to overcome these drawbacks, selective stimulation of effector immune cells is necessary which can be achieved by complexing IL-2 with certain anti-IL-2 monoclonal antibodies (mAb), as outlined in the next section.

IL-2/anti-IL-2 mAb Complexes

IL-2/anti-IL-2 mAb complexes consist of IL-2 bound to a specific mAb that can direct the cytokine to cells expressing the dimeric or the trimeric IL-2R. $CD8^+$ T cells and NK cells are preferentially stimulated by IL-2 complexed to CD122-biased mAbs, such as S4B6 or JES6–5H4 for murine IL-2 and NARA1 or MAB602 for human IL-2 (Figure 1).[55–57] Seven daily injections of mice with IL-2/S4B6 complexes showed 20 to 100-fold expansion of $CD8^+$ T cells, particularly $CD44^{high}$ $CD122^{high}$ memory-phenotype $CD8^+$ T cells, while $CD4^+$ $Foxp3^+$ T_{regs} were only increased by 2- to 5-fold.[55] IL-2/S4B6 complexes where shown to interfere with the interaction of IL-2 with CD25, thus disfavoring the binding and consumption of IL-2 by $CD25^+$ cells.[11] These effects are further enhanced by an extended *in vivo* half-life of IL-2 by its association with a full mAb, in this case S4B6, leading to a significant increase in IL-2 availability for effector T cells and NK cells.[56] Moreover, decreased binding of IL-2 complexes to $CD25^+$ endothelial cells reduced endothelial cell damage and VLS.[21,56]

The above-mentioned advantages make IL-2/S4B6 complexes a promising strategy for treatment of advanced malignancies. Several studies using various preclinical cancer models, such as B16-F10 melanoma, MC38 colon carcinoma, LLC-1 Lewis lung carcinoma, BCL1 B cell leukemia, MCA205 sarcoma, and TRAMP-C1 prostate carcinoma have demonstrated potent anti-tumor effects of IL-2 complexes alone or in combination with other therapies, including a Toll-like receptor ligand, an agonist anti-OX40 mAb, ACT, and peptide vaccination.[21,58–63]

Recently, a CD122-directed anti-human IL-2 antibody, termed NARA1, has been generated and characterized.[57] As determined by its crystal structure, the binding site of NARA1 overlaps with the CD25-binding epitope

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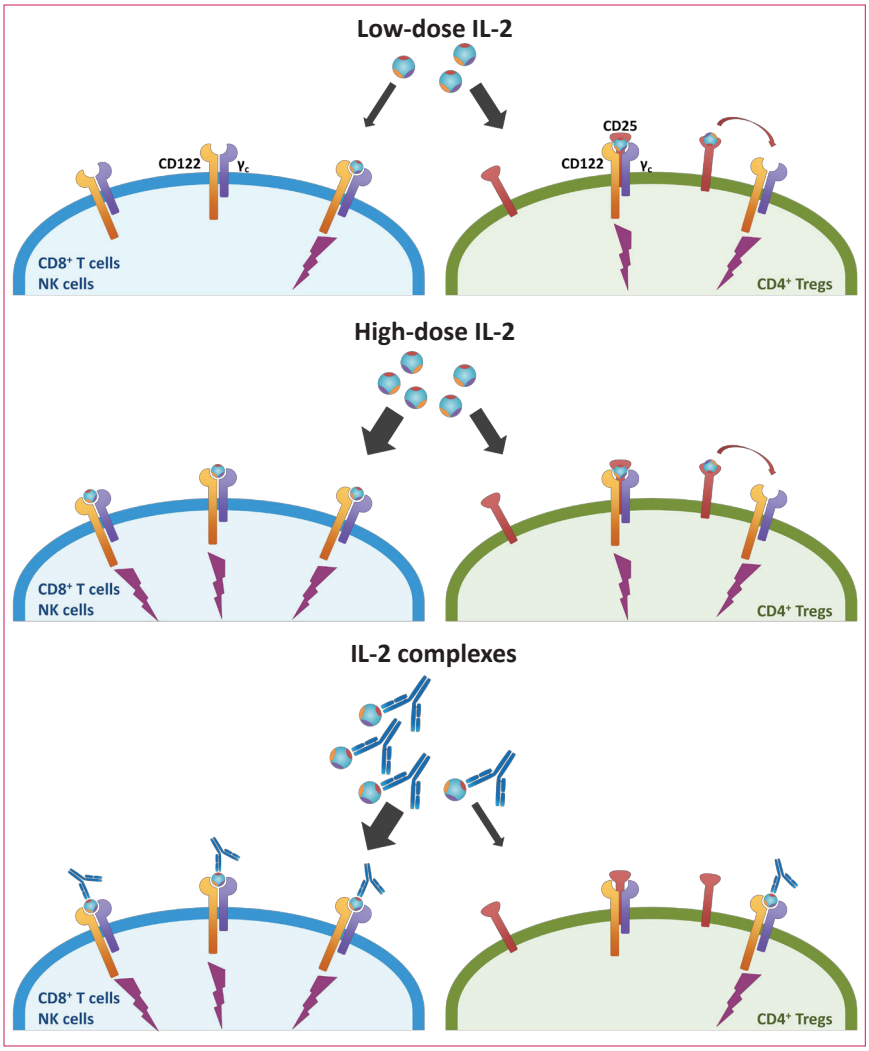

Figure 1. Stimulation of immune cells by IL-2 and IL-2 complexes.

Depicted are CD122^{high} (in blue; e.g., CD8$^+$ T cells or NK cells) versus CD25^{high} CD122 intermediate immune cells (in green; e.g., CD4$^+$ regulatory T cells, T_{regs}) stimulated by low-dose IL-2, high-dose IL-2, or IL-2 complexes. The constitutive and high CD25 expression on T_{regs} endows them with a selective advantage for the binding of available IL-2. Thus at low IL-2 doses, most of the cytokine is bound and consumed by CD25^{high} cells, whereas CD8$^+$ T and NK cells are less affected by these concentrations. With higher doses of IL-2, once T_{regs} are saturated, CD8$^+$ T and NK cells can bind the remaining cytokine. By contrast, association of IL-2 with a CD122-biased anti-IL-2 monoclonal antibody, such as NARA1 (in dark blue), leads to the formation of IL-2 complexes, in which IL-2 is preferentially directed to CD122^{high} immune cells, such as CD8$^+$ T cells and NK cells, leading to their preferential stimulation and expansion.

of IL-2. Therefore, when IL-2 is bound to NARA1, forming hIL-2/NARA1 complexes, association of IL-2 with CD25 and hence the trimeric IL-2R is disfavored. In line with studies on murine IL-2/S4B6 complexes, injection of hIL-2/NARA1 complexes to mice resulted in vigorous proliferation and expansion of CD8$^+$ T cells and NK cells, while counts of CD4$^+$ CD25$^+$ Foxp3$^+$ T_{regs} increased only minimally. In preclinical studies, hIL-2/NARA1 complexes displayed robust anti-tumor properties in syngeneic B16-F10 melanoma, both in the intradermal and the metastatic pulmonary model. Also, in the *Tyr: : N-RasQ^{61}K Ink4a$^{-/-}$*spontaneous melanoma model, tumor-free survival was increased in animals receiving hIL-2/NARA1 complex treatment, which showed reduced counts of skin melanoma nodules and lung metastases at the time of sacrifice. The anti-tumor response was dependent on CD8$^+$ T cells, which were increased in numbers in tumor-draining lymph nodes and within the tumor. The ratio of intratumoral T_{regs} to CD8$^+$ T cells was in favor of CD8$^+$ T cells. CD8$^+$ T cells isolated from tumors efficiently produced IFN-γ and expressed low levels of immune checkpoint molecules, including programmed cell death protein-1 (PD-1), T cell immunoglobulin and mucin domain-3 (TIM-3), and lymphocyte activation gene-3 (LAG-3), along with high expression of CD62L and CD44, all indicative of functional memory T cells (Figure 2).[57] Moreover, the combination of hIL-2/NARA1 complexes with an inhibitor of the histone methyltransferase enhancer of zeste homolog 2 (Ezh2) resulted in superior tumor control, compared to monotherapy, in several mouse models of melanoma,[64] including the *Tyr::N-RasQ^{61}K Ink4a$^{-/-}$* spontaneous melanoma model, RIM-3 melanoma (which is a tumor cell line derived from *Tyr::N-RasQ^{61}K Ink4a$^{-/-}$*mice),[65] and the intradermal B16-F10 model. This was due to reversing of adaptive resistance mechanisms to tumor immunotherapy by Ezh2 inhibition, thereby maintaining the tumor cells in an immunogenic state.[64]

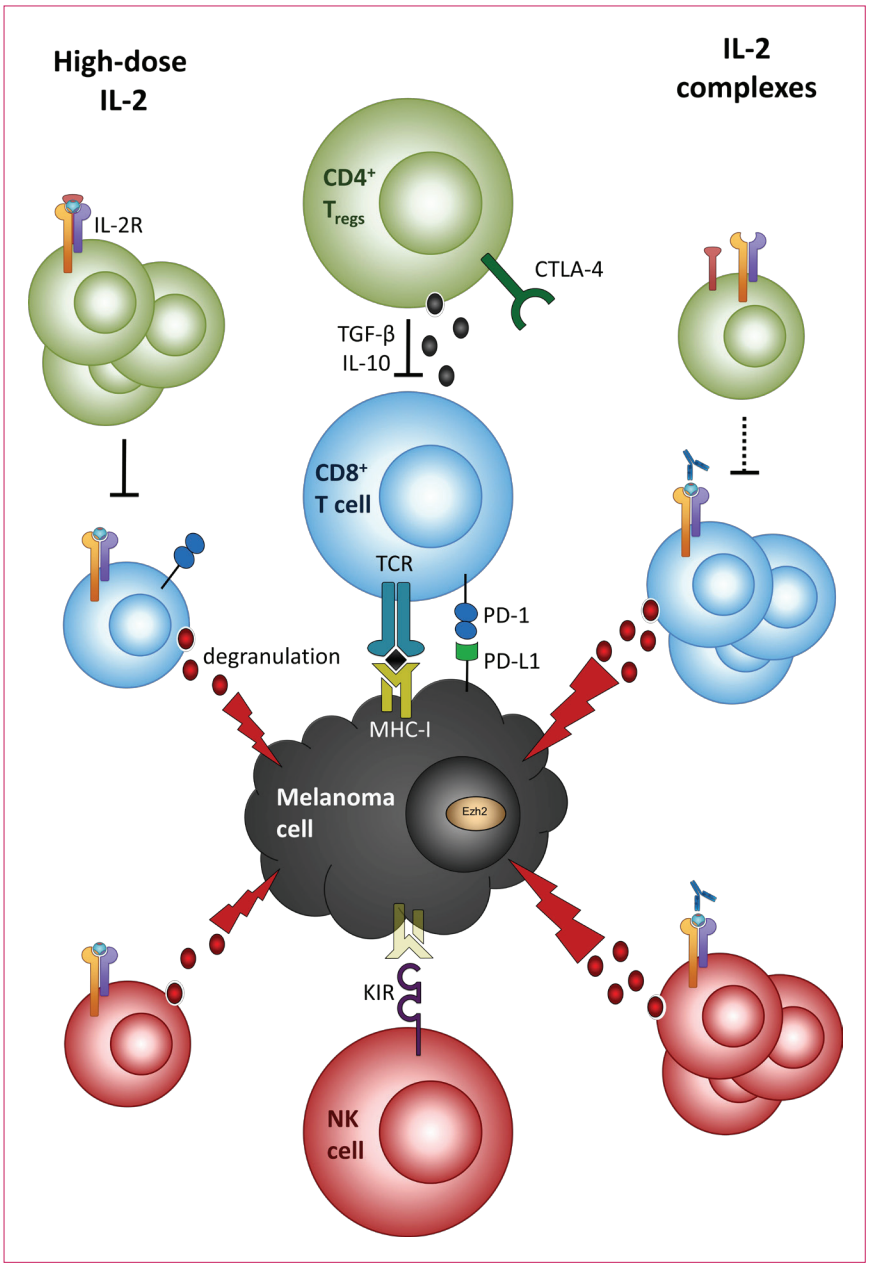

Figure 2. IL-2 complex immunotherapy.

$CD8^+$ T (blue) and NK (red) cells are able to identify and attack melanoma cells. NK cells sense a lack of major histocompatibility complex class I (MHC-I) molecules on melanoma cells, which triggers the degranulation of effector molecules causing lysis and/or apoptosis of melanoma cells. $CD8^+$ T cells recognize, via their T cell receptors (TCR), tumor antigens presented by MHC-I molecules on the surface of melanoma cells, leading to the release of cytotoxic molecules, such as perforin and granzyme B. Conversely, suppressive immune cells are also present in the tumor microenvironment, including regulatory T cells (T_{regs}; green) and myeloid-derived suppressor cells (not shown). These cells secrete immunosuppressive cytokines, such as IL-10 and transforming growth factor (TGF)-β, and express inhibitory molecules, such as cytotoxic T-lymphoctye antigen 4 (CTLA-4), which altogether limit effector functions of $CD8^+$ T cells and NK cells. Moreover, the anti-tumor activity of $CD8^+$ T cells is also regulated by the expression of inhibitory receptors, among others by programmed cell death protein-1 (PD-1). The interaction with its ligand PD-L1, which can be expressed by melanoma cells, inhibits T cell activity. High-dose IL-2 immunotherapy stimulates $CD8^+$ T cells and NK cells as well as T_{regs}. IL-2 complexes preferentially increase counts and activity of $CD8^+$ T cells and NK cells over T_{regs}, leading to prolonged immune mediated tumor control. Moreover, $CD8^+$ T cells also appear fitter upon IL-2 complex stimulation, as determined by increased degranulation and decreased expression of PD-1 and other inhibitory receptors.

Concluding Comments

The ability of IL-2 to strongly activate and program $CD8^+$ T cells can be harnessed for cancer immunotherapy. The setbacks of IL-2 therapy such as the short *in vivo* half-life and the stimulation of immunosuppressive $CD25^+$ regulatory cells and endothelial cells has inspired the development of improved IL-2 formulations. These strategies rely on selective stimulation of IL-2Rs that are differently expressed on $CD8^+$ T cells and NK cells versus T_{regs}. Association of IL-2 with specific anti-IL-2 antibodies not only directs IL-2 to selected IL-2Rs but also increases its potency (Figure 2).[55–57] Other strategies of selective IL-2 immunotherapy consist in the design of IL-2 muteins where specific amino acid substitutions are introduced to favor or disfavor the binding of IL-2 to certain IL-2R subunits (reviewed in[11]). These IL-2 muteins, however, appear to be immunogenic, thus eliciting anti-drug antibodies that interfere with the activity of IL-2 muteins.[11] This is not the case with hIL-2/NARA1 complexes, which use natural IL-2. Clinical testing will determine the benefits of hIL-2/NARA1 complexes, given either alone or in combination with other immunotherapies, anti-cancer therapies, or epigenetic modifier drugs.

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