Review

Application of IL-2 therapy to target T regulatory cell function

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Interleukin-2 (IL-2) was originally discovered as a growth factor for activated T cells *in vitro*. IL-2 promotes $CD8^+$ T cell growth and differentiation *in vivo*, but has little effect on $CD4^+$ T cell function. Regulatory T cells (Treg cells) express all three chains (CD25, CD122, and CD132) of the IL-2 receptor complex and are dependent on IL-2 for survival and function. Exogenous IL-2 can augment Treg cell numbers *in vivo* and may have therapeutic value in the treatment of autoimmune and inflammatory diseases. Complexes of IL-2 with different IL-2 antibodies can target delivery to cells expressing all three receptor chains (Treg cells and activated T effector cells) or to cells expressing just CD122 and CD132 (NK cells and memory phenotype $CD8^+$ T cells).

IL-2 and T regulatory cells

Since the discovery of $CD4^+CD25^+$ Treg cells in 1995 [1] and the identification of Foxp3 as the major transcription factor which controls many of their functions [2,3], it has become apparent that augmentation of either the numbers of Treg cells or their suppressor function would prove to be useful in the treatment of autoimmune diseases, graft rejection, and graft versus host disease (GVHD). A number of studies in animal models strongly supported this hypothesis. The main experimental tool to demonstrate the utility of this approach has been augmentation of Treg cell numbers by cellular biotherapy with polyclonal or antigen-specific Treg cells [4]. One of the unique properties of the majority of Treg cells that express Foxp3 is the co-expression of CD25 (IL-2Rα), as well as the other two components of the IL-2R complex IL-2Rβ (CD122) and IL-2Rγ (CD132, or the γ_c common chain). Although IL-2 is not required for the generation of $Foxp3^+$ T cells in the thymus [5] as other cytokines utilizing the γ_c chain (IL-7 and IL-15) can mediate this function, IL-2 is absolutely required for the homeostasis and maintenance of Treg cell in the periphery [6]. These results have raised the possibility that the administration of IL-2 may augment Treg cell numbers and function and may be of therapeutic benefit in the treatment of autoimmune and inflammatory diseases. Two recent publications [7,8] indicating the potential benefit of chronic low-dose IL-2 administration in alleviating chronic GVHD and chronic hepatitis C (HCV)-mediated vasculitis in human have confirmed the value of this approach. Here, I will review the history of IL-2 as an immunotherapeutic agent focusing on its remarkable transformation/evolution from a cytokine initially used to augment T effector (Teff) cells in oncology to an agent targeted to enhancing the major population of T suppressor cells, $CD4^+CD25^+Foxp3^+$ Treg cell.

IL-2 as a T cell growth factor and activator

IL-2 was originally discovered as a growth factor for T cells *in vitro* [9]. The gene for IL-2 was cloned in 1983 [10], and its crystal structure was solved in 1992 [11]. IL-2 is a monomeric secreted glycoprotein with a molecular weight of 15 kDa. It exists as a globular structure with four α-helices in a configuration typical of Type I cytokines. Responses to IL-2 are mediated through its interaction with the high affinity trimeric IL-2R complex (CD25, CD122, and CD132). CD122 and CD132 can form a dimeric low affinity receptor which binds IL-2 with lower affinity, and is crucial for signal transduction [12]. Absence of either chain leads to the abrogation of IL-2 signaling. Stimulation of the receptor complex by IL-2 induces activation of Stat5 [13].

Recombinant IL-2 was shown to have potent antitumor effects in several mouse tumor models. The first application of IL-2 for therapeutic use in human was to expand cells from peripheral blood, termed lymphokine-activated killer cells (LAK cells) that were administered in combination with IL-2 for the treatment of malignancies [14]. These studies were followed by the use of IL-2 alone to treat tumors. Treatment with a regimen of high dose bolus IL-2 led to a durable and complete regression of disease in ~8% of patients with these cancers. The Food and Drug Administration (FDA) ultimately approved high dose bolus IL-2 for the treatment of renal carcinoma and melanoma. Nevertheless, it was never clear why only a low percentage (5–15%) of patients responded to this therapy; furthermore, the mechanism of action of IL-2 in responders has not been elucidated. Significant toxicity was noted with this form of therapy including the development of the vascular or capillary leak syndrome (VLS). VLS leads to egress of intravascular fluid followed by volume depletion causing a drop in blood pressure. VLS correlates with the duration and dose of IL-2. Recent studies [15] have demonstrated that IL-2-induced pulmonary edema results from direct binding of IL-2 to $CD31^+$ lung endothelial cells that express low to intermediate levels of the trimeric IL-2 receptor complex.

IL-2 has also been used to boost immune responses in patients with advanced HIV [16] and induced a significant rise in the $CD4^+$ T cell count compared to patients treated with anti-retroviral therapy alone. Cell expansions of both

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naïve and memory T cell populations were secondary to an increase in T cell survival. $CD4^+$ T cells expanded with IL-2 express intermediate levels of CD25 as well as moderate levels of Foxp3 and may represent Treg cell [17]. Despite the increases in $CD4^+$ cell count, there was no clinical benefit, as measured by the reduction in the risk of opportunistic diseases or death in IL-2 treated patients compared to those patients that received anti-retroviral therapy alone [17].

Some of the beneficial effects of IL-2 in treatment of patients with metastatic melanoma or renal carcinoma were likely mediated by enhancement of $CD8^+$ T cell function and/or numbers. The $CD8^+$ T cell response to an acute systemic infection entails vigorous expansion of antigen-specific cells followed by a contraction phase in which 90–95% of the cells die by apoptosis. The cells remaining after the contraction phase become memory T cells. Naïve $CD8^+$ T cells are programmed to become memory cells in the early phase of the $CD8^+$ response. Initial studies [18] demonstrated that excessive IL-2 administration during the expansion phase was detrimental to the survival of rapidly dividing T effector (Teff) cells, whereas IL-2 administration during contraction led to increased cell survival. By contrast, Williams *et al.* [19] used mixed bone marrow chimeras between wild type (WT) and CD25-deficient bone marrow donors. CD25-deficient $CD8^+$ Teff and memory cells lacking CD25 were generated and normally maintained after acute infection, but their secondary expansion after pathogen re-challenge was severely compromised. Exposure to IL-2 during the initial expansion phase of an anti-viral $CD8^+$ T cell response was crucial for endowing memory $CD8^+$ T cells with efficient proliferative capacity following secondary challenge.

Kalia *et al.* [20] demonstrated that a very complex relationship existed between CD25 expression, IL-2, and the differentiation of $CD8^+$ T cells *in vivo* during infection. CD25 expression was uniformly induced on antigen-specific $CD8^+$ T cells early (days 1–2) after infection, was rapidly upregulated by T cell receptor (TCR) signals, and was maintained by a positive feedback loop via enhanced IL-2 signals. CD25 induction increased with increasing rounds of cell division, but was downregulated on all cells by day 8. On day 3.5 post-infection, a subpopulation downregulated CD25 expression, so that one could isolate $CD25^{lo}$ and $CD25^{hi}$ $CD8^+$ T cell populations. When both $CD25^{lo}$ and $CD25^{hi}$ subsets were transferred into infection-matched recipients, both populations differentiated into potent killer cells, but the $CD25^{hi}$ cells were more advanced in their effector differentiation, underwent more proliferation, but were more prone to apoptosis. The $CD25^{lo}$ cells differentiated into long-term functional memory cells and exhibited more IL-2 production. Thus, cells that rapidly downregulate CD25 early in the response preferentially contribute to the long-lived functional memory pool.

Effects of IL-2 on Treg cells

Whereas there is little doubt from the experiments described above that IL-2 can play a major role in the expansion/differentiation of $CD8^+$ T cells *in vivo*, its effects on $CD4^+$ T cells are less clear. The prevailing view of IL-2 as a cytokine needed for the expansion of $CD4^+$ T cells *in vivo* was rapidly challenged by studies in mice with a deficiency in IL-2, CD25, or CD122 expression that developed many manifestations of systemic autoimmunity and fatal lymphoproliferative diseases [21]. It was originally proposed based on *in vitro* studies that this syndrome was secondary to a lack of IL-2 mediated activation-induced cell death (AICD) [22]. However, experimental evidence that IL-2-induced AICD suppresses T cell responses *in vivo* is limited. Furthermore, autoimmunity in IL-2-deficient mice is mediated by the adaptive immune response, as disease can be eliminated when IL-2-deficient mice are bred to RAG-deficient mice. The finding that normal lymphocytes could correct the autoimmune disease of mice deficient in IL-2 signaling suggested that the major action of IL-2 is not cell intrinsic [21].

The numbers of $Foxp3^+$ Treg cells in the periphery of IL-2-, IL-2Rα- and IL-2Rβ-deficient mice are substantially decreased or almost absent. Treatment of normal mice with anti-IL-2 or with CTLA-4Ig to inhibit co-stimulatory signals also leads to a rapid decline in the number of $Foxp3^+$ T cells [23]. IL-2 is not only required for the survival of $Foxp3^+$ Treg cell in the periphery, but also appears to be critical for maintenance of their suppressive functions [24]. Foxp3 expression in Treg cell is required to establish a gene expression program that renders Treg cell critically dependent on paracrine IL-2 signaling that represses of IL-2 production by Treg cell and induces IL-2Rα. Treg cells are also the first cell population responding to IL-2 produced by Teff cells during immune responses [25]. Taken together, these experiments indicate that the major end product of IL-2 signaling is growth limiting via its effects on Treg cells rather than promoting T cell expansion.

The discovery of the effects of IL-2 on Treg cells raised the question of the effects of high dose IL-2 on Treg cells in cancer patients treated with high dose IL-2. Ahmadzadeh and Rosenberg [26] demonstrated that IL-2 treatment resulted in a 6-fold increase in the frequency of $CD25^{hi}$ cells in the peripheral blood compared with pretreatment levels and the expanded Treg cells were suppressive *in vitro*. IL-2-mediated expansion of Treg cell may contribute to the lack of objective response to IL-2 therapy in the majority of treated melanoma and renal cell carcinoma patients.

IL-2 delivery models

If IL-2 is to be used as a drug to augment Treg cell numbers and function, several questions need to be addressed about how to optimally deliver this agent. One of the major problems with the therapeutic administration of IL-2 is that following an i.v. injection, unbound IL-2 has a very short half-life in the serum of mice (<3–5 min). Subcutaneous or intraperitoneal injections can slightly prolong the half-life. One solution to prolonging cytokine half-life was the observation that monoclonal IgG anti-cytokine antibodies (mAb), which have a longer half-life than soluble cytokine receptors, could prolong the half-life of different cytokines [27]. Injection of complexes composed of two molecules of cytokine and one molecule of neutralizing anti-cytokine prolonged the half-life of IL-3, IL-4, and IL-7. It appeared from these studies that neutralizing anti-cytokine antibodies were superior in prolonging