

Interleukin-2 and autoimmune disease occurrence and therapy

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Abstract. – BACKGROUND: Interleukin-2(IL-2), also called T-cell growth factor and primarily produced by antigen-activated T cells, is a kind of lymphoid factor with immunoregulatory effect which can promote T-cell-dependent immune responses. IL-2 was first used as a therapeutic approach to boost immune responses in patients with invasive cancer or advanced HIV disease.

OBJECTIVE: The purpose of the review is to refer the mechanism of autoimmune disease caused by IL-2 deletion and the application of IL-2 in curing autoimmune disease.

STATE OF THE ART: IL-2 signal plays a key role in promoting the development, homeostasis and the function of the regulatory T cells. The deletion of IL-2 *in vivo* causes T cell-mediated autoimmune diseases. Now it is being considered as a kind of medicine inhibiting immune responses.

PERSPECTIVES: Further studies with controlled clinical trials will be needed to prove the potential of IL-2 as a therapeutic strategy for autoimmune diseases.

CONCLUSIONS: The decreased production of IL-2 in patients with autoimmune disease leads to immune defects, such as decreased production of Treg cells, decreased AICD and cytotoxicity. Combination therapy based on IL-2 may prove to be beneficial in curing the immunological disorders.

Key Words:

Interleukin-2 (IL-2), T-cell immune, Autoimmune disease.

can bind to the high affinity receptor on target cell membrane which consists of three subunits including IL-2R α (CD25), IL-2R β (CD122), and IL-2R γ (CD132)². Of them, IL-2R β and IL-2R γ play an important role in transmitting intracellular signals. IL-2R α can increase the affinity of its receptor by 10 to 100 folds, though not participating in the signal transduction due to its short cytoplasmic domain^{3,4}. Therefore, IL-2 does not produce biological effects in solely binding to IL-2R α , but mediates the activation of target cell in binding to IL-2R β . IL-2R γ can't binds to IL-2 singly, but is necessary for signal transduction and the combination of α , β and γ subunits. Moreover, high-affinity $\alpha\beta\gamma$ is constitutively expressed in CD4+ regulatory T cells and in recently antigen-activated T cells^{5,6}, while low-affinity $\beta\gamma$ is prominent on memory CD8+ T cells and NK cells. IL-2 acts in an autocrine or paracrine way on these cells, many of which up-regulate CD25 expression⁷.

Although β and γ chains lack kinase activity, they can be phosphorylated after binding to kinases JAK1 (Janus kinase gene 1) and JAK3. Then their SH2 domains recruit STAT5a/STAT5b. The later is phosphorylated by JAKs and translocated into nucleus as a heterodimeric STAT complex, which further regulates gene transcription by binding to target DNA sequences⁸. Other signaling pathways, such as MAPK and PI-3K, trigger signal transduction by phosphorylating tyrosine residues within the cytoplasmic tail of IL-2R β chain.

Barmeyer *et al*⁹ found that T cells and NK cells were present in newly-born mice with the deletion of IL-2. Four weeks later, the hyperplasia of lymph node and intestinal lymphoid tissue and splenomegaly occurred, at the same time the concentration of autoantibody in peripheral blood increased. Nine weeks later, 25%-50% mice died of severe hemolytic anemia, the others suffered from severe inflammatory bowel dis-

Introduction

Interleukin-2(IL-2), also named T-cell growth factor, plays an important role in T cell biology and can promote T-cell-dependent immune responses¹. IL-2 is a kind of 4-bundle α -helical protein with the molecular mass of 15 kDa. It is primarily produced by antigen-activated T cells and

eases and other autoimmune diseases. Further research showed that the deletion of IL-2 caused the lack of CD4+CD25+regulatory T cells, which resulted in the occurrence of T-cell mediated autoimmune diseases¹⁰.

IL-2 and Autoimmune Disease Occurrence

The research on *IL-2*-deficient mice showed that long-term lack of IL-2 signal resulted in an excessive proliferation of autoreactive T lymphocytes, further the occurrence of autoimmune diseases.

IL-2 and Development and Function of Treg Cells

Generally speaking, there are three types of regulatory T cells (Treg cells): IL-10-induced Tr1 cells, TGFβ-induced Th3 cells and CD4+CD25+Treg. Treg cells with immunosuppressive properties towards a variety of immune cells can inhibit T-cell proliferation, and they are characterized with preferential expression of Treg specific transcription factor FOXP3 and constitutive expression of IL-2Rα chain¹¹. Alteration of *FOXP3* results in a decrease in the number of Treg cells, and the occurrence of multi-organ autoimmunity¹². Meanwhile, Treg cells dependent on IL-2 inhibit T cell proliferation and violate homeostasis by producing lymphatic regional signal. Decreased Treg cells leads to a lack of this kind of signal, the binding of IL-2 to low-affinity receptor and proliferation of autoreactive T cells, and finally, autoimmune disease is caused. Decreased IL-2 brings about a decrease in the number of Treg cells and excessive lymphoproliferation, triggering the occurrence of autoimmunity. The low number of Treg cells in *IL-2/IL-2R* knockout mice evidenced that Treg cells are not completely dependent on IL-2 for development¹³. In addition, the stable expression of *FOXP3* is critical for establishing and maintaining Treg lineage¹⁴. Mice lacking *FOXP3* are completely short of Treg cells and suffer from serious autoimmune diseases.

Reportedly, mice with a lack of IL-2 or IL-2R lacked CD4+CD25+Treg, while transgenic T cells with inserted *IL-2R* could have a stable expression of CD4+CD25+, keeping homeostasis, which showed that IL-2 played an important role in preventing the occurrence of autoimmune disease¹⁵. Another research showed that *IL-2R*-deficient CD4+CD25+Treg was not amplified in peripheral lymphatic system of wild mice¹¹, which indicated that IL-2 was necessary for the amplifi-

cation and development of CD25+CD4+Treg generated by the thymus. If impaired IL-2R activated Stat5 in mice, a normal quantity of peripheral Treg cells was generated¹⁶. Meanwhile, these mice were protected from lethal autoimmunity but symptoms of organ-specific autoimmunity occurred to them as they aged. The findings above showed that the susceptibility that leads to autoimmune disease might increase, though the low mount of IL-2R could maintain relatively normal generation of Treg cells. In conclusion, *IL-2/IL-2R* deletion affects development, function and homeostasis of Treg cells.

IL-2 and Activation-Induced Cell Death

After the activation of naive T cells by antigenic stimulation through T-cell receptor (TCR), antigen specific T cells proliferated and differentiated into effector T cells, which eliminated antigen, then, the process of Activation-Induced Cell Death (AICD) destroyed the excess effector T cells to maintain the balance among immune cells. IL-2 is required for this kind of controlled apoptotic mechanism, together with costimulation through CD95, tumor necrosis factor receptor 1 (TNFR1), and other costimulatory molecules¹⁷.

Through up-regulating Fas ligand and TNF receptor, down-regulating cysteine aspartic enzyme inhibition factor (C-FLIP) and promoting the synthesis of Interferon receptor (IFN-r), IL-2 induced apoptosis of sensitized T cells. However, *IL-2/IL-2R*-deficient mice lacked the expression of Interferon receptor (IFN-r), which blocks AICD. Furthermore, IL-2 in CD8+ T cells modulated AICD by down-regulating IL-2Rγ, a receptor chain which was associated with cell survival¹⁸. Previous study showed that due to reduced level of intracellular TNFα and up-regulation of cyclooxygenase2 (COX2)¹⁹, autoreactive T cells lose regulatory role and become pathologic T cells, causing lymph nodes and splenomegaly. Consequently, systemic lupus erythematosus (SLE) patients were more resistant to AICD. Roifman²⁰ holds that in a patient with mutation of IL-2Rα, T cells abnormally proliferated and huge amounts of lymphocytic infiltration of tissues was observed, in conjunction with tissue atrophy and inflammation. *IL-2Rα*-deficient thymocytes fails to down-regulate bcl-2, and subsequently, apoptosis in the thymus is significantly reduced, leading to the expansion of autoreactive T cell and then the occurrence of autoimmune diseases.

IL-2 and Cytotoxicity

Cytotoxic T lymphocyte (CTL) development was decreased with the absence of IL-2, which indicated that IL-2 is necessary for the development of CD8⁺ T cell and NK cell cytotoxicity. Moreover, it is significant for these cell types to fight infection and for cytotoxic T lymphocytes (CTLs) cells to destruct virally infected cells and intracellular parasitic pathogen-infected target cells. One research showed that IL-2 up-regulated perforin transcription, and suppressed re-expression of memory CTL markers Bcl6 and IL-7R²¹. However, in mice infected by lymphocytic choriomeningitis virus, *IL-2Ra*-deficient effector CD8⁺T cells up-regulated Bcl6 and down-regulated perforin and granzymeB, the hallmarks of CD8⁺ T cell cytotoxicity²², leading to decreased CTL. Thus, inflammation influences differentiation of effector and memory CTL, whereas persistent stimulation of IL-2 promotes generation of effector CTL at the expense of memory CTL development²¹. Transgenic expression of IL-2 could increase the expansion of CTLs²³. Another research demonstrated that IL-2 up-regulated the costimulatory molecule CD70 which was necessary for expansion of CD8⁺T cells. When CD70 was blocked, CTL expansion did not occur²⁴. CD8⁺T cells could express costimulatory molecule CD27 and preferentially secrete IL-2²⁵. Recently, it has been found that IL-2 production induced by CD27 stimulates the survival of CD8⁺ T cell in an autocrine manner in virally infected mice²⁶. In addition, some researchers treated mice with IL-2 complex, and found that CTL effect was enhanced *in vivo*²⁷. These evidences prove that IL-2 signal is necessary for the development of cytotoxicity.

IL-2 and The Therapy of Autoimmune Disease

IL-2 has strong proliferative effects on T cells, and was firstly used as a therapeutic approach to boost immune responses in patients with invasive cancer or advanced HIV disease²⁸.

The Therapeutic Effect of Low-Dose IL-2 on Autoimmune Disease

Treg cells were capable of forming the highest affinity receptor complex for IL-2, due to their constitutive expression of CD25. Hence, they became sensitive to very low level of IL-2, which supported the rationale of examining the potential of low-dose IL-2 for “Treg-only enhancing treatment”²⁹. In the study on patients with chron-

ic myelogenous leukemia after allogeneic hematopoietic stem cell transplantation(HSCT), it was showed that low-dose IL-2 treatment resulted in a 1.9 median fold increase in the number of CD4⁺CD25⁺ cells in peripheral blood as well as a 9.7 median fold increase in *FOXP3* expression in CD3⁺T cells, and that this effect seemingly increased as the treatment went³⁰. Moreover, it has been proved that a low-dose IL-2 treatment promoted Treg cell survival and prevented diabetes occurrence in NOD mice³¹.

The therapeutic Effect of Anti-IL-2-IL-2 Complex on Autoimmune Disease

At first, researchers treated cancer with high doses of IL-2, but the short half-life of purified IL-2 and the high toxic of high-dose IL-2 limited its application in cancer treatment³². Therefore, in recent years, scientists adopted the combination of IL-2 with a carrier protein, such as bovine serum albumin (BSA), gelatin or even an irrelevant immunoglobulin chain, which have successfully prolonged the half-life of IL-2 and reduced its side effects³⁰.

It has been pointed out that treating cancer with Treg cells did not work well. Subsequently, a new therapy method came into being, that is, IL-2 was coupled with different anti-IL-2 monoclonal antibodies (mAb) and then preferentially acted on CD25 or CD122 by varying the mAb, which is specific and effective in treating autoimmune disease^{33,34}. Recently, it was demonstrated that IL-2/ mAb_{CD122} complex treatment produced 40-fold effect than soluble IL-2 treatment did; after the half-life of IL-2 was prolonged and the interaction of IL-2 with CD25 was blocked³⁵. Liu *et al* pointed out that anti-IL-2-IL-2 complex could cure autoantibody-dependent disease via proliferation of Treg cells³⁶. Meanwhile, IL-2 and Anti-IL-2 complex anti-IL-2-IL-2 complex could activate IL-2R $\beta\gamma$ + effector T cell, which honored a powerful anti-tumor effect to effector T cell. Therefore, the complex might improve tumor immune therapeutic strategies based on IL-2³⁷.

The Combination of IL-2 with Rapamycin for the Treatment of Autoimmune Disease

At present, another way to efficiently treat autoimmune disease with IL-2 is to selectively target IL-2 to Treg cells by using specific medication to selectively modulate biochemical pathways in Treg cells or effector T cells. Rapamycin is not only a kind of common immunosuppres-

sive drug but also is a kind of proliferation signal inhibitors. It can run through cell membrane easily, then combine with the intracellular immunophilin FK506-binding protein (FKBP12), forming FK506-binding protein-12-rapamycin complex which targets IL-2 to downstream mTOR, and inhibits proliferation of effector T cells³⁸. Previous experiments showed that Treg cells responded to rapamycin differently from T effector (Teff) cells. After treated with rapamycin, Treg cells could up-regulate anti-apoptotic molecules, and down-regulate pro-apoptotic molecules and then the balance between Treg cell and effector T cells was altered³⁹. It was recently showed that rapamycin was not only able to increase Treg cells, but also improve their specific suppressive capability against effector T cells *in vivo*⁴⁰. Therefore, these findings promoted the application of the therapy combining IL-2 with rapamycin. In humans, treating CD4+ T cells with both IL-2 and rapamycin *in vitro* led to an increase in the number of FOXP3+ T cells⁴¹. In addition, this kind of combination therapy with IL-2 to increase the number of Treg and improve its function, and mTOR inhibitors to block the generation of effector T cells, proved to be beneficial in curing immunological disorders⁴².

The Combination of IL-2 with Treg Cells for the Treatment of Autoimmune Disease

Generally, cell therapy is very promising. To obtain sufficient Treg cells is a major challenge in treating autoimmune diseases, due to the very low abundance of Treg cells. Therefore, proliferation and differentiation of Treg cells *in vitro* was recently the focus of clinical trials. Moreover, researchers have highlighted that the instability and heterogeneity of Treg cells is also a major problem in cell therapy. In addition, the loss of function of massively injected Treg population and their subsequent likely conversion into pathogenic T cells throws doubt upon the future of Treg immunotherapy. Fortunately, IL-2 can stabilize the function of FOXP3+ Treg cells, so IL-2 therapy in combination with Treg infusion can represent a plausible alternative⁴³. Indeed, the combination of low dose IL-2 with Treg infusion can significantly improve the medical effect of HSCT by increasing the number of Treg cells *in vivo*⁴⁴. Blazar et al⁴⁵ proposed that it was possible to use clinical-grade lentiviral vectors to redirect Treg cells to the target cells, and to prevent Treg cells from converting into effector T cells, which kept the infused Treg cells expressing Foxp3.

Conclusions

IL-2 signal plays a key role in promoting development, homeostasis and function of regulatory T cells. IL-2 affects multiple signal pathways, and its deficiency causes the multifaceted dysregulation of immune response. The decreased production of IL-2 in patients with autoimmune disease triggers various immune defects, such as decreased production of Treg cells, decreased AICD and decreased cytotoxicity of CTL. The decreased cytotoxicity of CTL would make patients more susceptible to intracellular infection. Therefore, to ensure the amount and function of IL-2 may help to realize the potential of IL-2 as an immunotherapeutic effect and ensure clinical application of IL-2.

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