

INTERLEUKIN 2 (IL2)

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GLOSSARY

Cytokine A term used for products of cells that act as hormones, serving to convey messages from one cell to another.

Janus Kinases A class of tyrosine-specific kinases that function as second messengers, and act intracellularly to signal molecules in the cytoplasm and nucleus by catalyzing the phosphorylation of tyrosine residues on target molecules.

Interleukin A term used to mean "between leukocytes", that was coined to describe the first cytokines that were involved with the regulation of the immune and inflammatory systems.

Signal Transducers and Activators of Transcription (STAT)

Molecules that act as second messengers by receiving signals from the cytoplasmic domains of cell surface receptors, becoming activated and transforming into activators of transcription.

I. Introduction

Interleukin 2 (IL2), originally termed T Cell Growth Factor (TCGF), was the first cytokine to be isolated, purified, and characterized at the molecular level. IL2 is prototypic of the interleukins as well as the hematopoietic cytokines and hormones such as growth hormone and prolactin; it is a small globular glycoprotein, composed of four amphipathic antiparallel α helices. The IL2 receptor (IL2R) was also the first of the cytokine receptors to be identified and characterized. The IL2R is comprised of 3 distinct chains (α, β, γ), each of which participates in the formation of the binding site for IL2. The α chain contributes a very rapid association rate to IL2 binding, while the β, γ heterodimer contributes a slow dissociation rate, making for a very high affinity binding site ($K_d = 10$ pM). Functionally, IL2 is confined to the immune system: only antigen-activated T cells produce it, and lymphocytes are the only cells that express IL2Rs. IL2 promotes the proliferation, differentiation and survival of mature lymphocytes, especially T cells. Consequently, IL2 is obligatory for a physiologic antigen-specific acquired cellular immune response, and it also can promote innate host defenses by

activating Natural Killer (NK) cells. *In vivo*, IL2 regulates the tempo, magnitude and duration of the T cell immune response. As well, because the size of the memory cell pool is dependent upon IL2, the degree of protective immunity is determined in large part by the availability of IL2. Accordingly, IL2 is presently in use therapeutically for the augmentation of the immune system in the treatment of cancer and infectious diseases. Moreover, if a deficiency of IL2R triggering occurs, either as a result of genetic defects, or pharmacologically, profound immunodeficiencies can be produced. For example, genetic deficiencies of IL2, or IL2R α or β chains, results in immunodeficiency early after birth, with a later accumulation of activated T cells that do not function properly. It is significant that these phenotypic changes cannot be compensated by other cytokines. Mutations of the IL2R γ chain results in severe combined immunodeficiency. Given these findings, it is not surprising that pharmacological agents, and therapeutic monoclonal antibodies reactive with the IL2R that block the IL2-IL2R system, are potent immunosuppressives and are the main agents employed to prevent organ transplant rejection.

II. Structures

Human IL2 is a small (15.5 kDa) globular glycoprotein of 133 amino acids. There are 4 antiparallel amphipathic α helices, with one intrachain disulfide bond essential for molecular integrity and activity. The high affinity IL2R is comprised of

three noncovalently linked type I transmembrane chains of 55 kDa (α chain), 75 kDa (β chain), and 65 kDa (γ chain). The α chain has only 13 intracellular amino acid residues and does not participate in signaling, while the β and γ chains have large intracellular portions and are responsible for signaling the interior of the cell. The IL2R binds IL2 with an extremely high affinity ($K_d = 10$ pM), owing to a rapid association rate contributed by the α chain ($k = 10^7 \text{ M}^{-1}\text{s}^{-1}$), and a slow dissociation rate ($k' = 10^{-4} \text{ s}^{-1}$) contributed by the $\beta\gamma$ chain heterodimer.

III. Functions

The IL2 concentrations that bind to IL2Rs are identical to the concentrations that promote a T cell proliferative response, or the $K_d = EC_{50}$. Thus, the IL2 binding and biological response curves are coincident, and there are no "spare" receptors. Moreover, there are a finite number of IL-IL2R interactions that must occur before a cell is triggered to undergo cell cycle progression. Accordingly, the concentration of IL2 available, the density of IL2Rs and the duration of their interaction determine the biological effects of IL2, and each individual cell responds in a quantal (all-or-none) fashion. The number and duration of intermolecular interactions determine the "strength" of the IL2-IL2R signal delivered to the cell. The signaling molecules triggered by the IL2-IL2R interaction include the Janus tyrosine-specific kinases, JAK1 and JAK3, which are activated by the stabilized IL2-IL2R complex. Subsequently, the JAKs

catalyze the phosphorylation of themselves as well as the β and γ chains of the IL2R, which then serve as docking sites for the Signal Transducers and Activators of Transcription (STAT) 5a and 5b. In addition to the JAK/STAT pathway, the IL2-IL2R interaction activates the Ras/Raf/MAPK and the PI-3 kinase/Akt pathways, which then are responsible for activating transcription factors that are responsible for activating the expression of specific genes that promote cell survival, proliferation and differentiation. Some of the important genes expressed as a consequence of IL2 signaling are known; for example Bcl2 and BclX, which are important in promoting cell survival, and cyclin-D2, which is important in promoting progression through the cell cycle. However, many others are yet to be discovered.

IV. IL2 Regulation of the Immune Response

IL2 is the principle cytokine responsible for activating the proliferation differentiation and survival of peripheral mature T cells once they have become activated by antigen. In particular, IL2 stimulates a marked proliferative expansion of CD8+ T cells, which are responsible for recognizing and reacting with intracellular infections, such as those caused by viruses. As well, this subset of T cells, which acquire the capacity to lyse infected target cells (cytolytic T cells, CTL), is also active in the destruction of transplanted organs. By comparison, the other major subset of T cells, which

express the CD4 surface molecule, differentiate into "helper" cells (Th cells), by virtue of their capacity to secrete large amounts of cytokines and also to express surface helper molecules. Thus, CD4+ T cells help CD8+ T cells by secreting IL2. Although CD8+ T cells can also produce IL2, CD4+ T cells produce approximately 80% of the IL2 released during an immune reaction. In experimental viral infections, there is a rapid release of IL2, which promotes a massive expansion of CD8+ T cells, as much as 100,000-fold, within just a few days. However, in mice that have had the IL2 genes deleted, the proliferative expansion is only ~ 10% of that observed in wild-type mice. The degree to which antigen-specific T cells expand after a primary antigenic stimulation determines the eventual size of the "memory" T cell population. Therefore, IL2 is responsible for the size of this population and the efficacy of the immune response to reinfection.

IL2 is also responsible for the differentiation of CTL to become cytolytic and of Th cells to become helper cells. IL2 augments the expression of genes that encode cytolytic molecules, such as perforin and the granular proteases (Granzymes), and the cytokine molecules that define the so-called Th1 and the Th2 helper T cell subsets.

IL2 also appears to maintain T cell homeostasis by generating feedback down-regulatory effects, thereby limiting the proliferation and

differentiation initiated upon the encounter with antigen.

activation of most of the NK cells and the attendant toxicity.

V. IL2 Therapy

Pharmacokinetics

Pharmacodynamics

The pharmacodynamics of IL2, or how IL2 affects the cells and tissues of the body, are based upon the type and distribution of the IL2Rs. High affinity, trimeric (α,β,γ) IL2Rs are expressed only transiently on antigen-activated T cells and B cells, while they are constitutively expressed by ~ 10% of NK cells. Because the affinity of the heterotrimeric IL2Rs is very high (10 pM), only very low IL2 concentrations (< 100 pM) are necessary to saturate them. By comparison, 90% of NK cells express the β and γ chains of the IL2R, but not the α chain, so that the K_d is 100-fold higher. This 100-fold difference in affinity, combined with the small number of recently antigen-activated T cells and B cells (<1%), vs. NK cells (~ 10^9 cells/ μ L), accounts for the main difference in the effects of high doses of IL2 as have been used in cancer therapy, vs. low doses used in infectious diseases such as Human Immunodeficiency Virus (HIV) infection. High IL2 doses result in systemic IL2 concentrations that saturate most of the IL2Rs expressed by NK cells, which leads to their activation and their secretion of pro-inflammatory cytokines, resulting in severe systemic toxicity. In contrast, if the IL2 doses are lowered, and the systemic IL2 concentration remains < 100 pM, only the high affinity IL2Rs are saturated, thereby avoiding the

The pharmacokinetics of IL2, or what the body does with the drug, are dependent upon the characteristics of the IL2 molecule. Since IL2 is a small (15.5 kDa) globular glycoprotein, it passes freely between capillary and lymphatic endothelial cells, so that after IV administration, it distributes into total extracellular space, which in a normal adult is about 15 L. This α decay phase, due to distribution, occurs with a half-time of ~ 10 minutes. Therefore, within 40 minutes, >94% of the initial peak concentration is dissipated. Subsequently, there is a β decay due to renal excretion, which has a half-time of ~ 3 hours. After subcutaneous administration, there is a half-time of ~ 1 hour for absorption, followed by renal excretion, so that peak plasma concentrations occur at ~ 2-3 hours, and detectable plasma concentrations are still present after 12-16 hours. Therefore, low plasma IL2 concentrations can be maintained by once or twice daily subcutaneous injections. After a subcutaneous or intradermal injection of IL2, there is a characteristic and classic delayed-type hypersensitivity (DTH) reaction that develops within 24 hours. The cardinal signs of inflammation (i.e. *rubor, calor, dolor, tumor*) are due to the IL2-activation of NK cells and T cells, which then produce pro-inflammatory cytokines, leading to

the extravasation of plasma and cells at the site of IL2 injection.

Clinical Results

In cancer treatment, IL2 is used in very high doses (i.e. 150 million U/day) for short treatment intervals of 3-5 days. At these doses, IL2 causes acute systemic inflammation, with high fever, malaise, myalgia, fatigue and hypotension. This therapy eventually results in an anti-tumor response in ~ 15% of subjects, and ~ 5% of patients with renal cell carcinoma and malignant melanoma achieve a long-term, complete response.

The treatment of HIV infection is the other major disease category where IL2 has been used. In patients with chronic infection and low circulating CD4+ T cells, doses that are ~ 10-fold lower than those used in cancer therapy have been found to be effective in increasing the concentration of CD4+ T cells. However, these doses are still quite toxic and can only be tolerated for ~ 5 days.

Low dose daily IL2 administration is now being tested in conjunction with therapeutic HIV vaccines while antivirals are also administered, to augment HIV-specific immunity. This has become an important new area for immune-based therapies because the antiviral drugs available are effective in suppressing viral replication, but they do not cure the infection, so that when discontinued the virus begins replicating immediately, and a viral relapse

occurs almost universally. Consequently, the new therapeutic approaches are designed to augment HIV immune reactivity, by boosting the number and function of HIV-specific T cells, especially CD8+ CTL. A similar approach is planned to test IL2 for the treatment of chronic Hepatitis C Virus (HCV) infection.

VI. Immunosuppressive Therapy

The mechanisms of action of the most commonly used immunosuppressive drugs are focused on the prevention of either IL2 production or action. Thus, glucocorticoid hormones, which have been widely used as immunosuppressives and anti-inflammatory agents for the past 50 years, inhibit the transcription of IL2 and other cytokine genes. Similarly, the drugs cyclosporin A and FK506 also inhibit IL2 gene expression, although by blocking slightly different transcriptional activating pathways.

Recently, a monoclonal antibody reactive with the IL2R α chain has been found to be very effective in blocking the IL2-IL2R interaction and attenuating the rejection response to renal and cardiac allografts. Also, in combination with Rapamycin, which blocks IL2R signaling, these antibodies have recently been shown to prevent the rejection of pancreatic islet cell grafts used for the treatment of type I diabetes mellitus.

VII. Summary

IL2 is the major mature T cell growth, differentiation and survival factor (cytokine) operative in the immune system. It promotes the proliferative expansion and function of antigen-selected T cell clones, and is responsible for the generation of protective immunity and immunologic memory. As a small globular glycoprotein that interacts with high affinity stereospecific cell surface receptors, it mediates its effects by activating tyrosine-specific kinases, and the transcriptional activation of specific genes. In the clinic it is being used to augment immune responses for the treatment of cancer and infectious diseases, and drugs that block the production or action of IL2 are effective immunosuppressives.

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