

# Signal Transduction by IL-2 and its Receptors as Target in Treatment of Rheumatoid Arthritis

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**Abstract:** Rheumatoid arthritis (RA) is a chronic and destructive arthropathy with systemic features, the etiopathogenesis of which remains unclear. It is characterized by relapsing and remitting inflammation and hyperplasia of synovial cells. Proinflammatory cytokines, such as interleukin-2 (IL-2), play an important role in maintaining cartilage damage and severe destruction of the joints due to an uncontrolled activation of cellular immunity. An imbalance between proinflammatory and anti-inflammatory mediators is likely to contribute to the chronicity of the disease. Therefore, insight into the activation state of T-cells in different stages of the disease may be important to understand pathogenetic mechanisms underlying RA and could be a lead for the design of future therapeutic strategies. Because of the central role of the IL-2/IL-2 receptor (IL-2R) system in mediation of the immune system, monitoring and manipulation of this system has important diagnostic and therapeutic implications. New approaches in RA therapy with anticytokine agents, which block cytokines and their receptors, are now used as antirheumatic drugs in clinical practice.

**Key Words:** Rheumatoid arthritis, cytokines, IL-2, cyclosporin A, anti-CD25 mAb.

## DESTRUCTIVE EFFECTS OF RHEUMATOID ARTHRITIS

Rheumatoid arthritis is a systemic autoimmune disease characterized by acute and chronic inflammation of the synovial joints causing their destruction and loss of function [1]. Early in the course of the disease several changes in joint structures take place. Joint effusion and inflammation of the synovium occur producing a soft tissue swelling that is easily detected during evaluation of the patient. Shortly after the disease, edema begins to be seen in cells in the synovium. As the disease progresses, the synovium may grow considerably larger, eventually forming a tissue, which is called pannus. Pannus can be considered as the most destructive element affecting joints in the patient with RA. It destroys articular cartilage as well as the soft subchondral bone once the protective articular cartilage is gone. The major cell types present in pannus are T-lymphocytes and macrophages, whereas the minor ones are fibroblasts, plasma cells and endothelial cells.

In RA the composition of synovial fluid is also affected, resulting in poor lubrication of the joint and provision of nutrients. The major cell type in synovial fluid is neutrophils. Ultimately, digestants formed in the fluid, attack the surrounding tissue. The destruction of the bone eventually leads to laxity in tendons and ligaments. Under the strain of daily activities and other forces, these alterations in bone and joint structure result in the deformities frequently seen in patients with RA. Bone destruction occurs at areas where the hyaline cartilage and the synovial lining do not adequately

cover the bone. If the disease progresses to a more advanced stage, the articular cartilage may lose its structure and density resulting in an inability to withstand the normal forces placed on the joint. In these advanced cases, muscle activity causes the involved ends of the bones to be compressed together resulting in further bone destruction. Thus, joint destruction can progress to the degree that joint motion is significantly limited and joints can become markedly unstable. Furthermore, disease is accompanied with many extra-articular features, such as neuropathy, scleritis, vasculitis, lymphadenopathy, pericarditis, splenomegaly, arteritis, and rheumatoid nodules [2].

## CYTOKINES AND IMMUNOPATHOLOGY OF RA

Although the etiology of the disease is not completely known, extensive studies have contributed to our understanding of the molecular mechanisms underlying the disease. According to the model proposed by Feldmann *et al.* [3], a variety of immune responses can contribute to the pathogenesis of RA. The unique pathophysiologic elements of RA arise from the interactions between the variety of leucocytes that invade the joint, and the native cellular components of joint tissue.

The presentation of an appropriate antigen to an immunogenetically susceptible host is believed to be the event that initiates a complex series of steps, which ultimately result in chronic inflammatory synovitis. As soon as helper T-cells presented with an antigen, they begin to proliferate and become activated. The overactive T-cells in RA mistake the body's own collagen as an antigen and trigger a series of immune responses to destroy the false enemy.

The antigen receptor on the T-cell surface interacts with the processed antigen exposed in the proper MHC on the

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surface of APC. The binding of the antigen to the TCR is essential for T-cell activation under physiological conditions. The TCR is a multi-component structure consisting of the clonotypic  $\alpha$  and  $\beta$  chains and the invariant CD3 subunits  $\gamma$ ,  $\delta$ ,  $\epsilon$ , and  $\zeta$ . The complete assembly of all components is required for cell surface expression and for antigen receptor function. The  $\alpha$  and  $\beta$  subunits of CD3 complex most likely transduce to the cytoplasm the activating signals originating from antigen recognition by the TCR. In addition, the two transmembrane proteins CD4 and CD8 (named co-receptors), expressed on helper and cytotoxic T-cells, participate in the interaction between the T-cell and the APC by binding to the MHC class II and I molecules, respectively.

CD28 is a surface glycoprotein expressed on T cells and interacts with two natural ligands (CD80 and CD86). These ligands, which are expressed by APC, are capable of co-stimulatory responses *via* CD28 [4]. This pathway is responsible for a tolerant state known as T-cell anergy. IL-2 seems to mediate prevention of anergy.

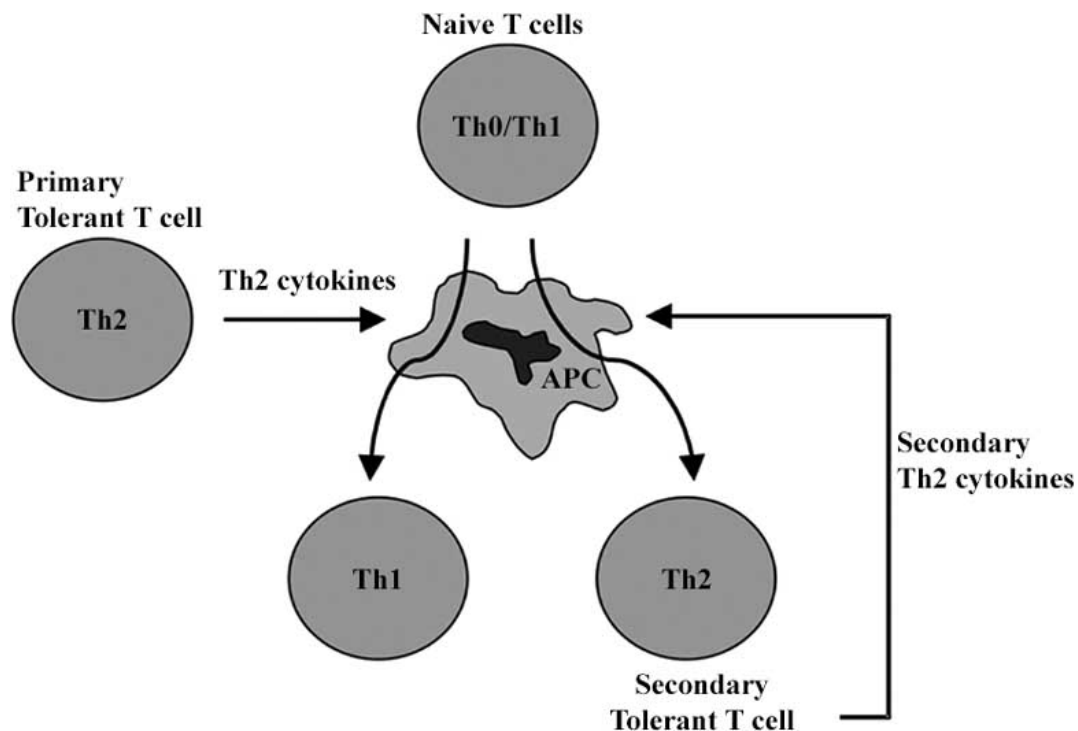
Co-stimulation of the TCR and the CD28 results in tyrosine phosphorylation of the TCR by membrane-associated protein kinases. As shown in Fig. (1), upon such activation, naive T-helper cells become Th0. With further stimulation Th0 cells deviate either towards Th1 or Th2 phenotype, since they have both Th1 and Th2 characteristics. Th-cells secrete or stimulate the production of powerful immune factors called cytokines. Cytokines are a group of low molecular weight glycoproteins important for cell-to-cell signalling. Th1 and Th2 cells are classified based on the pattern of cytokines that they secrete. For example, Th cells that secrete mainly IL-2 and INF- $\gamma$  are termed as Th1 cell,

whereas those that secrete mainly IL-4, IL-10, and IL-13 are known as Th2 cells. The initial critical stimulators that favour Th1 versus Th2 responses are largely unknown. However, it is stated that intracellular viral antigens favour Th1 and extracellular bacterial antigens favour Th2. Additionally, low concentration of the antigen favor Th1 whereas high concentration favour Th2 phenotype [5].

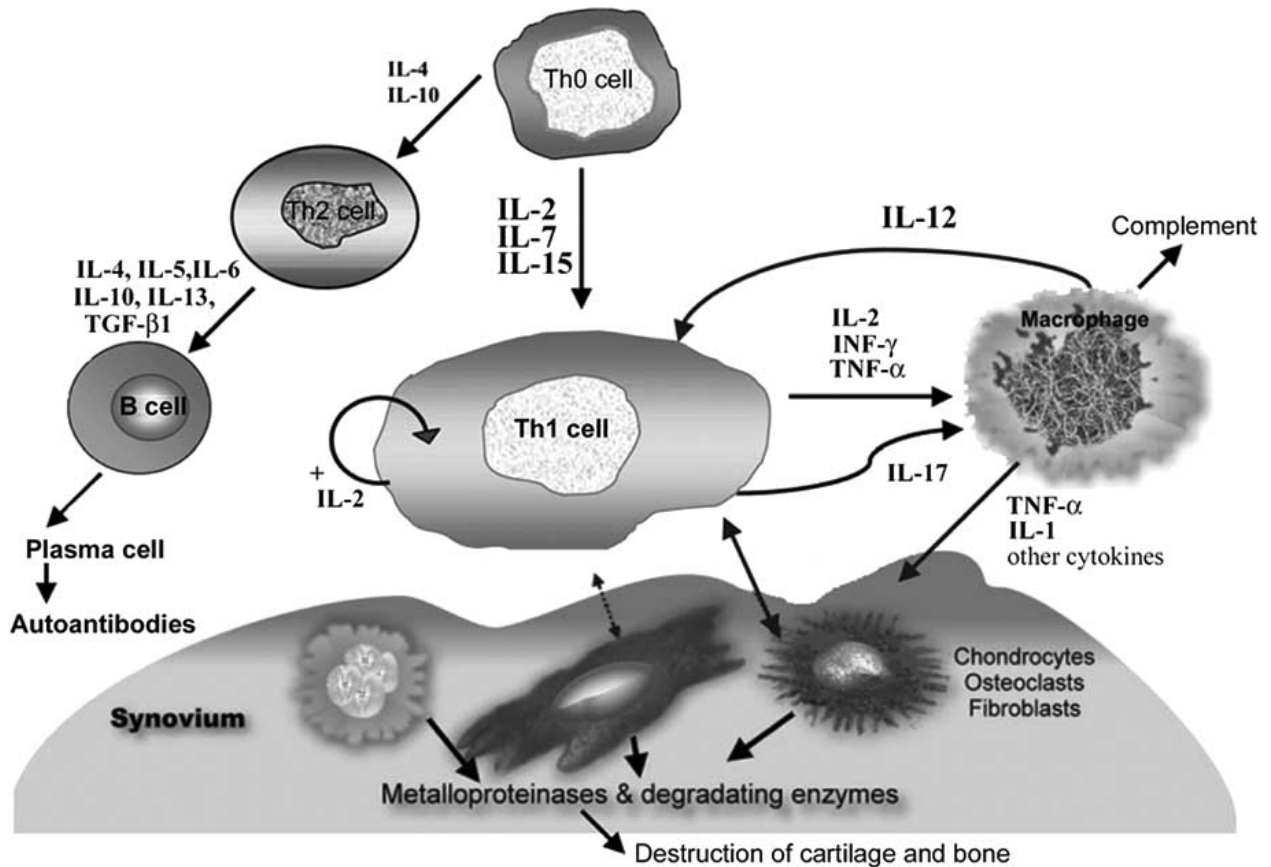
Th1 and Th2 secreted cytokines activate different arms of the immune system. Thus, Th1 cytokines activate macrophages, NK cells, cell mediated immunity, inflammation and the secretion of certain Ig isotypes, whereas Th2 cytokines tend to favor isotype switching in the humoral immune response. In addition, Th2 cytokines depress macrophage activation and cell mediated immunity. In the case of RA, Th2 cells stimulate B-cells to produce IgG-derived autoantibodies, which are directed against the body's own cells.

Cytokines are involved in various cellular responses, such as activation, proliferation and growth. In small amounts, cytokines are indispensable for healing. If overproduced, however, they can cause serious damage, including inflammation and injury in the joints during the RA process [6]. They may be responsible for inflammation, that occurs in parts of the body beyond the joints, including fever, shock, and even damage to organs, such as the liver.

As shown in Fig. (2), cytokines separate their cellular responses into Th1 and Th2 pathways. Experiments in both animal models of arthritis and humans suggested that Th2-type cytokines, such as IL-4, IL-5, IL-6, IL-10, IL-13 and TGF- $\beta$  1, could protect against arthritis, whereas Th1-type cytokines, such as IL-2, IL-12, IL-15, TNF and IFN- $\gamma$ , could be pro-inflammatory. RA initiation and perpetuation is dependent on Th1 lymphocyte response. Aberrant overpro-



**Fig. (1).** Naive T helper cells secreting restricted Th1 and Th2 patterns of cytokines, which differentially regulate infectious diseases and autoimmunity.



**Fig. (2).** Cytokine profile in RA synovium: Activated T cells stimulate monocytes, macrophages and synovial fibroblasts to produce cytokines and to secrete matrix metalloproteinases and degrading enzymes. Activation occurs via paracrine and autocrine pathways. T lymphocytes stimulate also B cells to produce autoantibodies including rheumatoid factor.

duction of proinflammatory cytokines by inflammatory cells leads to persistent up-regulation of various molecules responsible for the inflammatory and destructive processes in the joints of patients with RA. This overproduction of cytokines is regulated via specific signaling pathways and transcription factors.

### SIGNAL TRANSDUCTION IN RA

T lymphocytes utilize a variety of surface receptors to transmit environmental signal across the plasma membrane and initiate biochemical events leading to responses such as proliferation, anergy, cytokine secretion and death. The TCR complex, CD28 and IL-2R, each couple to distinct sets of cytoplasmic signaling events to modulate the biological responses of the T cells. Deficiency or defective function of proteins involved in cell signalling through these receptors is associated with murine and human diseases.

As shown in Fig. (3), the initial interaction requires cell-to-cell contact between antigen-loaded MHC molecules on APC and the TCR-CD3 complex on T-cells. Engagement of the TCR-CD3 complex induces CD154 expression predominately on CD4 T-cells that in turn activates the APC through CD40 engagement, leading to strong activation of their antigen presentation efficacy. This is caused partly by upregulation of the expression of CD80 and CD86 on the

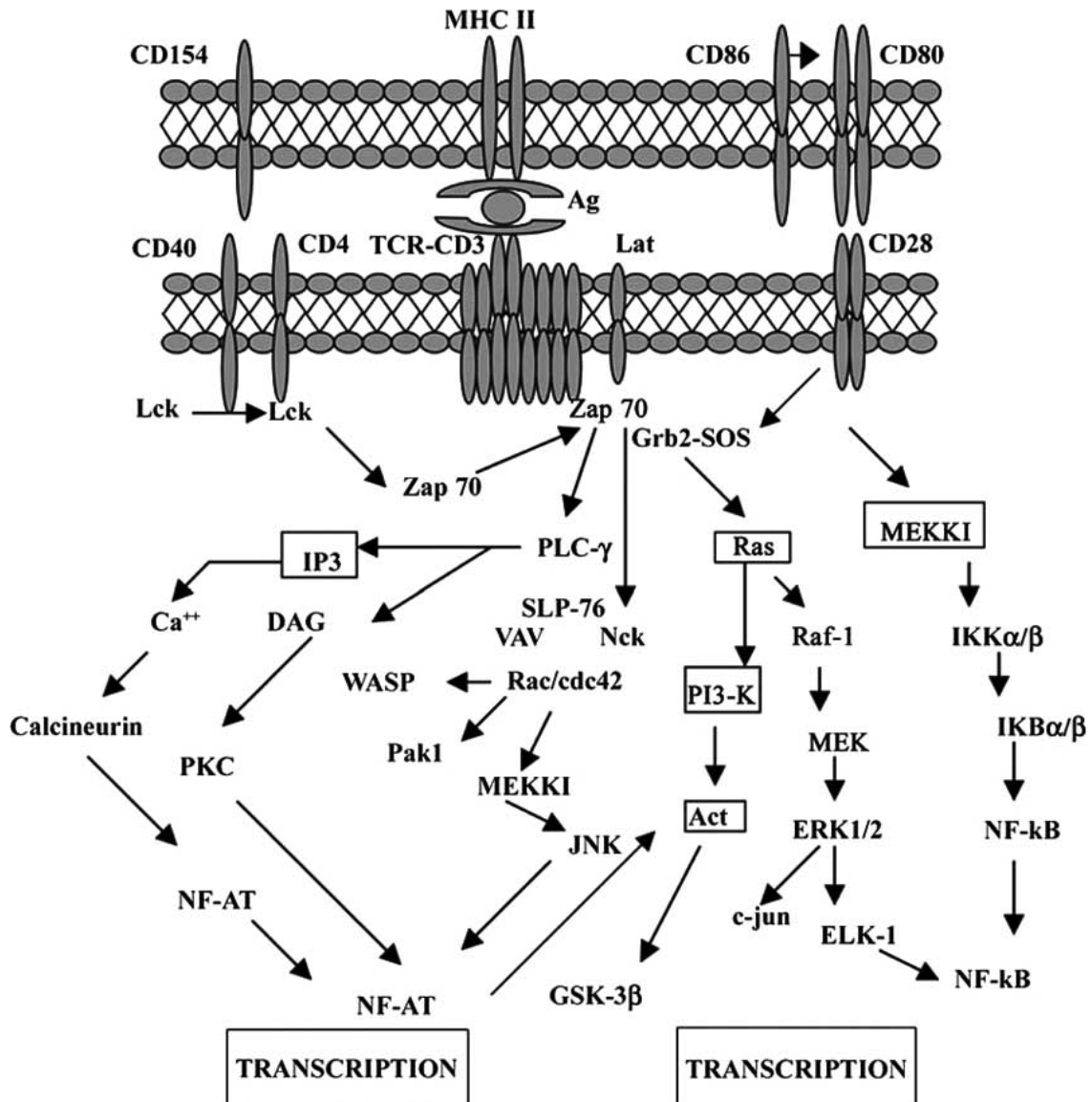
APC, both of which are ligands for the important CD28 co-stimulatory molecule on T cells. Furthermore, CD40 engagement leads to secretion of various cytokines and chemokines, which have important effects on both APC and T cell activation and maturation.

Co-stimulation of the TCR and the CD28 on cell surfaces results in tyrosine phosphorylation of the TCR by membrane-associated protein kinases. PLC- is then recruited to these phosphorylated sites on the TCR, due to its Src homology 2 domains, therefore positioning PLC- in the vicinity of the plasma membrane. Hydrolysis of phospholipids by PLC- activates the phosphoinositol-signaling pathway that generates two second-messengers: IP3 and DAG. IP3 triggers the release of  $Ca^{++}$  from intracellular storage sites, whereas DAG induces activation of PKC [7].

Signal transduction systems are typically arranged as networks of sequential protein kinases. Cytokine signals may be conducted into the nucleus at the transcription level via pathways, such as MAPK pathway, PI-3K pathway, JAK-STAT signaling system and Ras pathway.

### IL-2 and IL-2R Mode of Action

IL-2, a pro-inflammatory cytokine, was first recognized twenty-seven years ago and since then, its spectrum of biological activities and clinical uses has continued to



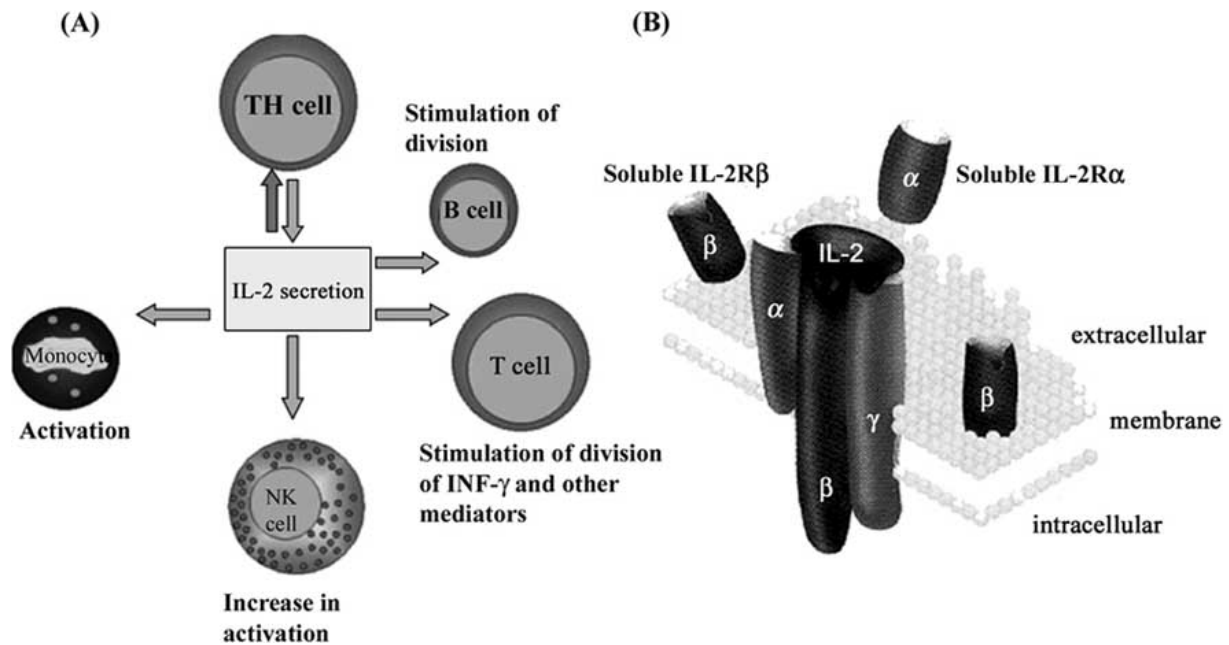
**Fig. (3).** Signal transduction pathways associated with activation of lymphocytes: An interaction between the antigen-loaded MHC II molecules on APC and the TCR-CD3 complex triggers the signal for activation of T cells. The activated TCR passes the signal on to different associated tyrosine kinases, therefore initiating pathways such as MAPK, PI-3K, Ras and IP3.

unfold. Its primary role was to promote cell survival, particularly in T cells [8]. Its central role on cell activation in immune system is shown in Fig (4A). More recently, IL-2 role was characterized as a negative regulator of the immune system. Initially this concept was supported by the surprising finding that IL-2-deficient mice suffer severe immune dysregulation and consequent autoimmunity, but they do not experience major defects in immune development or activation [6]. Probably, part of the autoimmunity syndrome in IL-2-deficient individuals arises because IL-2 triggers activation induced-cell death in antigen-activated T cells via the Fas-FasL apoptotic pathway [9]. Thus, IL-2 serves as an important regulator of immune homeostasis following antigen clearance. Studies on CIA indicated that, although IL-2 is a pro-inflammatory cytokine in established disease, it can play an anti-inflammatory role in early stages of the disease [10].

### IL-2R Structure

IL-2 has a primary role in promoting cell survival, particularly in T cells. The biological activities of IL-2 are mediated through the binding of IL-2 to its cellular receptor Fig (4B). The high affinity IL-2R is comprised of three distinct subunits: IL-2R (also known as CD25 and Tac antigen; human chromosome 10), IL-2R (p75, CD122; human chromosome 22) and IL-2R (p64; X chromosome) [11].

The IL-2R chain (70 kDa) is expressed constitutively in NK cells and cytotoxic T-cells, but not in T-helper cells and is further induced upon T-cell activation. The chain (64 kDa) is expressed constitutively in monocytes and lymphoid cells. In contrast, IL-2R (55 kDa) is normally never expressed in the absence of its hematopoietin receptor family



**Fig. (4).** **4A:** Schematic diagram showing that interleukin 2 (IL-2) is a potent cytokine that can lead to activation of monocytes and NK cells as well as to stimulate the division of T and B cells. **4B:** Schematic representation of the IL-2 receptor with its three distinct subunits (IL-2R $\alpha$ , IL-2R $\beta$  and IL-2R $\gamma$ ). Soluble forms of subunits  $\alpha$  and  $\beta$  (sIL-2R $\alpha$  and sIL-2R $\beta$ ) are also contributed to IL-2 signaling during inflammation. (Reprinting with permission from R&D).

partners, IL-2R $\alpha$  and IL-2R $\beta$  in lymphoid cells [12]. IL-2R $\alpha$  expression is exquisitely regulated in several types of immune cells, and can be induced by IL-2, IL-4, IL-5, and IL-10. Soluble forms of this receptor may modulate IL-2 signaling, particularly during inflammatory conditions [10].

### The IL-2R Chain

Although IL-2R $\alpha$  is an important affinity modulator that it is essential for proper responses *in vivo* [13], it does not possess intrinsic transmembrane signalling capacity [14,15], due to its short cytoplasmic tail. In contrast, IL-2R $\beta$  and IL-2R $\gamma$  together are necessary for effective signal transduction, and they serve physically to connect the receptor complex to cytoplasmic signaling intermediates by initiating a proliferative signal. Binding studies revealed that low affinity IL-2 receptors (i.e. IL-2R $\alpha$ ) outnumber high-affinity IL-2 receptors (i.e. IL-2R $\alpha$ /IL-2R $\beta$ /IL-2R $\gamma$ ) in activated T and B cells, indicating that at least some IL-2R $\alpha$  subunits exist "alone" in the membrane [12]. The expression of IL-2R $\alpha$  can be induced by IL-2 [16], IL-4 [17], IL-10 [18] and other inflammatory cytokines. Therefore, IL-2R $\alpha$  plays a critical role in regulating responses to IL-2 by controlling the affinity of the IL-2R for its ligand [19], even though this chain does not contribute directly to signal transduction.

### The IL-2/15R Cytoplasmic Tail

IL-2R $\beta$  is an indispensable component for the ligand internalization and the signal transduction via the high affinity IL-2R.

### The Membrane Proximal Region (Box1 and Box2)

Most of the signals that have been mapped to specific regions of the IL-2R are connected through IL-2R $\beta$ . IL-2R $\beta$

contains conserved motifs in its membrane proximal cytoplasmic tail termed Box1 and Box2. A serine rich domain termed the "S region", that overlaps Box1 and Box2, was found to be highly significant in signaling [20]. Box1 and Box2 were found to be interaction sites for the JAKs [21]. JAK activation occurs immediately following receptor dimerization, when JAK1 and JAK3 become phosphorylated on tyrosine residues located within the activation loops of their kinase domains [22,23,24]. Another protein tyrosine kinase, named Syk, is associated with the S region [25].

### The Central Cytoplasmic Region

IL-2R $\beta$ , also, contains two distal domains in its central cytoplasmic region, named the "A" and "H" regions. Signaling in these two regions depends on the specific tyrosine residues encoded within them, which initiate multiple signaling pathways. The IL-2R $\beta$  chain contains six potential sites of tyrosine phosphorylation, four of which are found in the A region and two in the H domain. Within the A region, only one of the four tyrosine residues appears to be involved in signaling, namely the most membrane-proximal residue (Tyr-338) [26]. Tyr-338 can link the receptor to multiple signaling pathways, such as the MAPK pathway, the PI-3K pathway and the STAT pathway [27].

### Signaling Via the MAPK Pathway

Tyr-338 serves as a site for the adaptor molecule Shc [28]. Shc connects receptors to the MAPK pathway utilizing the adaptor Grb2 and the nucleotide exchange factor SOS, engaging the Ras-Raf-MAPK pathway and eventually triggering upregulation of genes, such as *c-fos*. Activation of MAPK pathway is not sufficient for IL-2-dependent proliferation. Therefore additional signals are required.

### Activation of the PI-3K Pathway

Cytokines activate PI-3K, leading to the transient accumulation of its lipid products in cell membrane. These lipids serve as second messengers to regulate the location and activity of an array of downstream effector molecules. IL-2R activates PI-3K by inducing phosphorylation of p85 on tyrosine and subsequently recruiting PI-3K to the cell membrane [29]. However, reports differ on how the IL-2R activates PI-3K. Enhancement of TCR-dependent proliferation by CD28 signaling in normal mouse T cells is only partially inhibited by PI-3K inhibitors [30]. On the other hand, retroviral expression of constitutively active Akt kinase rescues IL-2 production in T cells of CD28-deficient mice [31]. Akt is activated in a PI-3K dependent manner and mediates anti-apoptotic signals. PI-3K signaling is implicated in proliferative and anti-apoptotic outcomes [32].

### Signal Transduction Via the JAK-STAT Pathway

This pathway enables a very direct signal transduction from the membrane to the cell nucleus. STAT 5 proteins are essential mediators of IL-2 signaling in T cells. Experiments in STAT5 / mutant peripheral T cells, were showed to be deficient in proliferation. Moreover they failed to express genes controlling cell cycle progression [33]. JAKs bind to cytoplasmic section of the IL-2 receptor and phosphorylate the STATs, which are induced to form dimmers through reciprocal Src homology 2 domain-phosphotyrosine interactions. Unlike JAK1 and JAK2, which require activation loop tyrosines for catalytic activation, JAK3 does not require the analogous tyrosines for its function [34]. STAT dimmers migrate into the nucleus where they activate transcription of target genes. STAT-5A and STAT-5B isoforms exist as two closely linked genes. Mice with a targeted deletion of STAT-5A have a normal thymic development but reduced numbers of spleenocytes. This may be due to a reduced proliferative response of these cells to IL-2 [35]. STAT-5 can be recruited to multiple tyrosines within IL-2R (Tyr-338, Tyr-392 and Tyr-510) [36,37].

### The Membrane-Distal Region

IL-2R C-terminal region, known as H domain, contains Tyr-392 and Tyr-510 that are involved in signaling. These two tyrosines, when phosphorylated, can activate STAT-5, which targets the gene for IL-2R [35] and FasL. The latter is involved in activation-induced cell death triggered by IL-2 in activated peripheral cells [9].

### **The Common Chain ( $\gamma$ ) Cytoplasmic Tail**

The  $\gamma$  chain as a member of the cytokine receptor superfamily contains conserved regions as well as Box1 and Box2 domains. Ligand internalisation seems to be dependent upon the gamma-chain [38,39]. It is suggested that the  $\gamma$  and  $\beta$  chains may interact through their cytoplasmic domains to initiate an IL-2 proliferative signal. Binding of the ligand causes two receptors to form a dimer. The dimer seems to activate JAK3 [40], which phosphorylates certain tyrosine (Tyr) residues on one or another of several STAT proteins. These, in turn, form dimmers, which enter the nucleus and bind to specific DNA sequences in the promoters of genes

that begin transcription. The importance of  $\gamma$  receptor chain is depicted by studies on JAK3 knockout mice [41].

The  $\gamma$  chain is shared by a number of receptors including IL-4, IL-7, IL-9 and IL-15. IL-2 seems to play a pivotal role, *in vitro*, in enabling the synthesis of Th2 cytokines, such as IL-4, IL-10. By up-regulating production of anti-inflammatory Th2 cytokines during a primary response, IL-2 may play a critical role in limiting Th1 mediated responses, too [42].

### **Interleukin 15 (IL-15)**

IL-15 induces T cell proliferation and promotes the activity of cytotoxic effector and NK cells. IL-2 and IL-15 trigger highly similar signaling cascades [43]. In order to signal, IL-15 uses the IL-2R and  $\gamma$  chains. Furthermore, IL-15 induces the expression of IL-2R  $\gamma$  chain. The IL-15R also employs a third subunit that acts as an affinity modulator, termed IL-15R $\alpha$  and does not appear to contribute to signal transduction [44].

IL-15, along with IL-2, enhances CD154 expression on activated CD4 T cells [45]. This produces a highly regulated feedback mechanism and underscores the interdependence of CD28 and CD40 signal pathways during T cell-APC interaction. This finding is important in patients with RA, with an elevated CD154 (CD40 ligand) expression on their CD4 cells [46]. STAT-3 and STAT-5 are used by both IL-2 and IL-15 in activated T cells.

IL-15 recruits and expands T cells in the synovial membrane, where newly employed T cells can produce TNF- $\alpha$  directly or through cell contact with macrophages [46]. However, IL-15 appears not to have a direct effect on macrophages, which represent the predominant source of TNF- $\alpha$  *in vivo* [47]. On the other hand, TNF- $\alpha$  enhances the capacity of IL-15 to induce cell contact-mediated macrophage activation.

### **Immunosuppressive Agents in RA**

Anti-rheumatic therapy currently used for the treatment of RA is often limited. Therefore, alternative therapeutic regimens need to be considered. Increased understanding of the signal transduction pathways involved in inflammatory cytokine production and signaling has provided a number of intracellular molecular targets that can be exploited for the development of novel therapeutic agents for the treatment of RA.

Cyclosporins are a family of hydrophobic cyclic undecapeptides with a remarkable spectrum of diverse biological activities. The first member of this class to be discovered was CsA. Today, about thirty members of this family have been isolated from natural sources, differing from each other in their amino sequences.

CsA as a potent immunosuppressive agent, is used to reduce the threat of transplant rejection by T-cells. This fungal cyclic peptide was also proved to be very effective on the treatment of RA. The effectiveness of CsA results from specific and reversible inhibition of T-cell proliferation. T-helper cells are the main target, but T-suppressor cells may also be inhibited. CsA also suppresses lymphokine production and release, including IL-2 [48].

### CsA Mechanism of Action

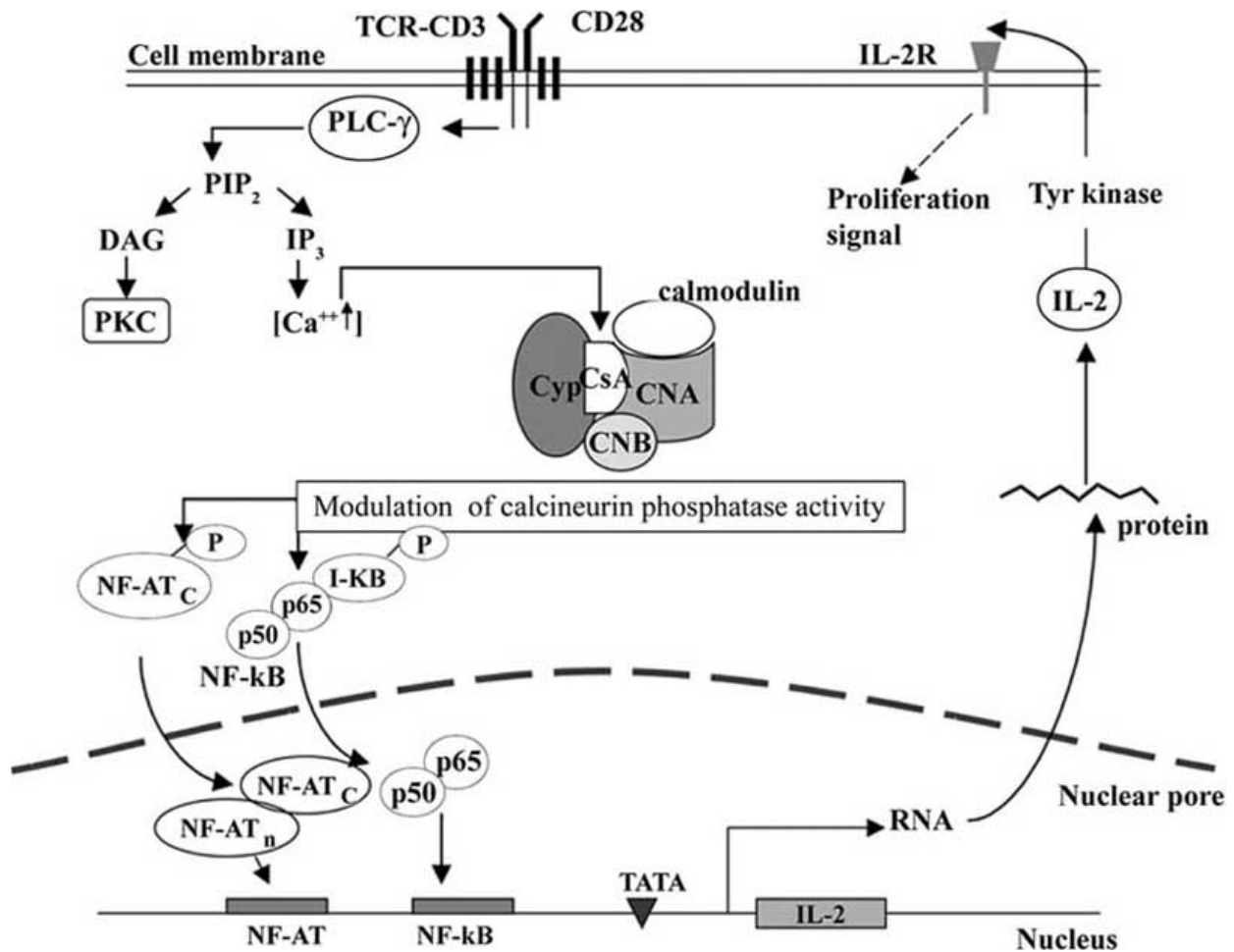
CsA neoral mode of action involves a selective inhibition of CaN, which activates the NF-AT required for IL-2 expression [49,50,51] Fig (5). Upon entering T cells, CsA binds with high affinity to its immunophilin, called cyclophilin (Cyp) [52,53], and inhibit its PPIase activity (receptor protein for immunosuppressive drugs) [54]. Cyp/CsA complex share a common cellular target phosphatase calcineurin [55,56].

CaN phosphatase activity is highly dependant on the interaction of its catalytic subunit, CNA, its regulatory subunit, CNB, calmodulin and  $Ca^{++}$  [57]. Engagement of the TCR with its cognate ligand induces the elevation of calcium concentration and activation of calmodulin. Activated calmodulin interacts with CaN and releases the auto-inhibitory domain of CaN from its active site, leading to activation of its phosphatase activity. The calcium-activated CaN binds to NF-AT and dephosphorylates the nuclear localization signal masking domain of NF-AT [58], resulting in nuclear localization signal exposure and nuclear import of the nuclear factors by importins. CaN is co transported with

NF-AT into the nucleus, where it continues to bind to NF-AT via sites containing nuclear export signals. The exportin CRM1 cannot bind or export NF-AT until calcium signaling ends and CaN dissociates from NF-AT.

Recently, it was found that calcium ionophore induce the transcription of the gene for CD154 in T-cells requiring the calcineurin-dependant transcription factor NF-AT [59]. CaN acts synergistically with calcium/calmodulin-dependent kinase IV to upregulate CD154 promoter activity. The promoter activity is inhibited by CsA, via action of CAN, thus blocking the nuclear translocation of NF-AT family and preventing the subsequent gene expression in activated T cells. CsA maintains a reversible inhibition in the  $G_0$  and  $G_1$  phase of the cell cycle.

The immunosuppressive CsA prevents transcription of T-cell derived cytokines via the NF-AT/NF-kB pathway [60,61]. Therefore, CsA inhibits synthesis of IL-2 and TNF- from monocytes and IL-2/3/4/8/13 and INF- from lymphocytes [62]. In PBMC experiments, *in vitro*, CsA inhibited the IL-15-induced expression of CD69 and the IL-15 or IL-2 induced synthesis of IL-17. It is also inhibits



**Fig. (5).** A simplified view of the signaling pathway, in which an increase in intracellular calcium leads to activation of the phosphatase activity in calcineurin. Activated calcineurin cleaves an inhibitory phosphate residue from NF-AT. Calcineurin is co-translated with NF-AT to the nucleus, where it stimulates the transcription of IL-2 and other genes. The CsA/Cyp complex inhibits the phosphatase activity of calcineurin and disrupts the signal transmission to NF-AT.

aggrecanase-mediated degradation of ECM upon IL-1 stimulation.

CsA decreases IL-15 and TNF- $\alpha$  production by fibroblast-like synoviocytes in an IL-10 dependent manner [63]. At the same time, it increases transcription and synthesis by T cells and macrophages of TGF- $\beta$  1, which down-regulates the T cell response [64]. CsA increase levels of IL-10, which results in inhibition of proinflammatory cytokines, including INF- $\gamma$ , IL-2, IL-12 and TNF- $\alpha$  [65]. This is maintained by blocking the activation of NF- $\kappa$ B, which is critical for the production of these cytokines [66].

### Anticytokine Therapy

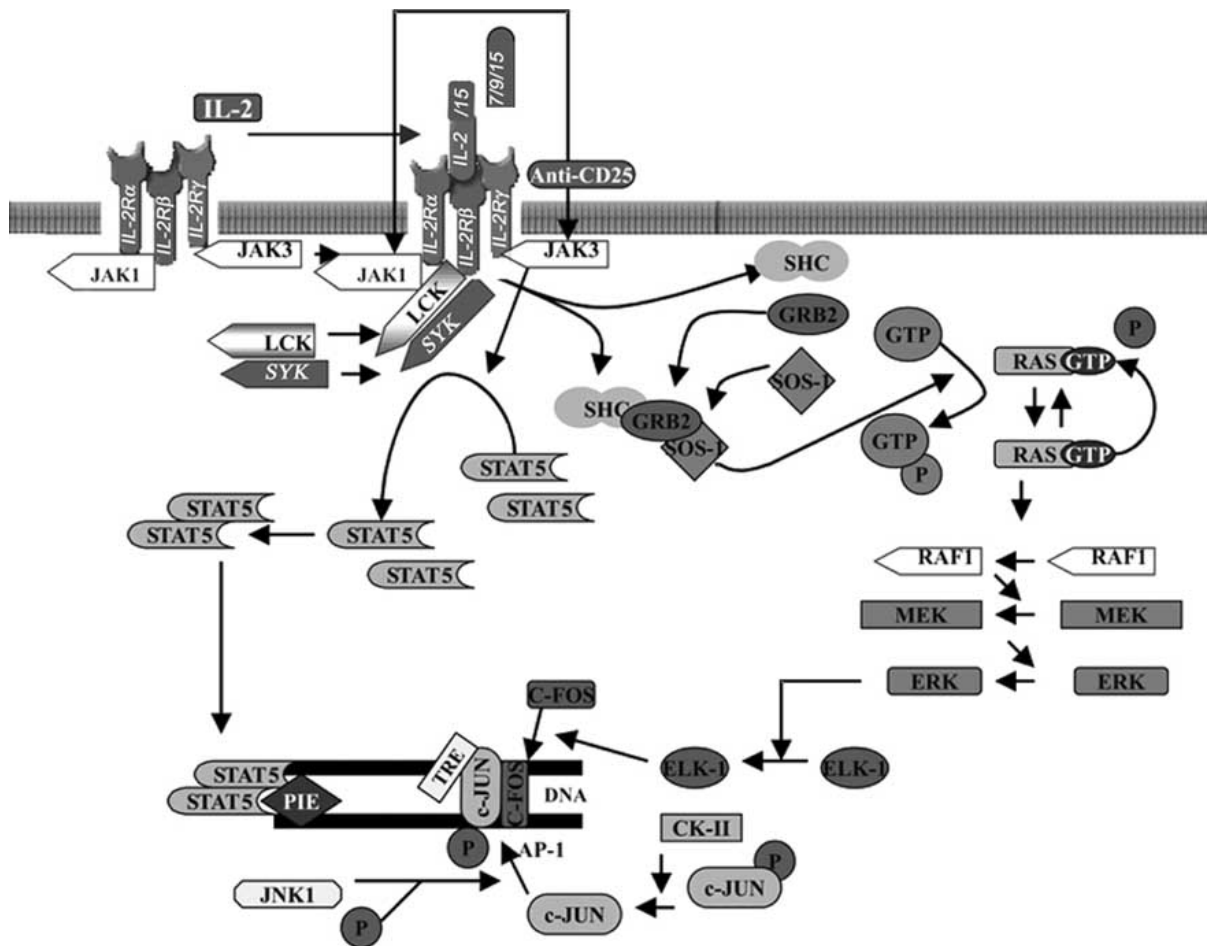
Cytokines can be either pro-inflammatory or anti-inflammatory. The outcome of RA is highly dependent on the imbalance between pro-inflammatory and anti-inflammatory cytokines, in favor of the pro-inflammatory ones. This has resulted in the investigation of therapies with compounds that either block the effects of pro-inflammatory cytokines (immunosuppressants) or enhance the effect of anti-inflammatory cytokines. Anticytokine therapy may include the use of mAbs antagonistic cytokines, soluble cytokine receptors, cytokine receptor antagonists, somatic

gene transfer or other approaches. Hopefully the study of cytokines and their interactions will lead to the development of new strategies that will benefit RA patients [67].

### Blockade of IL-2R by Specific Antibodies

Humanized mAbs that recognize the  $\alpha$  chain of the IL-2R have been used to prevent allograft rejection since this chain is expressed by T cells, which are activated by interaction with alloantigens, but not by T resting cells. Antibodies to IL-2R  $\alpha$  subunit inhibit proliferation of antigen activated T cells and prevent the generation of cytotoxic T cells [68]. IL-2R  $\alpha$  is expressed on T cells only after they are activated, probably as a result of interaction with a foreign antigen [69].

Anti-CD25 mAb, as a selective IL-2R antagonist, provides highly targeted inhibition of T lymphocyte activation by IL-2 [70] Fig. (6). It is a chimeric (murine/human) mAb, which is designed to specifically block and suppress the CD25 (IL-2R  $\alpha$  chain). When CsA and anti-CD25 mAb work together against RA, they believed to have an additive or synergistic effect. Thus, while CsA suppresses IL-2 production, anti-CD25 mAb blocks stimulation of T lymphocytes by IL-2.



**Fig. (6).** Strategy for targeting the IL-2R  $\alpha$  chain (CD25), as a means of achieving therapeutically desirable immunosuppression in RA: Anti-CD25 mAb blocks IL-2 binding to its receptor. Moreover, the IL-2 gene transcription is inhibited by inhibition of JAK1, which prevents the subsequent tyrosine phosphorylation of STAT5 through mechanisms targeting JAK3.

Antibodies to the IL-2R have been used successfully to treat lymphomas and to prevent transplant rejection [71]. Prophylaxis with IL-2R blockers reduces the incidence of acute rejection episodes significantly [72,73].

Until now it was unknown whether treatment with the anti-CD25 mAb is affected other chains of the IL-2R complex. However, recent studies demonstrated that anti-CD25 mAb prevents the tyrosine phosphorylation of JAK-1, JAK3 and STAT-5 and suggests that anti-CD25 mAb treatment influences IL-2R downstream events. Recently it was found that therapy with anti-CD25 mAb not only blocks the expression of  $\alpha$  chain but also down-regulates the expression of the common IL-2/IL-15 receptor  $\beta$  chain [74] in PBMC and inhibits the IL-2/IL-15 pathway. On the other hand, it was found that treatment with anti-CD25 mAb did not affect the  $\gamma$  chain of IL-2R complex [75].

### ABBREVIATIONS

APC	=	Antigen presenting cell
CIA	=	Collagen-induced arthritis
CsA	=	Cyclosporin A
CaN	=	Calcineurin Ca <sup>++</sup> dependent phosphatase
CNA	=	Calcineurin A
CNB	=	Calcineurin B
Cyp	=	Cyclophilin
DAG	=	Diacylglycerol
ECM	=	Extracellular matrix
IFN-	=	Interferon gamma
IL	=	Interleukin
IL-2R	=	Interleukin-2 receptor
IP-3	=	Inositol 1,4,5 triphosphate
JAKs	=	Janus kinases
mAb	=	Monoclonal antibody
MAPK	=	Mitogen-activated protein kinase
MHC	=	Major histocompatibility complex
NF-AT	=	Nuclear factor of activated T-cells
NF- B	=	Nuclear factor- B
NK	=	Natural killer (cell)
PBMC	=	Peripheral blood mononuclear cell
PLC-	=	Phospholipase-gamma
PI-3K	=	Phosphoinositide 3 kinase
PKC	=	Protein kinase C
RA	=	Rheumatoid arthritis
STAT	=	Signal transducer and activator of transcription
TCR	=	T-cell receptor

TGF 1 = Transforming growth factor beta1

TNF- = Tumor necrosis factor alpha

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