

SIGNAL TRANSDUCTION PATHWAYS IN RHEUMATOID ARTHRITIS

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ABSTRACT:

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease, primarily affecting the synovium leading to the destruction of cartilage and bone. Many inflammatory responses observed in RA synovium including the activation of cytokine and adhesion molecules can be traced to specific transcription factors and signal transduction pathways. Two of the most important mediators involved in pathophysiology of RA are tumor necrosis factor alpha (TNF – α) and interleukin

1-beta (IL-1 β) and they represent an attractive therapeutic target for novel anti-inflammatory drugs.

This review discusses the pathogenesis and highlights the role of signal transduction pathways as key mediators of disease activity of this disabling disease. A review into the signal pathways gives us scope for designing better therapeutic modalities in future to improve the quality of life.

Key words : Rheumatoid arthritis, signal transduction, cytokines

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INTRODUCTION:

Rheumatoid arthritis (RA) is a chronic inflammatory arthritis autoimmune disease, primarily located in the synovial joints leading to the destruction of cartilage and bone ⁽¹⁾. It's clinical course is highly variable with spontaneous disease fluctuations. Rheumatoid arthritis affects 0.5 – 1 % of the population and is more frequent in women as compared to men ⁽²⁾. Although, the cause of RA is still remains unknown, both genetics and environmental factors can play an inciting role in the development of this disease. It is an autoimmune disorder and has been associated with human leukocytes antigen (HLA) DR4 subtype ⁽³⁾. A specific amino acid sequences, the so called shared epitope confers a higher risk for developing destructive lesions in rheumatoid arthritis ⁽⁴⁾. A majority of RA patients are characterized by the presence of serological abnormalities. The autoantibodies such as the presence of rheumatoid factors and antibodies to anti-citrullinated proteins can be demonstrated in blood group donors several years before the onset of symptoms ⁽⁵⁾.

Cytokines are proteins with small molecular weight secreted mainly by cells of the immune system, which act as key mediators in physiological functions such as host defense and inflammatory responses. The physiological functions of these proteins turns pathological on their overproduction. The pathological role of the cytokines in RA has been demonstrated by Feldmann M and Maini RN ⁽⁶⁾. The proinflammatory cytokines tumor necrosis factor (TNF- α) and interleukin 1-beta (IL-1 β) are the two most important mediators involved in the pathophysiology of several chronic inflammatory diseases including RA.

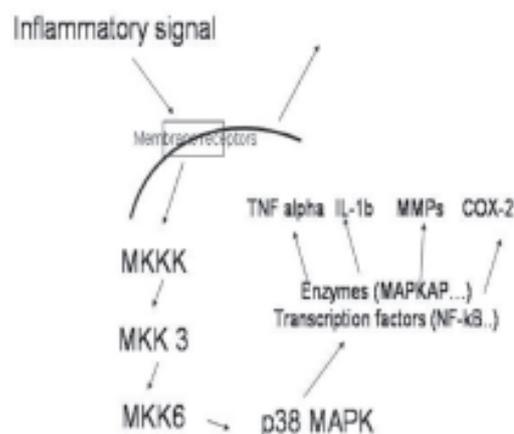
The extracellular stimuli such as stress and inflammatory cytokines convert an extracellular signal to intracellular

response. The kinases are the key regulators of cell function and gets activated by phosphorylation ⁽²⁾. The main signal transduction pathways implicated in RA include the mitogen-activated protein kinase (MAPK) pathway, nuclear factor-kappa B (NF- κ B) and Jannus kinases signal transducer and activator of transcription (JAK / STAT) pathway.

Mitogen-activated protein kinase (MAPK)

Mitogen-activated protein kinase are a family of ubiquitously distributed enzymes which intervene in signal transduction pathways from many cell surface receptors and participate in the regulation of number of physiological as well as pathophysiological cellular processes including cell growth, differentiation and apoptosis ⁽⁷⁾. The three main MAP kinase families are the extracellular signal regulated protein kinases (ERK), the C-Jun amino terminal (JNK) and the p38 kinases. The ERK are activated by growth factors and mitogenic stimuli and participate in the control of cell proliferation and differentiation ⁽⁸⁾. In contrast, the JNK and p38 kinases are activated by environmental stress factors including the proinflammatory signals and are often referred to as the stress activated protein kinase. p38 kinases, a 38 kDa protein is a member of the MAP kinase family gets activated occurs in response to different proinflammatory stimuli such as osmotic shock, endotoxin, UV light or cytokines ⁽⁹⁾ that leads to transcription of target genes. The key position occupied by p38 kinase is shown in Figure 1.

Figure 1: Representation of inflammatory signal transduction showing the key position of p38 MAP Kinase



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AP-Activating protein kinase, COX-2- Cyclo-oxygenase - 2
IL-1beta-Interleukin - 1beta , MAPK-Mitogen activated
protein kinase

MAPKK-MAPK kinase, MAPKKK-MAPK kinase kinase.

MMP-Matrix metalloproteinases, NF-kB-Nuclear factor
kappa B

TNF-alpha-Tumor necrosis factor alpha.

p38 MAPK pathway and RA

It has been demonstrated that MAPKK3/MKK3 (mitogen-activated protein kinase kinase) and MAPKK6/MKK6 make independent contributions to p38 MAPK activation in rheumatoid synovial fibroblast after cytokine stimulation⁽⁶⁾ and both must be blocked for maximal inhibition. In inflammation, the most important route for activation is via TNF- α and IL-1 ligation to extracellular membrane receptors and the activated p38 can phosphorylates a number of downstream kinases and transcription factors which leads to regulation of gene expression at the transcriptional and translational levels. The studies on synovial tissue of RA and osteoarthritis patients demonstrated that the activation of MAP kinase families is more intense in RA. p38 activation was feature observed in the synovial lining layer and in synovial endothelial cells⁽⁷⁾.

p38 inhibitors

p38 MAP kinase, occupies a central position in the inflammatory signal transduction pathway, is a target for the development of new inflammatory medications, since inhibition of this enzyme provides a mechanism for controlling the overproduction of TNF α and IL-1 β . A number of compounds representing a variety of structures such as pyridinyl imidazole, pyrimidinyl imidazoles, diarylureas and diaryl ketones have been developed as potential inhibitors for p38 kinases. The crystallographic studies have indicated that these inhibitors bind at ATP binding site of p38 kinase by competing with ATP and thereby inhibiting the phosphorylation process. The usage of these inhibitors to a certain extent is restricted due to side effects such as liver toxicity as it interferes with hepatic cytochrome P450 enzymes⁽⁸⁾. SB 203580 was investigated in adjuvant induced arthritis in Lewis rats and murine collagen induced arthritis⁽⁹⁾. RPR 200765 A has reduced the incidence and progression in the rat streptococcal cell wall arthritis⁽¹⁰⁾. Vertex has investigated a number of compounds with VX- 745 reaching phase II trials for RA⁽¹¹⁾ in humans but has been withdrawn due to side effects. One of the most promising p38 MAPK inhibitors for treatment of RA is SC10-469, now in phase II clinical trials⁽¹²⁾.

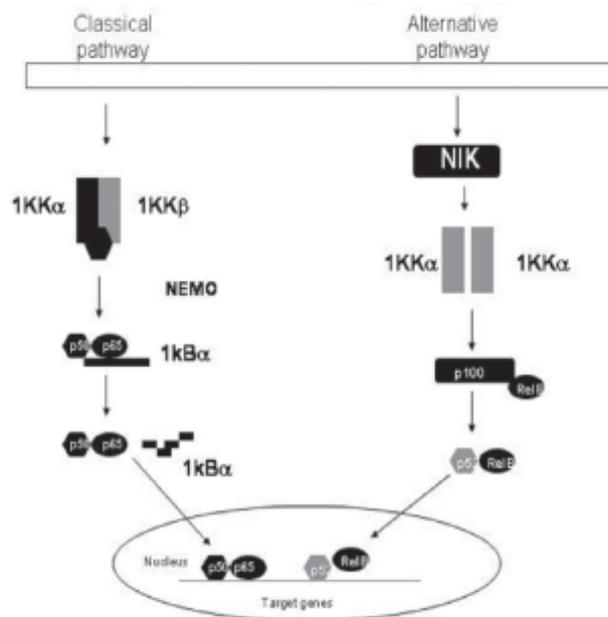
Nuclear factor kappa β (NF-kB)

It is a factor identified by Baltimore and his colleagues in 1986 in nucleus of B cells and is expressed ubiquitously in cytoplasm of all cells . It is being considered as the master switch for self defense responses⁽¹³⁾ and regulates expression of genes encoding cytokines, cell adhesion molecules, cell cycle regulators and apoptosis inhibitors. NF-kB is predominantly localized in the cytoplasm complexed with

the inhibitory 1kB proteins. The activation of NF-kB by classical / alterative pathway leads to the degradation of the inhibitory protein and with translocation of NF-kB to the nucleus and induce expression of target genes. Figure 2 highlights the mechanism of action of NFKB in signaling pathway.

NF-kB and RA

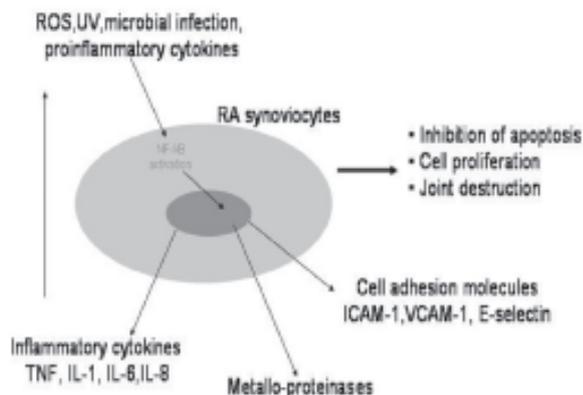
Figure 2 : Two different NF-KB signaling pathways



The classical pathway is activated by a large number of agonists such as tumor necrosis factor and interleukin -1. The alterative pathway is activated by a limited number of agonists that are involved in secondary lymphoid organogenesis, mature B-Cell function and adaptive immunity.

NF- κ B- Nuclear factor kappa B, IKK- Inhibitor of NF-kB, NEMO – NF-kB essential modulator, NIK – NF-kB inducing enzyme, p50, p65, p100, RelB, p52 – Components of NF-kB.

The pathophysiological features of RA can be explained by activation of limited number of transcription factor and its activation signal such as NF-kB as shown in Figure 3. NF-kB induces gene expression of cell growth promoting factors such as cyclin D1 and c-myc besides causing upregulation of inflammatory cytokines. The nuclear factor acts as a major determinant of RA pathophysiology by causing hyperproliferation of synovial tissues. Drugs such as acetylsalicylic acid, ibuprofen, sulfasalazine, dexamethazone, and aurothiomalate have shown to block NF-kB activation cascade and the discovery that various non steroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids (GC) are potent inhibitors of NF-kB has dramatically influenced the understanding of molecular mechanism underlying the anti -inflammatory action of drugs⁽¹⁴⁾. Oxidative stress, an important contributor to pathogenesis of RA causes the activation of this factor through reactive oxygen species⁽¹⁵⁾.

Figure: 3 Involvement of NF- κ B in the RA pathophysiology

NF- κ B induces gene expression of inflammatory mediators such as cytokines and cell adhesion molecules which has a positive feedback to perpetuate and expand the inflammatory response.

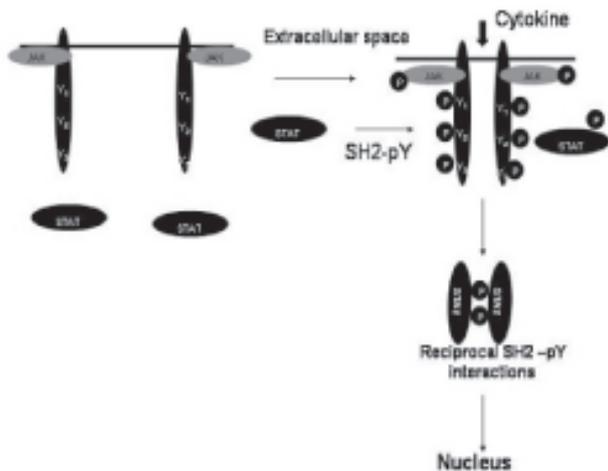
ICAM -1 – intercellular circulating adhesion molecule 1
– Interleukin

NF- κ B – Nuclear factor kappa B, ROS – reactive oxygen species.

UV – ultraviolet, VCAM – Vasculare circulating adhesion molecule

Jannus kinase pathway – JAK / STAT pathway

The third pathway (JAK /STAT) has received less attention among RA researchers⁽¹⁶⁾. This pathway is utilized by many cytokines and growth factors that regulate gene expression and cellular activation, proliferation and differentiation⁽¹⁷⁾. To date four known mammalian JAKs has

Figure: 4 Cytokine activation of JAK-STAT pathway

The activation of JAK-STAT pathway by cytokines leads to dimerization, activation of receptor-associated JAK kinases and phosphorylation of receptor cytoplasmic domains (Y_1, Y_2, Y_3). This leads to recruitment of STAT protein which are then phosphorylated, dimerized and translocated to the nucleus to activate gene transcription.

JAK-STAT – Jannus-activated kinase / Signal transducer and activator of transcription.

Y_1, Y_2, Y_3 – Receptor cytoplasmic domain tyrosine residues.

been identified fro JAK 1- JAK 4 which helps in activation of cytoplasmic transcription factors termed as signal transducers and activators of transcription (STATs) which binds to DNA. The cytokine activation of Jannus-activated signal is represented in the Figure 4.

JAK /STAT and RA

The STAT – 3 DNA binding activity has been demonstrated in freshly isolated RA synovial fluid⁽¹⁸⁾. IL -6 was the major STAT -3 activating factor present in RA synovial fluid⁽¹⁹⁾. A study by Yollota et al indicated the activation of STAT-1 in synovial fluid cells by IL-6. The function of STAT in RA synovitis as pathogenic or protective is not yet clear⁽²⁰⁾. A study by van der Pouw Kraan⁽²¹⁾ suggest that this depends on the cell type and possibly on the state of the disease.

JAK inhibitors

Janus kinases are activated by interferon γ and other cytokines also play a significant role in the pathogenesis of RA⁽²²⁾. Recently developed small molecules of molecular weight less than 1KDa⁽²³⁾ are being tested for efficacy in the preclinical and clinical trials. These agents have target impact on various intracellular factors and intracellular signaling pathways. The selective inhibitors of JAK kinases are already available and inhibitors of STATs have not yet been successfully developed. Pfizer developed inhibitors (CP-690, 550) to Jak 3 which are in phase II clinical trials in RA patients⁽²⁴⁾. A multicentre study is currently in progress to compare the effect of five dosing regimens of Jak-3 inhibitor with adalimumab and disease modifying anti-rheumatic drugs in patients with active RA (<http://clinicaltrials.gov>).

Our laboratory data with respect to key mediators in RA, showed a significantly high plasma protein levels of TNF α and IL-11 β which was statistically significant (p value <0.001) as compared to healthy groups. The plasma protein levels of TNF α and IL -1 β in RA patients undergoing treatment were found to be significantly (p < 0.001) higher than the healthy group supporting the role of the above markers in the pathogenesis of the disease⁽²⁵⁾. A significant positive correlation (p < 0.05) between these two molecules in RA group further suggested that these two molecules influence the production of each other⁽²⁶⁾. Genetic analysis revealed no significant differences between RA and control groups when the genotypes of both – 308 TNF - α promoter region and + 3953 IL-1 β exon 5 polymorphism were analyzed. These results suggest that factors other than variation in gene promoter are responsible for the elevated levels seen in the blood of RA and polymorphism at + 3953 IL -1 β exon 5 region may not have any association with RA disease.

A knowledge of these signaling pathways is of particular importance to rheumatologists because cytokines clearly regulate the inflammatory and immune responses. Numerous studies have proven the crucial role of these molecules in inflammatory pathologies, the synergistic effects observed by blocking these mediators will provide additional value. Patients have benefitted from drugs targeting these pathways

and several newer drugs with better efficacy are in the stage of clinical trials. To conclude, an in depth knowledge of cytokine signaling pathways with beneficial intervention of regulatory mechanism may provide a unique way to treat such chronic inflammatory diseases.

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