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■ REVIEW ■

Nuclear Factor κB (NF-κB) Pathway as a Therapeutic Target in Rheumatoid Arthritis

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Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by persistent joint swelling and progressive destruction of cartilage and bone. Current RA treatments are largely empirical in origin and their precise mechanism of action is uncertain. Increasing evidence shows that chronic inflammatory diseases such as RA are caused by prolonged production of proinflammatory cytokines including tumor necrosis factor (TNF) and interleukin 1 (IL-1). The nuclear factor κB (NF-κB) plays an essential role in transcriptional activation of TNF and IL-1. NF-κB is induced by many stimuli including TNF and IL-1, forming a positive regulatory cycle that may amplify and maintain RA disease process. NF-κB and enzymes involved in its activation can be a target for anti-inflammatory treatment. Aspirin and sodium salicylate inhibit activation of NF-κB by blocking IκB kinase, a key enzyme in NF-κB activation. Glucocorticoids suppress expression of inflammatory genes by binding glucocorticoid receptor with NF-κB, and increasing expression of inhibitory protein of NF-κB, IκBα. Sulfasalazine and gold compounds also inhibit NF-κB activation. Continuing advances in our understanding of action mechanism of antirheumatic agents will benefit the future development of RA regimens with greater efficacy and less toxicity.

Key Words: *Tumor necrosis factor; NF-κB; Arthritis, rheumatoid; Interleukin 1; Monokines; Anti-inflammatory agents; Antirheumatic agents*

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease that leads to chronic inflammation in the joints, functional disability and increased mortality. Although the immune and inflammatory mechanisms that constitute the process of RA have been well documented, its etiology and pathogenesis are largely unknown. Recent studies revealing correlation between some genetic background with increased susceptibility, and environmental factors that predispose susceptible individuals to RA have been the focus of intensive research. RA is characterized by persistent synovial inflammation and irreversible destruction of cartilage and bone in the majority of patients. However, the clinical course of the disease varies greatly between individual patients, ranging from spontaneous remission in some to relentless progression in others, leading to severe joint destruction, physical impairment, and death.

There are a number of currently available antirheumatic drugs, although none of them seems to cure RA or can be used preventively to reduce the risk of contracting the disease. These treatments are commonly divided into two groups: first the non-steroidal anti-inflammatory drugs (NSAID) of the aspirin type and corticosteroids which suppress acute inflammation without altering the progression of the underlying chronic inflammation and second those agents which may modify the clinical course of RA.

This latter class of drugs, including auranofin, parenteral gold, hydroxychloroquine, minocycline, sulfasalazine, azathioprine, penicillamine, methotrexate, cyclosporin, chlorambucil, and cyclophosphamide, is usually used after it has become apparent that a RA patient's arthritis has become established, thus named "second-line" drugs. They are also referred to as "slow-acting" or "disease-modifying" antirheumatic drugs because of their protracted onset of action, or their ability to induce a decrease in general inflammatory activity, respectively. These second-line treatments are empirical in origin, and their disease-modifying mechanisms have not been clearly demonstrated. However, large-scale, multi-center studies

for up to 20 years showed that increased second-line drug use was strongly associated with a better long-term disability index, especially in patients with more active disease at onset (1).

Over the past decade extensive studies revealed the pivotal role of proinflammatory cytokines, such as tumor necrosis factor (TNF) and interleukin 1 (IL-1), in synovial proliferation and joint destruction in RA. Cytokines are proteins or glycoproteins of relatively small size produced by immunologically competent cells during host response to infection, injury or inflammation. Cytokines modify the proliferation, death, migration, and behavior of many cell types by acting on the cells via cell surface receptors. In the inflamed rheumatoid synovium, TNF and IL-1 produced primarily by macrophages and fibroblasts appear to directly contribute to tissue damage and perpetuate inflammatory response by recruiting activated immune and inflammatory cells to the inflamed joint. Expression of these cytokines by various environmental signals is primarily regulated by changing the transcription rate of the gene, a process controlled by sequence-specific DNA-binding proteins called transcription factors. Nuclear factor κB (NF-κB) is a particularly important transcription factor in the expression of genes involved in immune and inflammatory responses (2). Recently accumulated evidences demonstrated that a number of antirheumatic drugs block the action of NF-κB, implying NF-κB or molecules involved in its activation as important targets for the action of these drugs.

MACROPHAGE-DERIVED CYTOKINES IN RHEUMATOID INFLAMMATION

Macrophages have been recognized as playing a crucial role in chronic inflammatory diseases, including RA, and gained widespread attention from a finding that showed cytokines produced by monocyte/macrophages constitute a major portion of cytokine profile in rheumatoid synovium (3). Although the T lymphocyte is likely to play an essential role in the initiation of rheumatoid synovitis, the levels of lymphokines such as lymphotoxin and interferon γ (IFN-γ) in RA synovial tissue or fluid are low, and the cell proliferation and expression of cell surface markers of synovial T cells indicate that they are not in active state (4). Activated macrophages are enriched in the rheumatoid synovium and are also found in the destructive pannus tissue, reflecting their central role as an effector cell in RA pathogenesis (5).

Two macrophage-derived cytokines IL-1 and TNF are recognized as key mediators in the pathogenesis and maintenance of rheumatoid synovitis. Both IL-1 and TNF are produced during the acute phase of RA and their levels are correlated with the number of infiltrating macrophages (6). RA synovial fluid containing either of these cytokines and recombinant IL-1 or TNF are injurious to normal cartilage in vitro. Moreover, the injection of IL-1 and TNF into animal joints leads to tissue damages that have been associated with RA. This cartilage destruction is due to reduced proteoglycan synthesis, enhanced proteoglycan degradation, and induction of cartilage-degrading metalloproteinases in synovial macrophages and fibroblasts (7). Further evidence supporting the importance of IL-1 and TNF in inflammatory arthritis was derived from studies on cytokine inhibition in various animal models of RA. Neutralizing antibodies against murine IL-1 suppressed both inflammation and cartilage destruction in antigen-induced arthritis in mice (8), and the naturally occurring competitive inhibitor of IL-1 called IL-1 receptor antagonist (IL-1ra) blocks tissue destruction in various animal models of arthritis, including collagen-induced arthritis in mice (7, 9). In similar animal models of RA, the administration of monoclonal antibodies against TNF or recombinant soluble TNF receptors either prior to or after the onset of arthritis led to an attenuation of the severity of the inflammation and joint destruction (10-13).

Studies with dissociated synovial membrane cells from RA patients suggested that TNF plays a pivotal role in RA pathogenesis through the induction of a proinflammatory cytokine cascade (14). Production of IL-1, IL-6, IL-8 and granulocyte macrophage colony stimulating factor (GM-CSF) from RA synovial cells was shown to be inhibited in vitro with anti-TNF antibodies (15). As observed in murine collagen arthritis model, clinical trials in which monoclonal anti-TNF antibody (such as infliximab) were administered to patients with long-standing and active RA by intravenous infusions showed dramatic remissions in arthritic symptoms (e.g. joint swelling, tenderness, pain, and morning stiffness) and a reduction in serum levels of C-reactive protein (CRP)(16, 17). Another approach to decrease TNF activity in RA patients was the administration of TNF receptors fused with the Fc portion of human IgG (such as etanercept). It has been shown to be effective when used alone as well as administered in combination with methotrexate in patients with established RA, resulting in a better response in approximately 40% more patients than the patients given placebo (18, 19).

Further evidence supporting the importance of TNF in RA has been provided by the spontaneous development of arthritis in transgenic mice modified to express high level of human TNF constitutively (20). These animals developed chronic inflammatory polyarthritis, pannus formation, and cartilage and bone destruction, which was correlated with the expression of human TNF

mRNA in the joints and the level of human TNF in serum. Histologic evaluation of arthritic joints revealed that there were many features in common with the pathology of RA. Administration of anti-human-TNF antibodies to these arthritic mice suppressed the disease, demonstrating that the overexpression of TNF was indeed the cause of the disease. Another interesting observation in these mice was that blocking IL-1 receptor with anti-murine IL-1 receptor antibody also prevented the development of arthritis, showing that the arthritogenic effects of TNF appeared through IL-1 in these animals (21).

The evidence obtained thus far indicates that TNF and IL-1 satisfy the criteria for identifying mediators of joint tissue damage in RA. These cytokines are present in arthritic synovial tissues and are injurious to normal cartilage in vivo and in vitro. Tissue damages elicited by TNF, IL-1, antigens, or pathogens in animals and progression of joint inflammation in patients with RA can be prevented by specific antibodies or antagonists of these cytokines. Furthermore, spontaneous development of arthritis in animals overexpressing TNF indicate that articular tissues are relatively more susceptible to the pathogenic effect of these cytokines compared to other tissues.

NF-κB-MEDIATED EXPRESSION OF TNF AND OTHER INFLAMMATORY GENES

The direct involvement of TNF and other proinflammatory cytokines in the development and maintenance of RA suggests that blocking their expression in the synovial tissue can offer therapeutic benefits. The TNF gene expression is under complex control. TNF is produced mainly from activated macrophages in response to a wide range of immune cell- and pathogen-derived molecules including cytokines, endotoxin (also called lipopolysaccharide, LPS), viruses, parasites, and fungi. Although the precise factors activating macrophages in the rheumatoid synovium are unknown, cytokines produced by activated T cells or macrophages, immune complexes, products of complement activation and possibly bacterial endotoxin might be involved (7). It was also suggested that direct contact between activated T cells and macrophages may be more important than the release of soluble factors (22). Human TNF is synthesized as a proprotein of 233 amino acids with a molecular mass of 26 kDa. The pro-TNF is cleaved by a specific metalloprotease (named TNF-α converting enzyme, TACE) to yield a mature form of 17 kDa comprising 157 amino acids. Under physiological conditions, TNF forms a noncovalently bound homotrimer (23).

TNF synthesis is known to be regulated at both the transcriptional and post-transcriptional levels. The p38 mitogen-activated protein kinase pathway controls translation possibly by actions on the 3' untranslated region (24). The human TNF 5' upstream region/promoter contains binding sites for multiple transcription factors, including NF-κB, AP-1, C/EBP, Egr-1 and NF-AT (25-28). Although there is strong evidence that shows TNF expression in murine macrophages is dependent on a number of NF-κB-binding sites (29), the situation in human monocytes/macrophages is more complex and cooperation between NF-κB and other factors appears to be necessary for maximal induction of TNF gene (25-27). A number of NF-κB-like binding motifs are located in the 5' flanking region of TNF promoter and some of them bind NF-κB proteins present in the nuclear extract of endotoxin-stimulated monocytes. Endotoxin activates NF-κB in human monocytes, and TNF expression is suppressed by specific inhibitors of NF-κB activation in the same cells (30). In human synovial cells NF-κB was also shown to be essential for TNF production. Transfer of gene encoding the natural inhibitor of NF-κB, called IκB, suppressed spontaneous production of TNF by 75% in rheumatoid joint cell cultures (31). In a similar experiment, TNF production from LPS-stimulated human monocytes was inhibited by 80%, indicating that TNF expression depends mainly on NF-κB activation in both types of cells.

NF-κB is a transcription factor that is recognized to play a pivotal role in the regulation of inflammation (2, 32). It was initially discovered as a regulator of κ light-chain gene expression in B cells, but subsequent studies revealed that it is present in many different cell types. The activated form of NF-κB is a heterodimer, which usually consists of two proteins, a p50 subunit and a p65 subunit (also called RelA). In unstimulated cells, NF-κB is retained in the cytoplasm through an interaction with inhibitory proteins known as IκB (IκB-α, β and ε). Many stimuli induce NF-κB activity, including TNF, IL-1, activators of protein kinase C, viruses, bacterial LPS, ionizing radiation and oxidants. These signals cause phosphorylation and subsequent degradation of IκB proteins via the ubiquitination-proteasome pathway. The release of NF-κB from IκB leads to translocation of NF-κB into the nucleus, where it binds to specific sequences in the promoter regions of target genes. NF-κB can activate transcription of many genes involved in immune and inflammatory responses, including TNF, IL-1, IL-6, IL-8, granulocyte-macrophage colony-stimulating factor, inducible nitric oxide synthase, intercellular adhesion molecule 1, E-selectin, and the MHC class I and II molecules (2, 32).

The phosphorylation of IκB is likely to be a central

point at which diverse signals converge to activate NF-κB. Biochemical studies showed that the activity of IκB kinase (IKK) was associated with a high molecular weight (approximately 700 kDa) complex in the cytosolic extracts (33, 34). Isolated enzyme was able to phosphorylate two conserved N-terminal serine residues of IκB-α and IκB-β required to activate NF-κB in vivo. IKK in the cell was activated by various inflammatory signals that induce NF-κB. Two kinases, designated IKK-α (or IKK-1) and IKK-β (IKK-2), have been cloned and shown to be part of multi-subunit IKK complex (34-38). Both IKK-α and IKK-β are Ser/Thr kinases of similar structure and contain an N-terminal kinase domain followed by a leucine zipper region and a C-terminal helix-loop-helix domain (32). Studies with cultured cells suggested that both kinases are involved in NF-κB activation after binding of TNF or IL-1 to the cell surface receptors. Moreover, recombinant IKK-α and IKK-β expressed independently in insect cells and purified to apparent homogeneity were able to phosphorylate IκB-α at specific serine residues in in vitro assays, indicating that each kinase can directly phosphorylate IκB proteins (39, 40). However, examination of mice in which either IKK-α or IKK-β gene was disrupted by gene targeting revealed that IKK-β is activated, and activates NF-κB in response to proinflammatory cytokines, whereas IKK-α participates in the transduction of signals during the embryonic development of the skin and skeletal systems (41-44). Other subunits of the IKK complex were also characterized by biochemical analysis and molecular cloning and they include mitogen-activated protein kinase/ERK kinase kinase 1 (MEKK1), NF-κB-inducing kinase (NIK), IKK-γ and IKK-complex-associated protein (IKAP) (35, 45, 46). These subunits were shown to play roles in the transmission of upstream signals to IKKα and IKKβ by directly phosphorylating them or by acting as scaffold proteins that aid association of various IKK components (45, 46).

Activation of NF-κB has also been suggested to play an important role in the regulation of hyperplasia in the RA synovium by blocking apoptosis of synovial cells. In contrast to the normal synovium, the rheumatoid synovium is characterized by massive infiltration of inflammatory cells accompanied with hyperplasia and fibrotic changes in synovial tissues (47). This process of disease progession results in the formation of tumor-like structure called pannus, which destroys joint bone and cartilage. Studies with cultured cells in which NF-κB was inactivated or overexpressed showed a protective function of NF-κB against the cytotoxicity of TNF and other agents (48-51). NF-κB activation leads to expression of several survival genes that prevent apoptosis in a number of cell types (52, 53). Inhibition of NF-κB by expression of IκB-α in synoviocytes potentiated TNF- and Fas ligand-induced cytotoxicity, and in vivo suppression of NF-κB enhanced apoptosis in the synovium of experimentally-induced arthritis of rats (54).

ANTIRHEUMATIC DRUGS THAT REGULATE NF-κB ACTIVATION AND TNF SYNTHESIS

Proinflammatory cytokines TNF and IL-1 produced by stimulated macrophages play pivotal roles in synovial inflammation and joint destruction in RA. Expression of these cytokines is upregulated by transcription factor NF-κB, which can be induced by many factors (cytokines, antigens, immune complexes, and activated T cells) present in the rheumatoid synovium. Activation of NF-κB also leads to expression of many genes that encode proteins involved in immune and inflammatory responses, amplifying and perpetuating the process of RA. These findings suggest that NF-κB is an obvious target for antirheumatic treatment. Studies with a number of therapeutic agents for RA, such as salicylate, glucocorticoids, sulfasalazine, and gold compounds, demonstrated that they indeed block one or another step of NF-κB pathway.

The salicylates, or aspirin-like drugs, are among the most frequently prescribed medicine and are widely used to relieve inflammation in RA. The antipyretic, anti-inflammatory and analgesic activities of these compounds have been attributed to their capacity to inhibit prostaglandin synthase (cyclooxygenase) (53). However, interference with prostaglandin synthesis does not explain their therapeutic efficacy completely: nonacetylated salicylates are effective anti-inflammatory drugs when given in adequate doses, although they only weakly inhibit prostaglandin synthase (56). Moreover, the anti-inflammatory effect of aspirin requires much higher doses than those needed to inhibit prostaglandin synthesis. Aspirin and sodium salicylate were shown to inhibit activation of NF-κB by preventing degradation of IκB in relatively high concentrations (57). Cell treatment with these agents resulted in suppression of IKK activity, and they inhibited IKK-β but not IKK-α kinase activity (58). The inhibition was due to binding of aspirin and salicylate noncovalently to IKK-β to reduce ATP binding. These results demonstrate that the clinical effects of aspirin-like drugs that do not depend on inhibiting prostaglandin synthesis are mediated in part by their inhibition of IKK-β, thereby preventing NF-κB activation of proinflammatory genes.

Glucocorticoids are potent anti-inflammatory and immunosuppressive agents which have been widely used in the treatment of chronic inflammatory diseases such as

RA. They inhibit synthesis of many cytokines and cell surface molecules involved in inflammatory and immune responses, but the molecular mechanism underlying this activity has been poorly understood. Glucocorticoids induce target genes by binding to glucocorticoid receptors in the cytoplasm. The activated receptors then move to the nucleus and bind to specific DNA motifs called glucocorticoid-response elements in steroid-responsive genes, leading to increased transcription. Transcriptional repression of inflammatory genes by glucocorticoid, however, is mediated by some other mechanism because there is no glucocorticoid-response elements in their promoter regions. Treatment of cells with a synthetic glucocorticoid, dexamethasone, blocked induction of NF-κB-mediated gene expression, and glucocorticoid-dependent gene expression was in turn reduced by overexpression of p65 subunit of NF-κB (59, 60). Glucocorticoid receptor and NF-κB seem to directly interact with each other and they reciprocally inhibit DNA binding and the ability of increasing gene activity. Glucocorticoids also increase transcription of IκB-α gene, thereby increasing synthesis of IκB-α protein (61, 62). Increased IκB-α inhibits activation and nuclear translocation of NF-κB and induces dissociation of NF-κB from its binding sites on target genes (62), which terminates NF-κB-dependent expression of inflammatory genes. Inhibition of NF-κB by glucocorticoids suggests that NF-κB is a primary target of glucocorticoids for their anti-inflammatory actions.

Sulfasalazine was developed as an antirheumatic drug, but its actual therapeutic mechanism remains unknown. It was synthesized to combine an anti-inflammatory (5-aminosalicylic acid) and an antibacterial (sulfapyridine) moieties. After oral administration, about 70% of sulfasalazine is degraded and reduced by colonic bacteria to 5-aminosalicylic acid and sulfapyridine. The remaining 30% of sulfasalazine is absorbed in its unaltered form along with sulfapyridine. In clinical studies, sulfapyridine was suggested to be responsible for the efficacy of sulfasalazine observed in RA (63). Treatment of cultured colon cells with sulfasalazine, but not 5-aminosalicylic acid or sulfapyridine, blocked NF-κB activation induced by TNF, endotoxin, or phorbol ester (64). This inhibition was associated with suppression of IκB-α phosphorylation and its subsequent degradation, suggesting that sulfasalazine blocks IKK or an upstream signal. Although the target of sulfasalazine in NF-κB activation pathway is not clear and the antirheumatic effect of sulfapyridine remains unanswered, these results suggest that therapeutic effect of sulfasalazine on RA is in part due to the suppression of NF-κB activation.

Gold compounds, comprised of elemental gold and a sulfur-containing ligand, have been used in the treatment of RA, inducing definite improvement of clinical conditions in a majority of RA patients (65). Parenteral gold compounds, such as sodium aurothiomalate and aurothioglucose, are water-soluble and administered by the intramuscular route, while a hydrophobic gold compound auranofin can be taken orally. Although their mechanism of antirheumatic action is not clearly understood, synovial monocytes/macrophages are considered to be the most likely targets for action by gold compounds. Parenteral gold is selectively concentrated within inflamed synovial tissues, and gold-rich deposits are formed in synovial macrophages during chrysotherapy (66, 67). Administration of aurothiomalate results in reduced accumulation of inflammatory monocytes and macrophages in the RA synovial membranes, and significant inhibition of IL-1, IL-6 and TNF expression in these cells (68). Auranofin inhibited endotoxin-induced IL-1 and TNF production in cultured human monocytes and mouse macrophages by reducing mRNA levels of these cytokines, suggesting that it blocks some common step in the signal pathways for the transcriptional activation of IL-1 and TNF genes (69). Treatment of macrophages with auranofin suppressed endotoxin-induced activation of NF-κB and IκB degradation (70). This inhibition seems to be due to the blocking of IKK activity by gold because hydrophilic gold salts were able to reduce kinase activity of isolated IKK. Gold salts and other metal compounds were also shown to inhibit the in vitro binding of NF-κB to DNA, presumably by replacing zinc that is a necessary component of NF-κB for its DNA binding activity (71). These results demonstrate that the inhibition of NF-κB activation and NF-κB-DNA binding by gold compounds is responsible for the reduced expression of proinflammatory cytokines in synovial tissues and their antirheumatic effect.

Chloroquine and methotrexate are widely used in the treatment of RA because they are relatively well tolerated in comparison with other second-line agents of similar potency. Antimalarial drugs chloroquine and hydroxychloroquine were shown to block the production of TNF in animals undergoing hemorrhagic shock and in endotoxin-stimulated macrophages (72, 73). Chloroquine does not suppress induction of TNF mRNA and NF-κB activity in LPS-stimulated macrophages, nor production of 26-kDa precursor protein of TNF (74). These results indicate that the inhibitory effect of chloroquine appears at post-translational levels and possibly by blocking conversion of pro-TNF to mature protein. Administration of methotrexate was also shown to lead to a profound decrease in synovial fluid TNF level in an animal model of RA (75). Some of anti-inflammatory effects of methotrexate were suggested to be mediated by cellular accumulation of adenosine deaminase and AMP deaminase inhibitors, resulting in increased concentrations of adeno-

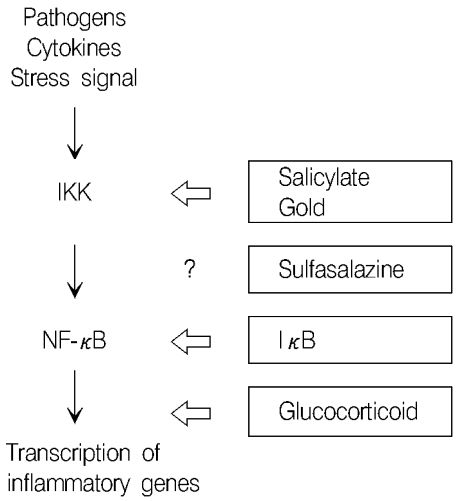

Fig. 1. Inhibition of NF-κB pathway by various anti-inflammatory agents. IKK, IκB kinase.

sine in the extracellular space (76). Adenosine, acting at one or another of its receptors on monocyte/macrophages, inhibits production of TNF and other cytokines (77, 78). Stimulation of adenosine receptor with specific agonists results in the suppression of mRNA levels of TNF, IL-6, and IL-8, and alters nuclear AP-1, but not NF-κB activity (79). These results suggest that there is an adenosine-sensitive pathway other than NF-κB that is critical for the induction of TNF in monocyte/macrophages.

CONCLUSIONS

The studies summarized above indicate that modulating the activation of NF-κB in the synovial cells (Fig. 1) is one of the mechanisms by which a number of antirheumatic drugs inhibit inflammation in RA. NF-κB increases the expression of the genes for proinflammatory cytokines, chemokines, enzymes and adhesion molecules, leading to coordinated stimulation of inflammatory response. IL-1 and TNF induced by activated NF-κB may amplify and perpetuate the disease, possibly by prolonged activation of NF-κB. Activation of NF-κB in the inflamed synovium may also protect the cells against apoptosis, resulting in hyperplasia and destruction of joint tissue. Therapeutic intervention specifically designed to target elements important to this vicious cycle should offer the potential for improved efficacy with reduced toxicity. Recent clinical studies with agents directed against TNF prove the promise of this approach. Understanding action mechanisms of antirheumatic drugs rather than empiricism will greatly benefit treatment decisions for RA patients and development of novel strategies to treat RA.

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