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**TARGETING NF- κ B: A PROMISING MOLECULAR THERAPY IN
INFLAMMATORY ARTHRITIS**

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Abstract

The Nuclear Factor-kappaB (NF-κB) family of transcription factors is intimately involved in the regulation of the inflammatory responses that play a fundamental role in the damage of articular tissues. Thus, many studies have examined the important contributions of components of the NF-κB signaling pathways to the pathogenesis of various rheumatic diseases, and their pharmacologic modulation. Currently available therapeutic agents including non-steroidal antiinflammatory drugs, corticosteroids, nutraceuticals and disease-modifying anti-rheumatic drugs, as well as novel specific small molecule inhibitors have been employed. In addition, promising strategies such as improved antisense DNA therapy and RNA interference have shown encouraging results. However, further research will be needed before NF-κB-aimed strategies become an effective therapy for inflammatory arthritis.

Key words: Inflammatory Arthritis, Rheumatoid Arthritis, Osteoarthritis, NF-κB,

Introduction

In the last few years, novel molecular approaches have provided invaluable insights into the multitude of complex anabolic and catabolic signals that act upon diverse cells from articular tissues. Many of these important processes play a key role in the pathogenesis of inflammation and tissue destruction, crucial components of numerous articular diseases (1,2). The precise interplay of these signaling pathways is essential for the activation of the cellular gene expression machinery. A large number of transcription factor families have been implicated as critical regulators of gene expression in the setting of the inflammatory process (3). This review focuses on the nuclear factor κ B (NF- κ B) signaling pathways, emphasizing their role in inflammation and damage to articular tissues, their modulation with therapeutic agents currently in use, and potential future strategies.

NF- κ B

The NF- κ B proteins are a family of ubiquitously expressed transcription factors that play an essential role in most immune and inflammatory responses. These transcription factors also have an important role in the protection of cells from apoptosis and in the process of intercellular signaling during normal vertebrate development. NF- κ B was first described as a B cell-specific transcription factor but has been subsequently shown to exist in all mammalian cell types.

In mammals, the NF- κ B family consists of five members: RelA (p65), RelB, c-Rel, NF- κ B1 (p50 and its precursor p105), and NF- κ B2 (p52 and its precursor p100). They share a 300-amino acid domain that is designated the Rel homology domain (RHD)

which mediates their dimerization, interaction with the inhibitory κ B (I κ B) proteins, DNA binding and nuclear translocation. RelA (p65), RelB, and c-Rel contain a C-terminal transcriptional activation domain (TAD) that positively regulates gene expression. In contrast, p50 and p52 lack TADs, therefore, they may repress transcription unless bound to other NF- κ B family member containing a TAD or to other proteins capable of recruiting coactivators (4-8). Although the NF- κ B family members form a variety of homodimers and heterodimers, the most prevalent activated form is the heterodimer RelA (p65) and p50. Each dimer activates its own characteristic set of genes and different dimers can bind to the same or distinct sites in NF- κ B-dependent gene promoters regulating the transcription of their corresponding response genes in a cell-type and stimulus-type manner (9-10).

NF- κ B function and regulation

NF- κ B is present in the cytoplasm of all mammalian cells in an inactive form associated with the I κ B proteins, which include I κ B α , I κ B β , I κ B ϵ , I κ B γ , Bcl-3, and the precursor proteins p100 and p105 (4-6). The I κ B proteins typically contain C-terminal ankyrin repeats that are crucial for their interaction with the NF- κ B proteins, and an N-terminal leucine-rich nuclear export-sequence, that is important for the shuttling of I κ Bs between the cytoplasm and nucleus. The shuttling of I κ Bs is an important mechanism to retain the I κ B-NF- κ B complex in the cytoplasm of unstimulated cells. The I κ B proteins can also act as NF- κ B cofactors that ultimately either inhibit or enhance NF- κ B binding to DNA. Thus, I κ Bs have both cytoplasmic and nuclear roles in regulating NF- κ B pathways (4,6,8,11).

The phosphorylation of I κ Bs is performed by the specific serine/threonine kinase I κ B kinase (IKK). The IKK complex consists of at least three subunits, including the kinases IKK α and IKK β (also called IKK-1 and IKK-2, respectively) and the associated regulatory subunit IKK- γ /NEMO (NF- κ B essential modulator). IKK β is the dominant kinase in the canonical pathway of NF- κ B activation, whereas IKK α appears to also play a significant role. Thus, a new IKK α function has been recently described; the regulation of histone function which in turn causes the activation of the NF- κ B canonical pathway. Conversely, IKK α has a unique role in the activation of the non canonical pathway. IKK- γ /NEMO has no known kinase activity, however, is crucial for IKK complex activation **(8,12-15)**.

A broad range of stimuli including the cytokines TNF- α and IL-1 β , chemokines, bacterial and viral products, and free radicals activate the NF- κ B dimers by triggering the canonical signaling pathway that leads to the IKK β phosphorylation-induced degradation of I κ Bs (I κ B α , I κ B β and I κ B γ), followed by its ubiquitination by the E3 ubiquitin ligase complex (SCF ^{β TrCP}), and its consequent degradation by the 26S proteasome. The mechanism through which cytokines activate the IKK complex is not fully known. At least two hypotheses have been postulated: one proposes that the activation of TAK1 (TGF- β -activated kinase-1) or MAP kinase kinase ERK1 (MEKK1) enhances IKK activity, whereas the second suggests that the linkage of IKK to the receptors localized in the cell membrane originates its autophosphorylation and further activation. Upon stimulation, interaction of NEMO with receptor-interacting protein (RIP) family members and TNF Receptor Associated Factor (TRAF) proteins in an ubiquitin-dependent manner would occur in either case. Although phosphorylation of IKK β is a

key event in the canonical pathway, the ubiquitination and subsequent degradation of the multiple factors involved on its regulation are also crucial mechanisms required for NF- κ B activation (8,11,13).

The degradation of I κ B exposes a nuclear localization signal on the NF- κ B proteins, which then become able to translocate into the nucleus and stimulate the transcription of specific target genes. It has been described that NF- κ B regulates more of than 150 genes, including those involved in immunity and inflammation, anti-apoptosis, cell proliferation and the negative feedback of the NF- κ B signal (11). A partial list of genes relevant to the inflammatory response whose expression is stimulated by NF- κ B activation is shown in **Table 1**.

In turn, IKK α activates the NF- κ B non-canonical pathway by phosphorylating precursor p100, followed by its polyubiquitination by SCF ^{β TrCP} and further proteasomal-processing to mature p52. TRAF family members and NF- κ B-inducing kinase (NIK) also play an essential role in the non-canonical pathway. Indeed, NIK phosphorylates IKK α . This process is generally slower than the canonical pathway and leads to a delayed activation of p52-containing complexes, such as p52/RelB. The activation of the non-canonical pathway is restricted to certain TNF receptor (TNFR) superfamily members such as lymphotoxin β receptor (LT β R), B-cell activating factor (BAFF), CD40 ligand, CD27, CD30 or Receptor Activator of Nuclear Factor- κ B (RANK). These molecules are involved in lymphoid organ formation, in B cell development, survival and homeostasis, and in osteoclastogenesis. Furthermore, the non canonical pathway is also activated by the oncogenic viruses EBV and HTLV1 (8,10,16).

A sequential activation of both canonical and non-canonical NF- κ B pathways generated by few inducers has been described. Thus, an initial activation of the canonical pathway is followed by the activation of the non-canonical NF- κ B pathway. Therefore, the orchestrated expression of their specific and/or common NF- κ B target genes, in a cell-type and stimulus-type specific fashion, contributes to the pleiotropic biological functions of this ubiquitous transcription factor **(16)**.

Role of the NF- κ B in inflammatory arthritis

Although NF- κ B plays an essential beneficial role in normal physiology, inappropriate regulation of NF- κ B activity has been implicated in the pathogenesis of several inflammatory diseases including rheumatic diseases such as rheumatoid arthritis (RA), osteoarthritis (OA), spondyloarthropaties (SpA), systemic lupus erithematosus (SLE), crystal induced-arthropaties, etc. **(17) (Table 2)**.

It has been shown that NF- κ B is involved in the differentiation and activation of immune cells including macrophages; dendrytic cells (DCs), granulocytes, as well as osteoclasts and chondrocytes **(18)**. Thus, differentiation of monocyte precursors to macrophages requires NF- κ B-dependent transcription of anti-apoptotic genes. Mature macrophage expression of the canonical NF- κ B pathway is very important for establishing innate and adaptive immunity through its microbial activation via Toll-like receptors (TLRs), antigen-processing/presentation and lymphocytes co-stimulation **(19)**. The development of DCs is mainly enhanced by RelB and its presence is required for antigen processing and presentation in these cells, however, impairment of the canonical NF- κ B pathway also inhibited DCs maturation.and survival **(20,21)**. During granulocyte

differentiation, the canonical NF- κ B pathway is activated in the setting of orchestrated chemokines/cytokines interaction (22). Mature normal granulocytes express NF- κ B-dependent anti-apoptotic genes and lack p52 and RelB (18,23). Intact, both canonical and non-canonical NF- κ B signaling pathways are also crucial for T and B cell differentiation and homeostasis (18,24). The development of lymphoid tissues, particularly lymph nodes and Peyer's patches, is influenced by both canonical and non-canonical NF- κ B pathways, however the non-canonical pathway related to LT β R is particularly relevant in these processes (25,26). The beneficial and harmful roles of NF- κ B are diagrammatically shown in **Figure 1**.

NF- κ B in rheumatoid arthritis. There is a very important NF- κ B activation in synovial tissue from patients with RA. Indeed, NF- κ B activation is significantly higher in RA than in OA, although p50 and p65 NF- κ B are abundant in both rheumatoid and osteoarthritic synovium (27). RA and SpA synovial tissues show that the numbers of cells expressing NF- κ B1 at the cartilage-pannus junction is significantly higher than in other areas; a similar finding was observed in the number of cells expressing RelA in RA synovium, but no in SpA synovium. Furthermore, the numbers of NF- κ B1+ and RelA+ cells in OA synovium were similar to those observed at the non-cartilage-pannus junction sites in all inflammatory tissues studied (28). In patients with RA and OA, immunoreactive IKK is abundant in primary fibroblast-like synoviocytes (FLS) and both IKK α and IKK β are constitutively expressed at the mRNA level. Following TNF- α and IL-1 stimulation of RA FLS, IKK β activation is a key event for NF- κ B mediated induction of IL-6, IL-8, ICAM-1 and collagenase gene expression (29).

Animal models of inflammatory arthritis also support the concept that NF- κ B plays a very active role in the development and progression of arthritis in vivo. NF- κ B activation prior to the onset of clinical manifestations of arthritis has been found in both, murine type II collagen-induced arthritis (CIA) and rat adjuvant induced arthritis (AIA). In the first model, NF- κ B expression correlated better than AP-1 expression with collagenase-3 (MMP-13) and stromelysin (MMP-3) levels, however, both transcription factors were activated before onset of clinical arthritis and metalloproteinase gene expression (30). Also, a shift to nuclear NF- κ B localization was shown in chondrocytes during cartilage destruction in the early stage of arthritis in DBA/1 mice immunized with type II collagen (31). In the second model, expression of activated NF- κ B p65 was found in the synovial lining layer and surrounding the blood vessels in the inflamed synovium, being stronger in the injected hindpaw than that in the noninjected one (32). In addition, intraarticular gene transfer of IKK β caused arthritis in normal rats, characterized by severe paw swelling, inflammatory histologic changes, increased IKK activity and enhanced NF- κ B DNA binding activity. Thus, these experiments confirm that IKK β activation is a crucial event in the initiation of synovitis (33).

NF- κ B family members play also a pivotal role in osteoclastogenesis and inflammation-induced bone loss observed in RA. The OPG/RANK/RANKL triad is particularly relevant to bone homeostasis. Thus, osteoclasts express RANK and osteoblasts express RANKL and its soluble decoy receptor osteoprotegerin (OPG) that blocks RANK binding to its ligand RANKL (34). Furthermore, mice deficient for RANK or RANKL show lack of precursor cells differentiation to osteoclast, leading to osteopetrosis (35,36). The p50/p52 double knock-out mice has shown similar osteopetrotic

phenotype (37). In addition, recent experiments in mice have demonstrated that IKK β activation is indispensable in the signal transduction from RANK to NF- κ B. Absolute absence of IKK β activity, and not of IKK α activity, leads to lack of osteoclastogenesis and bone unresponsiveness of IKK β -deficient mice to inflammation (38). Therefore, the canonical NF- β pathway is crucial for osteoclastogenesis *in vivo* and its specific inhibition represents a logical alternative strategy to the current therapies. In contrast, the role of the non-canonical NF- β pathway is less evident *in vivo*; nevertheless, it has been recently shown to be crucial for the antigen-mediated periarticular bone erosion that accompanies inflammatory arthritis in several murine models (39,40).

NF- κ B in juvenile rheumatoid arthritis. Since juvenile rheumatoid arthritis (JRA) shares many pathogenetic mechanisms with other autoimmune diseases, the activation of NF- κ B is also thought to play a relevant role in its molecular pathophysiology. Thus, NF- κ B p65 nuclear expression and activation have been detected in the synovial tissue and fluid cells from polyarticular JRA and RA patients (41). Also, increased mRNA and protein expression of RANK and RANKL have been found in synovial dendritic cells of the joints from children with oligoarticular and polyarticular JRA. RANK/RANKL interactions may contribute to the survival of inflammatory articular cells, as well as to erosions and osteoporosis in JRA (42).

NF- κ B in spondyloarthropathies. Few studies have directly addressed the role of NF- κ B activation in SpA. Initially, NF- κ B activation was found to be exclusively mediated by p50/p50 homodimers in synovial T cells from patients with reactive arthritis (ReA) and ankylosing spondylitis (AS), in contrast with the predominance of p50/65

heterodimers in rheumatoid synovial T cells (43). Recently, another study found that p65 DNA-binding was decreased during the course of infliximab therapy whereas p50 DNA-binding remained elevated in lymphocytes from AS patients (44). The differential activation of NF- κ B subunits p50 and p65 might provide insights into the NF- κ B role in the pathogenesis of SpA and in the effects of anti-TNF- α therapy in AS.

In addition, the involvement of NF- κ B activation in psoriatic arthritis (PsA) has recently been studied with growing interest. Indeed, cells expressing active NF- κ B p65 were primarily localized to lining layer and perivascular macrophages in PsA synovial membrane. Expression of NF- κ B p65 was equal in lining layer from both PsA and RA patients, but lower in PsA than RA sublining. However, the histologic findings did not correlate with clinical parameters of disease (45). In a further study, phosphorylated I κ B α expression and histological severity scores significantly decreased following six months of etanercept treatment in PsA synovium (46).

NF- κ B in crystal induced arthropathies. NF- κ B activation has been described in the response of articular cells to monosodium urate (MSU), calcium pyrophosphate dihydrate (CPPD) and basic calcium phosphate (BCP) crystals. Indeed, transcriptional activation of important inflammatory mediators such as IL-8, chemokines and iNOs by NF- κ B and AP-1, as well as ERK 1/2 signaling are essential for the mononuclear phagocyte response to CPPD and MSU crystals (47-49). NF- κ B and AP-1 have also been shown to mediate the effects of the BCP crystals in human fibroblasts (50).

NF- κ B in septic arthritis. NF- κ B activity has been studied in some studies of septic arthritis. Surprisingly, the clinical course of septic arthritis was not ameliorated in a

murine model of *S. aureus*-induced arthritis systemically treated with antisense oligonucleotides (ODN) to p65 NF- κ B, alone or in combination with antibiotics. However, the bacterial burden in the kidneys and IL-6 levels were significantly increased. These findings suggested that p65 antisense therapy approach may not be suitable for treatment of septic arthritis because it leads to increased bacterial burden overpassing the potential anti-inflammatory benefit of these compounds (51). In contrast, several studies suggest a beneficial effect of NF- κ B inhibition in other septic conditions. Indeed, parthenolide (PAR), an NF- κ B inhibitor found in medicinal herbs, blocked LPS-induced osteolysis in the mouse calvarium model. NF- κ B-dependent osteoclastogenesis and osteoclastic bone resorption were inhibited by PAR. Enhanced apoptosis of osteoclasts and their precursor cells was observed as well in a dose-dependent manner in this bacteria-induced bone destruction model (52).

NF- κ B in systemic lupus erythematosus: NF- κ B activation in SLE has been shown in *in vitro* and *in vivo* studies. SLE T cells have shown decreased expression of p65-Rel A heterodimer expression associated with a low level of IL-2 promoter activity and altered c-Rel expression and nuclear import (53-55). Moreover, SLE T cells increased IL-2 promoter activity to normal levels following transfection with cDNA encoding the NF- κ B p65 subunit (54). A recent study has been carried out in a mouse model that overproduces BAFF, developing a SLE-like disease. BAFF enhanced long-term B cell survival primarily through the non-canonical NF- κ B pathway, while it promoted immunoglobulin class switching and generation of pathogenic antibodies through the classical pathway. These findings demonstrate that both NF- κ B signaling pathways are important for development of lupus-like disease associated with BAFF

overproduction (56). Remarkably, another study has shown that administration of DCB-3503, a NF- κ B inhibitor, for 10 weeks nearly abrogated inflammatory skin disease with little effect on histologic kidney disease in MRL/Fas(lpr) mice (57).

NF- κ B in osteoarthritis. NF- κ B signaling pathways mediate critical events in the inflammatory response by chondrocytes, leading to progressive extracellular matrix damage and cartilage destruction (17,58). Numerous studies have examined the effects of NF- κ B on chondrocyte functions. The NF- κ B and the MEK1/2 kinase pathways were found to mediate inhibition of type II collagen and link protein gene expression by TNF- α as well as upregulation of MMP-1, MMP-3 and MMP-13 RNA/protein expression induced by TNF- α or IL-1 β in articular chondrocytes (59-61). NF- κ B has also been shown to mediate fibronectin fragment induced-chondrocyte activation and increased expression of IL-6, IL-8, MCP-1, growth-related oncogenes and MMP-13 by human articular chondrocytes (62,63). Furthermore, NF- κ B production was increased with donor age in IL-1 β stimulated human articular chondrocytes (63). Finally, a study performed in bovine chondrocytes showed that DNA binding of NF- κ B and AP-1 was significantly higher in hypoxic and reoxygenated chondrocytes treated with IL-1 β than in normoxic chondrocytes (64).

It is also known that NF- κ B signal pathways are employed by mechanical signals for transcriptional regulation of proinflammatory genes that are involved in catabolic events in chondrocytes. Mechanical strains of low magnitude prevent nuclear translocation of NF- κ B, resulting in inhibition of proinflammatory gene expression. In contrast, mechanical strains of high magnitude induce this translocation, and thus cause

proinflammatory gene induction. Furthermore, mechanical overload induces similar intracellular events to those generated by proinflammatory cytokines in arthritis (65,66).

Besides its anti-inflammatory effects, it has been suggested that NF- κ B may also play a role in chondrocyte apoptosis. Under certain conditions NF- κ B exerts prosurvival effects in articular cartilage. Thus, in human chondrocytes NF- κ B activation partially mediates the anti-apoptotic effects of IL-1 β against death receptor CD-95 (FAS/APO-1) (67). In contrast, other studies have described NF- κ B involvement in apoptotic events in articular chondrocytes. For example, it has been shown that NF- κ B activation mediates the apoptotic effect of NO in articular chondrocytes (17,68,69).

Inhibition of NF- κ B by pharmacologic agents

An increasing number of NF- κ B inhibitors, including several clinically important anti-inflammatory drugs, have been reported (70) as illustrated in **Figure 2**. Glucocorticoids are potent inhibitors of the NF- κ B pathway through several mechanisms (71). Glucocorticoids induce the transcription of the I κ B α gene through the glucocorticoid receptor (GR), causing an increased cytosolic retention of NF- κ B (72,73). Glucocorticoids may also inhibit the NF- κ B DNA binding activity through direct interaction between GR and components of the NF- κ B binding sites in various gene promoters (74). The activated GR can also interact with NF- κ B by direct protein-protein binding, preventing the activation of the NF- κ B pathway in certain types of cells (75). Lastly, competition can occur between GR and NF- κ B, limiting amounts of the coactivators CREB-binding protein (CBP), CBP-associated factor (p/CAF) and steroid receptor coactivator-1(SRC-1)

(76). Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, salicylate, ibuprofen, indomethacin, diclofenac, and sulindac inhibit IKK β activity, preventing I κ B α phosphorylation, consequently blocking the activation of the NF- κ B pathway **(77,78)**. It has also been shown that sulfasalazine suppresses I κ B phosphorylation, probably owing to the effects of its anti-inflammatory metabolite, 5-aminosalicylic acid **(79)**.

The immunosuppressive agents cyclosporin A and tacrolimus (FK-506) also inhibit the NF- κ B pathway. Cyclosporin A inhibits the protease activity of the 20S proteasome complex preventing I κ B α degradation in murine macrophages, Jurkat lymphoma cells, and in mouse and human T lymphocytes **(80)**. FK506 blocks translocation of c-Rel from the cytoplasm to the nucleus in both B and T cells, and Jurkat cells, leading to a decreased expression of IL-2 and its receptor **(81)**. Several other agents have also been described to inhibit NF- κ B including vitamin C, vitamin E, curcumin, flavonoids, lactacystin, thalidomide, leflunomide, pyrrolidine dithiocarbamate, glucosamine, diacehrein and resveratrol **(17)**.

In addition, since the nonclassical anti-inflammatory activity of estrogen has been attributed to interference with NF- κ B signaling by multiple mechanisms, agents that target estrogen receptors (ER) and show selective inhibition of NF- κ B activity have been synthesized recently. Thus, WAY-169916, a small molecule ER ligand that inhibits NF- κ B transcriptional activity but is devoid of conventional estrogenic activity, has demonstrated beneficial effects in two models of inflammatory disease: the HLA-B27 transgenic rat model of inflammatory bowel disease (IBD) and the Lewis rat AIA model. In both models, a near complete reversal in hindpaw scores was observed as well as marked improvement in the histological scores. In the Lewis rat AIA model, WAY-

169916 also markedly suppressed induction of serum acute phase proteins. Furthermore, WAY-169916 also suppressed TNF- α -mediated inflammatory gene expression in FLS from RA patients. Therefore, this class of compounds might have a potential utility in the treatment of inflammatory arthritis **(82,83)**.

Novel therapeutic strategies aimed at the specific inhibition of key elements in the NF- κ B pathway activation are being developed, causing great expectation regarding their potential effects as arthritis treatments **(84-87)**. For example, proteasome function inhibitors, decoy oligonucleotides, and peptides that inhibit nuclear localization of NF- κ B have been utilized to inhibit NF- κ B signaling in animal models **(88,89)**. Daily oral treatment with PS-341 (bortezomib), a proteasome inhibitor recently approved by the FDA for the treatment of multiple myeloma, decreases significantly NF- κ B activity in rats with streptococcal cell wall-induced polyarthritis. This decrease is associated with lower serum levels of IL-1, IL-6 and NO metabolites **(90)**. Decoy oligodeoxynucleotides (ODN), short double stranded DNA containing the consensus binding sequence of NF- κ B were introduced by intraarticular injection into the hind joints of CIA rats. In these experiments, NF- κ B decoy ODN decreased the severity of hind-paw swelling, suppressed IL-1 and TNF- α in the arthritic synovium, and abrogated joint destruction as evidenced by histologic and radiographic studies **(91)**. In a similar approach, the same investigators injected NF- κ B decoy ODN into the knee joints of anterior cruciate ligament transection (ACLT) OA model rats. Histopathological findings from knee joints injected with the naked NF- κ B decoy ODN showed a statistically significant amelioration as assessed by the Mankin 95 criteria, compared with either a scrambled decoy ODN or physiological

buffer administration. Also, naked NF- κ B decoy ODN significantly inhibited the levels of IL-1 β or TNF- α in the synovium and the cartilage, compared with the scrambled decoy ODN (92). Small peptides called protein transduction domains (PTDs) and cell penetrating peptides (CPPs) able to transport much larger molecules such as oligonucleotides, peptides, full-length proteins, bacteriophages, etc. across cellular membranes of almost all tissues have also been employed, modifying cellular function in the absence of ectopic gene expression (93). One example is BMS-205820, another novel selective NF- κ B inhibitor. It contains a synthetic PTD carrying two nuclear localization sequences (NLS) capable of blocking NF- κ B nuclear localization. This inhibition resulted in a decrease of cell surface protein expression, cytokine production, and T cell proliferation, and showed efficacy in a mouse septic shock model as well as in an IBD mouse model (94).

IKK β has become a particularly appealing target for therapeutic intervention in RA and OA because of its crucial role in NF- κ B pathway activation. Administration of IKK β resulted in a potent increase of cytokine production in numerous cell types including synoviocytes and chondrocytes. Thus, in AIA rats, intraarticular gene therapy delivering a dominant-negative IKK β (IKK β dn)–adenovirus construct inhibits NF- κ B translocation; consequently, cytokine-induced IL-6, IL-8 and ICAM-1 expressions are suppressed (28). In the same experimental model, recombinant adeno-associated virus 5 (rAAV5) carrying IKK β injected intraarticularly, significantly reduced paw swelling, IL-6 and TNF- α levels in early arthritis. No significant effect was found on cartilage and bone destruction, however. Remarkably, this genetic construct also reduced IL-6 production following TNF- α stimulation in whole human RA synovial tissue biopsies *ex*

vivo, representing a promising step in the development of gene therapy for human arthritis (95).

Gene constructs that overexpress I κ B or express an engineered protein without the sites for phosphorylation (I κ B super repressor) have also been used. However, there have been technical difficulties for their appropriate intracellular delivery, therefore, viral or non-viral vectors are necessary to carry them into the cell. A chimeric molecule that contains the super-repressor I κ B α (srI κ B) fused to the human immunodeficiency virus Tat protein PTD (Tat-srI κ B α) has been examined in a rat model of pleuresy. This chimeric molecule showed a good effect, causing reduced cellular infiltration as well as increased apoptosis of leukocytes in the sites of inflammation and decreased levels of the proinflammatory cytokines TNF- α and IL-1 β in the exudates (96). Recently, a peptide corresponding to the NEMO-binding domain (NBD) of IKK β linked to *Drosophila* Antennapedia protein (Antp) has been shown to inhibit NF- κ B signaling in animal models and rheumatoid tissue cultures. Intra-articular injection of the NBD peptide in the rat AIA model, reduced severity of arthritis, radiological damage, synovial cellularity and TNF- α and IL-1- β expression. NBD is able to block the interaction of IKK α and IKK β with the regulatory subunit IKK γ (NEMO). Because of its highly defined site of action, NBD inhibits only activated but not basal levels of NF- κ B and is unlikely to affect other essential kinases, in contrast to other small-molecule NF- κ B inhibitors (97). Also, adenovirus transferring I κ B α into late stage OA synovial cells were shown to regulate the spontaneous expression of an array of proinflammatory cytokines, chemokines and MMPs. Thus, the gene therapy delivered to synovial cells could be a potential option for OA treatment (98,99).

Also, new studies with small molecule inhibitors have further strengthened the role of IKK β . One of these small molecules, sc-514 inhibits I κ B phosphorylation/degradation and p65 NF- κ B phosphorylation/transactivation induced by IL-1 in RA synovial fibroblasts in a dose-dependent manner (100). Another IKK β inhibitor, BMS-345541, was administered to treat murine CIA in both prophylactic and therapeutic dosing regimens. Prophylactic BMS-345541 showed a dose-dependent efficacy reducing the incidence of arthritis, clinical disease severity and IL-1 β mRNA levels and blocking inflammation and joint destruction evaluated histologically. Therapeutic BMS-345541 reduced clinical and histological end points in animals with preestablished disease, showing a dose-dependent effect. Furthermore, use of high doses resulted in clinical remission of the disease (101). ML120B, a novel IKK β inhibitor, inhibited paw swelling in a dose-dependent manner and offered significant protection against cartilage and bone erosion in the AIA rat model. Using novel *in vivo* imaging techniques, the association between the inhibition of NF- κ B activity and the dampening of chronic inflammatory processes was documented in arthritic joints (102,103).

Dehydroxymethylepoxyquinomicin (DHMEQ), a novel small selective inhibitor of NF- κ B translocation, decreased the severity of clinical arthritis and improved radiographic and histopathologic scores in murine CIA, as well as suppressed proinflammatory cytokines expression and cell proliferation in RA FLSs. Interestingly, in further experiments performed in the animal model, DHMEQ significantly suppressed osteoclastogenesis and NFATc1 expression along the inner surfaces of bone lacunae and the eroded bone surface in arthritic joints. Serum levels of RANKL, OPG and M-CSF were not affected by the treatment. However, DHMEQ neither suppressed spontaneous

expression of RANKL nor M-CSF in culture of RA FLSs. Thus, DHMEQ may suppress RA associated-osteoclastogenesis, through NFATc1 downregulation (104,105). In an interesting cellular therapy approach, attempting to influence the antigen-specific immune response rather than to produce a broad antiinflammatory effect, dendritic cells (DCs) treated with the NF- κ B inhibitor BAY 11-7082 were injected intraarticularly into methylated bovine serum albumin (mBSA) induced-arthritic joints of C57BL/6 mice. DCs exposed to mBSA and further treated with BAY 11-7082 suppressed inflammation and erosion, showing potential as antigen-specific therapy for autoimmune inflammatory arthritis (106).

Other new promising therapeutic strategies to target specific proteins of the NF- κ B pathway include improved antisense therapy and RNA interference. Locked Nucleic Acid-Antisense (LNA), morpholino oligonucleotides, and particularly, RNA interference have been developed in recent years (107-110). RNA interference, a general post-transcriptional gene silencing mechanism, is initiated by a double stranded RNA which after being introduced into cells is cleaved into 21 or 22nt dsRNA fragments. These fragments called small interfering RNA (siRNA) induce the formation of a ribonucleoprotein complex (RNAi silencing complex) that mediates sequence-specific cleavage of the targeted transcript mRNA by the antisense RNA strand, thus promoting mRNA degradation of a specific mRNA (109,110). Indeed, siRNA targeting of NF- κ B p65 subunit has shown promising results, decreasing significantly the expression of COX-2, iNOS and MMP-9 mRNA/protein levels in rat chondrocytes stimulated with IL-1 β and TNF- α (111). Furthermore, in a recent *in vivo* study, an adenoviral vector carrying a siRNA targeting NF- κ B p65 subunit inhibited early changes in an OA rat model. IL-1 β

and TNF- α synovial fluid levels, cartilage degradation and synovial inflammation were all reduced in the early stages of this experimental OA model (112).

Conclusions

The NF- κ B family of transcription factors plays a crucial role in the distinctive inflammatory processes characteristic of certain rheumatic diseases leading to bone and cartilage destruction, and articular damage. Therefore, NF- κ B inhibition is a rational objective in the treatment of rheumatic diseases. NSAIDs, glucocorticoids, nutraceuticals, natural products and certain disease-modifying anti-rheumatic drugs (DMARDs) have been described to decrease NF- κ B activation. Yet, novel therapeutic strategies targeting key elements in the NF- κ B pathway including IKK, 26S proteasome, p65 and p50 subunits have been and continue to be developed, and small molecule inhibitors, chimeric molecules, improved antisense therapy and RNA interference are part of the new approaches to block the NF- κ B pathways.

Thus, NF- κ B transcription factors appear as a very attractive target for treatment of arthritis; however, some concerns about the systemic and indiscriminate blockade of its numerous beneficial effects, as well as technical problems for local delivery of a potential agent through gene therapy still remain. Further *in vivo* studies will increase our understanding of the true significance of NF- κ B inhibition and provide the foundations for the development of effective therapy for various joint diseases.

Abbreviations

AF-1/BF-1: activation function 1/brain factor 1; **AP-1:** activator protein 1; **A20:** deubiquitinase A20; **Bax:** BCL2-associated X protein; **Bcl-2:** B-cell lymphocyte/leukemia-2; **c-FLIP:** cellular fllice inhibitory protein; **c-IAP:** cellular

inhibitor of apoptosis protein; **COX-2**: ciclooxigenase 2; **CXCL1**: chemokine (C-X-C motif) ligand 1; **DNA**: deoxyribonucleic acid; **EBV**: Epstein-Barr virus; **ENA-78**: epithelial cell-derived neutrophil-activating protein 78; **ERK 1/2**: extracellular signal-regulated kinase 1/2; **GM-CSF**: granulocyte macrophage colony stimulating factor; **HTLV1**: human T-lymphotropic virus 1; **ICAM-1**: intercellular adhesion molecule 1; **IKK**: I κ B kinase; **IL-1 β** : interleukin-1 beta; **IL-2**: interleukin-2; **IL-6**: interleukin 6; **IL-8**: interleukin 8; **IL-12**: interleukin-12; **INF- γ** : interferon-gamma; **iNOS**: inducible nitric oxide synthase; **MAPK**: mitogen activated protein kinase; **MCP-1**: methyl-accepting chemotaxis protein 1; **M-CSF**: macrophage colony stimulating factor; **MEK 1/2**: MAP kinase ERK 1/2; **MHC-I**: major histocompatibility complex 1; **MIP-1 α** : macrophage inflammatory protein alpha; **MMP**: metalloproteinase; **NAFTc1**: nuclear factor of activated T cell 1; **NO**: nitric oxide; **RANTES**: Regulated upon activation, normal T-cell expressed and secreted; **RNA**: ribonucleic acid; **TLR-2**: toll-like receptor 2; **TNF- α** : tumor necrosis factor-alpha; **TRAF**: TNF-receptor-associated factor; **VCAM-1**: vascular cell adhesion molecule 1.

REFERENCES

1. Firestein GS. Evolving concepts of rheumatoid arthritis. *Nature* 423: 356-361, 2003.
2. Pelletier JP, Martel-Pelletier J, Abramson S. Osteoarthritis, an inflammatory disease. Potential implication for the selection of new therapeutic targets. *Arthritis Rheum* 44: 1237-1247, 2001.
3. Firestein GS, Manning AM. Signal transduction and transcription factors in rheumatic disease. *Arthritis Rheum* 42: 609-621, 1999.
4. Li Q and Verma IM. NF- κ B regulation in the immune system. *Nat Rev Immunol* 2: 725-734, 2002.
5. Silverman N, Maniatis T. NF- κ B signaling pathways in mammalian and insect innate immunity. *Genes Dev* 15: 2321-2342, 2001.
6. Ghosh S, Karin M. Missing pieces in the NF- κ B puzzle. *Cell* 109: S81-S96, 2002.
7. Bouwmeester T, Bauch A, Ruffner H, Angrand PO et al. A physical and functional map of the human TNF- α /NF- κ B signal transduction pathway. A physical and functional map of the human TNF- α /NF- κ B signal transduction pathway. *Nat Cell Biol* 6: 97-105, 2004.
8. Hayden MS, Ghosh S. Shared principles in NF- κ B signaling. *Cell* 132: 344-362, 2008.
9. Udalova IA, Mott R, Field D, Kwiatkowski D. Quantitative prediction of NF- κ B DNA-protein interactions. *Proc Natl Acad Sci U S A* 99: 8167- 8172, 2002.
10. Dejardin E, et al. The lymphotoxin- β receptor induces different patterns of gene expression via two NF- κ B pathways. *Immunity* 17: 525-535, 2002.
11. Yamamoto Y, Gaynor RB. I κ B kinases: key regulators of the NF- κ B pathway. *Trends Biochem Sci* 29: 72-79, 2004.
12. Yamaoka S, et al. Complementation cloning of NEMO, a component of the IKK complex essential for NF- κ B activation. *Cell* 93: 1231-1240, 1998.
13. Häcker H, Karin M. Regulation and function of IKK and IKK-related kinases. *Sci STKE* (357): re13, 2006.
14. Yamamoto Y, Verma UN, Prajapati S, Kwak YT, Gaynor RB. Histone H3 phosphorylation by IKK- α is critical for cytokine-induced gene expression. *Nature* 423: 655-659, 2003.
15. Anest V, Hanson JL, Cogswell PC, Steinbrecher KA, Strahl BD, Baldwin AS. A nucleosomal function for I κ B kinase- α in NF- κ B-dependent gene expression. *Nature* 423: 659-663, 2003.
16. Dejardin E. The alternative NF- κ B pathway from biochemistry to biology: pitfalls and promises for future drug development. *Biochem Pharmacol* 72: 1161-1179, 2006.
17. Roman-Blas JA, Jimenez SA. NF- κ B as a potential therapeutic target in osteoarthritis and rheumatoid arthritis. *Osteoarthritis Cartilage* 14: 839-848, 2006.
18. Uwe S. Anti-inflammatory interventions of NF- κ B signaling: potential applications and risks. *Biochem Pharmacol*; 75: 1567-1579, 2008.
19. Groesdonk HV, Schlottmann S, Richter F, Georgieff M, Senftleben U. *Escherichia coli* prevents phagocytosis-induced death of macrophages via classical NF- κ B signaling, a link to T-cell activation. *Infect Immun* 74: 5989-6000, 2006.

20. Hayden MS, West AP, Ghosh S. NF-kappaB and the immune response. *Oncogene* 25: 6758-6780, 2006.
21. Zanetti M, Castiglioni P, Schoenberger S, Gerloni M. The role of relB in regulating the adaptive immune response. *Ann N Y Acad Sci* 987: 249-257, 2003.
22. Theilgaard-Mönch K, Jacobsen LC, Borup R, Rasmussen T, Bjerregaard MD, Nielsen FC, Cowland JB, Borregaard N. The transcriptional program of terminal granulocytic differentiation. *Blood* 105: 1785-1796, 2005.
23. McDonald PP, Bald A, Cassatella MA. Activation of the NF-kappaB pathway by inflammatory stimuli in human neutrophils. *Blood* 89: 3421-3433, 1997.
24. Horwitz BH, Zelazowski P, Shen Y, Wolcott KM, Scott ML, Baltimore D, Snapper CM. The p65 subunit of NF-kappa B is redundant with p50 during B cell proliferative responses, and is required for germline CH transcription and class switching to IgG3. *J Immunol* 162: 1941-1946, 1999.
25. Weih F, Caamaño J. Regulation of secondary lymphoid organ development by the nuclear factor-kappaB signal transduction pathway. *Immunol Rev* 195: 91-105, 2003.
26. Ware CF. Network communications: lymphotoxins, LIGHT, and TNF. *Annu Rev Immunol* 23: 787-819, 2005.
27. Handel ML, McMorro LB, Gravalles EM. Nuclear factor-kB in rheumatoid synovium: localization of p50 and p65. *Arthritis Rheum* 38: 1762-1770, 1995.
28. Benito MJ, Murphy E, Murphy EP, van den Berg WB, FitzGerald O, Bresnihan B. Increased synovial tissue NF-kappa B1 expression at sites adjacent to the cartilage-pannus junction in rheumatoid arthritis. *Arthritis Rheum* 50: 1781-1787, 2004.
29. Aupperle K, Bennett B, Han Z, Boyle D, Manning A, Firestein G. NF-kB regulation by IκB kinase-2 in rheumatoid arthritis synoviocytes. *J Immunol* 166: 2705-2711, 2001.
30. Han Z, Boyle DL, Manning AM, Firestein GS. AP-1 and NF-kappaB regulation in rheumatoid arthritis and murine collagen-induced arthritis. *Autoimmunity* 28: 197-208, 1998.
31. Eguchi J, Koshino T, Takagi T, Hayashi T, Saito T. NF-kappa B and I-kappa B overexpression in articular chondrocytes with progression of type II collagen-induced arthritis in DBA/1 mouse knees. *Clin Exp Rheumatol* 20: 647-652, 2002.
32. Tsao PW, Suzuki T, Totsuka R, Murata T, Takagi T, Ohmachi Y, Fujimura H, Takata I. The effect of dexamethasone on the expression of activated NF-kappa B in adjuvant arthritis. *Clin Immunol Immunopathol* 83: 173-178, 1997.
33. Tak PP, Gerlag DM, Aupperle KR, van de Geest DA, Overbeek M, Bennett BL, Boyle DL, Manning AM, Firestein GS. Inhibitor of nuclear factor kappaB kinase beta is a key regulator of synovial inflammation. *Arthritis Rheum* 47: 1897-1907, 2001.
34. Theoleyre S, Wittrant Y, Tat SK, Fortun Y, Redini F, Heymann D. The molecular triad OPG/RANK/RANKL: involvement in the orchestration of pathophysiological bone remodeling. *Cytokine Growth Factor Rev* 15: 457-475, 2004.
35. Dougall WC, Glaccum M, Charrier K, Rohrbach K, Brasel K, De Smedt T, Daro E, Smith J, Tometsko ME, Maliszewski CR, Armstrong A, Shen V, Bain S, Cosman D, Anderson D, Morrissey PJ, Peschon JJ, Schuh J. RANK is essential for osteoclast and lymph node development. *Genes Dev* 13: 2412-2424, 1999.

36. Kong YY, Yoshida H, Sarosi I, Tan HL, Timms E, Capparelli C, Morony S, Oliveira-dos-Santos AJ, Van G, Itie A, Khoo W, Wakeham A, Dunstan CR, Lacey DL, Mak TW, Boyle WJ, Penninger JM. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature* 397: 315-323, 1999.
37. Iotsova V, Caamaño J, Loy J, Yang Y, Lewin A, Bravo R. Osteopetrosis in mice lacking NF-kappaB1 and NF-kappaB2. *Nat Med* 3: 1285-1289, 1997.
38. Ruocco MG, Karin M. Control of osteoclast activity and bone loss by IKK subunits: new targets for therapy. *Adv Exp Med Biol* 602: 125-134, 2007.
39. Aya K, Alhawagri M, Hagen-Stapleton A, Kitaura H, Kanagawa O, Novack DV. NF-(kappa) B-inducing kinase controls lymphocyte and osteoclast activities in inflammatory arthritis. *J Clin Invest* 115: 1848-1854, 2005.
40. Vaira S, Johnson T, Hirbe AC, Alhawagri M, Anwisye I, Sammut B, O'Neal J, Zou W, Weilbaecher KN, Faccio R, Novack DV. RelB is the NF-kappaB subunit downstream of NIK responsible for osteoclast differentiation. *Proc Natl Acad Sci U S A* 105: 3897-3902, 2008.
41. Sioud M, Mellbye O, Førre O. Analysis of the NF-kappa B p65 subunit, Fas antigen, Fas ligand and Bcl-2-related proteins in the synovium of RA and polyarticular JRA. *Clin Exp Rheumatol* 16: 125-134, 1998.
42. Varsani H, Patel A, van Kooyk Y, Woo P, Wedderburn LR. Synovial dendritic cells in juvenile idiopathic arthritis (JIA) express receptor activator of NF-kappaB (RANK). *Rheumatology* 42: 583-590, 2003.
43. Collantes E, Valle Blázquez M, Mazorra V, Macho A, Aranda E, Muñoz E. Nuclear factor-kappa B activity in T cells from patients with rheumatic diseases: a preliminary report. *Ann Rheum Dis* 57: 738-741, 1998.
44. Eggert M, Seeck U, Semmler M, Maass U, Dietmann S, Schulz M, Dotzlaw H, Neeck G. An evaluation of anti-TNF-alpha-therapy in patients with ankylosing spondylitis: imbalanced activation of NF kappa B subunits in lymphocytes and modulation of serum cortisol concentration. *Rheumatol Int* 27: 841-846, 2007.
45. Danning CL, Illei GG, Hitchon C, Greer MR, Boumpas DT, McInnes IB. Macrophage-derived cytokine and nuclear factor kappaB p65 expression in synovial membrane and skin of patients with psoriatic arthritis. *Arthritis Rheum* 43: 1244-1256, 2000.
46. Lories RJ, Derese I, Luyten FP, de Vlam K. Activation of nuclear factor kappa B and mitogen activated protein kinases in psoriatic arthritis before and after etanercept treatment. *Clin Exp Rheumatol* 26: 96-102, 2008.
47. Liu R, O'Connell M, Johnson K, Pritzker K, Mackman N, Terkeltaub R. Extracellular signal-regulated kinase 1/extracellular signal-regulated kinase 2 mitogen-activated protein kinase signaling and activation of activator protein 1 and nuclear factor kappaB transcription factors play central roles in interleukin-8 expression stimulated by monosodium urate monohydrate and calcium pyrophosphate crystals in monocytic cells. *Arthritis Rheum* 43: 1145-1155, 2000.
48. Jaramillo M, Godbout M, Naccache PH, Olivier M. Signaling events involved in macrophage chemokine expression in response to monosodium urate crystals. *J Biol Chem* 279: 52797-52805, 2004.

49. Jaramillo M, Naccache PH, Olivier M. Monosodium urate crystals synergize with IFN-gamma to generate macrophage nitric oxide: involvement of extracellular signal-regulated kinase 1/2 and NF-kappa B. *J Immunol* 172: 5734-5742, 2004.
50. McCarthy GM, Augustine JA, Baldwin AS, Christopherson PA, Cheung HS, Westfall PR, Scheinman RI. Molecular mechanism of basic calcium phosphate crystal-induced activation of human fibroblasts. Role of nuclear factor kappa B, activator protein 1, and protein kinase c. *J Biol Chem* 273: 35161-35169, 1998.
51. Gjertsson I, Hultgren OH, Collins LV, Pettersson S, Tarkowski A. Impact of transcription factors AP-1 and NF-kappaB on the outcome of experimental *Staphylococcus aureus* arthritis and sepsis. *Microbes Infect* 3: 527-534, 2001.
52. Yip KH, Zheng MH, Feng HT, Steer JH, Joyce DA, Xu J. Sesquiterpene lactone parthenolide blocks lipopolysaccharide-induced osteolysis through the suppression of NF-kappaB activity. *J Bone Miner Res* 19: 1905-1916, 2004.
53. Wong HK, Kammer GM, Dennis G, Tsokos GC. Abnormal NF-kappa B activity in T lymphocytes from patients with systemic lupus erythematosus is associated with decreased p65-RelA protein expression. *J Immunol* 163: 1682-1689, 1999.
54. Herndon TM, Juang YT, Solomou EE, Rothwell SW, Gourley MF, Tsokos GC. Direct transfer of p65 into T lymphocytes from systemic lupus erythematosus patients leads to increased levels of interleukin-2 promoter activity. *Clin Immunol* 103: 145-153, 2002.
55. Burgos P, Metz C, Bull P, Pincheira R, Massardo L, Errázuriz C, Bono R, Jacobelli S, González A. Increased expression of c-rel, from the NF-kappaB/Rel family, in T cells from patients with systemic lupus erythematosus. *J Rheumatol* 27: 116-127, 2000.
56. Enzler T, Bonizzi G, Silverman GJ, Otero DC, Widhopf GF, Anzelon-Mills A, Rickert RC, Karin M. Alternative and classical NF-kappa B signaling retain autoreactive B cells in the splenic marginal zone and result in lupus-like disease. *Immunity* 25: 403-415, 2006.
57. Choi JY, Gao W, Odegard J, Shiah HS, Kashgarian M, McNiff JM, Baker DC, Cheng YC, Craft J. Abrogation of skin disease in LUPUS-prone MRL/FASlpr mice by means of a novel tylophorine analog. *Arthritis Rheum* 54: 3277-3283, 2006.
58. Roman-Blas JA, Stokes DG, Jimenez SA. Modulation of TGF-beta signaling by proinflammatory cytokines in articular chondrocytes. *Osteoarthritis Cartilage* 15: 1367-1377, 2007.
59. Seguin CA, Bernier SM. TNFalpha suppresses link protein and type II collagen expression in chondrocytes: Role of MEK1/2 and NF-kappaB signaling pathways. *J Cell Physiol* 197: 356-369, 2003.
60. Liacini A, Sylvester J, Li WQ, Zafarullah M. Inhibition of interleukin-1-stimulated MAP kinases, activating protein-1 (AP-1) and nuclear factor kappa B (NF-kappa B) transcription factors down-regulates matrix metalloproteinase gene expression in articular chondrocytes. *Matrix Biol* 21: 251-262, 2002.
61. Liacini A, Sylvester J, Li WQ, Huang W, Dehnade F, Ahmad M, Zafarullah M. Induction of matrix metalloproteinase-13 gene expression by TNF-alpha is mediated by MAP kinases, AP-1, and NF-kappaB transcription factors in articular chondrocytes. *Exp Cell Res* 288: 208-217, 2003.

62. Pulai JI, Chen H, Im HJ, Kumar S, Hanning C, Hegde PS, Loeser RF. NF-kappa B mediates the stimulation of cytokine and chemokine expression by human articular chondrocytes in response to fibronectin fragments. *J Immunol* 174: 5781-5788, 2005.
63. Forsyth CB, Cole A, Murphy G, Bienias JL, Im HJ, Loeser RF Jr. Increased matrix metalloproteinase-13 production with aging by human articular chondrocytes in response to catabolic stimuli. *J Gerontol A Biol Sci Med Sci* 60: 1118-1124, 2005.
64. Martin G, Andriamanalijaona R, Grassel S, Dreier R, Mathy-Hartert M, Bogdanowicz P, Boumediene K, Henrotin Y, Bruckner P, Pujol JP. Effect of hypoxia and reoxygenation on gene expression and response to interleukin-1 in cultured articular chondrocytes. *Arthritis Rheum* 50: 3549-3560, 2004.
65. Agarwal S, Deschner J, Long P, Verma A, Hofman C, Evans CH, Piesco N. Role of NF-kappaB transcription factors in antiinflammatory and proinflammatory actions of mechanical signals. *Arthritis Rheum* 50: 3541-3548, 2004.
66. Dossumbekova A, Anghelina M, Madhavan S, He L, Quan N, Knobloch T, Agarwal S. Biomechanical signals inhibit IKK activity to attenuate NF-kappaB transcription activity in inflamed chondrocytes. *Arthritis Rheum* 56: 3284-3296, 2007.
67. Kuhn K, Hashimoto S, Lotz M. IL-1 beta protects human chondrocytes from CD95-induced apoptosis. *J Immunol* 164: 2233-2239, 2000.
68. Kim SJ, Hwang SG, Shin DY, Kang SS, Chun JS. p38 kinase regulates nitric oxide-induced apoptosis of articular chondrocytes by accumulating p53 via NFkappa B-dependent transcription and stabilization by serine 15 phosphorylation. *J Biol Chem* 277: 33501-33508, 2002.
69. Kim SJ, Chun JS. Protein kinase C alpha and zeta regulate nitric oxide-induced NF-kappa B activation that mediates cyclooxygenase-2 expression and apoptosis but not dedifferentiation in articular chondrocytes. *Biochem Biophys Res Commun* 303: 206-211, 2003.
70. Yamamoto Y, Gaynor R. Therapeutic potential of inhibition of the NF- κ B pathway in the treatment of inflammation and cancer. *J Clin Invest* 107; 135-142, 2001.
71. De Bosscher K, Vanden Berghe W, Haegeman G. Mechanisms of anti-inflammatory action and of immunosuppression by glucocorticoids: negative interference of activated glucocorticoid receptor with transcription factors. *J Neuroimmunol* 109: 16-22, 2000.
72. Auphan N, DiDonato JA, Rosette C, Helmberg A, Karin M. Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis. *Science* 270: 286-290, 1995.
73. Scheinman RI, Cogswell PC, Lofquist AK, Baldwin AS Jr. Role of transcriptional activation of I kappa B alpha in mediation of immunosuppression by glucocorticoids. *Science* 270: 283-286, 1995.
74. De Bosscher K, Vanden Berghe W, Vermeulen L, Plaisance S, Boone E, Haegeman G. Glucocorticoids repress NF-kappaB-driven genes by disturbing the interaction of p65 with the basal transcription machinery, irrespective of coactivator levels in the cell. *Proc Natl Acad Sci USA* 97: 3919-3924, 2000.

75. De Bosscher K, Schmitz ML, Vanden Berghe W, Plaisance S, Fiers W, Haegeman G. Glucocorticoid-mediated repression of nuclear factor-kappaB-dependent transcription involves direct interference with transactivation. *Proc Natl Acad Sci USA* 94: 13504-13509, 1997.
76. Sheppard KA, Rose DW, Haque ZK, Kurokawa R, McInerney E, Westin S, Thanos D, Rosenfeld MG, Glass CK, Collins T. Transcriptional activation by NF-kappaB requires multiple coactivators. *Mol Cell Biol* 19: 6367-6378, 1999.
77. Tegeder I, Pfeilschifter J, Geisslinger G. Cyclooxygenase-independent actions of cyclooxygenase inhibitors. *FASEB J* 15: 2057-2072, 2001.
78. Takada Y, Bhardwaj A, Potdar P, Aggarwal BB. Nonsteroidal anti-inflammatory agents differ in their ability to suppress NF-kappaB activation, inhibition of expression of cyclooxygenase-2 and cyclin D1, and abrogation of tumor cell proliferation. *Oncogene* 23: 9247-9258, 2004.
79. Wahl C, Liptay S, Adler G, Schmid RM. Sulfasalazine: a potent and specific inhibitor of nuclear factor kappa B. *J Clin Invest* 101: 1163-1174, 1998.
80. Meyer S, Kohler NG, Joly A. Cyclosporine A is an uncompetitive inhibitor of proteasome activity and prevents NF-kappaB activation. *FEBS Lett* 413: 354-358, 1997.
81. Venkataraman L, Burakoff SJ, Sen R. FK506 inhibits antigen receptor-mediated induction of c-rel in B and T lymphoid cells. *J Exp Med* 181: 1091-1099, 1995.
82. Chadwick CC, Chippari S, Matelan E, Borges-Marcucci L, Eckert AM, Keith JC Jr, Albert LM, Leathurby Y, Harris HA, Bhat RA, Ashwell M, Trybulski E, Winneker RC, Adelman SJ, Steffan RJ, Harnish DC. Identification of pathway-selective estrogen receptor ligands that inhibit NF-kappaB transcriptional activity. *Proc Natl Acad Sci U S A* 102: 2543-2548, 2005.
83. Keith JC Jr, Albert LM, Leathurby Y, Follettie M, Wang L, Borges-Marcucci L, Chadwick CC, Steffan RJ, Harnish DC. The utility of pathway selective estrogen receptor ligands that inhibit nuclear factor-kappa B transcriptional activity in models of rheumatoid arthritis. *Arthritis Res Ther* 7: R427-438, 2005.
84. Firestein GS. NF-kB: Holy Grail for Rheumatoid Arthritis? *Arthritis Rheum* 50: 2381-2386, 2004.
85. Bacher S, Schmitz ML. The NF-kappaB pathway as a potential target for autoimmune disease therapy. *Curr Pharm Des* 10: 2827-2837, 2004.
86. Feldmann M, Andreakos E, Smith C, Bondeson J, Yoshimura S, Kiriakidis S, Monaco C, Gasparini C, Sacre S, Lundberg A, Paleolog E, Horwood NJ, Brennan FM, Foxwell BM. Is NF-kappaB a useful therapeutic target in rheumatoid arthritis?. *Ann Rheum Dis* 61 Suppl 2: ii13-18, 2002.
87. Smolen JS, Steiner G. Therapeutic strategies for rheumatoid arthritis. *Nature Rev Drug Dis* 2: 473-488, 2003.
88. Epinat JC, Gilmore TD. Diverse agents act at multiple levels to inhibit the Rel/NF-kappaB signal transduction pathway. *Oncogene* 18: 6896-6909, 1999.
89. Elliott PJ, Zollner TM, Boehncke WH. Proteasome inhibition: a new anti-inflammatory strategy. *J Mol Med* 81: 235-245, 2003.
90. Kawakami A, Nakashima T, Sakai H, Hida A, Urayama S, Yamasaki S, Nakamura H, Ida H, Ichinose Y, Aoyagi T, Furuichi I, Nakashima M, Migita K,

- Kawabe Y, Eguchi K. Regulation of synovial cell apoptosis by proteasome inhibitor. *Arthritis Rheum* 42: 2440-2448, 1999.
91. Tomita T, Takeuchi E, Tomita N, Morishita R, Kaneko M, Yamamoto K, Nakase T, Seki H, Kato K, Kaneda Y, Ochi T. Suppressed severity of collagen-induced arthritis by in vivo transfection of nuclear factor kappaB decoy oligodeoxynucleotides as a gene therapy. *Arthritis Rheum* 42: 2532-2542, 1999.
 92. Hashimoto H, Tomita T, Kunugiza Y, Nampei A, Morishita R, Kaneda Y, Yoshikawa H. NF- κ B Decoy Oligodeoxynucleotides Suppressed the Progression of Osteoarthritis in rat arthritis. *Arthritis Rheum* 48: S630, 2003.
 93. Tilstra J, Rehman KK, Hennon T, Plevy SE, Clemens P, Robbins PD. Protein transduction: identification, characterization and optimization. *Biochem Soc Trans* 35: 811-815, 2007.
 94. Fujihara SM, Cleaveland JS, Grosmaire LS, Berry KK, Kennedy KA, Blake JJ, Loy J, Rankin BM, Ledbetter JA, Nadler SG. A D-amino acid peptide inhibitor of NF-kappa B nuclear localization is efficacious in models of inflammatory disease. *J Immunol* 165: 1004-1012, 2000.
 95. Tas SW, Adriaansen J, Hajji N, Bakker AC, Firestein GS, Vervoordeldonk MJ, Tak PP. Amelioration of arthritis by intraarticular dominant negative Ikk beta gene therapy using adeno-associated virus type 5. *Hum Gene Ther* 17: 821-832, 2006.
 96. Blackwell NM, Sembi P, Newson JS, Lawrence T, Gilroy DW, Kabouridis PS. Reduced infiltration and increased apoptosis of leukocytes at sites of inflammation by systemic administration of a membrane-permeable IkappaBalpha repressor. *Arthritis Rheum* 50: 2675-2684, 2004.
 97. Tas SW, Vervoordeldonk MJ, Hajji N, May MJ, Ghosh S, Tak PP. Local treatment with the selective IkappaB kinase beta inhibitor NEMO-binding domain peptide ameliorates synovial inflammation. *Arthritis Res Ther* 8: R86, 2006.
 98. Amos N, Lauder S, Evans A, Feldmann M, Bondeson J. Adenoviral gene transfer into osteoarthritis synovial cells using the endogenous inhibitor IkappaBalpha reveals that most, but not all, inflammatory and destructive mediators are NFkappaB dependent. *Rheumatology* 45: 1201-1209, 2006.
 99. Bondeson J, Lauder S, Wainwright S, Amos N, Evans A, Hughes C, Feldmann M, Caterson B. Adenoviral gene transfer of the endogenous inhibitor IkappaBalpha into human osteoarthritis synovial fibroblasts demonstrates that several matrix metalloproteinases and aggrecanases are nuclear factor-kappaB-dependent. *J Rheumatol* 34: 523-533, 2007.
 100. Kishore NA, Sommers C, Mathialagan S, et al. A selective IKK-2 inhibitor blocks NF-kappa B-dependent gene expression in interleukin-1 beta-stimulated synovial fibroblasts. *J Biol Chem* 278: 32861-32871, 2003.
 101. McIntyre KW, Shuster DJ, Gillooly KM, Dambach DM, Pattoli MA, Lu P, et al. A highly selective inhibitor of I kappa B kinase, BMS-345541, blocks both joint inflammation and destruction in collagen-induced arthritis in mice. *Arthritis Rheum* 48: 2652-2659, 2003.
 102. Schopf L, Savinainen A, Anderson K, Kujawa J, DuPont M, Silva M, Siebert E, Chandra S, Morgan J, Gangurde P, Wen D, Lane J, Xu Y, Hepperle M, Harriman G, Ocain T, Jaffee B. IKKbeta inhibition protects against bone and cartilage

- destruction in a rat model of rheumatoid arthritis. *Arthritis Rheum* 54: 3163-3173, 2006.
103. Izmailova ES, Paz N, Alencar H, Chun M, Schopf L, Hepperle M, Lane JH, Harriman G, Xu Y, Ocain T, Weissleder R, Mahmood U, Healy AM, Jaffee B. Use of molecular imaging to quantify response to IKK-2 inhibitor treatment in murine arthritis. *Arthritis Rheum* 56: 117-128, 2007.
 104. Wakamatsu K, Nanki T, Miyasaka N, Umezawa K, Kubota T. Effect of a small molecule inhibitor of nuclear factor-kappaB nuclear translocation in a murine model of arthritis and cultured human synovial cells. *Arthritis Res Ther* 7: R1348-1359, 2005.
 105. Kubota T, Hoshino M, Aoki K, Ohya K, Komano Y, Nanki T, Miyasaka N, Umezawa K. NF-kappaB inhibitor dehydroxymethylepoxyquinomicin suppresses osteoclastogenesis and expression of NFATc1 in mouse arthritis without affecting expression of RANKL, osteoprotegerin or macrophage colony-stimulating factor. *Arthritis Res Ther* 9: R97, 2007.
 106. Martin E, Capini C, Duggan E, Lutzky VP, Stumbles P, Pettit AR, O'Sullivan B, Thomas R. Antigen-specific suppression of established arthritis in mice by dendritic cells deficient in NF-kappaB. *Arthritis Rheum* 56: 2255-2266, 2007.
 107. Jepsen JS, Wengel J. LNA-antisense rivals siRNA for gene silencing. *Curr Opin Drug Discov Devel* 2: 188-194, 2004.
 108. Heasman J. Morpholino oligos: making sense of antisense? *Dev Biol* 243: 209-214, 2002.
 109. McManus MT, Sharp PA. Gene silencing in mammals by small interfering RNAs. *Nat Rev Genet* 3: 737-747, 2002.
 110. Huppi K, Martin SE, Caplen NJ. Defining and assaying RNAi in mammalian cells. *Molecular Cell* 17: 1-10, 2005.
 111. Lianxu C, Hongti J, Changlong Y. NF-kappaBp65-specific siRNA inhibits expression of genes of COX-2, NOS-2 and MMP-9 in rat IL-1beta-induced and TNF-alpha-induced chondrocytes. *Osteoarthritis Cartilage* 14: 367-376, 2006.
 112. Chen LX, Lin L, Wang HJ, Wei XL, Fu X, Zhang JY, Yu CL. Suppression of early experimental osteoarthritis by in vivo delivery of the adenoviral vector-mediated NF-kappaBp65-specific siRNA. *Osteoarthritis Cartilage* 16: 174-84, 2008.

Table 1

Partial list of NF-κB-induced genes
<ul style="list-style-type: none">• GENES THAT ENCODE MOLECULES INVOLVED IN IMMUNITY:<ul style="list-style-type: none">Cytokines: TNF-α, IL-1β, IL-2, IL-6, IL-8, IL-12, INF-γ, GM-CSFAdhesion molecules: e-selectin, ICAM-1, VCAM-1Chemokines: CXCL1, ENA-78, eotaxin, IL-8, MIP-1α, MCP-1, RANTESReceptors: CD-3g, CD-40, CD-48, CD68, MHC-I, TLR-2Inducible enzymes: COX-2, iNOS • GENES THAT ENCODE MOLECULES INVOLVED IN CELL PROLIFERATION, APOPTOSIS AND CELL CYCLE:<ul style="list-style-type: none">Anti-apoptosis: AF-1/BF-1, c-IAP-1, c-IAP-2, c-FLIP, Bcl-2, TRAF-1, TRAF-2Apoptosis: Bax, caspase 11, Fas, FasLProliferation: c-myc, cyclin D1, ephrin A1, E2F3a • GENES THAT ENCODE MOLECULES INVOLVED IN TISSUE DAMAGE:<ul style="list-style-type: none">Extracellular matrix degradation: cathepsyn B, cathepsyn K, MMP-1, MMP-3, MMP-13 • GENES THAT ENCODE MOLECULES INVOLVED IN NEGATIVE FEEDBACK OF NF-κB:<ul style="list-style-type: none">IκBα, IκBβ, A20

Table 2

Rheumatic Disorders associated with NF-κB activation
<ul style="list-style-type: none">• Rheumatoid arthritis• Juvenile rheumatoid arthritis• Osteoarthritis• Systemic lupus erythematosus• Spondyloarthropaties• Crystal induced arthropaties• Septic arthritis

FIGURE LEGENDS

Fig 1. Beneficial and harmful role of NF- κ B in inflammatory arthritis.

Fig 2. NF- κ B signaling pathways. Many current therapeutic agents and future strategies block the canonical NF- κ B pathway at different steps: 1) **I- κ B phosphorylation:** NSAIDs (aspirin, salicylate, ibuprofen, sulindac), 5-ASA, IKK β inhibitors, NBD peptide. 2) **Protease activity of the 26 S proteasome complex:** Bortezomib*, Cyclosporin A, sc-514, lactacystin. 3) **Reduction of levels of NF- κ B subunits p65, p50, c-Rel and others:** siRNA. 4) **Nuclear translocation of NF- κ B subunits p65, p50, c-Rel and others:** FK-506, BMS-205820, I- κ B super repressor, Tat-srI κ B α , DHMEQ*. 5) **NF- κ B DNA binding:** Glucocorticoids, NF- κ B ODN, NF- κ B morpholinos. *Some therapeutics agents also block the non-canonical pathway NF- κ B pathway.

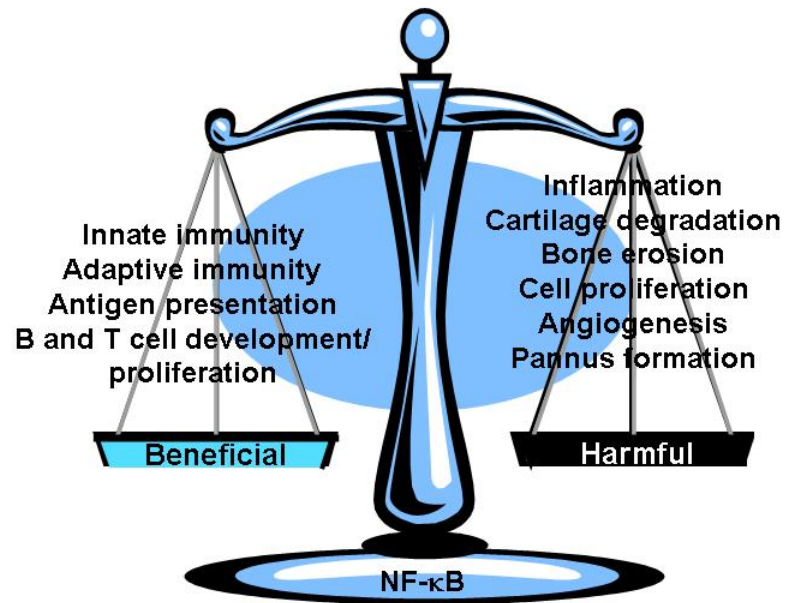


Fig 1. Beneficial and harmful role of NF-κB in inflammatory arthritis

Fig 2

