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Review
NF-κB as a potential therapeutic target in osteoarthritis and rheumatoid arthritis

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Summary

The family of nuclear factor-kappaB (NF-κB) transcription factors is intimately involved in the regulation of expression of numerous genes in the setting of the inflammatory response. Since inflammatory processes play a fundamental role in the damage of articular tissues, many *in vitro* and *in vivo* studies have examined the contribution of components of the NF-κB signaling pathways to the pathogenesis of various rheumatic diseases, in particular, of osteoarthritis (OA) and rheumatoid arthritis (RA). Inflammation, cartilage degradation, cell proliferation, angiogenesis and pannus formation are processes in which the role of NF-κB is prominent. Consequently, large efforts have been devoted to the study of the pharmacologic modulation of the NF-κB pathways. These studies have employed currently available therapeutic agents including non-steroidal anti-inflammatory drugs, corticosteroids, nutraceuticals and disease-modifying anti-rheumatic drugs, as well as novel small molecule inhibitors targeted to specific proteins of the NF-κB pathways. In addition, promising strategies such as improved antisense DNA therapy and RNA interference have been examined with encouraging results. However, since NF-κB also plays a crucial beneficial role in normal physiology and technical problems for effective gene therapy still remain, further research will be needed before NF-κB-aimed strategies become an effective therapy for joint diseases, such as OA and RA.

Key words: Cartilage, Osteoarthritis, Rheumatoid arthritis, NF-κB, Transcription factors.

Introduction

A multitude of complex autocrine and paracrine anabolic and catabolic signals act upon diverse cells from articular tissues and the precise interplay of these signaling pathways is essential for the activation of their gene expression machinery. In the last few years, novel molecular approaches have provided invaluable insights into these important processes, many of which play a key role on the pathogenesis of inflammation and tissue destruction, crucial components of numerous articular diseases[1–5].

The signaling pathways transduce extracellular signals from the cell surface to the nucleus. A signaling molecule or ligand outside the cell interacts with a specific receptor on the extracellular surface of the cell membrane. The ligand binding is followed by the interaction of the intracellular domains of the receptor with intracellular pathway components. This interaction initiates a cascade of protein–protein interactions that expands the signal inside the cell and transfers it to the nucleus, where binding or modification of the activity of transcription factors (TFs) plays a crucial role in the process of activation or repression of gene expression[6,7].

Once a signal has been transduced from the cell surface to the nucleus, TFs respond, interacting with specific DNA-binding elements present in the promoters and often in the first introns of genes to induce their expression or repression at the level of mRNA synthesis. A number of TF families, including those for activator protein 1 (AP-1), nuclear factor-kappaB (NF-κB), signal transducer and activator of transcription (STATs), Smads, p53 tumor suppressor, Sps, and others have been implicated as critical regulators of gene expression in the setting of the inflammatory process[8–10]. This review focuses on the NF-κB signaling pathways, emphasizing their role in inflammation and damage to articular tissues and their modulation with therapeutic agents currently in use, and potential future strategies.

NF-κB

The NF-κB proteins are a family of ubiquitously expressed TFs that play an essential role in most immune and inflammatory responses. These TFs also have an important role in the protection of cells from apoptosis and in the process of intercellular signaling during normal vertebrate development. However, the extracellular signals that activate NF-κB in development have not been fully elucidated.

In mammals, the NF-κB family consists of five members: RelA (p65), RelB, c-Rel, NF-κB1 (p50 and its precursor p105), and NF-κB2 (p52 and its precursor p100). They form a variety of homodimers and heterodimers, each of which activates its own characteristic set of genes, and share a 300-amino acid domain that is designated the Rel homology domain which mediates their DNA binding, dimerization and nuclear translocation[11–15]. Although, the most prevalent activated form is the heterodimer RelA (p65) and p50, different dimers can bind to the same or distinct sites in NF-κB-dependent promoters regulating the transcription of response genes in a cell-type and stimulus-type manner[16,17].

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NF-κB function and regulation

NF-κB is present in the cytoplasm of almost all mammalians cells in an inactive form associated with the inhibitory κB proteins (IκB), which include IκBα, IκBβ, IκBε and IκBγ[11–13]. The IκB proteins typically contain C-terminal ankyrin repeats that are crucial for their interaction with the NF-κB proteins, and an N-terminal leucin-rich nuclear export sequence, that is important for the shuttling of IκB between the cytoplasm and nucleus. The shuttling of IκBα is an important mechanism to retain the IκBα–p50–p65 complex in the cytoplasm. IκBα is also involved in the removal of NF-κB proteins from the nucleus. Thus, IκB has both cytoplasmic and nuclear roles in regulating the NF-κB pathway[11–13,18].

A broad range of stimuli including the cytokines, tumor necrosis factor-alpha (TNF-α) or interleukin (IL-1β) chemokines, bacterial and viral products, UV radiation and free radicals activate the NF-κB dimers by triggering a signaling pathway that leads to the phosphorylation of IκB, its ubiquitination by $E3^{I\kappa B}$ ubiquitin ligase complex, and its consequent degradation by 26S proteasome. The phosphorylation of IκB is performed by the specific serine/threonine kinase IκB kinase (IKK). The IKK complex consists of at least three subunits, including the kinases IKKα and IKKβ (also called IKK-1 and IKK-2, respectively) and the associated regulatory subunit IKK-γ/NF-κB essential modulator (NEMO). Despite the structural similarity between IKKα and IKKβ, IKKβ is the dominant kinase involved in the activation of NF-κB proteins, whereas IKKα plays a partially redundant role in NF-κB activation. IKK-γ/NEMO has no known kinase activity, however, is crucial for the IKK complex activation[14,18,19].

The mechanism through which cytokines activate the IKK complex is not fully known. At least two hypotheses have been postulated: one proposes that mitogen activated protein kinase kinase kinase (MAPKKK) activation 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. Although phosphorylation of IKK is a key event in the NF-κB pathway, the ubiquitination and subsequent degradation of the multiple factors involved on its regulation are also crucial mechanisms required for NF-κB activation[18,20–23].

While IKKβ activates the NF-κB canonical pathway through phosphorylation of IκB, IKKα activates the NF-κB non-canonical pathway by phosphorylating p100, leading to further p52 activation. NF-κB-inducing kinase (NIK-1) also plays an essential role in the non-canonical pathway, inducing the p100 processing. Furthermore, a new IKKα function has been recently described; the regulation of histone function which in turn causes the activation of the NF-κB canonical pathway. The histone proteins assembled with the DNA form the nucleosomes, fundamental packing units of chromatin. The chromatin limits the DNA-binding protein access to the immediate early-response gene promoters, such as NF-κB response genes. Histone modification occurs as a result of diverse stimuli, causing changes in the structure of chromatin and allowing further interaction between certain sequences of gene promoters and TFs, such as NF-κB, coactivators and other components of the transcription machinery[23–26].

The degradation of IκB exposes a nuclear localization signal on the NF-κB proteins, which then are able to translocate into the nucleus and stimulate the transcription of specific genes. It has been described that NF-κB regulates more of than 150 genes, including those involved in immunity and inflammation, anti-apoptosis, cell proliferation and the negative feedback of the NF-κB signal[14]. NF-κB positively regulates genes encoding cytokines (e.g., TNF-α, IL-1β, IL-6, IL-2, IL-12, interferon (IFN)-γ, granulocyte macrophage colony stimulating factor (GM-CSF)), cell adhesion molecules (e.g., E-selectin, vascular cell adhesion molecule (VCAM)-1, intercellular adhesion molecule (ICAM)-1), chemokines (e.g., IL-8, macrophage inflammatory protein (MIP)-1α, methyl-accepting chemotaxis protein (MCP)-1, RANTES (regulated upon activation, normal T-cell expressed and secreted), eotaxin), receptors (e.g., major histocompatibility complex (MHC)) and inducible enzymes (e.g., cyclooxygenase (COX)-2, inducible nitric oxide synthase (iNOS)). NF-κB also increases the expression of molecules important in regulation of cellular proliferation, apoptosis and cell-cycle progression, such as cellular inhibitor of apoptosis protein 1 (c-IAP1), c-IAP2, TNF-receptor-associated factor 1 (TRAF1), TRAF2, B-cell lymphocyte/leukemia-2 and its homologs (AF-1/BF-1 activation function 1/brain factor 1, IEX-1L immediate early gene X1L), Fas, c-myc and cyclin D1[18]. A partial list of genes relevant to the inflammatory response whose expression is stimulated by NF-κB activation is shown in Table I.

Gene knockout studies show different functions for members of the NF-κB family. Mice lacking p65 (Rel A) die at 15 or 16 days of gestation, due to hepatic apoptosis. Embryonic fibroblasts from these animals fail to increase IKK and macrophage colony stimulating factor (M-CSF) mRNA levels following TNF-α stimulation, although their basal levels are similar to those in controls[27]. Gene knockout of p50 results in a phenotype close to normal, without any alteration in the hepatic inflammatory response to ischemia/reperfusion; but displays a variety of specific immune defects of lymphocyte B function and of the unspecific response to infections. The animals also show deficits in specific cognitive tasks, such as a remarkably low level of anxiety-like behavior[28,29].

Role of the NF-κB in arthritis

Although NF-κB plays an essential beneficial role in normal physiology, inappropriate regulation of NF-κB activity has been implicated in the pathogenesis of several diseases including inflammatory and rheumatic diseases (rheumatoid arthritis (RA), osteoarthritis (OA), atherosclerosis, asthma, multiple sclerosis, chronic inflammatory demyelinating polyradiculoneuritis, inflammatory bowel disease,

Table I
Partial list of NF-κB-induced genes

Genes that encode molecules involved in inflammation and immunity:	
Cytokines	TNF-α, IL-1β, IL-2, IL-6, IL-8, IL-12, IFN-γ, GM-CSF
Adhesion molecules	e-selectin, ICAM-1, VCAM-1
Chemokines	eotaxin, IL-8, MIP-1α, MCP-1, RANTES
Receptors	CD-3G, CD-40, CD-48, MHC-I, TLR-2
Inducible enzymes	COX-2, iNOS
Genes that encode molecules involved in cell proliferation, apoptosis and cell cycle:	
Anti-apoptosis	AF-1/BF-1, c-IAP-1, c-IAP-2, c-FLIP, Bcl-2, TRAF-1, TRAF-2
Apoptosis	Bax, caspase 11, Fas, FasL
Proliferation	c-myc, cyclin D1, ephrin A1, E2F3a
Genes that encode molecules involved in negative feedback of NF-kB:	
	IkBα, IkBβ, A20

Helicobacter pylori-associated gastritis, systemic inflammatory response syndrome), diabetes type 2, cancer, acquired immuno-deficiency syndrome, central nervous system-related disease conditions, euthyroid sick syndrome and cachexia (Tables II and III). The beneficial and harmful roles of NF-κB are diagrammatically shown in Fig. 1[30–33].

The p50 and p65 NF-κB are abundant in rheumatoid and osteoarthritic synovitis. However, NF-κB activation is higher in RA than in OA[34]. Synovial tissues from patients with RA and spondyloarthropathies (SpA) show that the numbers of cells expressing NF-κB1 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 not in SpA synovium. Furthermore, the numbers of NF-κB1+ and RelA+ cells in OA synovium were similar to those observed at the non-cartilage–pannus junction sites in all inflammatory tissues studied[35]. In patients with RA and OA, immunoreactive IKK is abundant in primary fibroblast-like synoviocytes and IKKα and IKKβ are constitutively expressed at the mRNA level. Following TNF-α and IL-1β stimulation of these cells, IKKβ activation is a key event for NF-κB mediated induction of IL-6, IL-8, ICAM-1 and collagenase gene expression[36].

Animal models of inflammatory arthritis also support the concept that NF-κB plays a very active role in the development and progression of arthritis *in vivo*[37,38]. NF-κB activation prior to the onset of clinical manifestations of arthritis has been found in both, murine type II collagen-induced arthritis and rat adjuvant-induced arthritis. In the first model, NF-κB expression correlated with collagenase-3 (metalloproteinase (MMP)-13) and stromelysin 1 (MMP-3) levels better than AP-1 expression level, however, both TFs were activated before onset of clinical arthritis and MMP gene expression[39]. Also, a shift to nuclear NF-κB localization was shown in chondrocytes during cartilage destruction in the early stage of arthritis in DBA/1 mice immunized with type II collagen[40]. In the second model, expression of activated NF-κB 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[41]. In addition, intra-articular gene transfer of IKKβ caused arthritis in normal rats, characterized by severe paw swelling, inflammatory histologic changes, increased IKK activity and enhanced NF-κB DNA-binding activity. Thus, these experiments confirm that IKK activation is a crucial event in the initiation of synovitis[31,42].

Table II
Partial list of diseases associated with NF-κB activation

(1) Diseases with associated inflammation:
RA, OA, and other rheumatic diseases
Atherosclerosis
Asthma
Multiple sclerosis
Chronic inflammatory demyelinating polyradiculoneuritis
Inflammatory bowel disease
Systemic inflammatory response syndrome
(2) Infectious diseases:
AIDS
Helicobacter pylori-associated gastritis
(3) Endocrine diseases:
Type 2 diabetes mellitus
Euthyroid sick syndrome
(4) Cancer:
Solid: breast, colon, kidney, liver, lung, prostate, ovary
Hematologic: acute lymphocytic leukemia, acute myeloid leukemia, Hodgkin's lymphoma, multiple myeloma
(5) Other:
Chronic heart failure
Alzheimer disease
Cachexia
Neuropathic pain

Table III
Rheumatic disorders associated with NF-kB activation

• RA
• OA
• SpA
• Psoriatic arthritis
• Crystal induced arthropathies
• Septic arthritis
• Juvenile RA
• Systemic lupus erythematosus

Role of NF-κB in articular cartilage destruction

NF-κB signaling pathways mediate critical events in the inflammatory response by chondrocytes, leading to progressive extracellular matrix damage and cartilage destruction. Indeed, our own observations (shown in Fig. 2) have evidenced the potent activation of NF-κB in articular chondrocytes following stimulation with two proinflammatory cytokines, IL-1β and TNF-α, which play a prominent role in the catabolism of the articular cartilage. Furthermore, our studies also showed that costimulation with both IL-1β and TNF-α causes a synergistic potentiation of NF-κB activation in articular chondrocytes. Numerous other studies have examined the effects of NF-κB on chondrocyte functions. In rat prechondrocytes as well as in articular chondrocytes, the NF-κB and the MAP kinase ERK 1/2 kinase pathways were found to mediate inhibition of type II collagen and link protein gene expression by TNF-α[43,44]. Studies performed in human OA chondrocytes and chondrosarcoma cells have shown that NF-κB, as well as MAP kinases, mediates MMP-1, MMP-3 and MMP-13 RNA/protein expression induced by TNF-α or IL-1β. These results were achieved employing various specific inhibitors of the

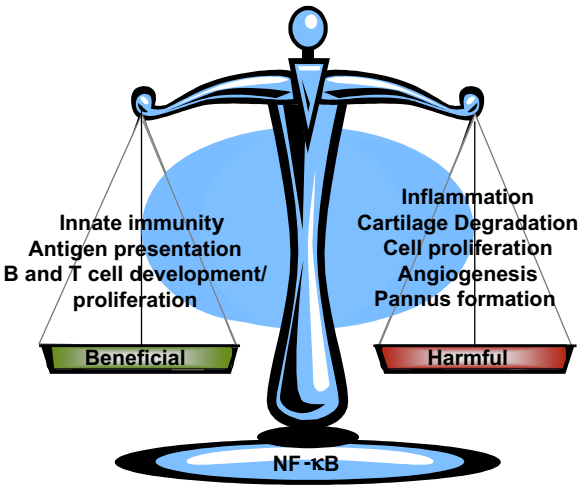

Fig. 1. Beneficial and harmful effects of NF-κB.

NF-κB and MAPK pathways suggesting that inhibition of TNF-α and IL-1β signal transduction employing these agents could be a potential therapeutic strategy aimed to reduce articular cartilage degradation by MMPs in arthritis[45,46]. Persistent activation of NF-κB in cytokine-stimulated bovine chondrocytes requires nitric oxide (NO) presence to sustain p65 nuclear translocation, while NF-κB immediate activation does not[47].

NF-κB has also been shown to mediate fibronectin fragment induced-chondrocyte activation and increased expression of proinflammatory cytokines, chemokines as well as MMPs such as IL-6, IL-8, MCP-1, growth-related oncogene α (GRO-α), GRO-β, GRO-γ and MMP-13 by human articular chondrocytes[48,49]. Furthermore, NF-κB participates in the receptor for advanced glycation end (RAGE) signaling-induced increase in MMP-13 expression in monkey and human articular chondrocytes[50]. In addition, NF-κB production was increased with donor age in IL-1β-stimulated human articular chondrocytes[49]. Finally, a study performed in bovine chondrocytes under hypoxic and normoxic conditions showed that DNA binding of NF-κB and AP-1 was significantly higher in hypoxic and reoxygenated chondrocytes treated with IL-1β than in normoxic chondrocytes[51].

It is also known that NF-κB 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-κB, 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[52,53]. In fact, experiments performed in chondrocytes isolated from rabbit articular cartilage grown on flexible membranes have shown that cyclic tensile strain (CTS) of low magnitude (4–8% equibiaxial strain) inhibited IL-1β-dependent NF-κB nuclear translocation, whereas CTS of high magnitude (15–18% equibiaxial strain) induced rapid nuclear translocation of NF-κB subunits p65 and p50, and reproduced the actions of IL-1β[54].

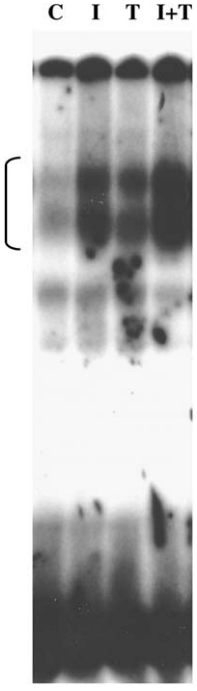

Fig. 2. NF-κB activation in articular chondrocytes. Nuclear extracts from bovine articular chondrocytes were subjected to an electromobility shift assay with a probe containing a consensus site for NF-κB. A dramatic increase in the NF-κB DNA-binding activity in the cells treated with either IL-1β (lane I) or TNF-α (lane T) compared with control cells (lane C) is observed. Furthermore, treatment with both proinflammatory cytokines, IL-1β and TNF-α together (lane I + T) resulted in greater increase in the NF-κB DNA-binding activity compared with either IL-1β (lane I) or TNF-α alone (lane T).

Besides its anti-inflammatory effects, it has been suggested that NF-κB may also play a role in chondrocyte apoptosis. Under certain conditions NF-κB exerts prosurvival effects in articular cartilage. Thus, in human chondrocytes NF-κB activation partially mediates the anti-apoptotic effects of IL-1β against death receptor CD-95 (FAS/APO-1)[55]. Further experiments demonstrated that NF-κB-dependent mechanisms oppose CD95-induced apoptosis. These NF-κB-dependent mechanisms include interference with caspase 3 activation, likely through regulation of cytosolic concentrations of XIAP-1[56]. The inhibition of proteasome and RNA synthesis of NF-κB sensitizes human articular chondrocytes to NO-induced apoptosis, as well[57,58]. In contrast, other studies have described NF-κB involvement in apoptotic events in articular chondrocytes. For example, it has been shown that NF-κB activation mediates the apoptotic effect of NO in articular chondrocytes. The signaling pathway involved in this process is quite complex and has been partially elucidated. This pathway involves the activation of p38 kinase by NO. Activated p38 in turn causes inhibition of the kinase activities of protein kinase Cζ (PKCζ). The inhibition of PKCζ results in activation of NF-κB which subsequently activates caspase 3-induced apoptosis, through activation of p53[59,60].

Inhibition of NF-κB by pharmacologic agents

An increasing number of NF-κB inhibitors, including several clinically important anti-inflammatory drugs, have been reported (illustrated in Fig. 2). Glucocorticoids are potent inhibitors of the NF-κB pathway through several proposed mechanisms[61–64]. Glucocorticoids induce expression of IκB, causing an increased cytosolic retention of NF-κB[65,66]. Glucocorticoids may also inhibit the NF-κB DNA-binding activity through direct interaction between the glucocorticoid receptor and components of the NF-κB binding sites in various gene promoters[67]. The activated glucocorticoid receptor can also interact with NF-κB by direct protein–protein binding, preventing the activation of the NF-κB pathway in certain types of cells[68]. Lastly, competition can occur between the glucocorticoid receptor and NF-κB, limiting amounts of the coactivators CREB-binding protein (CBP), CBP-associated factor (p/CAF) and steroid receptor coactivator-1[69]. Non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin, salycilate, ibuprofen and sulindac inhibit IKK activity significantly, preventing IκB phosphorylation, consequently blocking the activation of the NF-κB pathway[70]. It has also been shown that sulfasalazine suppresses IκB phosphorylation, probably owing to the effects of its anti-inflammatory metabolite, 5-aminosalicylic acid[71].

The immunosuppressive agents cyclosporin A and tacrolimus (FK-506) also inhibit the NF-κB pathway. Cyclosporin A inhibits the protease activity of the 20S proteasome complex

preventing IκBα degradation in murine macrophages, Jurkat lymphoma cells, and mouse and human T lymphocytes[72,73]. FK506 blocks translocation of c-Rel from the cytoplasm to the nucleus in both B and T cells, and Jurkat cells, leading a decreased expression of IL-2 and its receptor[74,75]. Several other agents have also been described to inhibit NF-κB including vitamin C[76], vitamin E[77], curcumin[78], flavonoids[78], lactacystin[79], thalidomide[80], leflunomide[81], pyrrolidine dithiocarbamate[82], glucosamine[83] and diacehrein[84].

Novel therapeutic strategies (Fig. 3) aimed at the specific inhibition of key elements in the NF-κB pathway activation have been developing in the last few years, causing great expectation regarding their effects as arthritis treatments[85–89]. Proteasome function inhibitors, decoy oligonucleotides, and peptides that inhibit nuclear localization of NF-κB have also been utilized to inhibit NF-κB signaling in animal models[90,91]. Daily oral treatment with PS-341 (bortezomib), a proteasome inhibitor newly approved by the FDA for multiple myeloma, decreases significantly NF-κB 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[92]. Decoy oligodeoxynucleotides (ODN), short double stranded DNA containing the consensus binding sequence of NF-κB which competes for binding with native NF-κB and its consensus sequences in the promoter of target genes, were introduced by intra-articular injection into the hind joints of rats with type II collagen-induced arthritis. In these experiments, NF-κB decoy ODN decreased the severity of hind-paw swelling, suppressed IL-1β and TNF-α in the arthritic synovium, and abrogated joint destruction as evidenced by histologic and radiographic studies[93]. In a similar approach, the same investigators injected NF-κB decoy ODN into the knee joints of anterior cruciate ligament transection OA model rats. Fluorescein isothiocyanate (FITC)-labeled NF-κB decoy ODN was located mostly in the nuclei of superficial synovial lining cells at 2 days after the injection. Histopathological findings from knee joints injected with the naked NF-κB decoy ODN showed a statistically significant amelioration as assessed by the Mankin 95 criteria, compared with either a scrambled decoy ODN or phosphate buffered saline administration. Also, naked NF-κB decoy ODN significantly inhibited the levels of IL-1β or TNF-α in the synovium and the cartilage, compared with the scrambled decoy ODN[94]. BMS-205820 is another novel, potent, and selective NF-κB inhibitor. It contains a cell-permeable peptide carrying two nuclear localization sequences capable of blocking NF-κB 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 a mouse model of inflammatory bowel disease[95].

IKKβ has become a particularly appealing target for therapeutic intervention in RA and OA, because of its crucial role in the NF-κB pathway activation. Administration of IKKβ resulted in a potent reduction of cytokine production in numerous cell types including synoviocytes and chondrocytes. Thus, in rats with adjuvant-induced arthritis, intra-articular gene therapy delivering a dominant-negative IKKβ adenovirus construct inhibits NF-κB translocation; consequently, cytokine-induced IL-6, IL-8 and ICAM-1 expressions are suppressed[36]. Genetic constructs that overexpress IκB or express an engineered protein without the sites for phosphorylation (IκB 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. Recently, a new technical approach has been developed by delivering a chimeric molecule, which contains the super-repressor IκBα (srIκB) fused to the membrane-transducing domain of the human immunodeficiency virus Tat protein (Tat-srIκBα) in a rat model of pleurisy. This chimeric molecule showed a good effect with 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-1β in the exudates[96]. Also, new studies with small molecule inhibitors have further strengthened the role of IKKβ. One of these small molecules, SC-514 inhibits IκB phosphorylation/degradation and p65 NF-κB phosphorylation/transactivation induced by IL-1β in RA synovial fibroblasts in a dose-dependent manner[97]. Another IKKβ inhibitor, BMS-345541, was administered to treat murine type II collagen-induced arthritis 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-1β 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[98].

Other new promising therapeutic strategies to target specific proteins of the NF-κB pathway include improved antisense therapy and RNA interference. Locked nucleic acid-antisense (LNA) is a class of nucleic acid analogs which contain a gapmer LNA with a central DNA or a phosphorothioate-DNA segment flanked by LNA gaps. These chemical modifications lead to improved binding affinity to complementary DNA or RNA and improved biostability, consequently, better pharmacological properties[99]. Morpholino oligonucleotides are DNA analogs that block mRNA translation in a sequence-specific manner. Their chemical structures consist of an ODN which has been modified, containing a six-membered morpholino ring instead of a deoxyribose sugar. In addition, the charged phosphodiester internucleotide linkage is replaced by an uncharged phosphorothioate. These changes have improved their stability and pharmacological properties[100,101]. 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[102–104]. Indeed, siRNA targeting of NF-κB 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-1β and TNF-α[105].

Conclusions

The NF-κB family of TFs plays a crucial role in the distinctive inflammatory processes characteristic of certain rheumatic diseases, such as OA and RA, leading to cartilage destruction and articular damage. NF-κB is abundant in rheumatoid and OA synovium, however, its activation is higher in RA than in OA. IKK, a key enzyme in the activation of the canonical NF-κB signaling pathway, is also abundantly expressed in RA and OA fibroblast-like synoviocytes.

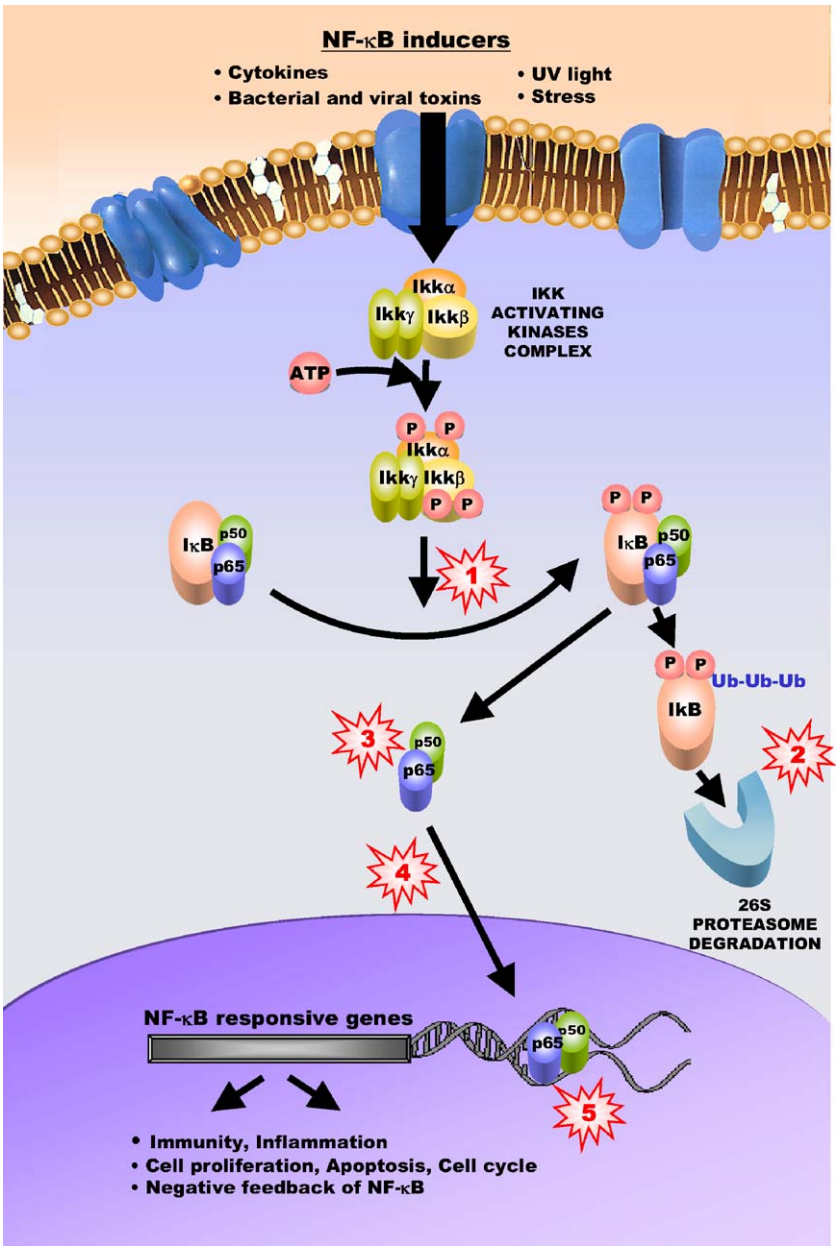

Fig. 3. NF-κB signaling pathway. Many current therapeutic agents and future strategies block the NF-κB pathway in different steps:

(1) **I-κB phosphorylation**: NSAIDs (aspirin, salycilate, ibuprofen, sulindac), 5-aminosalicylic acid, SC-514.
(2) **Protease activity of the 26S proteasome complex**: Bortezomib, Cyclosporin A, sc-514, lactacystin.
(3) **Disminution of levels of NF-κB subunits p65, p50, c-Rel and others**: siRNA.
(4) **Nuclear translocation of NF-κB subunits p65, p50, c-Rel and others**: FK-506, BMS-205820, I-κB super repressor, Tat-srIκBα.
(5) **NF-κB DNA binding**: Glucocorticoids, NF-κB ODN, NF-κB morpholinos.

Animal models of arthritis, including murine type II collagen-induced arthritis and rat adjuvant arthritis, support the essential role of NF-κB, and of IKK in particular, on MMP gene expression and the development of inflammatory and histological changes of arthritis.

In articular chondrocytes, NF-κB activation mediates the response to important proinflammatory cytokines, namely, IL-1β and TNF-α, as well as to fibronectin fragments and mechanical signals. NF-κB also participates in the RAGE signaling. Important NF-κB-mediated outcomes of the inflammatory response in human articular chondrocytes are the decrease in the expression of chondrocyte specific genes (collagen type II, link protein gene), and the increase in the expression of MMPs (MMP-1, MMP-3, MMP-13), cytokines (IL-6, IL-8) and chemokines. Interestingly, NF-κB production is increased with donor aging and under hypoxic conditions in IL-1β-stimulated articular chondrocytes. NF-κB is also involved in the regulation of apoptosis in articular chondrocytes, exerting primarily anti-apoptotic effects.

Therefore, NF-κB inhibition is a rational objective in the treatment of rheumatic diseases such as RA and OA. NSAIDs, glucocorticoids, nutraceuticals, natural products and certain disease-modifying anti-rheumatic drugs have been described to decrease NF-κB activation. Yet, novel therapeutic strategies targeting key elements in the NF-κB pathway including IKK, 26S proteasome, p65 and p50 subunits have been and continue being developed, and small molecule inhibitors, chimeric molecules, improved antisense therapy and RNA interference are part of the new approaches to block the NF-κB pathways.

Thus, NF-κB appears as a very attractive target for treatment of RA and OA; 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-κB and provide the foundations for the development of effective therapy for various joint diseases, including OA and RA.

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Glossary of abbreviations

NF-κB*:* nuclear factor-kappaB.
OA*:* osteoarthritis.
RA*:* rheumatoid arthritis.
NSAIDs*:* non-steroidal anti-inflammatory drugs.
DMARDs*:* disease-modifying anti-rheumatic drugs.
RNA*:* ribonucleic acid.
TF*:* transcription factors.
DNA*:* deoxyribonucleic acid.
AP-1*:* activator protein 1.
STAT-1*:* signal transducer and activator of transcription 1.
IκB*:* inhibitory κB.
IKK*:* IκB kinase.
NIK-1*:* NF-κB-inducing kinase 1.
MAPK*:* mitogen activated protein kinase.
TNF-α*:* tumor necrosis factor-alpha.
IL-1β*:* interleukin-1 beta.
IL-2*:* interleukin-2.
IL-6*:* interleukin 6.
IL-8*:* interleukin 8.
IL-12*:* interleukin-12.
IFN-γ*:* interferon-gamma.
GM-CSF*:* granulocyte macrophage colony stimulating factor.
VCAM-1*:* vascular cell adhesion molecule 1.
ICAM-1*:* intercellular adhesion molecule 1.
MIP-1α*:* macrophage inflammatory protein 1 alpha.
MCP-1*:* methyl-accepting chemotaxis protein 1.
RANTES*:* Regulated upon activation, normal T-cell expressed and secreted.
MHC-I*:* major histocompatibility complex 1.
TLR-2*:* toll-like receptor 2.
COX-2*:* cyclooxygenase 2.
iNOS*:* inducible nitric oxide synthase.
c-IAP*:* cellular inhibitor of apoptosis protein.
TRAF*:* TNF-receptor-associated factor.
c-FLIP*:* cellular flice inhibitory protein.
Bcl-2*:* B-cell lymphocyte/leukemia-2.
AF-1/BF-1*:* activation function 1/brain factor 1.
AIDS*:* acquired immuno-deficiency syndrome.
CNS*:* central nervous system.
SpA*:* spondyloarthropathies.
MMP*:* metalloproteinase.
MEK 1/2*:* MAP kinase ERK 1/2.
GRO*:* growth-related oncogene.
CTS*:* cyclic tensile strain.
NO*:* nitric oxide.
PKC*:* protein kinase C.
CBP*:* CREB-binding protein.
CAF*:* CBP-associated factor.
SRC-1*:* steroid receptor coactivator 1.
ODN*:* oligodeoxynucleotide.
ACLT*:* anterior cruciate ligament transaction.
PBS*:* phosphate buffered saline.
srIκB*:* super-repressor IκBα.
LNA*:* locked nucleic acid-antisense.