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Therapeutic approaches in bone pathogeneses: targeting the IKK/NF-κB axis

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Bone erosion is a major hallmark of rheumatoid arthritis and is executed solely by the bone-resorbing cell, the osteoclast. This cell arises from macrophage precursors and differentiates into the mature polykaryon after stimulation with the receptor activator of NF-κB ligand (RANKL) and macrophage colony-stimulating factor. Osteoclasts are recruited to sites of inflammation, or differentiate at these sites owing to elevated levels of circulating RANKL and other inflammatory cytokines secreted by cells in the inflamed tissue. Recent therapies to combat inflammatory bone erosion have focused on proximal and intracellular signaling molecules to attenuate osteoclast formation and activity. In this review, osteoclast differentiation, activation mechanisms, the role of the NF-κB pathway in inflammatory osteolysis and the relevant intervention approaches are presented briefly. The emphasis of this review will be on the RANKL–RANK–IκB kinase–NF-κB pathway and related antiosteolytic and anti-inflammatory modalities.

Keywords: bone erosion, cytokines, inflammatory arthritis, IKK, NBD, NEMO, NF-κB, osteoclast, osteoprotegerin, RANK, STAT6

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Inflammatory synovitis and subsequent destruction of joint cartilage and bone are hallmarks of rheumatoid arthritis (RA) [1]. Whereas the destruction of cartilage tissue results primarily from the action of tissue proteinases, focal bone erosion is almost exclusively the result of osteoclast action. Increased osteoclast activity as is obvious in numerous osteopenic disorders, including RA, leads to increased bone resorption and devastating bone damage. Several studies have established the fact that synovial tissue-residing cells secrete a broad range of inflammatory cytokines, and factors that directly or indirectly encompass a microenvironment supportive of osteoclast recruitment and activation [2,3]. These include interleukin (IL)-1, IL-6, transforming growth factor (TGF)-β, parathyroid hormone (PTH), inducible nitric oxidase synthase (iNOS), cyclooxygenase (COX)-2 and, most notably, members of the tumor necrosis factor (TNF) superfamily of cytokines. These latter cytokines include the receptor activator of NF-κB ligand (RANKL) and TNF-α, which activate the Rel/NF-κB family of transcription factors predominantly [4–7]. These transcription factors govern inflammatory and osteolytic processes [8–11] and are thus increasingly considered the centerpiece fueling inflammatory arthritic bone erosion and, as such, the focus for therapeutic intervention.

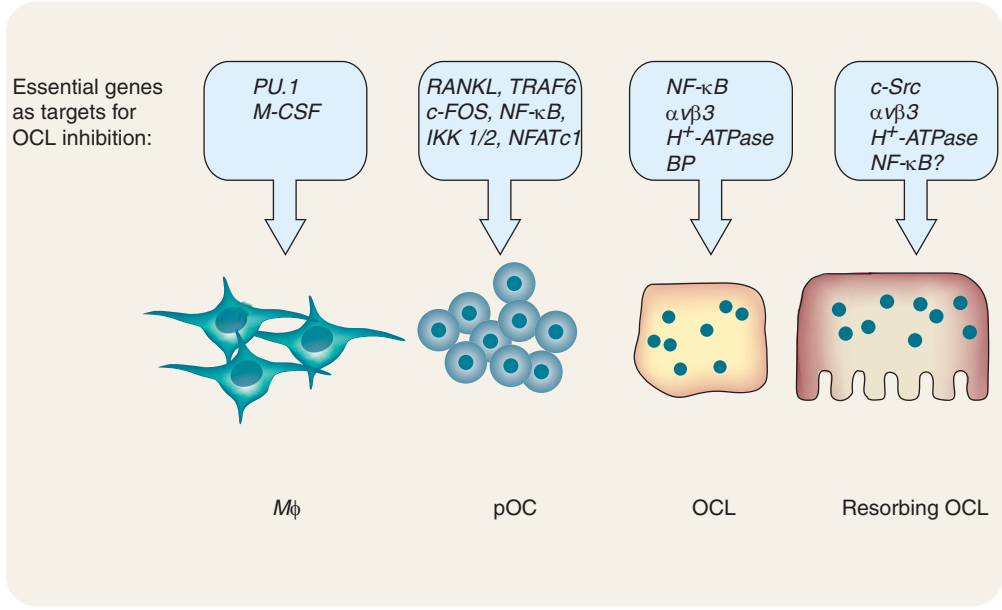
Osteoclast differentiation & activation

The bone loss component associated with RA has a devastating impact on human health. Thus, understanding the mechanisms involved in this process is particularly imperative. One key component in this response is the development and function of the sole bone-resorbing cell, the osteoclast [12].

Osteoclasts are required for skeletal development, bone resorption and remodeling throughout the lifespan of mammals. Osteoclast differentiation is controlled primarily by the stromal/osteoblast-derived proteins, RANKL and macrophage colony-stimulating factor (M-CSF) [12]. RANKL, a member of the TNF superfamily, binds to its transmembrane receptor, RANK and leads to the differentiation of bone marrow macrophages into multinucleated mature osteoclasts, a process that requires adhesion to the matrix by various cell-associated proteins, termed integrins [13–15]. Several genes, such as *PU.1*, *c-fms* (M-CSF receptor), *c-fos*, *RANK* and *NF-κB* (p50, p52 subunits), are critical for osteoclast differentiation. Other gene deletion studies implicated the proto-oncogene *c-Src*, the proton adenosine triphosphatase (*H^+-ATPase*) [12,13], nuclear factor for activated T cell (*NFAT*) c1, tartrate-resistant acid phosphatase (*TRAP*) and *cathepsin-k* genes [16–18] at later stages of osteoclast activation and function (Figure 1).

The principal function of osteoclasts is to resorb bone matrix. The primary event in this process is acidification of a defined and isolated extracellular resorptive microenvironment. This critical process is facilitated by adhesion to the matrix and formation of a tightly sealed zone beneath the osteoclast concurrent with polarization of the cell towards bone tissue. The polarization event is coupled with the translocation of a

Figure 1. Genetic regulation of osteoclast development.



The critical stages of osteoclast differentiation with corresponding stage essential genes are illustrated. *PU.1* and *M-CSF* are required for early-stage macrophage precursor formation; *RANKL*, *TRAF6*, *c-fos*, *NF-κB* and *IKK*s are essential for the differentiation of immediate osteoclast progenitors; other genes, such as the integrin *αvβ3*, *c-Src*, proton *ATPase*, *carbonic anhydrase* and more, play a crucial role in the differentiation of the mature multinucleated osteoclasts and their activation state. Certain genes, such as *NF-κB* participate at multiple differentiation stages.
IKK: IκB kinases; M-CSF: Macrophage colony-stimulating factor; NFATc1: Nuclear factor of activated T cells isoform c1; OCL: Osteoclast; pOC: Prefusion osteoclast-like cell; RANKL: Receptor activator of NF-κB ligand; TRAF: Tumor necrosis factor receptor-associated factor.

vacuolar proton pump, the vacuolar H^+-ATPase, to the ruffled border of the osteoclast. This event requires the assembly of microtubules and actin filaments, which provide structural tracks defining cellular polarization domains and the delivery of cargo vacuoles to and from these domains [19]. The ruffled membrane is a highly convoluted membrane structure juxtaposed to the bone and facilitates the movement of ions and molecules essential for the resorption process. Another important component required for proper acidification is the exportation of chloride ions. This is coordinated by energy-independent Cl^-/HCO_3^- exchangers that exist on basolateral osteoclast plasma membranes [20]. Coupling of chloride ions with secreted protons leads to the formation of hydrochloric acid in the resorptive microenvironment. The acidification step is critical for mineral mobilization and degradation of the organic phase of bone by acidic proteases and enzymes, such as procathepsin-κ and TRAP [12].

A major breakthrough in the regulation of osteoclastogenesis was achieved with the discovery of osteoprotegerin (OPG), a soluble protein of the TNF-receptor family [21–23]. OPG acts as a decoy receptor through binding to circulating RANKL and decreasing its bioavailability. Several studies have demonstrated that OPG is a potent inhibitor of bone loss, thus regulating bone density and mass in mice and humans [15,23–25]. As expected, overexpression or targeted deletion of the *OPG* gene in animals led to osteopetrosis or bone loss, respectively [15]. This secreted cytokine was also proven effective in blockade of metabolic, pathologic and tumor-induced bone loss. Subsequently, these functions led to the identification of the OPG target protein (i.e., RANKL) [22,26,27]. Gene targeting studies have shown that RANKL and its receptor RANK are required for osteoclastogenesis; as such, regulation of these factors determines overall osteoclastogenesis. In fact, mice deficient in these genes are osteopetrotic and lack osteoclasts [26,28]. Inhibition of RANKL by OPG was mimicked by the expression of soluble extracellular RANK protein which, essentially, in a similar way to OPG, neutralizes RANKL by sequestering it in an inactive complex. This approach was further

proven effective *in vitro* and *in vivo* by administration of Fc–RANK fusion protein to block bone loss pathologies [29,30].

Signaling by RANKL entails binding of the soluble ligand to its cognate receptor RANK to prompt transcription of osteoclastogenic genes (Figure 2). The primary signals are initiated by assembly of the signaling cascade at the cytoplasmic tail of RANK. To this end, assembly begins with recruitment of signaling and adaptor molecules, such as TNF receptor-associated factor (TRAF)-6 [7,31,32]. Subsequently, several downstream tyrosine and serine/threonine kinases, including NF-κB inducing kinase (NIK), IκB kinases (IKKs), c-Src, Akt/protein kinase B (PKB) and mitogen-activated protein extracellular signal-regulated kinase (MEKK)-1 are recruited to the complex and undergo activation. The most notably activated pathway by RANK is NF-κB [11,33]. Another major pathway transmitted by the activated RANK–TRAF6 axis is the mitogen-activated protein kinase (MAPK) pathway [34,35]. The functional relevance of these proteins to RANK-induced osteoclastogenesis has been established [7,36–38]. In this respect, interfering with NF-κB activation [9,39,40] or deleting certain NF-κB subunits (combined deletion of p50 and p52) arrests osteoclastogenesis [41,42]. Likewise, dominant negative forms of various MAPKs and selective inhibitors of the MAPK pathways inhibited osteoclastogenesis or reduced osteoclast survival [43–45].

In general, osteoclast deficiency leads to osteopetrosis, whereas excessive osteoclast activity under pathologic conditions leads to devastating bone loss diseases, such as osteoporosis, periarticular osteolysis, inflammatory arthritis, periodontitis and other forms of osteopenia. This hyperactivity of osteoclasts was established as the result of direct upregulation of RANKL-induced osteoclastogenesis by a network of pro-inflammatory cytokines and factors, notably TNF, which synergize with RANKL pre-existing signals in preosteoclasts [7,46,47]. Therefore, understanding key signal transduction pathways in osteoclastogenesis provides an important foundation towards the design of intracellular inhibitors in states of exaggerated osteoclast activity.

Overview of the IKK/NF-κB signaling pathway

The transcription factor NF-κB family comprises several members, including p50, p52, RelA/p65, RelB, c-Rel, the precursors NF-κB1/p105, NF-κB2/p100 (which undergo processing into p50 and p52, respectively) and the inhibitory subunits IκBα, IκBβ, and IκBε [11,33,48]. Under non-stimulated conditions, most NF-κB is bound to IκBα and retained in the cytosol in its inactive form [49,50]. Stimulation of the NF-κB pathway is mediated by diverse signal transduction cascades that lead to three major events. First, phosphorylation of the inhibitory IκBα by upstream kinases and the release of NF-κB. Second, translocation of NF-κB dimers to the nucleus, and last, NF-κB dimers bind to specific DNA elements and trigger transcriptional activity of several genes (Figure 2) [51] The signal is eventually terminated through NF-κB-directed IκBα resynthesis which binds and resequesters cytoplasmic NF-κB. Phosphorylation of IκBα occurs on N-terminal serine residues and is induced by the IKK complex. The predominant IKK complex found in most cells contains two catalytic subunits, IKK1 (also known as IKKα) and IKK2 (IKKβ), and a regulatory subunit, IKKγ/NF-κB essential modulator (NEMO) [48,52–54]. Whereas the catalytic serine kinases IKK1 and IKK2 were found to target serines 32 and 36 of the IκBα (and p100 processing by IKK1), the role of NEMO was identified as a regulatory subunit. NEMO contains several protein interaction motifs with no apparent catalytic domains but essential for staging the assembly of the IKK signalsome [55–58]. Aside from their classical NF-κB activation mode, IKKs, in particular IKK1, induce activation of p100NF-κB through a noncanonical pathway (Figure 2) resulting in the activation of p52–RelB dimers [59].

Role of IKK/NF-κB in basal bone biology, inflammation & inflammatory bone erosion

NF-κB is absolutely essential for osteoclastogenesis [42,60]. In fact, the combined deletion of two subunits of this transcription factor (i.e., p50 and p52) arrests osteoclast development and leads to osteopetrosis [41,42]. Recent findings further established that members of the IKK complex, namely IKK1, IKK2 and NEMO regulate osteoclasts directly. In this regard, genetic studies show that the deletion of IKK1 or IKK2 impedes osteoclastogenesis [61,62]. Furthermore, inhibition of NEMO binding to and activation of the IKK2 by a NEMO binding decoy peptide (termed NBD for NEMO-binding domain) arrests osteoclast formation and activation [63,64]. Several other studies have established that NF-κB is a legitimate target for modulating osteoclastogenesis and osteoclast activity. For

example, the administration of NF-κB inhibitors, such as dominant-negative forms of IκBα and inhibitors of IKKs, showed great promise in arresting osteoclasts [40,65].

Osteoclast recruitment and activation are induced markedly and coincide with elevated levels of NF-κB in states of inflammatory bone diseases [8,66,67]. This is apparently fueled by proinflammatory factors converging at inflammatory sites under the auspices of NF-κB. This transcription factor induces a wide range of genes among which are those encoding IL-1, IL-6, TNF, iNOS, COX-2 and other proinflammatory cytokines, some (such as TNF, IL-1 and IL-6) are capable of activating the NF-κB pathway directly, thus establishing a vicious positive autoregulatory loop that can amplify the inflammatory response and increase its duration. In this regard, activation of the NF-κB pathway has been shown to be involved in the pathogenesis of several inflammatory diseases, including all forms of arthritis [5,8,9,67–69]. Specifically, NF-κB subunits p50 and p65 have been localized to nuclei in synovial lining cells as well as mononuclear cells in the underlying regions. NF-κB binding to DNA is also much higher in RA compared with osteoarthritis controls, consistent with increased proinflammatory cytokine production in RA [70]. Furthermore, cytokines, such as TNF, IL-6 and RANKL, which activate NF-κB and induce bone loss and inflammation, are elevated in the synovial fluid of arthritic patients.

The pathogenic role of NF-κB has been also widely established in animal models of inflammatory arthritis. The incidences of increased NF-κB activity that correlate with early stages of RA in rodents support the concept that the transcription factor plays a key role in the development and progression of RA [8,70,71]. Numerous studies have established that NF-κB regulates RA directly through the use of decoy oligonucleotides and various IκBα and IKK dominant negative forms virally transferred into different rodent models of RA [72–78].

Therapeutic targets for bone loss

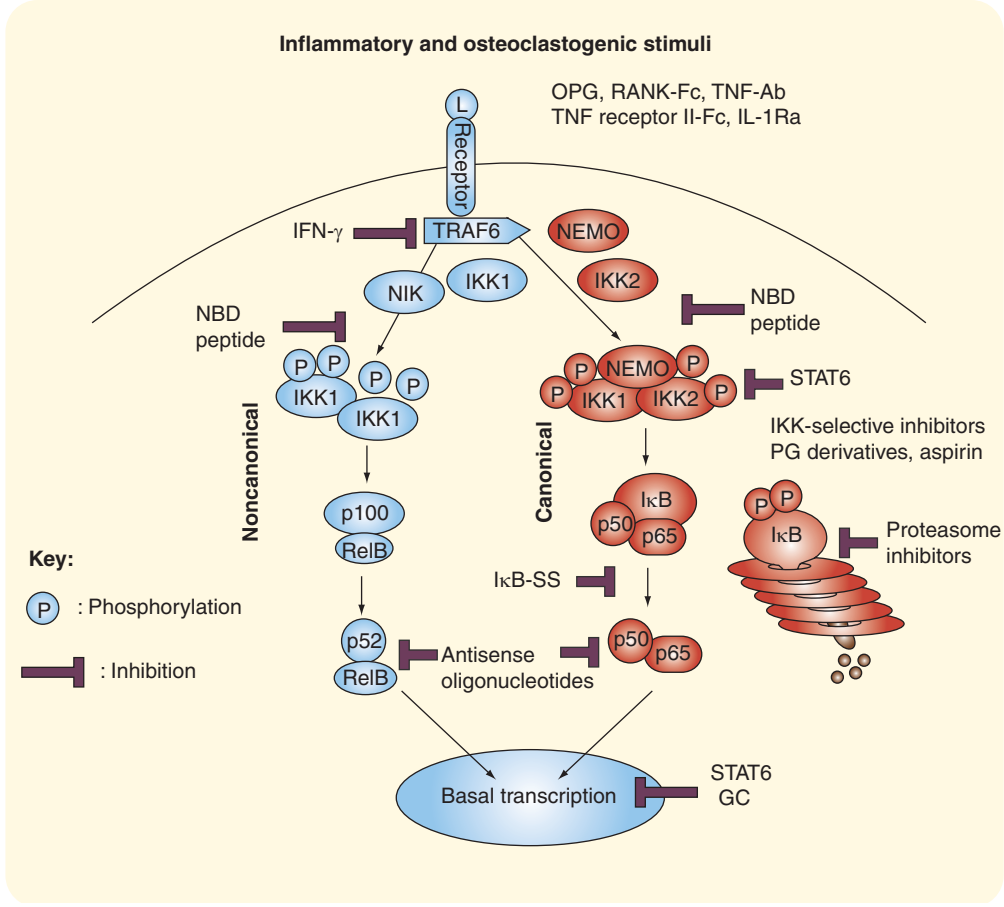
Proximal approaches: RANK/RANKL/OPG/TNF/TNF receptors

As osteoclasts, the centerpiece of osteolytic responses, depend upon circulating levels of RANKL (which is abundant in inflamed sites where it is secreted by synovial and activated T cells [79]), in experimental arthritis, targeting this mechanism has proved tangible [2,6,10,80].

A direct approach to target the final destructive phase of bone loss in inflammatory osteolytic diseases is the inhibition of osteoclast differentiation through the application of the RANKL decoy molecule, OPG. Studies with animal models and with *in vitro* osteoclast cultures have shown significant inhibition of osteoclastogenesis and reduced hallmarks of bone erosion [10,15,25,81,82]. In this regard, recent studies with animals have shown that RANKL-deficient mice subjected to autoimmune serum transfer-induced RA did not develop bone erosion, despite ongoing inflammation [83]. Moreover, treating animals with OPG at the onset of disease almost completely preserved cortical and trabecular bones compared with severe bone loss in joints from untreated rats. This was associated with a significant decrease in osteoclast numbers in OPG-treated animals. Cartilage destruction was less severe in the absence of RANKL (knockout animals) and in OPG-treated arthritic rats, probably owing to the preserved architecture of the subchondral bone structures that provide physical support for the articular cartilage [1,84,85].

Following years of investigating effectors of bone loss, proinflammatory cytokines (primarily TNF and IL-1) remain the centerpiece among factors mediating osteoclast differentiation, bone erosion and exacerbating inflammatory bone diseases. Osteoclast recruitment and activation is induced markedly by TNF and IL-1 *in vitro* and *in vivo* [5,7,46,86–90]. As TNF, IL-1 and RANKL are abundant in sites of inflammatory bone erosion and their signaling pathways overlap considerably, it was recognized that these potent osteoclast inducers synergistically orchestrate inflammatory bone loss. There is ample evidence to implicate TNF as a major mediator of inflammatory arthritis in experimental animals and patients with RA [91,92]. For example, TNF transgenic mice develop spontaneous joint destructive polyarthritis [93,94]. Thus, several approaches have been developed over the past decade to combat erosive arthritis through anti-TNF therapies. These approaches were based on targeting proximal moieties of the TNF system, primarily neutralizing circulating levels of the cytokine by TNF-binding proteins and soluble (non-signaling) receptors or blocking binding of TNF to respective receptors with monoclonal antibodies [91–93,95–98]. Three drugs that block the activity of TNF are available. Infliximab and adalimumab are antibodies against

Figure 2. NF-κB signaling pathway in osteoclasts and selected therapeutic targets.



L, including RANKL and TNF, binding to transmembrane receptors activates the NF-κB transduction pathway in osteoclasts. Signaling molecules and inhibitory steps are illustrated.
GC: Glucocorticoid; IFN: Interferon; IκB-SS: IκB super repressor; IKK: IκB kinases; IL-1Ra: Interleukin-1 receptor antagonist; L: Ligand; NBD: NEMO-binding protein; NEMO: NF-κB essential modulator; NIK: NF-κB inducing kinase; OPG: Osteoprotegerin; PG: Prostaglandin; RANKL: Receptor activator of NF-κB ligand; STAT: Signal transducers and activators of transcription; TNF: Tumor necrosis factor; TRAF: TNF receptor-associated factor.

TNF and etanercept is a fusion protein of the TNF receptor II. All of these agents improve clinical signs of RA, typically over 50% responsiveness, and reduce radiographic progression of RA significantly [91,99–104].

Similar to TNF, IL-1 has long been known as a potent inducer of osteoclastogenesis and a mediator of inflammation and RA [44,89,105]. Evidence for a key role of IL-1 in erosive arthritis was established in animals lacking the IL-1 decoy receptor, IL-1 receptor antagonist (IL-1Ra) (commercially known as anakinra). These mice developed RA owing to excessive IL-1 signaling. Typically, this soluble IL-1Ra molecule binds to circulating IL-1 and attenuates binding of the cytokine to its cognate cell surface receptor. Other findings showed that blocking IL-1 activity with IL-1Ra resulted in significant clinical and hematological responses in patients with juvenile idiopathic arthritis (JIA) [106,107]. Resolution of clinical symptoms including fever, marked leukocytosis, thrombocytosis, elevated erythrocyte sedimentation, anemia and arthritis were rapid and sustained. The efficacy of IL-1Ra in these children superseded TNF therapies in JIA mandating careful consideration of anti-RA therapeutic choices.

Another promising approach to directly lessen osteoclast activity is the use of bisphosphonates and selective estrogen-receptor modulators (SERMs). These compounds inhibit osteoclast function and induce osteoclast apoptosis [108–111]. Although initial clinical trials in RA failed to show retardation of joint destruction, recent

experimental data from TNF-mediated destructive arthritis indicate that high doses of bisphosphonates could be highly effective in the prevention of joint destruction [112]. Other studies using combined therapies of OPG and pamidronate show greater reduction of inflammatory bone erosion in the TNF transgenic mouse model of spontaneous destructive polyarthritis [25]. In summary, these approaches seem to directly target the destructive osteoclast-directed phase and only indirectly cause a moderate reduction in cartilage destruction. These observations support the concept that cartilage breakdown results largely from osteoclast-independent mechanisms, probably secreted metalloproteinases and other catalytic enzymes (Figure 2).

Inhibition of intracellular & signal transduction cascades

Signal transduction cascades induced by RANKL, TNF and IL-1 in osteoclasts are well studied and described above. Ample data point to a considerable signaling overlap between the various cytokines, which converges at the NF-κB and MAPK signal transduction pathways. Notably, ligation of RANKL, TNF or IL-1 to their respective receptors induces recruitment of adaptor proteins (TRAF2, TRAF6, cellular inhibitor of apoptosis [IAP]) and kinases (TGF-β activated kinase [TAK] 1, MEKK, IRAK, IKKs, c-Src tyrosine kinase and more) that direct the signaling cascades towards relevant inflammatory and osteoclastogenic transcriptional regulation [12,66]. One of the initial steps in the signal assembly is the formation of the IKK signalsome that catalyzes NF-κB machinery. This process is mediated by recruitment of IKKγ/NEMO by distal receptor-interacting adaptors to form a platform that facilitates upstream receptor-transmitted signals through IKK2 and IKK1, which in turn activate classical and nonclassical NF-κB pathways, respectively, through phosphorylation and proteolytic processing of the inhibitory protein IκBα and the NF-κB precursor p100 [49]. These events lead to the release and nuclear translocation of the various NF-κB subunits. Given the notion that NF-κB is central to inflammatory and osteoclastogenic responses, targeting various regulatory steps in the activation cascade of this transcription factor attracted considerable interest. A promising approach to block NEMO from binding to IKK2 and IKK1 was described recently in murine models of inflammation and spontaneous RA. A cell-permeable NBD derived from the carboxyl terminal domain of IKK2 binds efficiently to NEMO and attenuates activation of the IKK complex [113,114]. More intriguingly, this peptide inhibits osteoclastogenesis and ameliorates inflammatory bone erosion [63,64]. Several immunomodulatory and selective inhibitors of IKK2, IκBα and NF-κB subunits have been described. For example, thalidomide inhibits TNF-induced IKK2 in various cells and blocks TNF-stimulated osteoclasts [115,116]. However, toxic side effects may preclude usage *in vivo*. Other inhibitors of IKK activity, in the form of chemical compounds, have been designed recently and exhibit varying efficiencies [4].

Commonly used approaches to inhibit NF-κB activation in animal models of the inflammatory response, including RA, have centered for several years on the administration of dominant-negative forms of IKKs and IκBα, as well as using proteosome inhibitors to preserve the IκBα protein. In this regard, viral transfer and protein transduction of dominant-negative forms of IKK2 and IκBα were efficient in decreasing inflammation and arresting bone erosion in the joint of experimental models of RA [66,75]. Specifically, transduction of a dominant-negative form IκBα was sufficient to block osteoclast formation and activity [65,117]. More importantly, application of the IκBα protein to arthritic mice significantly blocked bone erosion associated with inflammatory arthritis [40]. Selective activation of NF-κB in normal rats by intra-articular transfer of a constitutively active *IKK2* gene induced synovial inflammation and clinical signs of arthritis. By contrast, transfer of a dominant-negative adenoviral IKK2 construct reduced NF-κB nuclear translocation and clinical synovitis in adjuvant arthritis (AA) in rats [69]. Similarly, in other studies using collagen-induced arthritis (CIA) or serum transfer-induced RA, direct administration of dominant-negative forms of IκBα reduced inflammatory signs of RA and inhibited tissue deterioration significantly [40]. Other studies used a direct approach to inhibit NF-κB-mediated arthritis. These include the blockade of NF-κB with decoy oligonucleotide, direct viral gene transfer of dominant-negative molecules upstream of NF-κB (such as super-repressor IκBα or its kinase, IKK) [8,40,69,76] and cell-permeable blocking peptides, as outlined below [63,64].

Several genes have been shown to be critical for osteoclast differentiation or function. Among these are *c-fms*, *c-fos*, *RANKL*, *NF-κB*, *c-Src*, nuclear factor of activated T cells isoform c1 (*NFATc1*) and the proton *H^+-ATPase*. Recent studies have unveiled that proinflammatory

cytokines, such as TNF, act directly on some of these genes and their products, in particular *c-src* and *NF-κB*, to accelerate osteoclast formation and cause a potent osteoclastic response [7,46,118,119]. Selective inhibitors of the c-src tyrosine kinase show great promise in halting osteoclast activity and future studies should be geared towards testing the effect of such inhibitors on inflammatory osteolysis [119–122] (Figure 2).

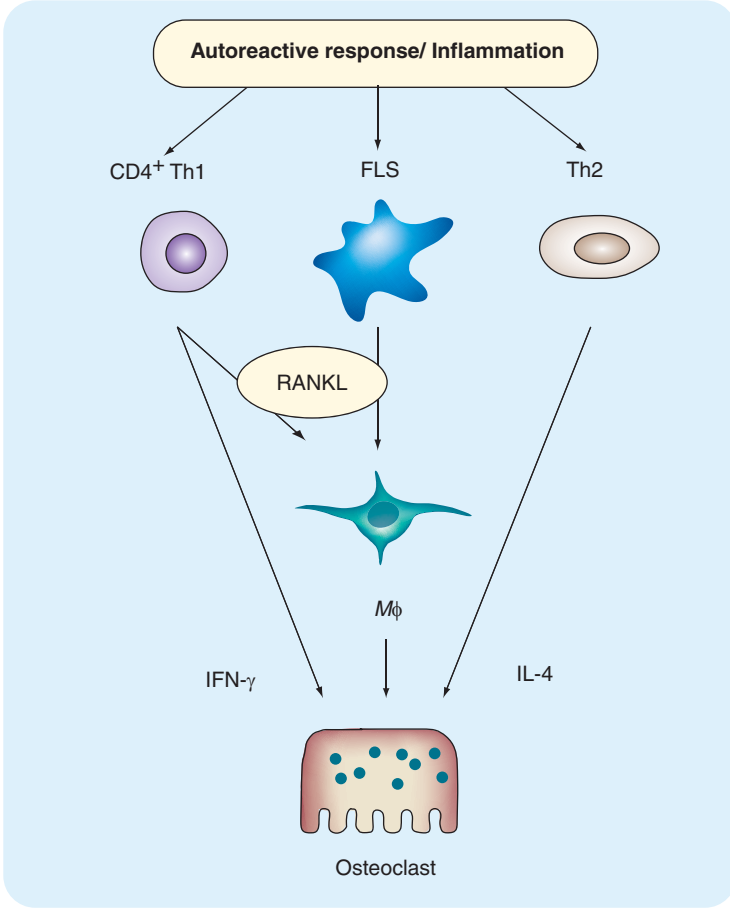
Anti-inflammatory approaches

Anti-inflammatory cytokines secreted by T lymphocytes, such as interferon (IFN)-γ, IL-4, -13, and -10, have also been shown effective in antagonizing proinflammatory and osteoclastogenic cytokine actions transmitted by T helper cell (Th)1 and fibroblast-like synoviocytes. In this regard, a recent finding documents that IL-4 mRNA was found more frequently in nonerosive compared with erosive disease [123]. Additionally, IL-4 adenoviral gene therapy has been shown to be effective in reducing inflammation, inhibiting proinflammatory cytokine secretion and sparing bone destruction in a model of adjuvant-induced arthritis (AIA) [124]. These findings provide indirect evidence that IL-4 has bone-sparing effects *in vivo* and participates in the resolution of inflammatory arthritis. Clarifying the molecular mechanisms underlying the antiresorptive action of these anti-inflammatory cytokines should unveil useful molecular targets. For example, the finding that IL-4 requires signal transducer and activator of transcription (STAT) 6 to block osteoclastogenesis [125], presents the latter transcription factor as a potential target for antierosive drug design. In fact, an active form of STAT6, termed STAT6-valine-threonine (VT), readily ameliorates bone erosion associated with spontaneous serum-induced arthritis [126].

IFN-γ is another major product of immune cells that potently inhibits bone resorption. Recent reports illustrated that IFN-γ interferes with RANK–RANKL signal transduction in osteoclasts and their precursors. It induces rapid degradation of TRAF6, a RANK adaptor protein [80,127]. This action results in the arrest of RANK downstream signals, such as the NF-κB and c–Jun N-terminal kinase (JNK) pathways. Another study reported that RANKL-induced secretion of IFN-γ by osteoclast precursors counterbalances bone resorption by blocking osteoclastogenesis in an autoregulatory fashion [127].

NF-κB transcription machinery also encodes anti-inflammatory cues, such as COX-2-mediated synthesis of anti-inflammatory cyclopentenone prostaglandins (cyPGs), which are involved in the resolution phase of inflammation [128]. These prostaglandin metabolites inhibit NF-κB transcriptional activity through induction of peroxisome proliferation-activated receptor (PPAR)-γ [129–132]. Moreover, cyPGs can inhibit activation of the NF-κB pathway directly by blocking IKK2 activity [133]. The compound 15-deoxy-prostaglandin (PG)-J2 (15dPGJ2) inhibits IκBα degradation through the inhibition of IKK activity [134]. The utility of these anti-inflammatory PG metabolites as antiosteoclastogenic factors is supported by a study showing that 15d-PGJ2 is a potent inhibitor of NF-κB in macrophages and also inhibits osteoclastogenesis [135] (Figure 3).

Figure 3. Cellular responses in inflammatory arthritis.



Pro-inflammatory signals, such as RANKL, are elicited by FLS and Th1 cells. Anti-inflammatory signals including IFN-γ and IL-4, are secreted by Th1 and Th2 cells and participate in the resolution of the inflammatory and osteolytic responses.
FLS: Fibroblast-like synoviocytes; IFN: Interferon; IL: Interleukin; RANKL: Receptor activator of NF-κB ligand; Th: T-helper cell.

Executive summary

Introduction

- The destruction of bone is a hallmark of rheumatoid arthritis (RA). This focal bone erosion is the result of increased osteoclast action. Synovial tissue-residing cells secrete a wide range of inflammatory cytokines and factors such as interleukin (IL)-1, IL-6, transforming growth factor (TGF)-β, parathyroid hormone (PTH), inducible nitric oxidase synthase (iNOS), cyclooxygenase (COX)-2 and members of the tumor necrosis factor (TNF) superfamily cytokines that directly or indirectly comprise a microenvironment supportive of osteoclast recruitment and activation. Members of the TNF family including receptor activator of NF-κB ligand (RANKL) and TNF-α activate the Rel/NF-κB family of transcription factors that govern inflammatory and osteolytic processes and are thus considered increasingly as the centerpiece that fuels inflammatory arthritic bone erosion and, as such, the focus for therapeutic intervention.

Osteoclasts & their role in inflammatory arthritis

- Osteoclasts arise from bone marrow macrophage precursors and their differentiation into the mature polykaryon requires RANKL and macrophage colony-stimulating factor (M-CSF). Osteoprotegerin (OPG), a soluble factor secreted by osteoblast and stromal cells, acts as a decoy receptor through binding to circulating RANKL and decreasing its bioavailability. This molecule has been used widely as a potent antiosteoclast therapy.
- Several genes such as *PU.1, c-fms* (M-CSF receptor), *c-fos, RANK* and *NF-κB* (p50, p52 subunits), the proto-oncogene *c-Src*, the proton *ATPase*, tartrate-resistant acid phosphatase (*TRAP*), and *cathepsin-k*, are critical for osteoclast differentiation. These genes have been targeted to modulate osteoclastogenesis.

Summary of the NF-κB system

- Members of the NF-κB family include p50, p52, RelA/p65, RelB, c-Rel, the precursors NF-κB1/p105, NF-κB2/p100 (which undergo processing into p50 and p52, respectively) and the inhibitory subunits IκBα, IκBβ, and IκBε. Inactive NF-κB is bound to IκBα and resides in the cytosol. Stimulation of the NF-κB pathway entails phosphorylation by IκB kinases (IKKs) and subsequent removal of IκBα, followed by the nuclear translocation of NF-κB dimers to the nucleus and initiation of transcriptional activity.

NF-κB axis is central to osteoclastogenesis & inflammatory responses

- Certain NF-κB family members are essential for osteoclast formation. Deletion of IKK1, IKK2, p50 and p52 arrests osteoclastogenesis. Similarly, inhibition of NF-κB essential modulator (NEMO) binding to IKKs inhibits osteoclasts.
- NF-κB induces a wide range of genes encoding proinflammatory cytokines and factors, including interleukin (IL)-1, IL-6, tumor necrosis factor (TNF), inducible nitric oxidase synthase (iNOS) and cyclooxygenase (COX)-2. NF-κB mediates inflammatory responses directly, including RA and inflammatory bone erosion.

Proximal approaches to inhibit inflammatory osteolysis

- Given that osteoclasts are primarily responsible for focal bone erosion associated with RA, proximal inhibition of this process is highly efficacious. OPG and RANK–Fc target RANKL directly and inhibit osteoclast formation. Other widely used approaches target proinflammatory cytokines such as TNF and IL-1, which propagate inflammatory osteolysis. Anti-TNF antibodies, soluble TNF receptor (nonsignaling) moieties and IL-1 receptor antagonist (anakinra) are also effective at inhibiting inflammatory bone erosion. A similar approach is the use of bisphosphonates and selective estrogen-receptor modulators (SERMs), which directly target and inhibit osteoclast activity and viability.

Targeting signaling pathways to combat inflammatory bone erosion

- The intracellular NF-κB signaling cascade by RANKL, TNF-α and IL-1 provide ample targets for therapeutic intervention. Upstream signaling targets include TNF receptor-associated factor (TRAF) 6, NEMO, IKK1,and IKK2. A promising approach described recently is the use of a NEMO-binding domain (NBD) derived from IKK1/2. Short peptides corresponding to this domain block osteoclasts and ameliorate bone erosion in mouse models of inflammatory arthritis.
- Selective inhibitors for IKKs and dominant-negative forms of IKKs and IκBα are also promising approaches to inhibit NF-κB-mediated responses. Other signaling molecules, such as the tyrosine kinase c-Src, have been exploited through the use of selective inhibitors.

Anti-inflammatory approaches

- Anti-inflammatory cytokines, such as interferon (IFN)-γ, IL-4, IL-13 and IL-10, have also been shown to be effective in antagonizing pro-inflammatory cytokine actions and osteoclastogenesis. IL-4 inhibits NF-κB activation and osteoclastogenesis in a signal transducer and activator of transcription (STAT) 6-dependent manner. Furthermore, active STAT6 ameliorates inflammatory osteolysis. IFN-γ targets TRAF6 for degradation, thereby attenuating NF-κB activation, arresting osteoclastogenesis and alleviating inflammatory responses.
- Prostaglandin metabolites termed cyclopentenone prostaglandins (cyPGs), inhibit IKK2 activity and NF-κB transcriptional activity through the induction of peroxisome proliferation-activated receptors (PPAR)-γ. These metabolites are potent inhibitors of inflammatory and osteoclastogenic processes.

Concluding remarks

- The central role of the NF-κB cascade in osteoclastogenesis and inflammatory responses positions this family of transcription factors as a target for therapeutic intervention in diseases associated with inflammatory bone erosion. However, selective inhibition remains limited due to the ubiquitous nature of the NF-κB pathway and its essential role for basic cellular functions as well as pathological cell responses.

Conclusion & future perspectives

Discoveries in the past decade have unveiled the role of a large number of genes that regulate osteoclastogenesis, with specific emphasis on the RANK–RANKL–OPG system. Concerted efforts have led to the design of successful antiresorptive therapies that should benefit patients suffering from bone loss pathologies. These types of therapies are more promising owing to their cell-specific approach. Unfortunately, the same does not apply when using anti-inflammatory approaches targeting signaling mechanisms, such as NF-κB. The approach of inhibiting the IKK/NF-κB signal transduction pathway has proved very useful in combating numerous forms of inflammatory responses, including RA. However, the ubiquitous nature of this pathway across cell types and its fundamental role in basal cellular functions limits its utility. For example, concerns related to toxicity and liver cell death in the absence of NF-κB are evident. Such cell death is likely to propagate in the presence of elevated levels of TNF-α, a hallmark of RA. To avoid such drawbacks, therapy design would have to rely on a better understanding of the molecular role of the various IKK/NF-κB components in RA. For example, short-term treatment with specific inhibitors of the NF-κB pathway that resembles regimens in animal models might reduce potential side effects.

Selective inhibition of certain candidate molecules, at levels that do not interfere with basal cell functions and acquire tissue specificity, would be ideal. For example, the precise role of IKK1 (noncanonical) versus IKK2 (canonical) pathways in inflammatory arthritis remains in its infancy. Recent work suggests that IKK1-mediated NF-κB signals may participate in the resolution of inflammatory responses [136,137]. Future studies might provide further insights as to the differential roles and thus the utility of either pathway. As it stands, antiresorptive therapy using OPG and RANK–Fc appears to be the most promising approach to alleviate erosive arthritis. Less promising are therapies directed against the inflammatory component of the diseases that might require combined therapies to ameliorate the disease.

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