Targeting p38 MAPK for the treatment of inflammatory arthritis

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Inflammatory bone destruction is a relatively frequent and incapacitating complication of rheumatoid arthritis and other chronic inflammatory joint diseases, and is a product of accelerated osteoclast recruitment and activation in bone under the aegis of cytokines produced in the inflammatory milieu. Over the past decade there have been major advances in our understanding of the mechanisms of this family of diseases. It is now clear that p38 mitogen-activated protein kinase plays an essential role in the production of proinflammatory cytokines and cytokine-induced osteoclastogenesis, thus providing a potential tool for preventing pathologic bone loss. This review outlines our current understanding of the mechanisms mediating inflammatory arthritis and highlights potential therapeutic strategies targeting p38 mitogen-activated protein kinase.

Destruction of the bone is a major complication of inflammatory arthritis, such as rheumatoid arthritis (RA), the most frequent of all chronic inflammatory joint diseases. RA is an autoimmune disease affecting approximately 1.0% of US adults, with a female:male ratio of 2.5:1 [1]. Its hallmark is progressive joint destruction and, if untreated, it often leads to permanent joint damage and eventual disability, which causes major morbidity. The etiology of RA is largely unclear, but insights into pathogenic pathways have accumulated significantly over the past decade. The combination of genetic susceptibility with environmental factors is considered pivotal in the initiation of an immunologic response against the synovium. In brief, activated cells in the innate immune system, once activated, act on cells of the adaptive immune system and elicit a battery of inflammatory responses [1,2].

Although inflammatory arthritis may progress by alternative pathways, the important common factors in the pathological process are overproduction of proinflammatory cytokines and excessive destruction of bone by osteoclasts near the sites of inflammation. The bone erosion seen in inflammatory arthritis is largely localized to the inflamed tissues, distinct from systemic, hormonally regulated bone pathologies, such as osteoporosis. These inflamed tissues produce proinflammatory cytokines, specifically TNF-α, IL-1 and IL-6, which are, in turn, involved in osteoclast differentiation signaling and bone resorbing activities [3]. Thus, inflammatory arthritis is the product of enhanced osteoclast recruitment and activation prompted by proinflammatory cytokines. The osteoclast, the principal and probably exclusive resorptive cell of bone, is abundant in affected joints of patients with these conditions [3]. Thus, understanding the mechanisms by which osteoclasts resorb bone, and the cytokines that regulate their differentiation and activity, provides mechanism-based candidate therapeutic targets to prevent inflammatory bone loss.

Osteoclastogenesis in inflammatory conditions

Osteoclasts are multinucleated cells formed by the fusion of mononuclear precursors of the monocyte/macrophage lineage under the influence of the specific osteoclastogenic cytokine, receptor activator of NF-κB ligand (RANKL) and macrophage colony-stimulating factor (M-CSF) [4]. RANKL, a member of the TNF superfamily, is a membrane-bound homotrimeric protein found on the surface of mesenchymal cells of osteoblast lineage, and exerts its effects by recognizing its receptor, RANK, on marrow macrophages. Thus, RANKL expression is the key to the pathogenesis of osteolytic conditions. Negative regulators of RANKL expression have been reported, including osteoprotegerin, a soluble ‘decoy’ receptor that is also expressed by stromal...
cells/osteoblasts and competes with RANKL for RANK [4] and IFN-γ, a cytokine produced by RANKL in osteoclast precursors, which inhibits osteoclast differentiation in an autocrine fashion [8].

In states of juxtaskeletal inflammation such as RA, the osteoclastogenic molecule RANKL is also produced in abundance by activated T lymphocytes and synovial fibroblasts, whether in joint or bone [9,10]. RANKL may also be cleaved from the cell membrane and then interact with RANK as a soluble ligand.

Production of proinflammatory cytokines plays a vital role in the pathogenesis of inflammatory arthritis, of which TNF-α is probably the dominant cytokine and the rate-limiting factor in that its blockade abolishes both the inflammatory and osteoclastogenic components of these diseases in many, but not all, RA patients [11-15]. The cytokine is primarily produced by activated T cells, macrophages and synoviocytes. TNF-α stimulates RANKL expression, a fundamental component of inflammatory arthritis [16,17]. Interestingly, this process is mediated, at least in part, by another major proinflammatory cytokine, IL-1, since the blockade of IL-1 signaling significantly inhibits TNF-induced RANKL production and osteoclastogenesis in vitro and in vivo [18], as well as TNF-mediated bone damage in an animal model [19]. Like TNF-α, the major sources of IL-1 are the activated cells of monocyte-macrophage lineage, and its production can be rapidly induced by bacterial lipopolysaccharides (LPS), TNF-α, IFN-γ, IL-1β and IL-6, and IL-1 itself. On the other hand, TNF-α also directly stimulates osteoclast recruitment, characterized by enhanced RANK expression and sensitization of precursor cells to RANKL [7]. In addition, at least one animal study has shown that TNF-α-induced inflammatory arthritis is also mediated, at least in part, by the stromal cell-produced osteoclastogenic cytokine M-CSF [20].

Inhibition of p38 MAPK as a new approach to the treatment of inflammatory arthritis

The recent advances in basic research in understanding the mechanisms of inflammatory arthritis lead to two major therapeutic approaches: anti-inflammatory agents and bone resorption-blocking agents. The representative therapeutic drugs in the first group include antibodies that bind specifically to a cytokine or cytokine receptor, such as infliximab and adalimumab (anti-TNF-α monoclonal antibodies), etanercept (TNF receptor-p75 Fc fusion protein) and anakinra (IL-1 receptor antagonist). The therapeutic agents in the second category mainly target RANKL-RANK interaction or osteoclast cytoskeleton molecules (i.e., denosumab, a monoclonal antibody against a RANKL epitope) [21]. Although the RANK-RANKL interaction in the formation of osteoclasts is an excellent therapeutic target for osteolytic destruction, anti-inflammatory effects, if any, have not been shown in this class of drugs. The success of the aforementioned anti-inflammatory agents, given alone or in combination with methotrexate, an antimetabolite, has been demonstrated by moderately reducing the progression of active disease, highlighting the central role of these proinflammatory cytokines as valid targets. However, all of the anticytokine agents available to date are protein-based drugs, of which their major disadvantages are poor stability, poor cellular penetration, poor cellular activity, a short half-life and high costs of manufacturing [22]. Therefore, the therapeutic strategy is to find small compounds that mimic the anticytokine effects of these drugs, yet evade breakdown in the digestive tract.

While a number of molecular targets have been identified for the development of such small molecular agents, p38 mitogen-activated protein kinase (MAPK), one of the key sensors of cellular stress (and a molecule known to play a crucial role in mediating osteoclastogenesis in inflammatory conditions), has received short shrift. There are four isoforms of the enzyme (p38α, p38β, p38γ, and p38δ), which differ in tissue distribution, regulation of kinase activation and subsequent phosphorylation of downstream substrates. P38α is believed to be the family member primarily responsible for regulation of inflammation [23]. Activation of p38 MAPK occurs within both the synovial lining layer and in juxtaposition to endothelial cells, and is induced chiefly by proinflammatory cytokines such as TNF-α and IL-1 [24]. p38, most notably its p38α isoform, is activated mainly within cells involved in the inflammatory process [23,25]. Activation of p38 induces synthesis of key inflammatory mediators such as TNF-α, IL-1, IL-6, IL-8 and COX-2, either via direct activation of gene transcription or via mRNA stabilization [26-31]. A microarray study has shown that...
approximately a third of the TNF-induced genes in fibroblast-like synoviocytes are regulated by the p38 signaling pathway [32]. Importantly, p38 has a multifaceted role in CD4+ T cells, which, in turn, have been implicated in initiating and driving sustained inflammation in autoimmune diseases such as RA [33]. Moreover, treatment with infliximab in patients with RA causes a marked reduction of activated p38 levels in CD4+ T cells, but not in macrophages, suggesting that a prolonged therapeutic benefit with these agents may be mediated by their effect on CD4+ T cells [34]. These findings make p38 an attractive anti-inflammatory drug target.

In the context of bone erosion, p38 mediates TNF-α-induced IL-1 upregulation in stromal cells and subsequent IL-1-induced RANKL production. In macrophages, which express p38α but not p38β, p38γ and p38α isoforms, the synthesis of the IL-1 functional receptor by either TNF-α, IL-1 or RANKL is mediated via p38 [35]. Moreover, the fact that in vitro RANKL-induced osteoclastogenesis is inhibited by a p38 inhibitor, SB203580, and that TNF-induced osteoclastogenesis in bone marrow cells derived from p38-gene-deficient mice was significantly less than that from control mice, suggest that p38 plays an important role in osteoclast differentiation [35–37]. While stromal cells/osteoblasts are largely present in the bone marrow, cells phenotypically resembling osteoblasts are also found in arthritic mice and humans [38,39], although it is not clear whether these cells produce significant amounts of cytokines. A major proportion of p38-mediated cytokine production in the joints is also likely derived from T cells and synoviocytes. As a consequence, tissue invasion into joint-articular bone can be effectively blocked by the use of p38 inhibitors, in addition to the improvement in clinical signs, cytokine production and articular cartilage damage, as evident in recent studies in models of experimental arthritis [40–42]. By contrast, deregulation of p38 signaling, such as found in CD44-deficient mice, leads to increased osteoclast formation and increased bone resorption upon challenge with TNF-α [43]. It has been recently demonstrated in a mouse inflammatory arthritis model that TNF-induced p38 activation triggers the formation of DKK-1, a regulatory molecule of the Wnt pathway, suggesting a key role for this molecule in the remodeling of joints and an important cross-talk between the bone anabolic Wnt and the bone catabolic RANKL pathway [44]. Importantly, the role of DKK1 in the development of osteolytic lesions in multiple myeloma was also shown in humans [45]. Moreover, treatment with a p38α inhibitor in murine models of multiple myeloma reduces the development of bone disease and increases survival [46].

These data impressively demonstrated the major importance of p38 for inflammation-mediated bone destruction, and suggest that inhibition of p38 might be a significant tool for reducing structural damage within the inflammatory milieu. Creating novel p38 inhibitors thus has two main goals:

- To prevent the synthesis of these cytokines rather than neutralize them after they are produced
- To block selective effects produced by TNF-α, IL-1 or other inflammatory mediators

Thus, p38 inhibitors should have a potential competitive advantage over the current anti-TNF or anti-IL-1 biologics [23]. Since p38 mediates osteoclastogenesis at multiple levels, the potential efficacy of p38 inhibitors would be expected to be greater than that from the inhibition of proinflammatory cytokines alone [47]. To date, numerous p38 inhibitors have been characterized, of which several compounds have been advanced in clinical trials [48].

Although p38 inhibitors have been extensively evaluated in preclinical models of arthritis, data in humans are far less inclusive. At this point, convincing proof of concept from clinical trials has not been achieved. The major issues that have interfered with the development of this class of drugs generally relate to preclinical and clinical toxicity. Studies with earlier p38α inhibitors, VX-745 and VX-702, have been limited by their side effects. A few other drugs, including SC10-469 and PH-797804, are currently under evaluation [49,50]. Several clinical trials with p38 inhibitors have been discontinued because of serious dose-limiting toxicities within the liver, skin and CNS. Second-generation p38 inhibitors that are unable to cross the blood–brain barrier are currently under development.

Furthermore, the specificity of the p38 inhibitors should be taken into consideration. For example, more recent studies have found that SB203580, the most used p38 inhibitor in vitro, also inhibits other protein kinases such as cyclin G-associated protein kinase, as well as receptor-interacting protein 2, c-Raf...
and GSK3 in vitro, with similar or even greater potency [51–53]. These results have further emphasized the need for considerable caution in using small-molecule inhibitors of protein kinases for assessment of the physiological roles of these enzymes, and also for pharmaceutical drug development.

Conclusion
Patients with inflammatory arthritis face complications of joint destruction, which leads to significant impairment in their quality of life. The differentiation and function of osteoclasts are accelerated under the aegis of cytokines produced in the inflammatory environment, in which TNF-α, IL-1 and RANKL play a central role. Small-molecular anticytokine agents may provide potential alternatives in preventing inflammation and bone loss. Blockade of the p38 MAPK signaling pathway may prevent inflammatory bone loss at multiple levels: inhibiting production of proinflammatory cytokines and preventing osteoclast formation via:
- Reducing osteoclast precursors
- Inhibiting TNF-α-induced and IL-1-induced RANKL expression
- Blocking RANKL-induced osteoclastogenesis

Although both the clinical utility and therapeutic index in humans is yet to be determined, we hope the emerging drugs targeting this signaling pathway offer novel therapy to patients suffering from inflammatory bone loss.

Future perspective
Alternative strategies are continuously being proposed to overcome some of the issues associated with clinical utilization, including development of newer drugs with greater specificity, and identifying alternative target molecules in the cascade. The hope thus remains that the emerging drugs targeting the p38 MAPK signaling pathway will offer novel therapy to patients suffering from inflammatory bone loss.

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Executive summary

- Patients with inflammatory arthritis face complications of joint destruction, which leads to significant impairment in quality of life.
- Rheumatoid arthritis (RA) affects approximately 1.0% of US adults.
- The hallmark of RA is progressive joint destruction and, if untreated, it often leads to permanent joint damage and eventual disability, which causes major morbidity.
- The differentiation and function of osteoclasts are accelerated under the aegis of cytokines produced in the inflammatory environment, in which TNF-α, IL-1 and receptor activator of NF-κB ligand (RANKL) play a central role.
- RANKL is produced by mesenchymal cells of osteoblast lineage, and is the key molecule to initiate osteoclastogenesis in physiological and pathological conditions.
- TNF-α stimulates RANKL expression, a fundamental component of inflammatory arthritis.
- IL-1 mediates, at least in part, TNF-induced RANKL production and osteoclastogenesis in vitro and in vivo.
- Small molecular anticytokine agents may provide potential alternatives in preventing inflammation and bone loss.
- These agents may have anticytokine effects of protein-based drugs, yet evade breakdown in the digestive tract.
- Blockade of the p38 MAPK signaling pathway may prevent inflammatory bone loss at multiple levels.
- p38 inhibitors potentially inhibit production of proinflammatory cytokines and prevent osteoclast formation via:
  - Reducing osteoclast precursors
  - Inhibiting TNF-induced and IL-1-induced RANKL expression
  - Blocking RANKL-induced osteoclastogenesis
- Issues related to toxicity have impeded drug development. Thus, the clinical utility and therapeutic index of p38 mitogen-activated protein kinase inhibitors in humans are yet to be determined.
- A new generation of this class of drugs that are unable to cross the blood–brain barrier are under development and are expected to bring fewer and less severe side effects.
- Cross-reactivity of other protein kinases should be taken into consideration.
Bibliography

Papers of special note have been highlighted as either of interest (+) or of considerable interest (++) to readers.


- Clearly written review on inflammatory osteolysis.


- First study that demonstrated TNF-induced osteoclastogenesis is receptor activator of NF-κB ligand (RANKL)-dependent.


- Important study providing evidence of TNF-α and RANKL-induced osteoclastogenesis and bone resorption.


- Important study providing evidence of TNF- and RANKL-mediated osteoclastogenesis and bone resorption.


- First evidence demonstrating that IL-1 mediates TNF-induced osteoclastogenesis in vitro and in vivo.


- Important study providing evidence of IL-1 mediating TNF-induced bone destruction in an animal model.


- Important study providing evidence of TNF-α and RANKL-mediated osteoclastogenesis and bone resorption.


One of the earliest studies demonstrating p38 MAPK-mediated signals are required for inducing osteoclast differentiation but not for osteoclast function. Endocrinology 143(8), 3105–3113 (2002).


Important study providing evidence of the effectiveness of p38 inhibitors in inflammatory bone destruction in an animal model.


Updated recent review on p38 MAPK as a therapeutic target of inflammatory osteolysis.