Published in final edited form as:

Pathol Res Pract. 2008 ; 204(10): 695–706. doi:10.1016/j.prp.2008.07.002.

Mechanisms Modulating Inflammatory Osteolysis: A Review with Insights into Therapeutic Targets

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Abstract

Inflammatory osteolysis is a relatively frequent and incapacitating complication of rheumatoid arthritis and multiple other inflammation-associated bone diseases. It is thought to operate through an ultimate common pathway of accelerated osteoclast recruitment and activation under the control of cytokines produced in the inflammatory environment. Over the past decade, there have been major advances in our understanding of the mechanisms of osteoclastogenesis. It is now clear that the interaction of receptor activator NF- κ B (RANK) and its ligand, RANKL, plays a central role in osteoclast formation and activity. Therefore, understanding osteoclastogenesis offers new pathways for potential therapeutic intervention in inflammatory osteolysis. The success of anti-tumor necrosis factor- α and interleukin-1 therapy highlights the central role that these specific cytokines play in this disease. This review outlines our current understanding of the mechanisms mediating inflammatory osteolysis and highlights potential therapeutic strategies.

Keywords

Bone; Osteolysis; Osteoclast; Interleukins; TNF- α ; RANK ligand

Introduction

Destructive erosion of bone or osteolysis is a major complication of inflammatory conditions such as rheumatoid arthritis (RA), periodontal disease, and periprosthetic osteolysis. RA is an autoimmune disease that affects approximately 1.0% of US adults, with a female to male ratio of 2.5 to 1. [1] Its hallmark is progressive joint destruction which causes major morbidity. The etiology of RA is largely unclear. The combination of genetic susceptibility with environmental factors is considered to play a role in the initiation of an immunologic response against the synovium. Periodontal disease is highly prevalent and can affect up to 90% of the world's population. It is well known as the leading cause of tooth loss in adults. [2] Despite its prevalence, little is known about the mechanism by which periodontal bone erosion occurs, although host response to pathogenic microorganisms present in the mouth appears to trigger the process. In addition, genetic and environmental factors are also thought to contribute to the cause of this disease. [2] Periprosthetic osteolysis is caused by chronic bone resorption around exogenous implant devices until

fixation is lost, [3] and is considered as resulting from an innate immune response to wear-debris particles, with little contribution by components of the acquired immune system. [4]

Although these conditions are initiated by distinct causes and progress by alternative pathways, the important common factor(s) in the pathological process of these diseases are overproduction of proinflammatory cytokines and excessive destruction of bone by osteoclasts near the site of inflammation. The bone erosion seen in these conditions is largely localized to the inflamed tissues, distinct from systemic, hormonally regulated bone pathologies, such as osteoporosis. These inflamed tissues found in many of these diseases also produce proinflammatory cytokines, i.e., TNF- α , IL-1 and IL-6, that are, in turn, involved in osteoclast differentiation signaling and bone resorbing activities. Thus, inflammatory osteolysis is the product of enhanced osteoclast recruitment and activation prompted by proinflammatory cytokines. The osteoclast, which is the sole bone-resorbing cell, is therefore central to the pathogenesis of inflammatory osteolysis. Understanding the mechanisms by which osteoclasts resorb bone and the cytokines that regulate their differentiation and activity, provides mechanism-based potential therapeutic targets to prevent inflammatory bone loss.

Osteoclastogenesis in physiological 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 NF- κ B ligand (RANKL), and macrophage colony-stimulating factor (M-CSF). [5] Mice genetically deficient in RANKL [6] or M-CSF [7] signaling do not form osteoclasts and thus develop the pathologic condition, osteopetrosis. 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, activating them to assume the osteoclast phenotype. [5] The production of RANKL is enhanced by osteoclast-stimulating agents such as parathyroid hormone. [8] M-CSF, which is also produced by marrow stromal cells and their derivative osteoblasts, supports the survival, proliferation and maturation of monocytes/macrophages to maintain a pool of osteoclast precursors in the bone marrow. Another important effect of M-CSF is to increase expression of RANK on the osteoclast precursors. Thus, regulation of RANKL expression is the key to the pathogenesis of osteolytic conditions.

Given that RANKL is the critical cytokine stimulating osteoclast differentiation, dissecting intracellular signals that follow RANK activation is of considerable interest. The known signaling events arising from ligation of RANK are similar to those of other members of the TNF receptor superfamily. RANKL activates NF- κ B signaling, the PI3K-Akt axis as well as all three MAPK pathways, namely ERK, JNK and p38. [9,10] While the Akt signaling pathway is crucial for survival of precursor and mature osteoclasts, the two events in RANK signaling critical for osteoclast differentiation are activation of both the NF- κ B complex and the transcription factor AP-1, a dimer composed of the c-fos and c-jun proteins, as demonstrated by gene-targeting studies. [11–14]

Osteoclasts resorb bone through a complicated mechanism. α v β 3 is probably the principle integrin mediating osteoclast function. This heterodimeric receptor is expressed when macrophage precursors commit to a bone resorptive phenotype. In the resorptive process, α v β 3 occupancy recruits the proto-oncogene c-Src, which activates, in turn, the tyrosine kinase Syk, the guanine nucleotide exchange factor Vav3, and the Rho GTPase Rac. Deletion of any of the aforementioned signaling molecules results in a disturbance of the osteoclast cytoskeleton and in bone-resorbing capacity. [15] These events occur in the context of the immunoreceptor, tyrosine-based, activation motif (ITAM) proteins Dap12 and

FcR γ . [16] Accordingly, in conjunction with ITAM-bearing proteins, Syk, c-Src, and $\alpha\text{v}\beta 3$ represent an essential signaling complex for the bone-resorbing osteoclast, and, therefore, each is a potential therapeutic target.

Another family of transcription factors, nuclear factors of activated T cells (NFAT) have recently been implicated in osteoclastogenesis. The distinguishing feature of NFAT is its regulation by Ca^{2+} and the Ca^{2+} /calmodulin-dependent serine phosphatase calcineurin. [17] Research to this purpose has demonstrated that ITAM proteins, when deleted in tandem, arrest terminal osteoclastogenesis due to failed expression of NFATc1. [18] The ITAM proteins Dap12 and FcR γ are stimulated by immunoreceptors TREM-2 and OSCAR, respectively, hence proving themselves crucial in osteoclastogenesis. [19,20] RANK activation also turns on the Ca^{2+} signaling pathway, including activation of calmodulin and its downstream effector, CaMKII. [21] Moreover, calmodulin appears to regulate osteoclast apoptosis through a mechanism involving interference with the death receptor Fas. [22] Consequently, NF- κ B, MAPK-activated transcription factors and the calcineurin-activated NFATs coordinately regulate osteoclast-specific gene transcription. These findings suggest that RANKL and M-CSF signaling pathways may not be sufficient for optimal osteoclastogenesis, and postulate the vital importance of the Ca^{2+} signaling cascade as the master regulators in osteoclast differentiation and function, and therefore position these molecules as potential anti-osteoclastic targets.

Strong evidence for a critical role of the transcription factors PU.1 and microphthalmia-associated transcription factor (MITF), a downstream target of p38 MAPK, in osteoclast differentiation is provided by the severe osteopetrotic and “osteoclastic phenotype” in mice lacking these genes. [23,24] Genetic analysis of MITF and PU.1 in mouse models support the critical interaction of these genes in osteoclast differentiation. [25] It appears that MITF, PU.1 and NFATc1, as nuclear effectors, may form a complex and integrate M-CSF/RANKL signals during osteoclast differentiation. [25,26] A schematic model of our current understanding of the integration of osteoclastogenic signaling pathways is illustrated in Figure 1.

The natural negative regulator of RANKL is the soluble molecule osteoprotegerin (OPG), which competes with RANK for RANKL as a “decoy” receptor. It, too, is a member of the TNF receptor superfamily and, like RANKL, is also expressed by osteoblast lineage cells. The importance of this molecule in bone homeostasis has been demonstrated by functional studies in transgenic and gene-deficient mice. [27,28] Importantly, the relative levels of RANKL and OPG are likely to be the key determinants of the rate of osteoclast formation *in vivo*. [29] In addition, it has been reported that interferon- β , a cytokine produced by RANKL in osteoclast precursors via c-Fos activation, inhibits osteoclast differentiation in an autocrine fashion. [30]

Osteoclastogenesis in inflammatory conditions

In states of juxta-articular inflammation such as RA, the osteoclastogenic molecule RANKL is also produced in abundance by activated T lymphocytes and synovial fibroblasts, [31,32] whether they are in joint or bone. RANKL may also be cleaved from the cell membrane and then interact with RANK as a soluble ligand. Additionally, these same activated synovial fibroblasts also express M-CSF and OPG. [33] Subsequently, blocking of RANKL with OPG at the onset of induced RA in mice prevents bone and cartilage destruction but had little effect on inflammation. [34] As mentioned, the RANKL/OPG ratio seems to dictate the rate of bone resorption in a variety of pathologic conditions and, in fact, many of the same resorptive agents enhance RANKL expression while concurrently suppressing OPG production. [29]

Production of proinflammatory cytokines plays a vital role in the pathogenesis of inflammatory osteolysis. TNF- α is probably the dominant cytokine extant in these conditions, and is the rate-limiting factor in that its blockade eliminates both the inflammatory and osteoclastogenic components of these diseases. [35–39] It is primarily produced by activated T cells, macrophages and synoviocytes.

TNF- α targets two distinct cell membrane receptors: type I receptor (p55) and type II receptor (p75) that are responsible for the divergent actions of this inflammatory molecule. However, the osteoclastogenic properties of TNF- α are mediated via the type I receptor. [15] TNF- α induces osteoclastogenesis at multiple levels. TNF- α acts on stromal and osteoblastic cells to enhance RANKL expression, a fundamental component of inflammatory osteolysis. [15,40] On the other hand, TNF- α also directly stimulates osteoclast recruitment, characterized by enhanced RANK expression and sensitization of precursor cells to RANKL. [41] While TNF-induced osteoclastogenesis requires at least constitutive levels of RANKL, a synergistic relationship exists between the two cytokines in directly inducing marrow macrophages to commit to the osteoclast phenotype. [42] Although others argue that the inflammatory cytokine is capable of osteoclastogenesis in the absence of RANK signaling *in vitro*, [43] TNF- α fails to induce meaningful osteoclastogenesis in RANK deficient mice. [44] Moreover, RANK-independent osteoclast formation was not found in an animal model of wear debris-induced osteolysis. [45] Importantly, TNF- α also contributes to inflammatory bone loss by enhancing osteoclastogenesis through a mechanism of activation of PIR-A, a costimulatory receptor for RANK, as demonstrated in an animal model. [46]

It is worth noting that TNF-induced inflammatory osteolysis is also mediated by the stromal cell-produced osteoclastogenic cytokine M-CSF. [47] Anti-c-Fms (M-CSF receptor) monoclonal antibody has been shown to selectively and completely arrest the profound pathological osteoclastogenesis in a mouse model demonstrating this condition. This same antibody also completely arrested osteolysis in TNF-injected mice with a nominal effect on macrophage number, confirming that inhibition of the M-CSF signaling pathway targets TNF- α . Therefore, M-CSF and its receptor, c-Fms, also present themselves as candidate therapeutic targets.

IL-1 also plays an essential role in the pathophysiology of inflammatory bone loss. [48–51] 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 , interferons- α , - β and - γ , and IL-1 itself. IL-1 exerts its biological effects by forming a complex with type I IL-1 receptor (IL-1RI) and IL-1R accessory protein (IL-1RAcP). IL-1RAcP, which has no affinity on its own, is recruited to the IL-1/IL-1RI complex and binds with high affinity and, subsequently, induces signal transduction. In contrast, type II IL-1 receptor (IL-1RII) lacks a cytoplasmic tail and, therefore, acts exclusively as a decoy receptor. A unique aspect of IL-1 biology is the naturally occurring soluble IL-1 receptor antagonist, IL-1Ra. IL-1Ra is structurally similar to IL-1 β and is capable of binding to IL-1RI with a complete blockade of receptor activation. [52, 53]

Reflecting sequential signaling of the cytokines TNF- α and IL-1, TNF- α induces stromal cell expression of IL-1 and IL-1RI. IL-1 mediates a substantial portion, but not all, of TNF- α -induced osteoclast recruitment in inflammatory osteoclastogenesis. [51] IL-1 stimulates RANKL production by marrow stromal cells and, in the context of constitutive RANKL, directly promotes osteoclast precursor differentiation in a TNF-independent manner. Thus, whereas IL-1 is downstream of TNF- α in the osteoclastogenic process, the reciprocal does not occur. In macrophages, the functional receptor of IL-1 is upregulated by RANKL, TNF- α and IL-1 itself, while its decoy receptor, IL-1RII, is suppressed by RANKL. IL-1 by itself

also promotes the bone-resorbing activity of osteoclasts. [54] Attesting to their interdependence, blockade of either TNF- α or IL-1 does not completely arrest the periarticular damage of inflammatory arthritis, whereas inhibition of the two cytokines in combination is substantially more effective. [50] Thus, the interdependence of TNF- α , RANKL and IL-1 in the generation of osteoclasts lends credence to the observation that combined blockade is most effective in preventing pathological bone loss in the inflammatory conditions.

It is notably important that p38 mitogen-activated protein kinase (MAPK) plays a crucial role in mediating osteoclastogenesis in inflammatory conditions. Activation of the p38 MAPK occurs within the synoviocytes embedded in 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. [55] The p38 MAPK, most notably its p38 α isoform, is activated mainly within cells involved in the inflammatory process. [56, 57] Activation of p38 induces synthesis of key inflammatory mediators such as TNF- α , IL1, IL6, IL8 and COX2, either via direct activation of gene transcription or via mRNA stabilization.[58–63] In addition, p38 mediates the synthesis of other compounds involved in inflammation, including chemokines and adhesion molecules; the metalloproteinases responsible for cartilage breakdown; and prostaglandins. [56, 64] In the context of bone erosion, p38 mediates TNF- α -induced IL-1 upregulation in stromal cells, and the subsequent IL-1-induced RANKL production. In macrophages, on the other hand, the synthesis of IL-1 functional receptor by either TNF- α , IL-1 and RANKL is mediated via p38. [51]

A number of other cytokines and inflammatory mediators have been shown to be able to stimulate osteoclast formation and bone resorption, both *in vivo* and *in vitro*. Among the cytokines are IL-6, IL-11, IL-17, leukemia inhibitory factor (LIF), and oncostatin M (OSM). Each may contribute to the bone loss in states of inflammation. On the other hand, other cytokines, including IL-4, IL-10, IL-12, IL-13, IL-18 and interferon- γ , in addition to OPG and interferon- β , appear to counteract the bone-resorbing factors by inhibiting osteoclast formation and/or periosteal bone resorption, either *in vitro* or *in vivo*, or both. [10,65] The effect of IL-7 in regulating osteoclast formation is controversial, since both stimulation and inhibition have been recognized. [66,67] Again, it is likely the balance between the stimulatory and inhibitory cytokines, which may in part translate into the RANKL/OPG ratio, is the determinant of the degree of osteoclastogenesis and subsequent bone loss. Mechanisms of RANKL over-production and the key factors and their pathways involved in regulating osteoclastogenesis in states of inflammation are summarized in Figures 2 and 3.

Current and potential future therapeutic targets for inflammatory osteolysis

The recent advances in basic research in understanding the mechanisms of inflammatory osteolysis lead to two major treatment approaches: anti-inflammatory agents and bone resorption-blocking agents.

Anti-inflammatory agents

The prominent role of TNF- α in driving inflammation has made it a target for biology-based therapeutics currently used for treatment of inflammatory osteolysis. There is also genetic evidence for a role for TNF- α in humans in the development of osteolysis after total hip arthroplasty. [68] As noted, IL-1 has been implicated as an important cytokine in these conditions. Its close link to TNF- α makes it another potential target (Figs. 2 and 3). The biotherapeutic targets of proinflammatory cytokines largely fall into two categories. The first group includes antibodies that bind specifically to a cytokine or cytokine receptor; and the second group is composed of soluble receptors that capture a cytokine before it binds to the cell membrane receptor. Numerous clinical trials of TNF-blocking agents have been

performed in early and late-stage RA and with different severity groups. [69] Representative therapeutic drugs in this group are Infliximab® and Adalimumab® (anti-TNF- α monoclonal antibodies), Etanercept® (TNF receptor-p75 Fc fusion protein). The efficacy of these agents, combined with Methotrexate, appear to be comparable although a randomized study has not yet been performed and is unlikely. [70]

Other recent FDA-approved therapeutic agents for RA include Abatacept® and Rituximab®. Abatacept® is the first member of a new class of drugs known as costimulatory blocking modulators. This class of molecules selectively modulate the CD80 or CD86-CD28 costimulatory signal required for full T-cell activation [71, 72] and, therefore, act upstream in the inflammatory cascade. Abatacept® has been shown to be effective in combination with Methotrexate and produce significant clinical and functional benefits in patients with RA who had had an inadequate response to anti-TNF- α therapy. [73, 74] However, the possibility of an increased risk of lung cancer has been suggested in patients receiving this therapy, markedly decreasing any attractiveness of this approach. [70] Rituximab® is a chimeric anti-CD20 monoclonal antibody approved for treatment of non-Hodgkin's lymphoma for many years. Recent clinical trials suggest that the combination of Rituximab® with Methotrexate yields superior efficacy when compared with monotherapy for RA. [75–77]

Anakinra® (an IL-1 receptor antagonist) has also been demonstrated to be effective in RA, but its specific place (i.e. before or after giving a TNF-blocking agent) is not yet defined. [70] Recently, a meta-analysis has been performed to adjust for differences between study characteristics, allowing indirect comparisons between treatments. [78] The results of this study suggest that TNF-antagonist treatment is more efficacious when compared to an IL-1 antagonist which, in turn, is better than placebo. TNF- α antagonists, as a class, appear to be no different from one to the other. Interestingly, despite the theoretical attractiveness of combining Anakinra® with an anti-TNF- α agent, this approach has not shown itself to be superior to anti-TNF- α therapy alone in patients with RA who have been unsuccessfully treated with Methotrexate. [74]

Novel therapeutic agents in this group are emerging on an ongoing basis. Interleukin-6 (IL-6) is a pleiotropic cytokine that regulates the immune response, inflammation, hematopoiesis and bone metabolism, and is constitutively overproduced in RA. This molecule is capable of inducing osteoclast formation and is thought to be responsible for joint destruction associated with RA. [79] Anti-IL-6 receptor monoclonal antibody (Tocilizumab®) has been tested in randomized controlled trials with promising results. [80, 81] Other targeting cytokines beyond TNF- α and IL-1 include IL-12 and IL-15 with continued efforts ongoing. [82, 83]

Another pathway by which inflammation can be sustained involves toll-like receptors (TLRs), recently identified as the receptors recognizing self versus non-self molecular patterns and a primary source of proinflammatory signals, thus serving as a link between the body's innate and adaptive immune response activities. TLR 2 and 9 are highly expressed on synovial fibroblasts and lymphocytes from patients with RA. [84, 85] These receptors can be activated by a wide range of molecules released in damaged synovial tissues, i.e., degraded extracellular matrix, antibodies, necrotic cell products, and heat-shock proteins, suggesting the therapeutic potential of TLR signaling blockade for RA. [86] Recently, the therapeutic potential of heat-shock protein 10, a molecule that inhibits TLRs 2 and 4, in patients with RA has been described in a double-blind, randomized trials with a small number of patients, with results similar to those of cytokine blockade with mild adverse effects. [87] Although the optimal dose range and route of administration needs to be identified, this preliminary study offers exciting potential future possibilities for therapy.

Bone resorption-blocking agents

The central role of RANKL in osteoclastogenesis makes this molecule an attractive therapeutic target for osteolytic conditions. Denosumab® (formerly known as AMG 162), a monoclonal antibody targeted against a RANKL epitope, appears to have a potent effect in increasing bone mineral density and decreasing bone resorption in postmenopausal women with low bone mass. [88] This class of molecules is likely to become the treatment of choice for blocking RANKL in inflammation-associated bone loss, yet their anti-osteolytic effect has yet to be fully assessed. AMG-0007, a recombinant OPG, has been shown to effectively suppress bone resorption with good tolerance in multiple myeloma and breast carcinoma patients in a phase I study. [89] However, the fact that OPG also binds to TNF-related apoptosis-inducing ligand (TRAIL) raises the issue of undesirable side effects of OPG-based drugs, because the cytokine may function as a survival factor for cancer cells. [90] Osteoclast cytoskeleton molecules, i.e., c-Src, are also currently anti-resorptive therapeutic targets under drug development. [91] Other potential osteoclast-specific targets include inhibitors for cathepsin K, [92] the osteoclast ATPase proton pump [93] and integrin $\alpha_v\beta_3$. [94–95] The immunoreceptor-calcium signal-NFAT and other costimulatory receptor pathways, as the master regulators of osteoclastogenesis, also opens a new avenue in therapeutic targeting for anti-bone resorbing drugs. [21, 46] Although the RANK-RANKL interaction in the formation of osteoclasts is an excellent therapeutic target for osteolysis, anti-inflammatory effects, if any, have not appeared in this class of drugs. Therefore, an ideal approach would be the combination of anti-inflammatory and anti-resorptive agents, which should lead to optimal remission of disease, as demonstrated in an arthritis model. [50]

Bisphosphonates are a class of molecules with a similar chemical structure to that of inorganic pyrophosphate and bind strongly to mineral crystals. Despite the extensive use of this class of drugs in the treatment of metabolic bone diseases associated with increased osteoclast activity, the precise mechanism responsible for their antiresorptive action remains poorly understood. A number of studies report reduced bone loss using bisphosphonates in animal models of conditions associated with inflammatory osteolysis. [96, 98] Although results from the few studies in humans have been disappointing, new-generation drugs have rekindled the hope that bisphosphonates may be beneficial, and meaningful clinical trials of such therapies are need for patients with these conditions. [99]

Small molecule anti-cytokine agents

All of the anti-cytokine 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. [100] The therapeutic strategy is, therefore, to find small compounds that mimic the anti-cytokine effects of these drugs yet evade breakdown in the digestive tract. Research to that end has been largely focused on seeking specific inhibitors to the intracellular signal transduction pathway(s) induced when ligands (cytokines) bind to their membrane receptors. While a number of molecular targets have been identified for the development of such small molecular agents, p38 occupies a central role in the regulation of IL-1, in the TNF- α signaling network and in RANKL-induced osteoclastic bone resorption as previously described. [57, 100, 101] Consequently, creating novel p38 inhibitors has two main goals theoretically: (1) to prevent the synthesis of these cytokines rather than neutralize them after they are produced; (2) to block some effects produced by TNF- α , IL-1 or other inflammatory mediators. As a result, p38 inhibitors have a potential competitive advantage over the current anti-TNF- α or anti-IL-1 biologics. [57] Because 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. [102]

In recent studies in models of experimental arthritis, p38 inhibitors have been demonstrated to significantly improve clinical signs, cytokine production and articular cartilage damage. More importantly, bone destruction was almost completely prevented or reversed by p38 inhibition. These data impressively demonstrated the major importance of p38 for TNF- α -mediated bone destruction and suggest that inhibition of p38 might be an important tool for reducing structural damage within the inflammatory environment. [103, 104] To date, numerous p38 inhibitors have been characterized, of which several compounds have been advanced in clinical trials. [101]

Although the rationale for p38 blockade is quite clear, the critical issues that have interfered with the development of p38 inhibitors are preclinical and clinical toxicities, i.e., an unusual inflammatory condition of the central nervous system in animals and hepatotoxicity in humans. [105] Second generation p38 inhibitors that are unable to cross the blood-brain barrier are currently under development. Furthermore, because p38 is essential for many processes in most cell types, it will be necessary to develop p38 inhibitors that have no or little effect on other signaling pathways, which may become an insurmountable problem.

Gene therapy as an alternative approach

Although anti-inflammatory agents are capable of ameliorating disease in a majority of patients with arthritis, these biologics must be given systemically in relatively high dosages to achieve constant therapeutic levels in the joints, which result in undesirable effects. On the other hand, systemic therapies targeting osteoclasts have a common concern - disturbing normal bone remodeling activity and thus adversely affecting the mechanical quality of bone. Research to this end has led to an alternative approach using a local gene-transfer technique to achieve more efficient and sustained delivery of inhibitors of inflammatory cytokines as well as other therapeutic agents, thus minimizing systemic exposure. Several gene therapy approaches have been established in preclinical animal models, using retroviral-based vectors and adeno-associated virus vectors. [106–108] The therapeutic targets include all of, but are not limited to, the previously cited molecules involved in the process leading to inflammatory bone loss. In light of the success of TNF- α and IL-1 blockade in clinical practice, inhibition of these molecules is still considered to be promising for future gene therapy approaches in these conditions. A number of cell types have been evaluated as potential delivery vehicles, included among these are antigen-specific T cells and dendritic cells, as well as synoviocytes. Ideally, mesenchymal stem cells may provide a new cell-mediated therapy as these cells are capable of differentiating into cartilage and bone and have been proposed as potent therapeutic means for tissue repair in arthritic disease. [109] However, it will be necessary to develop strategies to regulate their differentiation pathway *in vivo* as well as assuring their homing to areas of bone erosion for appropriate repair. [106] Although evidence in animal models convincingly supports the application of local gene therapy in these diseases, the potential risk of some viral vectors as illustrated by fatal systemic inflammation [110, 111] and the development of leukemia in X-SCID patients [112] have underscored the need for caution in adopting uncritically this attractive therapeutic strategy. Further extensive studies remain necessary to fully assess the coalition of cell-specific targeting, vector development, and disease-related transgene expression to optimize efficacy and safety.

Conclusions

Patients with RA and other inflammatory bone diseases face complications of joint destruction. The differentiation and function of osteoclasts, the culprits in these closely associated conditions, are accelerated under the control of cytokines produced in the inflammatory environment, in which TNF- α , IL-1 and RANKL play a central role. Understanding this complex process has been helpful in the clinical translation from mechanism to drug development. The approach to combine anti-inflammatory agents and osteoclast-targeted therapies should theoretically achieve optimal control of these diseases. Small molecular anti-cytokine agents and local gene transfer techniques may also provide potential alternatives in preventing inflammation and bone loss. However, investigation of these matters has only just begun. The exciting time in bone biology leads to numerous new potential drug targets and techniques, which are currently under development and are impossible to be covered in any limited review. The current clinical decision-making pathway largely depends upon the degree of disease, drug tolerance and reimbursement issues. Additional biological and clinical markers will also be needed to reliably access the responsiveness to any particular therapeutic modality now and in the future.

Acknowledgments

Supported by NIH grants CA93796, CA098543, AR046031, and the Haley's Hope Memorial Support Fund for Osteosarcoma Research at the University of Alabama at Birmingham, Birmingham, Alabama.

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Fig. 1. Schematic model of integration in osteoclastogenic pathways

Activation of the M-CSF receptor c-Fms promotes proliferation and survival of osteoclast precursors. RANK stimulation leads to activation of NF- κ B signaling, the PI3K-Akt axis as well as all three MAPK pathways, namely ERK, JNK and p38. While the Akt signaling pathway is crucial for survival of precursor and mature osteoclasts, the activation of both the NF- κ B complex and the transcription factor AP-1 is critical for osteoclast differentiation. Phosphorylation of ITAM stimulated by immunoreceptors leads to the activation of calcium signaling, an event also induced by RANK activation, which is critical for NFAT induction. NFAT may interact with other transcription factors induced by MAPK, MITF and PU.1, thus acting as nuclear effectors and leading to gene expression and optimal osteoclastogenesis.

Fig. 2. Mechanisms of RANKL over-production in inflammatory osteolysis

Signaling through p38 MAPK, TNF- α induces stromal-cell synthesis of IL-1, which upregulates its own functional receptor (IL-1RI) in a p38-dependent fashion, as well as other inflammatory cytokines (not shown). Occupancy of now abundant IL-1RI similarly activates p38, which promotes RANKL production. Activated T cells and synovial fibroblasts provide additional sources of RANKL in arthritis. Activation of TNF receptor stimulates the expression of M-CSF by stromal cells/osteoblasts and synovial fibroblasts.

Fig. 3. Important factors and their pathways involved in regulating osteoclastogenesis in states of inflammation

In macrophages, TNF- α enhances the synthesis of proinflammatory cytokines including IL-1, whose functional receptor is upregulated by the three key cytokines, each in a p38-dependent manner. P38 mediates the osteoclastogenic effects of these cytokines in different stages of the lineage. M-CSF upregulates its own receptor, c-Fms, and RANK in early stage of osteoclast differentiation.