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Review Article

Hylonome

RANK, RANKL and OPG in inflammatory arthritis and periprosthetic osteolysis

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Abstract

Elucidation of the receptor activator of nuclear factor kappa B (RANK), its ligand (RANKL) and osteoprotegerin (OPG) as the final effectors of bone resorption has transformed our understanding of metabolic bone diseases and revealed novel therapeutic targets. Activation of the RANK-RANKL signaling pathway is directly responsible for dramatic focal erosions that are observed in inflammatory arthritis and aseptic loosening of orthopaedic implants. While these conditions share many features common to all metabolic bone disorders (e.g., osteoclastic resorption), they exhibit several unique properties, which are highlighted in this review. Most important is the relative inability of bisphosphonate therapy to inhibit osteolysis in joint inflammation and periprosthetic joint loosening and the unexpected effectiveness of anti-cytokine therapy in both rheumatoid and psoriatic arthritis. Herein, we provide a review of the role of RANK, RANKL and OPG in erosive arthritis and periprosthetic osteolysis and discuss the potential of anti-RANKL therapy for these conditions.

Keywords: Rheumatoid Arthritis (RA), Psoriatic Arthritis (PsA), Aseptic Loosening, Tumor Necrosis Factor-alpha (TNFα), Osteoclast Precursors (OCP)

Erosive arthritis

Rheumatoid arthritis (RA) is an autoimmune disease that affects approximately 1% of the population and is characterized by joint pain, stiffness and inflammation[1,2]. Although the precise etiology of the disease remains elusive, various genetic, epigenetic and environmental factors are thought to play a role in the establishment and progression of RA.

It is now firmly established that the pro-inflammatory cytokine, tumor necrosis factor (TNF), is a central mediator of inflammation and matrix destruction in RA[3]. Although this review focuses on the role of RANK, RANKL and OPG in joint erosions, no discussion of RA would be complete without highlighting the remarkable success of anti-TNF therapy in the treatment of rheumatoid and psoriatic arthritis. For their seminal role in defining TNF at the apex of the pro-inflammatory cytokine cascade and their pioneering work in anti-TNF clinical trials, Drs. M. Feldmann and R.N. Maini received the 2003 Lasker Clinical Medical Research Award[4]. Beyond these specific achievements, the success of anti-TNF therapy via injection of a recombinant soluble receptor (etanercept) or antibody (infliximab, adalimumab) personifies the ultimate goal of biotechnology and dramatically underscores how complex autoimmune diseases can be effectively suppressed using a targeted therapeutic approach.

A central feature of the RA is the highly osteodestructive process, which leads to three forms of bone loss: i) focal bone loss at the joint margins and in underlying subchondral bone (periarticular osteopenia); ii) localized resorption at the site of synovial attachment to bone (erosions); and iii) generalized osteoporosis involving the axial and appendicular skeleton[5]. Of particular interest is the local bone erosion because this radiographic manifestation reflects underlying disease activity, is a key outcome measure, and is associated with an unfavorable prognosis[6-8].

Anton Weichselbaum first described the focal bone erosions in RA joints as "caries of the joint ends" in 1878[9]. The histopathology of bone erosions is unique in that the lesions

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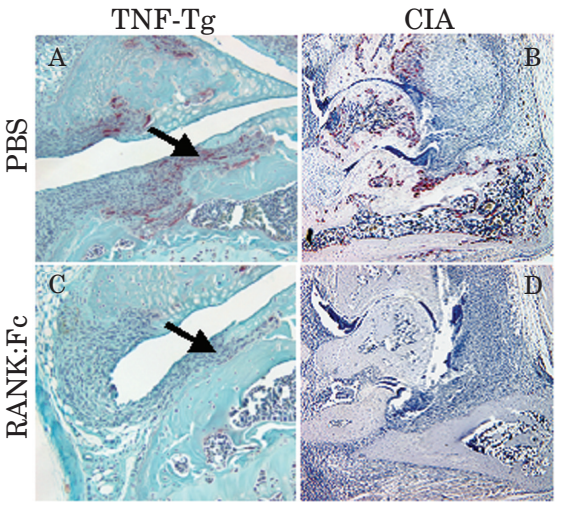

Figure 1. Efficacy of RANK blockade in mouse models of inflammatory arthritis. TNF-Tg mice (A+C) or mice with CIA (B+D) were treated with PBS (A+B) or high dose RANK:Fc (C+D). Histochemistry was performed on the knee joints (A+C) or ankles (B+D) by staining for TRAP (red). Arrows indicate the leading edge of the pannus, which mediates irreversible joint damage via subarticular erosions.

are eccentric and they emanate from the junction zone, where the bone, cartilage and synovial membrane are attached (Figure 1). Joint erosion is driven by the inflammatory synovial tissue or 'pannus', a hyperplastic, locally invasive tissue comprised of fibroblasts, monocytes and T lymphocytes, mast cells and numerous blood vessels. These cells produce vast array of inflammatory mediators, including cytokines (tumor necrosis factor (TNF)), interleukins (IL-1, IL-6, IL-17), prostaglandins (PGE2), reactive oxygen species (O_2^-, NO^-), and matrix metalloproteinases (MMPs) that destroy the extracellular matrix in the joint by direct and indirect mechanisms. The pannus is extremely vascular, providing portals of entry for effector cells to enter the joint from the circulation and perpetuate joint destruction via autocrine and paracrine pathways.

Remarkably, it took more than a century for investigators to formally characterize the chondroclasts and osteoclasts positioned at the leading edge of the pannus tissue. These effector cells arise from different lineages and mediate focal bone erosions in the RA joint[10]. In seminal studies, Bromley and Woolley noted the very aggressive nature of these rapidly eroding lesions and they highlighted the presence of a bi-directional attack on the cartilage and bone[11], whereby the invading pannus drives "outside-in" erosions and cutting cones arising in the bone marrow erupt through the subchondral bone to cause "inside-out" erosions. Subsequent studies by Gravallese and colleagues further characterized the presence of osteoclast precursors (OCP) and mature osteoclasts within resorption lacunae of local bone erosions by morphological features and molecular phenotype[12]. From these central studies, the theory that osteoclasts are responsible for bone erosions in RA achieved broad acceptance.

RANK, RANKL and OPG in RA

Following the discovery that osteoclastogenesis and bone turnover are ultimately regulated by the expression of the osteoclast differentiation factor, RANKL vs its soluble inhibitor OPG[13,14], researchers began to investigate the expression of these molecules in tissues from RA patients. These early studies revealed that RANKL mRNA was detected by RT-PCR in whole synovial tissues from patients with RA but not in synovial tissues isolated from healthy controls. RANKL was also detected in cultured adherent synovial fibroblasts and activated T lymphocytes derived from RA synovial tissue[15]. In a parallel study, Takayanagi et al found that RANKL mRNA was highly expressed in all tissues from RA patients, but not from patients with osteoarthritis (OA)[16]. They also demonstrated that cultured rheumatoid synovial fibroblasts efficiently induce osteoclastogenesis in the presence of vitamin D, via up-regulation of RANKL and decreased OPG expression. In these studies, osteoclastogenesis was inhibited by OPG in a dose-dependent manner.

Several factors have been identified that increase the ratio of RANKL to OPG expression tipping the balance in favor of osteolysis but TNF has emerged as a dominant regulator. TNF directly stimulates RANKL production by stromal cells[17], T lymphocytes[18], B lymphocytes[19], and endothelial cells[20]. TNF also induces the expression of macrophage-colony stimulatory factor (M-CSF) by stromal cells[21], which is the only other obligatory signal for osteoclastogenesis. TNF can promote RANKL expression by indirect mechanisms as well. For example, TNF-induced upregulation of prostaglandins, IL-1 or IL-17 can result in enhanced expression of RANKL[22-24].

Psoriatic arthritis

Joint damage is also very common in psoriatic arthritis (PsA), an inflammatory joint disease that occurs in 10-15% of psoriasis patients[25]. Gladman and colleagues noted that two-thirds of PsA patients manifest bone erosions radiographically on initial presentation to a rheumatologist[26]. Of all the known arthropathies, PsA lesions are renowned for their marked destruction of cartilage and bone, particularly the arthritis mutilans subset reported in 16% of PsA patients[26]. PsA joints often show extensive bone loss manifesting as eccentric erosions, frank tuft resorption and pencil-in cup deformities[27]. Histopathologically, many PsA patients have aggressive synovitis with marked synovial hyperplasia, extensive vascular proliferation with a tortuous morphology and pannus tissue penetrating deep into cartilage and bone[28]. In addition, osteoclasts are prominently situated at the bone-pannus junction and in bone marrow-

Model	Effect on Inflammation	Effect on Bone Erosion	Effect on Cartilage
Adjuvant arthritis in rats, OPG (1 mg/kg/day)[31]	–	+++	+++
K/BxN (serum transfer) in RANKL-/- mice[32]	–	+++	+
CIA in rats, OPG (3 mg/kg/day)[34]	–	++	+
CIA in mice, RANK:Fc (10 mg/kg/48hr)[36]	–	+++	+
hTNF-Tg mice, OPG (6.4 mg/kg/day)[37]	–	++	–
hTNF-Tg mice, RANK:Fc (10 mg/kg/48hr)[40]	–	+++	+
hTNF-Tg x cFos-/- mice[38]	–	+++	–
hTNF-Tg x RANK-/- mice[40]	++	+++	+++

Table 1. Role of RANK signaling in animal models of inflammatory arthritis.

derived cutting cones traversing the bone matrix[29].

Immunohistochemistry revealed the very striking spatial regulation of RANK, RANKL and OPG expression in PsA joints[29]. OPG expression was restricted to endothelial cells within the sub-synovial lining of the pannus. In contrast, intense RANKL immunoreactivity was identified in the outer lining of the synovium, where RANK positive monocytes, presumably osteoclast precursors (OCP) are also present. At the erosion front of the pannus, RANK positive multinucleated osteoclasts are found in resorption lacunae. Our interpretation of these data is that "outside-in" erosions occur as a result of OCP recruitment into the joint via the blood vessels in the synovium, where osteoclastogenesis is strongly inhibited by OPG. In response to the chemotactic cytokine stromal cell-derived factor-1 (SDF-1)[30], the OCPs migrate towards the RANKL-rich environment of the synovial lining, where they differentiate into active osteoclasts at the erosion front. In contrast, "inside-out" erosions are essentially void of all cell types except RANK positive monocytes and osteoclasts, and stromal cells. Presumably, these cells arise from precursors in the subchondral bone.

Lessons from animal models

Over the last decade, no technology has been more valuable to study the molecular pathogenesis of mammalian disease than genetically manipulated mice. By combining transgenic animals with highly selective protein inhibitors (antibodies and soluble receptors), rigorous *in vivo* gain and loss of function studies can be performed to elucidate the role of a particular gene in a disease state. Using established gene cloning methodologies and transgenic mouse models, investigators faithfully fulfilled Koch's postulates providing firm evidence that the RANK-RANKL signaling pathway is critical for osteoclast formation and bone resorption[14].

Currently there are several well-established rodent models of inflammatory-erosive arthritis; and the roles of RANK, RANKL and OPG have been investigated in many of them. These include adjuvant arthritis[31], serum transfer[32], collagen-induced arthritis[33-36] and the TNF-transgenic mouse[37-40]. Immunohistochemistry and *in situ* hybridization studies revealed similar findings to the human studies described above. More importantly, genetic ablation and *in vivo* blockade experiments unequivocally demonstrated that RANK signaling is required for the genesis and progression of erosive arthritis. The results of these studies are summarized in Table 1 and can be visualized in Figure 1. In all cases, disruption of RANK signaling significantly inhibited osteoclast formation and/or induced osteoclast apoptosis, and prevented or inhibited erosion of cartilage and bone. The importance of NFκB and AP-1 signaling immediately downstream of this RANK signal has also been confirmed in knockout mice[14], as both signals are required for osteoclast formation and bone resorption. Thus, these pathways also serve as attractive targets of therapeutic intervention and are under extensive investigation. In contrast, no published study has demonstrated a significant role for this pathway in synovial inflammation, indicating that these two events are now separable at the molecular level. The view that inflammation and matrix destruction can be approached independently may have important therapeutic implications because pushing the doses of anti-inflammatory agents to lessen joint destruction can be immunosuppressive resulting in an increased risk for bacterial and opportunistic infections[41].

Osteoclast Precursor (OCP) Frequency as a marker of erosive arthritis

One of the critical questions faced by physicians treating patients with RA is how aggressive to be with therapy in patients who present early in the disease course. In our preclinical[40,42] and clinical[29] studies of TNF and RANK signaling in erosive arthritis we made several interesting observations indicating that OCP frequency in the blood may be a mark-

er of erosive disease including: i) TNF induces the release of CD11b+ OCP from the bone marrow into the circulation resulting in a significant increase in OCP frequency, by a mechanism independent of RANK signaling. ii) The frequency of circulating OCP correlated with the presence of erosive disease and, iii) anti-TNF therapy swiftly and dramatically reduced OCP frequency. Future studies designed to elucidate the mechanism of OCP release from the bone marrow, to fully characterize the surface marker phenotype of these OCPs and to better understand how they home to sites of focal erosions are underway. Ultimately, OCPs may be an important biomarker used to predict which patients with early RA are at greatest risk for joint damage and would benefit from aggressive therapeutic interventions.

The current frontier: chondroprotection and bisphosphonates

A central controversy in the field is the great disparity in findings regarding the chondroprotective effects of RANK blockade observed in the animal studies outlined in Table 1. Some studies documented that the articular surface of inflamed joints with extensive pannus were protected from erosion and proteoglycan loss as a result of RANK blockade, while other studies failed to observe these effects. The discrepancy in these studies is confounded by our lack of knowledge of the role of RANK and RANKL signaling in cartilage. Although the expression of RANK and RANKL in articular cartilage has been documented[35,43,44], experiments designed to demonstrate a functional role for these molecules in chondrocytes have failed to produce significant findings.

Another major controversy centers on whether bisphosphonates inhibit bone resorption in erosive arthritis. Based on the consistent efficacy of bisphosphonates in osteoporosis and the ability of these drugs to induce osteoclast apoptosis, scientists and clinicians predicted that they would be effective in preventing bone erosions in inflammatory arthritis. Although only a few clinical trials exploring this hypothesis have been published, the data were largely negative[45-47]. In more recent studies, investigators have demonstrated that etidronate therapy did not prevent radiologic progression in patients with RA[48], while others reported that etidronate significantly decreased Larsen damage scores in RA[49]. In both studies etidronate decreased serum markers of bone turnover, suggesting a favorable effect on osteoporosis. The unimpressive results observed in clinical trials are consistent with animal studies that showed limited effects on erosion and osteoclast apoptosis in TNF-induced arthritis[37]. In our *in vitro* experiments, we found that TNF protects osteoclasts from alendronate-induced apoptosis *in vitro*[36]. We also observed that alendronate (10 mg/kg/day i.v. for 3 days) effectively induces OC apoptosis in the growth plate of hTNF-Tg mice, but OC in direct contact with synoviocytes at sites of focal bone erosion were unaffected. One explanation for these findings is that inflammatory cells residing in the pannus tissue deliver anti-apoptotic signals to osteoclasts at the bone-pannus junction but not to those cells located in the growth plate. Collectively, these studies suggest that standard regimens of first generation bisphosphonates were largely ineffective in preventing focal bone erosions in inflammatory arthritis. Future trials with more potent bisphosphonates at higher doses than traditionally prescribed in osteoporosis are warranted to determine if these agents can significantly retard osteolysis in the inflamed joint.

Aseptic loosening of total joint replacements

Unfortunately, a common outcome of irreversible joint destruction from arthritis, trauma, cancer or avascular necrosis is loss of function and debilitating pain which can be alleviated by total joint replacement (TJR). Current estimates indicate that there are approximately 1.5 million TJR surgeries performed each year[50]. The vast majority of procedures are for patients with severe osteoarthritis (OA) of the hip and knee. While TJR is considered to be one of the most successful surgical procedures in all of medicine, long-term outcomes are often limited by a condition known as "aseptic loosening". This condition, which takes 5-10 years to develop, is caused by chronic osteoclastic bone resorption around the implant until fixation is lost[51]. The current paradigm to explain aseptic loosening involves an inflammatory response to the wear debris particles produced by prosthetic implants[52-55]. These wear debris particles are phagocytosed by macrophages adjacent to the implant resulting in cell activation and the release of a diverse array of cytokines. This localized inflammatory response leads to the formation of a periprosthetic membrane with features similar to the synovitis of RA and PsA. Of particular interest, is the presence of osteoclasts that resorb bone at the bone-implant interface resulting in periprosthetic loosening.

RANK, RANKL and OPG in periprosthetic membranes

Periprosthetic membranes retrieved from patients with loose implants contain fibroblasts, macrophages, and a small number of T lymphocytes[56]. As many as 10^9 particles per gram of tissue can be recovered from periprosthetic membranes that are recovered during revision surgery[53,57]. The inflamed tissues produce a variety of factors including TNFα, IL-1, IL-6, prostaglandin, and peptides that stimulate osteoclasts to resorb bone through the induction of RANKL[56,58-61]. Since macrophages are the chief phagocytic cell ingesting wear debris particles, much attention has been focused on their role in cytokine production and osteoclast activation[62-64]. Indeed, macrophages located in the periprosthetic membrane are also OCP and, *in vitro* they differentiate into osteoclasts in response to: i) M-CSF and stromal cell derived factors[65], ii) RANKL alone[66], and iii) TNF and IL-1 in the absence of RANKL[67]. Immunohistochemistry and *in situ* hybridization studies of periprosthetic membranes indi-

cate that macrophages are a source of RANKL in these tissues[68,69]. Stromal cells and fibroblasts are also known to express this factor[70]. Formal proof demonstrating the cellular source of the functional RANKL involved in periprosthetic osteolysis remains to be demonstrated.

Wear debris-induced osteolysis *in vivo*

While *in vitro* and *in situ* studies provide important information on the biology of aseptic loosening, *in vivo* experiments are critical to determine the true function of a specific pathway in this process. To this end, rat[71,72] and canine[73] animal models have been developed to study wear debris-induced osteolysis. However, due to the availability of genetically defined mouse strains and the wealth of molecular probes, murine models have been most widely adopted[74-77]. Experimental studies in transgenic and knockout mice support the concept that wear debris particles stimulate osteolysis via NFκB activation, and TNF production[74,78,79]. Moreover, *in vivo* TNF blockade significantly inhibits but does not completely eradicate wear debris-induced osteolysis[80,81]. In contrast, disruption of RANK signaling via genetic ablation or high dose RANK:Fc treatment (10 mg/kg/48hr) completely eliminates osteoclasts and bone resorption in this model[82]. Similar effects were also achieved via OPG gene therapy[83-85]. Of particular importance was the finding that new bone formation on both resorbed and unresorbed surfaces was not suppressed by complete osteoclast depletion via RANK:Fc treatment, and that this newly synthesized bone had normal mineral content and matrix composition[82].

Towards a therapeutic intervention for aseptic loosening

Currently no therapies are approved for aseptic loosening. At this time clinical trial data on the effects of bisphosphonates in this condition are unavailable but unpublished reports and anecdotal evidence suggest that these agents are ineffective. In our view, the greatest limitation to the development of an effective intervention is the lack of an accurate and reliable outcome measure. Development of such an outcome measure is particularly challenging given that aseptic loosening takes years to develop and because progression is non-linear, small sampling of bone metabolites in urine or blood is not useful. Additionally, periprosthetic osteolysis involves a complex 3-dimensional lesion, rendering 1-dimentional (DEXA) or 2-dimensional (X-rays) radiology inaccurate. Therefore, we have developed 3-dimensional computerized tomography (3D-CT) methods to quantify periprosthetic osteolysis. We validated this approach by demonstrating the known correlation between polyethylene wear and osteolysis[86]. Based on the success of this technique and the efficacy of TNF blockade in an animal model, we performed a clinical pilot to evaluate the efficacy of etanercept in 20 patients with established periprosthetic osteolysis[87]. While this study was not powered to evaluate drug efficacy, it concluded that the technique could determine a significant effect of a drug that inhibited osteolysis by 50% in a placebo controlled trial with 83 patients in each arm. Thus, future trials are now being proposed to develop the first therapeutic intervention for aseptic loosening.

Conclusions

Focal bone resorption in erosive arthritis and aseptic loosening remains a unique form of osteolysis that poses significant therapeutic challenges not observed in metabolic bone disorders. In particular, extensive focal bone loss arising from the adjacent dense hyperplastic inflammatory tissue and the relative resistance of these conditions to bisphosphonates necessitates development of alternative treatment strategies. The elucidation of RANK, RANKL and OPG as the final effectors of osteoclastogenesis and bone resorption is a true breakthrough providing invaluable insights into the mechanisms that underlie pathologic osteolysis. Formal proof that RANK and RANKL are viable targets in inflammatory osteolysis comes from studies in animal models of arthritis and wear debris-induced osteolysis showing that osteoclastogenesis and bone resorption do not take place void of RANK signaling *in vivo* and are significantly inhibited by RANK or RANKL blockade. While additional studies are required to determine the toxicity of agents that block molecules in the RANK-RANKL signaling pathway, preliminary studies in animal models suggest that they are well-tolerated. Moreover, the absence of major adverse events in phase I clinical trials of osteoporosis with recombinant OPG and anti-RANKL provide additional support for the tremendous potential of this innovative treatment strategy.

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