

Pathogenic bone loss in rheumatoid arthritis: mechanisms and therapeutic approaches

Rheumatoid arthritis (RA) is a systemic inflammatory disorder with progressive joint destruction being its hallmark feature. Conventional treatments, such as DMARDs, aim to inhibit the inflammation; however, over the past decade, major advances in our understanding of bone metabolism has given us the ability to directly treat the bone loss in RA. Although modern anti-inflammatory therapies, such as anti-TNF- α treatment, have resulted in a remarkable improvement in the treatment of RA, these treatments do not directly target bone destruction in the joint. The aim of this review is to demonstrate that antiresorptive therapies can prevent structural joint destruction, particularly in the early stages of anti-inflammatory treatment. A number of novel approaches targeting bone resorption are also becoming available.

KEYWORDS: anti-inflammatory treatments ■ antiresorptive treatments ■ B ligand ■ NFATc1 ■ nuclear factor of activated T cells ■ osteoclasts receptor activator of nuclear factor ■ rheumatoid arthritis

Rheumatoid arthritis (RA) is a systemic inflammatory disorder characterized by destruction of the joint, and this has a major impact on its function. Progressive joint destruction is the hallmark feature of RA. The disease is characterized by joint inflammation, synovial hyperplasia and associated destruction of bone and cartilage [1]. It is the most common autoimmune disease in Australia and currently affects 1% of the world's population [201]. Despite the prevalence of this disease, complete understanding of the disease process is still lacking. Most studies have focused on inflammation in the soft tissues; however, research is now focusing on preventing bone and cartilage destruction. Conventional treatments for RA aim to inhibit the inflammation; however, over the past decade, major advances in our understanding of bone metabolism have enabled us to identify the mechanisms of joint destruction in RA. This knowledge, in combination with the recent development of therapies inhibiting bone loss, gives us the ability to directly treat the bone loss in RA. Although modern anti-inflammatory therapies have resulted in a remarkable improvement in the treatment of RA, this review aims to demonstrate that antiresorptive therapies, in combination with modern anti-inflammatory treatments, are needed to better prevent structural joint destruction and disease progression in RA.

Rheumatoid arthritis presents clinically as a symmetrical polyarthritis that targets many joints in the body. RA only affects synovial-lined joints and predominantly affects peripheral

joints in the hands and feet as well as the knees. Other synovial joints such as the ankles, hips, elbows and shoulders are relatively spared and the spine is rarely affected, apart from the atlanto-axial joint in the cervical spine. RA rarely, if ever, affects distal interphalangeal joints even though these are synovial joints. It is unclear why RA affects some synovial-lined joints frequently and others rarely or never. The primary joints affected include the metacarpo-phalangeal, proximal interphalangeal, mid-carpal, radio-carpal and distal radio-ulnar joints in the hands [2]. In the feet, the target sites include the metatarso-phalangeal, proximal interphalangeal and the intertarsal joints [2]. The disease results in pain, stiffness and swelling of the joints, which impacts on their functional capacity. Recurring inflammation leads to bone and cartilage destruction, affecting the individual's physical functioning, and causes both short and long-term morbidity [3]. The synovium is the major target for the inflammatory process where infiltration of the tissue occurs with multiple immune cells and cytokines resulting in tissue inflammation. The synovial tissue expands and forms a pannus that invades the bone and cartilage, destroying the tissue as it proceeds [4]. The inflammation of the synovial tissues promotes osteoclastogenesis that leads to both focal articular bone erosion at the site of pannus formation as well as systemic bone loss, similar to osteoporosis [4,5]. The invasion of inflammatory tissue into the subchondral bone involves many cell types including fibroblasts,

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lymphocytes and monocytes. Monocytes are the precursors of osteoclasts that resorb bone through the acidic dissolution of bone mineral and enzymatic destruction of bone matrix [6].

Resorption of the unmineralized cartilage does not appear to directly involve osteoclasts but seems to be due to proteases that are synthesized by the synovial fibroblasts, neutrophils and the chondrocytes [7]. The articular cartilage is avascular and nonmineralized, consisting of chondrocytes that are embedded in the extracellular matrix. The extracellular matrix consists of a type II collagen network that forms the structural backbone of the matrix along with other proteins [7]. There is a large amount of proteoglycan in cartilage that binds water and functions as a mechanical shock absorber [7]. The cartilage damage in RA can be attributed to a number of catabolic factors, such as pro-inflammatory cytokines, aggrecanases, matrix metalloproteinases and nitric oxide. The synovial fibroblasts express large amounts of matrix-degrading enzymes that invade the articular cartilage resulting in cartilage destruction [7]. Many of the factors produced by the inflamed synovium that are involved in the bone destruction also have an effect on chondrocyte functioning [8]. Inflammatory cytokines, IL-1 and TNF- α are thought to play a major role in cartilage loss [8]. Many studies have demonstrated that IL-1 stimulates chondrocytes to increase production of matrix metalloproteinases that degrade the cartilage [9,10].

While the localized destruction of the joint is the major focus of treatment, generalized bone loss, known as osteopenia, results in a loss of bone mineral density (BMD) and elevates the risk of osteoporotic fractures in patients with RA [11]. Clinical studies have evaluated BMD in the hands of patients with early RA and found an association between reduction in BMD and disease severity [12,13]. This increased systemic bone loss is attributed to the effects of pro-inflammatory cytokines released from the site of synovial inflammation that act systemically and result in a generalized bone loss [14–16]. RA has also been demonstrated to have an impact on increasing the risk of fractures as a result of the associated generalized bone loss. One study found an increased risk of fracture, especially of the hip (relative risk: 2.0) and the spine (relative risk: 2.4), in RA patients compared with age and sex-matched controls. Predictors included a long duration of RA, low BMI and use of corticosteroids which, apart from a long duration of RA, are known risk factors for osteoporotic

fracture [17]. It is important to note that other factors may contribute to the increased risk of fracture such as the development of osteoporosis owing to decreased mobility of the RA patient.

Mechanisms of disease

A major hallmark of inflammatory bone loss diseases, such as RA, is enhanced osteoclastic bone resorption without a corresponding increase in bone formation. Osteoclasts are the multinucleated cells derived from the monocyte/macrophage hematopoietic lineage that are responsible for resorbing bone [18–20]. The essential role that osteoclasts play in bone loss in RA has been demonstrated in animal studies showing that mice without osteoclasts are resistant to arthritis-induced bone loss [21,22]. The crucial role of osteoclasts in RA bone loss is also supported by studies of human tissue obtained from patients with RA [23–26]. In animal models of RA, and in human RA, large multinucleated osteoclastic cells resorbing subchondral bone have been detected at sites of bone erosion in the joints [27,28]. These osteoclasts form as a result of exposure to inflammatory cytokines present in synovial tissue, and understanding this process is the key to developing treatments for bone loss in RA. **FIGURE 1** demonstrates the inflammatory cytokines involved in both localized bone loss within the joint and generalized bone loss.

■ Osteoimmunology

The involvement of the immune system is fundamental to the progression of RA. In recent decades the involvement of the immune system in bone metabolism has become apparent (as reviewed by Takayanagi *et al.* [29]). Therefore, it is not surprising that bone loss in RA appears to be controlled by immune factors and cells. This relationship between the immune system and bone metabolism has been termed ‘osteoimmunology’, and an understanding of osteoimmunology is vital for developing ways of preventing bone loss in RA.

■ Macrophage colony stimulating factor

Macrophage colony stimulating factor (M-CSF) was one of the first factors to be recognized as playing an essential role in the process of osteoclast development. It is normally produced by osteoblasts or bone marrow stromal cells and transmits its signal by binding to its specific receptor, cFMS, which is a member of the tyrosine kinase receptor superfamily. This binding activates early transcription factors, such as c-fos,

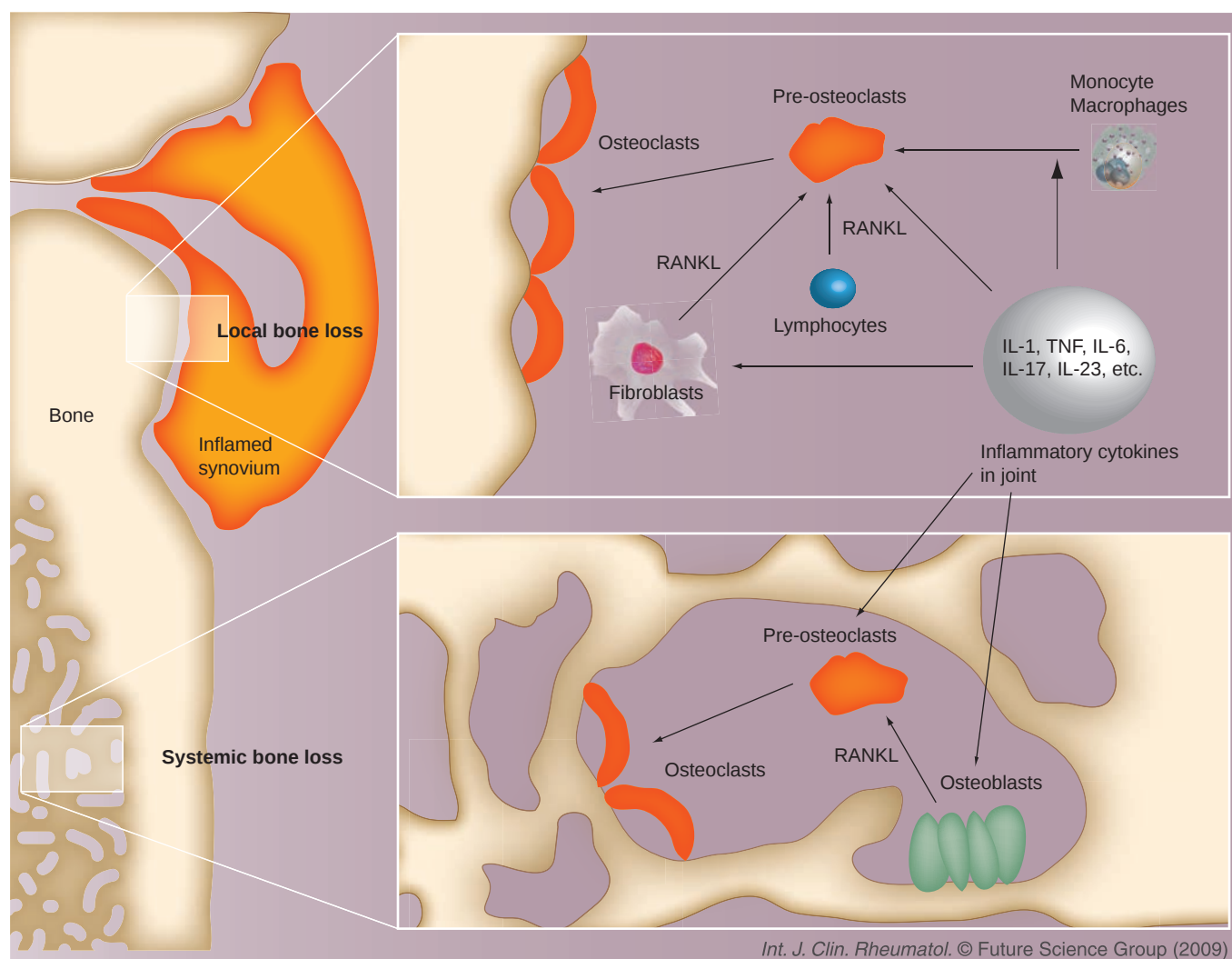


Figure 1. The pathogenesis of osteoclastogenesis in RA. Inflammatory cytokines in the RA joint stimulate osteoclast formation locally and externally to the bone in several ways. First, inflammatory cytokines can stimulate RANKL production by lymphocytes (e.g., IL-6 [181]) and fibroblasts (e.g., IL-6 [182]) in the joint. Second, they can activate monocytes (e.g., TNF- α [37], IL-23 [183]) that arrive at the inflamed joint to become macrophages and pre-osteoclasts (e.g., IL-1 [184], TNF- α [36]). Third, they may have a direct effect on pre-osteoclasts, usually in concert with RANKL, to directly promote osteoclast formation (e.g., IL-1 [185], IL-6 [186], IL-23 [187], TNF- α [185,188]). Systemic bone loss can also be a feature of RA and is stimulated by promoting osteoclast formation within the bone. In this situation, inflammatory cytokines act systemically to stimulate RANKL production by osteoblasts (e.g., IL-1 [189], IL-6 [190], IL-17 [191], IL-23 [187], TNF- α [60,189]) and may also directly promote osteoclast formation in concert with RANKL released by osteoblasts. RA: Rheumatoid arthritis; RANKL: Receptor activator of NF- κ B ligand.

which initiates osteoclastogenesis. The main role of M-CSF in osteoclast formation is to enhance the proliferation and survival of both precursor cells and mature osteoclasts [30]. The essential role of M-CSF (known as CSF-1 in mice) in osteoclastogenesis was demonstrated by the observation that M-CSF knockout mice (op/op) were osteopetrotic owing to the inhibition of bone resorption. Daily injections of M-CSF reversed the osteopetrosis, highlighting its importance in osteoclast formation [31]. Sarma and Flanagan also demonstrated that M-CSF induced substantial osteoclast formation and bone resorption in cultures of human bone marrow stromal cells [32].

■ Receptor activator of NF- κ B ligand/ receptor activator of NF- κ B pathway

Receptor activator of NF- κ B ligand (RANKL) is a membrane-bound protein of the TNF family that is expressed by osteoblasts, fibroblasts and activated T cells and is a key mediator in the process of osteoclast formation. In normal bone metabolism, RANKL is expressed by osteoblastic cells; however, in inflammatory disease states, it is also produced by immune cells, such as T lymphocytes [33]. RANKL is also involved in the hormone regulation of bone metabolism, since it is expressed in response to a variety of molecules including parathyroid hormone (PTH), vitamin

D3 and IL-11 [34,35]. RANKL binds to its receptor, receptor activator of NF- κ B (RANK), on the surface of osteoclast precursors and activates the c-jun terminal kinase that activates NF- κ B to stimulate the formation of osteoclasts that resorb bone [21]. RANKL has been demonstrated to be essential for osteoclast bone resorption [21,22] although, under some circumstances, TNF has been reported to induce osteoclast formation in the absence of RANKL [36]. However, according to others, TNF alone was insufficient to induce osteoclastogenesis, suggesting an essential role of RANKL in osteoclast formation [37]. The importance of RANKL in the process of osteoclast bone resorption was demonstrated using RANKL knockout mice that were found to exhibit an osteopetrotic phenotype owing to an absence of osteoclasts [21]. Conversely, excessive administration of recombinant RANKL in mice results in increased osteoclast formation that leads to bone resorption, and the mice develop osteoporosis [34].

Receptor activator of NF- κ B ligand has been demonstrated to form this important link between immunology and bone physiology since inflammatory cytokines, such as TNF- α and IL-1, stimulate the production of RANKL [38,39]. Previous studies have provided evidence for implicating RANKL in the pathogenesis of the bone destruction that occurs in RA. Elevated expression of RANKL has been found in the synovium of patients with RA, and therefore may contribute to the excessive bone loss observed [24,40,41]. We previously demonstrated higher levels of RANKL expression in the mononuclear aggregates and fibroblast-like cells in the subintimal regions of the synovial membrane from patients with active RA [42]. It was later confirmed that the high expression of RANKL is largely confined to sites of bone resorption at the pannus–bone interface and subchondral bone erosion [43]. FIGURE 2 clearly demonstrates the enhanced RANKL expression in RA synovium compared with normal synovium. Elevated expression of RANKL by inflammatory cells, such as synovial fibroblasts and activated T-cells, has also been demonstrated to be associated with RA [40–41,44,45]. Using murine spleen cells, peripheral blood mononuclear-derived T cells were demonstrated to promote osteoclast formation and activation *in vitro* [45]. In a rat model of adjuvant arthritis, local T cell activation was also found to be associated with RANKL expression and subsequent joint destruction [46]. The involvement of a variety of inflammatory cytokines in stimulating

RANKL expression [33–37] and enhancing the effects of RANKL [47] is thought to be important. However, the data strongly supports the view that it is the elevated levels of RANKL that largely results in enhanced osteoclast formation and enhanced bone resorption in RA.

■ Osteoprotegerin

Osteoprotegerin (OPG) is a soluble TNF receptor-like molecule that is a natural inhibitor of RANKL. It acts as a decoy receptor and prevents the binding of RANKL to RANK, thereby preventing osteoclastogenesis. OPG knockout mice have an osteoporotic phenotype and have a high incidence of fractures [48]. Conversely, overexpression of OPG in transgenic mice resulted in the development of severe osteopetrosis owing to the inhibition of osteoclastic bone resorption [49]. Administration of OPG to mice was shown to prevent TNF- α -mediated bone destruction by reducing the number of osteoclasts able to resorb bone [50]. Active osteoclasts can be isolated from the pannus joint from RA patients and there is a correlation between the ratio of RANKL mRNA:OPG mRNA and resorption *ex vivo* [24]. Furthermore, the level of OPG protein was markedly reduced in patients with RA [51], whilst higher levels of RANKL were expressed by lymphocytes and macrophages in tissues extracted from patients with active RA [41]. FIGURE 3 demonstrates the lower OPG expression levels observed in active RA tissue compared with normal tissue. These results were also confirmed by Petitt and colleagues [43] who demonstrated that OPG was expressed remotely in the synovium of RA patients and was limited at sites of bone erosion, especially in regions that were associated with RANKL expression. These results confirm that the imbalance between RANKL and OPG plays a major role in bone destruction in RA, and that OPG expression is a crucial determinant of the degree to which RANKL can stimulate osteoclast development [43].

■ Intracellular regulators of osteoclasts

Binding of RANKL to RANK activates a number of intracellular factors including TRAF-6, c-fos and calcium signaling pathways that regulate osteoclast differentiation and activation, as shown in FIGURE 1. These are all responsible for the induction and activation of nuclear factor of activated T cells-1 (NFATc1), which has been found to be the master transcription factor for osteoclastogenesis [52].

■ TRAF-6

TRAF-6 is a member of the TNF receptor factor (TRAF) family of proteins that plays an important role in linking the interaction of RANK on the cell membrane with the adaptor proteins downstream. Binding to TRAF adaptor proteins within the cytoplasmic domain of RANK is the initial step in the process of RANK signaling [53]. In a gene knockout study, TRAF-6-deficient mice were demonstrated to develop a severe osteopetrotic phenotype and to be defective in osteoclast formation owing to defective signaling from RANK upon RANKL binding [54]. The downstream targets of TRAF-6 are transcription factors including NF- κ B, activator protein-1 (AP-1) and NFATc1, cascades of mitogen-activated protein kinases (MAPKs) such as p38 stress kinase, c-jun N-terminal kinase (JNK), ERK and Pi3K/AKT pathways [53].

■ NF- κ B

NF- κ B is a pleiotropic transcription factor that is also critical in RANK signaling and the process of osteoclast formation and activation. It is also likely to be a significant factor in regulating synovial inflammation [55]. Its importance in osteoclastogenesis was confirmed by investigations using NF- κ B knockout mice, who developed osteopetrosis as a result of defective osteoclast formation [56]. NF- κ B proteins reside in the cytoplasm of nonstimulated cells and rapidly enter the nucleus when stimulated, for example, by RANKL. This classical NF- κ B is activated by the binding of RANKL to RANK, which activates the IKK kinase (IKK) complex [57]. The IKK complex consists of two catalytic sites – the IKK- α and IKK- β (IKK-1 and IKK-2) – and the associated regulatory subunit IKK- γ . The IKK- β is essential for NF- κ B activation, as it phosphorylates the inhibitor subunit IKB- α , which leads to its degradation and subsequent activation of NF- κ B [58]. In the alternate activation pathway, IKK- α is required for NF- κ B activation, resulting in phosphorylation and proteasome-induced processing of p100, which is cleaved to generate an active p52 product [57,59]. Both of these pathways that activate NF- κ B are important in the process of osteoclastogenesis.

■ AP-1 family: c-fos & c-jun

Interaction of RANK and RANKL activates the transcription factor complex AP-1. The AP-1 transcription factor is a dimeric complex that consists of the FOS family of proteins (c-fos, FOSB, FRA1 and FRA2), the JUN family of proteins (c-JUN, JUNB and JUND) and the

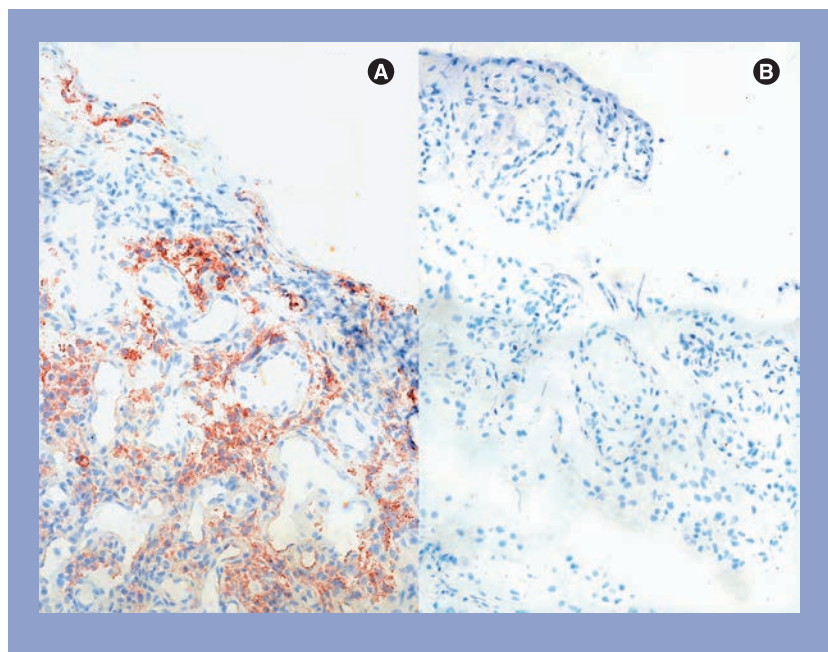


Figure 2. Expression of RANKL in synovium of an RA subject and a normal subject. RANKL expression detected with mAb 626/immunoperoxidase and AEC (red) in synovial tissues from (A) a patient with active RA and (B) a normal subject.

AEC: 3-amino-9-ethylcarbazole; mAB: Monoclonal antibodies; RA: Rheumatoid arthritis, RANKL: Receptor activator of NF- κ B ligand.

activating transcription factor (ATF) family of proteins [60]. C-fos is a transcription factor and is a member of the FOS family that has been implicated in a number of metabolic processes, including both the skeletal and immune systems. C-fos expression is induced by both M-CSF and the RANKL/RANK interaction. C-fos-deficient mice have been demonstrated to develop a severe osteopetrotic phenotype, demonstrating its importance in the process of osteoclast formation [61,62]. C-jun is a member of the JUN family of proteins, some of which play a key role in osteoclastogenesis. This has been demonstrated through studies with transgenic mice expressing dominant-negative c-jun, where it was demonstrated that severe osteopetrosis results from impaired osteoclastogenesis [63]. C-Jun was also demonstrated to play a crucial role in the regulation of the NFAT family; over expression of NFAT promoted osteoclast formation that could be suppressed by overexpressing dominant-negative c-jun [63].

■ Nuclear factor of activated T cells

NFATc1 is the key intracellular molecule that regulates terminal osteoclast formation (FIGURE 4) [64]. RANKL binding to RANK recruits TRAF adaptor proteins to activate key signaling cascades that promote the

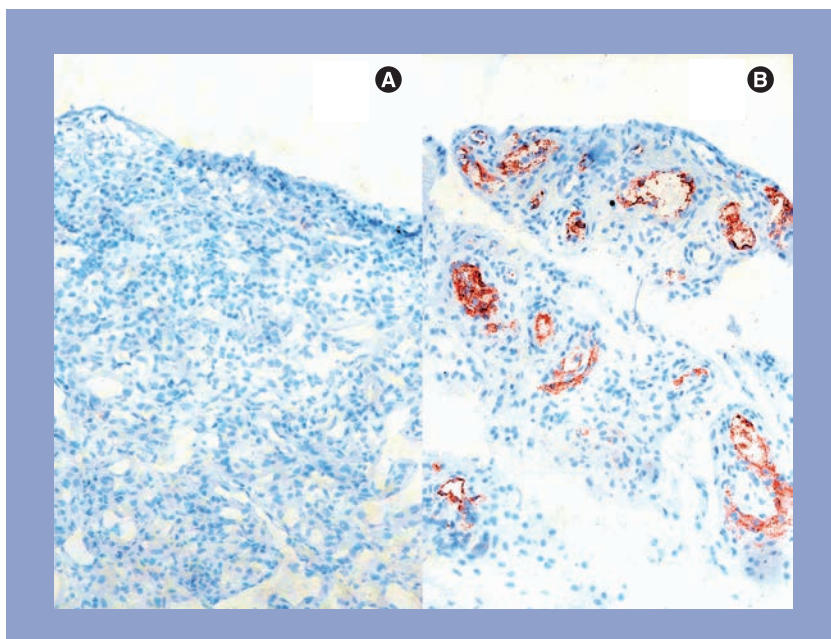


Figure 3. OPG expression in synovium of an RA subject and a normal subject. OPG staining (mAb 805) of synovial tissues from (A) a patient with active rheumatoid arthritis and (B) a normal subject. mAb: Monoclonal antibodies; OPG: Osteoprotegerin.

differentiation of monocytes into multinucleated mature osteoclasts (as reviewed by Asagiri and Takayanagi [64]). NFATc1 is the terminal factor that directly induces the expression of osteoclast genes such as the calcitonin receptor (*CTR*), cathepsin K (*Cath-K*), tartrate-resistant acid phosphatase (*TRAP*) and the $\beta 3$ integrin, in addition to the osteoclast-associated receptor (*OSCAR*) [65–67]. The ligand for OSCAR is yet to be identified. The essential requirement for NFATc1 is evident by its ability to induce osteoclast-specific genes in the absence of RANKL [68]. Furthermore, NFATc1-deficient embryonic stem cells are unable to differentiate into osteoclasts [69].

Animal models of disease

Many developments in understanding the process of bone metabolism and the mechanisms of joint destruction in RA have been made through the use of animal models. These models are widely used to investigate the mechanisms involved in the RA disease process and also play a critical role in the development of drugs for treating RA. The models used are known to share features with the human disease process and can therefore be used to investigate the mechanisms of actions and to determine any associated side effects of novel therapies. Many of the current treatments now on the market for RA (such as ciclosporin A, indomethacin, anakinra, celecoxib, etanercept,

cyclophosphamide and abatacept) were originally tested in animal models and their efficacy in these models results in progression to clinical studies. The models used to study RA include adjuvant-induced arthritis (AIA), collagen-induced arthritis (CIA), collagen antibody-induced arthritis (CAIA), K/BxN Tg mouse, hTNF α Tg mouse and the severe combined immunodeficiency (SCID) mouse model [4]. The advantages and disadvantages of using each of these models to investigate RA and test possible treatments are shown in Table 1. The most commonly used animal models are AIA in rats and the CIA and CAIA models in mice, as these have many features in common with human RA.

Rat adjuvant-induced arthritis model

The rat adjuvant-induced arthritis (AIA) model was the first animal model of RA and is now widely used to test new therapeutic agents. This is a T-cell- and neutrophil-dependent disease [70] that is induced by a single intradermal injection of complete Freund adjuvant, resulting in a rapid progressive onset of the disease 7 days later. The disease is characterized by severe joint inflammation and marked bone resorption with histology, taken at the completion of the study (usually 14 days after adjuvant injection), demonstrating neutrophil infiltration and subchondral bone destruction. Drugs currently approved for RA treatment that demonstrated therapeutic efficacy in the AIA model include methotrexate [71–75], ciclosporin A [76,77], gold compounds [78], penicillamine, prednisone, cyclophosphamide, indomethacin, anakinra [79,80], naproxen, celecoxib, flobufen and etanercept [80,81].

Collagen-induced arthritis model

Collagen-induced arthritis (CIA) is another commonly used arthritis model to test potential treatments. It is induced by immunization with heterogeneous type II collagen in complete Freund's adjuvant. Mice develop a polyarthritis with severe cartilage and bone destruction. This animal model of disease shares several clinical, histopathological and immunological features with the human RA condition and can therefore be effectively used to test therapies that target both inflammation and bone loss [70]. Drugs currently approved for RA treatment that demonstrated therapeutic efficacy in the CIA model include prednisone, cyclophosphamide, indomethacin, anakinra, etanercept and abatacept.

■ Collagen antibody-induced arthritis model

Collagen antibody-induced arthritis is a newer animal model that is a rapid, simple and versatile model of RA induced by systemic administration of a cocktail of type II collagen monoclonal antibodies that are directed to conserved auto-antigenic epitopes of collagen type II, either alone or in combination with lipopolysaccharide (LPS) [82,202]. LPS and the auto-antibodies to type II collagen have a synergistic action that induces arthritis [202]. This model is known to exhibit pathogenic features that are similar to those in human RA. It has previously been demonstrated to result in severe inflammation in the ankles and paws of mice [83], with clinically apparent arthritis occurring by day 4 and active arthritis peaking at day 6 [82,83]. Tissue from paw joints demonstrated marked pathological changes with synovial hyperplasia, a large number of infiltrating polymorphonuclear and mononuclear cells, extensive pannus formation at the cartilage–bone junction, severe cartilage destruction and bone erosion [83,84]. The development of CAIA was originally thought to be independent of a T- and B-cell mechanism; however, it has recently been demonstrated that T-cell activation is important for the development of CAIA [85]. It is likely that T cells play an important role in the prolongation of CAIA. An inhibitor of T-cell activation, ciclosporin A, was demonstrated to reduce the severity of arthritis developed using a CAIA model in both the

early and late phases of disease [85]. The ease of disease induction and the close resemblance to the human disease has resulted in this model becoming commonly used to investigate RA.

■ TNF transgenic inflammatory mouse model

Transgenic mice are commonly used to study the roles that particular genes play in the RA disease process. For example, in the TNF transgenic (TNFtg) mouse model, mice have a human TNF- α transgene modified in the 3' region, and they therefore express high levels of both soluble and membrane-bound TNF- α [86]. These mice develop a symmetrical polyarthritis approximately 4–6 weeks after birth. Multiple lines of TNFtg mice have been generated without any other obvious developmental defects [87]. Interestingly, no joints are spared, including the temporomandibular joint [87,88].

■ Measuring bone loss in animal models of RA

The animal models of RA are commonly used to test therapies that inhibit the bone destruction, so analysis techniques are required to quantitate the bone loss and to determine if antiresorptive drugs effectively suppress this bone loss. Many of the current methods used, such as histomorphometry and 2D radiography, are not effective at giving a true indication of bone loss and can also be prone to measurement errors. One of the most effective methods that is now commonly used to determine the

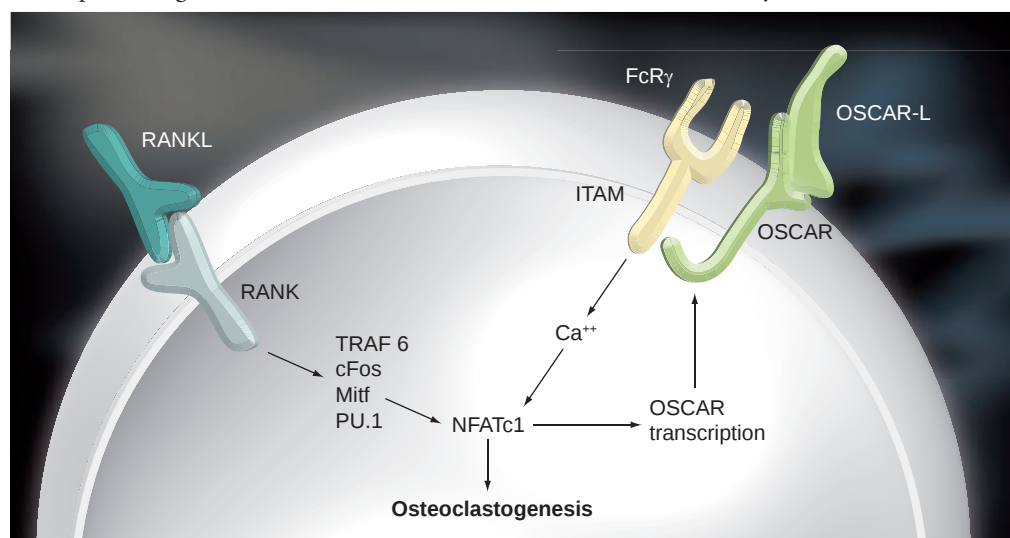


Figure 4. The important intracellular regulators of osteoclast formation. NFATc1 is the central intracellular molecule regulating terminal osteoclast formation. RANKL binds to its receptor, RANK, and the TRAF adaptor proteins are recruited, prompting the differentiation of monocytes into multinucleated mature osteoclasts via activation of key signaling cascades. RANK: Receptor activator of NF- κ B; RANKL: Receptor activator of NF- κ B ligand.

Table 1. The advantages and disadvantages of animal models of RA.

Model	Animals	Advantages	Disadvantages	Ref.
Adjuvant-induced arthritis	Rat	Reliable, rapid onset easily measurable polyarticular inflammation, marked bone resorption and cartilage destruction T cell and neutrophil dependent disease	Development of periostitis, bony ankylosis and most of the extra-articular manifestations	[53,71–75]
Collagen-induced arthritis	Rat, mouse	Shares both immunological and pathological features of human RA Can be either in rat or mouse Short onset time Marked cartilage destruction, bone resorption, periosteal proliferation, marked synovitis and periaricular inflammation Paws are characterized by extreme swelling and erythema T- and B-cell dependent and responses can be measured	Self limiting and not characterized by exacerbations and remissions The cell infiltrate is predominantly polymorphonuclear cells, in humans high proportion of mononuclear cells observed Mouse model has slower onset Preparation of CII is a time-consuming procedure Commercial sources of purified native CII are expensive	[71,72,76–80]
Collagen antibody-induced arthritis	Mouse	Rapid, simple and versatile model of RA that is induced by systemic administration of a cocktail of anti-type II collagen monoclonal antibodies alone or with a combination of lipopolysaccharide The severity of disease can also be controlled by changing the dose of the monoclonal antibody cocktail Develop synovial hyperplasia with a large number of infiltrating polymorphonuclear and mononuclear cells, extensive pannus formation at the cartilage-bone junction, severe cartilage destruction and bone erosion	Unclear whether it is T- and B-cell dependent	[79,81–84,202]
K/BxN Tg mouse	Mouse	Relatively fast and cost efficient model involving B and T cells Several mouse strains can develop arthritis without administration of any external antibody or antigen Mice spontaneously develop chronic progressive inflammation, occurs at 3 weeks of age then progressively evolves to chronic inflammation	Genetic modifications of mice can have unpredictable consequence to the immune system and also to other phenotypic features	[80,85,86]
hTNF α Tg model	Mouse	Relatively fast and cost efficient A simple model in which mice develop a deforming arthritis approx 4–6 weeks after birth Mice develop symmetrical polyarthritis Multiple lines of TNFtg mice have been generated without any obvious developmental defects No joints are spared from inflammation and destruction, also include temporomandibular joint	Genetic modifications of mice can have unpredictable consequence to the immune system and also to other phenotypic features	[86,87]
Streptococcal cell wall arthritis	Rat	A reliable well characterized model that is T-cell dependent Chronic, severe, erosive arthritis is induced There are spontaneous remissions and exacerbations similar to the human disease	The arthritis induced is apparently acute, as is not reported past 7 days, and no pannus or erosions were seen	[79,80]
Severe combined immunodeficiency	Mouse	Severe combined immunodeficiency mouse model is a feasible method for investigating cartilage destruction in RA Many of the features of RA last for at least 12 weeks		[86,88,89]
RA: Rheumatoid arthritis.				

extent of bone loss in animal models is micro-computed tomography (CT). This has many distinct advantages as it enables the detailed 3D micro architecture of bone to be examined. Various studies have used the method of micro-CT to quantitate the volume of bone loss in models of arthritis [84,89–91]. New technology has now been developed that allows quantitative live animal micro-CT analyses to be performed over the duration of a time-course experiment [92]. This allows investigators to follow live animals throughout the disease process and to monitor changes in bone loss before and after drug administration. Animals can be repeatedly scanned throughout a study and this means that they are able to act as their own controls. This allows investigators to determine the effects that drugs have on bone loss in individual animals, thereby reducing sources of error, as one major problem encountered in animal models of RA is the difference in susceptibility to disease development. The micro-CT scanners also have a high-resolution camera (9 or 18 μM) that produces more detailed scans and means that subtle changes in bone are able to be detected. Although a detailed analysis of cartilage destruction can not be investigated, this can be assessed via narrowing of the joint space.

Using the CAIA model and the new method of live animal micro-CT, we have been able to demonstrate severe pitting and a loss of bone mass (FIGURE 5). Abnormal bone growth also demonstrates fusion of the smaller wrist and foot bones, which can also be seen in this late stage of disease that is similar to human RA. Therefore, this model can be effectively used to test potential inhibitors of bone destruction.

Therapeutic approaches to treat RA

The main risk of bone erosion in RA occurs in the first 2 years, and earlier treatment targeted at the initial stages of bone loss is more likely to cause arrest [93]. The main goals of RA treatment are to relieve pain, control the inflammation and prevent joint destruction [94]. The following section is divided into two parts. First, conventional therapies for RA that tend to focus on reducing inflammation in the soft tissues, and second therapies that target the bone destruction of RA. This review will focus on the effects of these drugs on both localized bone erosion and generalized bone loss, with changes in bone markers and fractures only briefly discussed since less is known about these markers.



Figure 5. 3D models of left-hand side front mouse paws. (A) Control paw, no disease. (B) Paw with collagen-antibody induced arthritis.

■ Conventional RA therapies

NSAIDs

NSAIDs have long been the first line of treatments for pain and inflammation in RA. This group of drugs block inflammation in RA by preventing prostaglandin release through inhibition of the cyclooxygenase enzymes (COX) of which there are two isoforms – COX-1 and COX-2. Aspirin is one of the commonly used NSAIDs for the treatment of RA but it has been reported to have no effect on preventing joint destruction [94]. Since many of the traditional NSAIDs have been found to have adverse side effects, including major gastrointestinal selective COX-2 inhibitors such as celecoxib, more recently, rofecoxib and valdecoxib have been

used to treat RA [94]. Although conventional NSAIDs and selective COX-2 inhibitors have been demonstrated to reduce inflammation in RA, they can have ambiguous effects on bone. Under pathological conditions, prostaglandins such as prostaglandin E_2 can stimulate osteoclast-mediated bone resorption [95–97]. On the other hand, prostaglandins produced as a result of COX-2 stimulation are reported to increase bone formation by stimulating osteoblast formation and in association with hormones and growth factors, prostaglandins have also been demonstrated to play an important role in bone healing [98,99]. Although NSAIDs have been demonstrated to have many benefits in treating the symptoms of RA, this group of drugs generally appears not to have a major benefit on bone destruction in RA.

Modified NSAIDs could provide an alternative treatment to conventional NSAIDs for the treatment of RA. Nitrosylated flurbiprofen derivative HCT1026 has been demonstrated to inhibit bone resorption, both *in vivo* and *in vitro*. HCT1026 was demonstrated to strongly suppress osteoclast formation, activity and survival in murine osteoclast cultures by inducing osteoclast apoptosis; however, there was no effect on osteoblasts or macrophages. This derivative compound also inhibited RANKL-induced NF- κ B and extracellular signal-regulated kinase (ERK) pathways, whereas the parent compound, flurbiprofen, did not. Since this derivative is not a COX inhibitor due to the presence of a nitric oxide group, this reduces the side effects related to gastrointestinal toxicity [100].

Glucocorticoids

Glucocorticoids are a class of steroid hormones that bind to the cortisol receptors and have a variety of biological effects. They have been found to have anti-inflammatory effects and are commonly used in the treatment of RA. Glucocorticoids were originally found to be reliable and have rapid effects on controlling joint inflammation [94]. However, long-term use of glucocorticoids has been demonstrated to be associated with the induction of osteoporosis. Treatment with glucocorticoids has been found to be associated with an increased risk of fractures, particularly of the hip and vertebrae, and this is dose dependent and occurs rapidly after treatment is commenced [101,102]. Other side effects of glucocorticoid therapy include hypertension, peptic ulcer diseases, accelerated atherosclerosis, vascular disease and many others [94]. Glucocorticoids have also been found to have negative effects on bone,

suppressing osteoblasts and bone formation; however, they may exert a moderate beneficial effect through the suppression of inflammatory-induced bone erosions [7]. Glucocorticoids may play a role in suppressing early inflammation in RA; however, with their adverse side effects from long-term use and their inhibition of bone formation, they are not the most useful treatment to prevent structural damage in RA.

DMARDs

One of the most common conventional therapies now widely used for the effective treatment of RA are DMARDs. There are a variety of DMARDs used to control RA including sulfasalazine, antimalarials, penicillamine, gold, methotrexate (MTX), azathioprine, leflunomide and cyclophosphamide. These can be used as monotherapy or in combination with other therapeutic agents. DMARDs are small molecular weight, orally available drugs that have anti-inflammatory properties and are used for long-term control of the disease process [7]. MTX is a widely used DMARD and is the standard of care for patients with moderate-to-severe arthritis owing to the fact that it is safe and well tolerated at therapeutic doses [103,104]. It appears to act through the induction of adenosine, which has anti-inflammatory properties. MTX is often used in combination with other agents such as etanercept [105,106], adalimumab [107], anakinra [108], leflunomide [109] and rituximab [110] to treat RA. MTX effectiveness is achieved by providing an additional anti-inflammatory mechanism and/or by acting synergistically with other therapies [7]. DMARDs have resulted in improvements in symptoms in a substantial proportion of RA patients; however, not all patients respond favorably. There have also been issues relating to toxicity and adverse events [103] and for these reasons, patients tend to take time to find an effective DMARD that works well for them, and when they do find a suitable DMARD, it can take time to develop substantial anti-inflammatory effects. Unfortunately, during this time, irreversible bone and cartilage destruction can continue to occur. In addition, only a minority of patients continue with effective DMARD treatment over the long term, as the majority discontinue use as a result of adverse side effects [111].

Anti-TNF- α therapy

During the process of inflammation, a number of cytokines such as TNF- α , IL-1, IL-6 and IL-17 are involved in the differentiation and

activation of osteoclasts that are responsible for resorbing bone [112]. TNF- α , one of the major mediators involved in RA, is produced by activated macrophages as well as synoviocytes in the inflamed synovial tissue. TNF- α is reported to both directly and indirectly induce osteoclast formation and hence, it forms a link between the immune and bone systems. TNF- α -induced osteoclast formation may require both M-CSF and RANKL, and its resorptive effects may be due to its enhancement of RANKL activity [48] and its ability to stimulate RANKL production by osteoblasts [7]. TNF- α has also been demonstrated to induce osteoclast formation independently of the RANKL/RANK interaction [36]. The fact that TNF- α is able to induce osteoclast formation in both the absence and presence of RANKL has resulted in it being a target for therapies aimed at suppressing both the inflammation and bone destruction in RA.

Anti-TNF- α therapy has become increasingly popular for the treatment of RA owing to its rapid onset of action, which reduces joint destruction compared with the slower acting drugs. There are currently five TNF- α antagonists approved for RA treatment:

- Adalimumab – human monoclonal antibody
- Entanercept – TNF receptor (p75): FcIgG construct
- Infliximab – chimeric monoclonal antibody
- Golimumab (CNT0148) – fully human monoclonal anti-TNF- α antibody
- Certolizumab pegol (Cimzia®, UCB, Brussels, Belgium) – humanized anti-TNF- α antibody

All of these treatments work through inhibition of TNF- α activity with the main difference being in the method of delivery and the frequency of administration. However, the greater efficacy of treatment with TNF- α inhibitors comes at a much higher cost and with the fact that they do not work adequately on all patients [103]. In addition, continuous parental administration is associated with an increased risk of infection [103], with reactivation of TB being a major concern. As a result, anti-TNF- α therapies are not usually initiated until other less expensive treatments have been tried and erosions may have already occurred before anti-TNF- α therapy takes effect. Even treatments commenced at the onset of disease may not prevent joint damage as recent evidence has demonstrated that bone damage can still occur during anti-TNF- α therapy [113].

The effects of anti-TNF- α treatment on generalized bone loss have been investigated and small increases in BMD of the spine and hip have been found in patients on TNF blockers, while RA patients on MTX alone have small decreases in BMD [114,115]. However, many of these changes were not clinically significant. It has been suggested that the mechanisms underlying localized bone loss around a joint, bone erosions and generalized osteoporosis are due, at least in part, to TNF, and anti-TNF therapy may therefore have benefits on both localized and generalized bone loss [114,115].

Interleukins: IL-1, IL-3, IL-6, IL-12, IL-15, IL-17 & IL-23

In addition to TNF- α , other inflammatory cytokines such as IL-1, IL-6, IL-12, IL-15, IL-17 and IL-23 play a role in the pathogenesis of RA, and these have become targets for therapeutic agents. For example, IL-1 is an inflammatory cytokine that plays a key regulatory role in the disease process of RA. Like TNF- α , IL-1 has been demonstrated to increase expression of both RANKL and RANK and to facilitate osteoclast differentiation [116]. Anakinra, a recombinant IL-1 receptor antagonist (IL-1Ra), is currently approved for the treatment of RA [7]. The effectiveness of anakinra has been investigated as both a monotherapy and in combination with MTX [108]. It has been demonstrated to effectively reduce focal bone erosion in arthritis [108,117–119]. Novartis AG (Basel, Switzerland) have developed a fully humanized monoclonal antibody targeting IL-1 that neutralizes the activity of human IL-1 β , known as canakinumab (ACZ885). This is administered by the intravenous or subcutaneous route and is currently in Phase III clinical development with promising early results [120].

A number of new therapies are being developed against other cytokines involved in the inflammatory cascade. IL-3, a cytokine that is secreted by T-helper (Th) cells, stimulates the proliferation, differentiation and survival of pluripotent hematopoietic stem cells. In bone marrow stem cells isolated from mice, IL-3 was demonstrated to irreversibly inhibit osteoclast formation and resorption in a dose-dependent manner [121]. It was also demonstrated to inhibit TNF- α -induced bone resorption *in vitro* [121]. In a mouse model of monoclonal antibody/LPS-induced arthritis, pretreatment with IL-3 also resulted in less inflammation in the paws, indicating that IL-3 treated mice are resistant to monoclonal antibody/LPS-induced arthritis [121].

Other inhibitors of interleukins that are in clinical trials include tocilizumab, a humanized monoclonal antibody against the IL-6 receptor with several Phase II clinical trials suggesting that tocilizumab may be effective in treating RA [122]. Earlier this year, tocilizumab was approved for clinical use [123]. HuMaxIL-15 is a high-affinity fully human monoclonal antibody against IL-15 that has been tested in Phase I–II clinical trials and has resulted in substantial improvements in disease activity as well as being well tolerated [124]. A monoclonal antibody against IL-17 (AIN457) and a monoclonal antibody against p40 subunits of cytokines IL-12 and IL-23 – ustekinumab [125] – are also in clinical trials.

While these novel anticytokine therapies offer useful options for the treatment of RA, they do not directly inhibit osteoclast-mediated bone destruction so the bone and cartilage loss may continue to progress despite treatment. The involvement of a variety of inflammatory cytokines in stimulating RANKL expression [33–35] and enhancing the effects of RANKL [47] to induce the formation of osteoclasts that resorb bone is important. However, therapies that specifically target osteoclast bone resorption combined with therapies targeting inflammation, as discussed above, are likely to more effectively stop the joint damage and disease progression of RA.

■ Antiresorptive therapies

Despite the use of conventional and emerging therapies that target the inflammation, structural damage can still progress that results in permanent joint damage in RA patients. Therefore, there is a need for effective drug therapy that will preserve the joint structure [6]. A number of options have been suggested that may be used in combination with conventional anti-inflammatory treatments.

Bisphosphonates

Bisphosphonates (BPs) are a group of drugs that are known to inhibit osteoclast activity and are used to treat a wide variety of bone disorders, including osteoporosis, tumor-associated osteolysis and arthritis [112]. The first generation BPs were synthesized in the early 1970s and only exhibited weak activity against osteoclasts, whereas the later generation agents exhibit stronger antiresorptive effects [126]. There are two main classes of BPs – amino-BPs (alendronate, ibandronate, risedronate, pamidronate, zoledronic acid and icandronate) and nonamino-BPs

(etidronate, tiludronate and clodronate) [127]. The amino-BPs are known to have a stronger antiresorptive effect since they induce osteoclast apoptosis. The mechanism of action of BPs varies and some may be better suited to the treatment of systemic bone loss whereas others may be better for reducing focal bone erosions, such as that seen in RA [127]. In an AIA model, two BPs, etidronate and alendronate, were demonstrated to prevent both paw swelling and bone loss [128]. Itoh and colleagues showed that both clodronate and alendronate reduced systemic bone loss in a rat CIA, and clodronate also protected against focal bone loss [129]. Zoledronic acid is a new generation BP that is thought to suppress bone loss by reducing the life span of osteoclasts [130]. In a study using mice transgenic for human TNF that spontaneously develop arthritis, zoledronic acid was demonstrated to have no effect on inflammation, but a single dose significantly retarded bone erosion and repeated administration almost completely blocked bone erosion [131]. This was confirmed in another study using a CIA model of arthritis in which zoledronic acid effectively suppressed structural joint damage. It significantly reduced focal bone erosions and juxtaarticular trabecular bone loss, even though inflammation was slightly increased [132]. These observations of BPs having no effect on inflammation confirms the importance of combination therapy with anti-inflammatory agents such as DMARDs and antiresorptive therapy such as BPs. In a more recent study, zoledronic acid was tested in a small group of 39 patients with RA. Treatment was found to reduce the number of MRI hand and wrist bone erosions by 61% compared with the placebo [130]. Both animal and clinical studies indicate that BPs protect against generalized bone loss, but not all of them result in a significant reduction in focal bone damage [127]. The ability of a particular BP to protect the joint in RA also seems to be dependent upon the disease model used and also on the particular BP used.

Hormone replacement therapy

The role of hormones in the regulation of bone metabolism is well known and hormone replacement therapy has been demonstrated to be beneficial in preventing postmenopausal osteoporosis [133,134]. The use of hormone replacement therapy as a treatment for RA is very controversial as early studies indicated a protective effect of estrogen against RA [135], whereas more recent studies have disputed this observation [136]. Different effects have been reported with some

studies demonstrating a significant effect on the disease process whilst others show no effect. As a result of the potentially severe side effects, hormone replacement therapy is no longer recommended as a treatment for RA [137]. Animal studies using a rat CIA model demonstrated that estrogen treatment over a 28 day period delayed the time of disease onset and reduced the degree of disease, thus demonstrating a chondroprotective effect [138]. However, this is not supported by human studies where in a randomized controlled trial with 27,347 postmenopausal subjects, hormone therapy was demonstrated to have no significant effect on reducing the risk of developing RA, or on reducing the severity of RA [136]. Raloxifene is a selective estrogen receptor modulator that has been approved for the treatment of postmenopausal osteoporosis [139]. Using a CIA model of arthritis, raloxifene treatment (at 60 µg/mouse/day) was demonstrated to significantly reduce the frequency and severity of the disease, with very little joint destruction being observed in raloxifene-treated animals. Levels of the proinflammatory cytokine IL-6 were also significantly lowered in treated mice, indicating that raloxifene may also have an anti-inflammatory effect [139]. These results clearly demonstrate the conflicting and complex effects that hormone replacement can have on postmenopausal women.

RANKL/RANK interactions

The RANKL/RANK pathway is becoming an important target for the treatment of pathological bone loss. This was first tested with a single subcutaneous injection of OPG fused to the human IgG constant region in women with postmenopausal osteoporosis [140]. This single injection rapidly and profoundly reduced bone turnover for a substantial period of time, indicating that the inhibition of RANKL could be an effective treatment for bone loss diseases such as osteoporosis [140]. However some risks were identified and associated with chronic use of OPG, including the generation of anti-OPG antibodies, which could lead to cross reactivity with the endogenous OPG and would neutralize the OPG activity [141]. There was also the possibility of OPG binding to TNF-related apoptosis-inducing ligand (TRAIL), which could affect the normal defense mechanisms against tumors [141]. More recently, denosumab (formally known as AMG 162), a monoclonal antibody to RANKL, has been studied in clinical trials of postmenopausal women with low BMDs [142,143]. A single dose was found to

reduce bone resorption for up to 6 months [144]. Repeated injections resulted in significant increases in bone mass in the axial and peripheral skeleton, in trabecular and cortical bone. To date, no adverse side effects have been reported [143]. Injections of denosumab twice yearly with ongoing MTX treatment were found to inhibit structural damage in patients with RA with no increased rate of side effects [145]. At the 6 month time point, the increase in MRI erosion scores was significantly lower in the group treated with 180 mg of denosumab and there was also a significant difference in the modified sharp erosion score at 6 months. In addition, there sustained suppression of the bone turnover markers (serum CTX-1 and PINP) was observed. Little effect on cartilage was noted as cartilage turnover markers did not decrease at the 6 month time point [145]. Although denosumab significantly reduced bone destruction, denosumab did not, however, have an effect on RA inflammatory activity observed as RA flares, or on joint space narrowing, and had no significant effect on reducing cartilage erosion [145]. It was suggested that this lack of effect on joint space narrowing could be related to the specific action of denosumab, that is the inhibition of RANKL, or as a result of insufficient dosages used [145].

The role of the RANKL/OPG system in cartilage destruction is still not known. Inhibition of RANKL can protect the mineralized cartilage from osteoclast-mediated bone loss, but nonmineralized cartilage is not directly affected by osteoclasts [6]. In some studies, RANKL inhibition has been demonstrated to protect against proteoglycan loss and hence, cartilage damage, whereas in other studies, no protection was observed. Romas and colleagues found that while administration of OPG in a mouse CIA model markedly reduced bone erosions, the effects on cartilage were modest in the context of severe synovitis [146]. On the other hand, Kong and colleagues demonstrated that OPG largely preserved articular cartilage except at sites where cartilage was in direct contact with the pannus [47]. It is suggested that OPG has chondroprotective effects in the early disease phase, but once synovitis is established, this is no longer the case [146]. The effects of RANKL inhibition on cartilage could also be indirect, as protection from subchondral bone loss could also protect the cartilage attached to that bone from being destroyed [6,147]. This supports the concept that there is a relationship between the cartilage and bone. RANK and OPG are expressed by

chondrocytes and may be highly expressed in RA; however, RANKL was not able to activate human articular chondrocytes [148]. To date, the results of RANKL inhibitors such as denosumab indicate that inhibition of RANKL leads to increased BMD and decreased bone resorption. However, the use of RANK/RANKL inhibitors may have unwanted side effects, as they will inhibit both inflammatory and physiological bone resorption. It is still unclear whether RANKL inhibition has any positive effects on suppressing cartilage destruction.

Cathepsin K inhibitors

Cathepsin K is an enzyme found predominantly in osteoclasts and plays a role in bone resorption through its activity in the resorption lacunae. Since it is one of the few extracellular proteolytic enzymes capable of degrading native fibrillar collagen, it is thought to play an important role in joint destruction [149]. Cathepsin K is known to be highly expressed by synovial fibroblasts and macrophages in RA joints [150]. Since Cathepsin K is also thought to play an important role in osteoclast bone resorption, it has become a target for novel antiresorptive drugs. Cathepsin K inhibitors that are highly potent, selective and orally applicable have been developed and some have been demonstrated to inhibit bone resorption [151–153]. Selective Cathepsin K inhibitors have passed preclinical trials and are now in clinical trials for the treatment of osteoporosis [154].

IKK- β inhibition

The activation of IKK- β occurs downstream of RANK/RANKL ligation and IKK inhibitors may offer a selective and effective approach to inhibit NF- κ B activation. IKK- β is essential for NF- κ B activation in response to pro-inflammatory cytokines [58]. IKK- β phosphorylates the inhibitor subunit I κ B- α , which leads to its degradation and subsequent activation of NF- κ B. It was demonstrated that *in vitro*, bone marrow cells that were deficient in IKK- β did not form osteoclasts when stimulated with RANKL, confirming that inhibitors of IKK- β could reduce bone degradation in RA [58]. Oral administration of ML120B, a selective potent inhibitor of IKK- β , was demonstrated to inhibit paw swelling and offered significant protection against arthritis-induced cartilage and bone loss, using an adjuvant arthritis model. The general anti-inflammatory and chondro-protective effects may indicate that IKK- β activation is also involved with these aspects of the disease. In support of this, ankle joints obtained from

rats treated with ML120B also demonstrated a decrease in NF- κ B-regulated gene expression of the inflammatory cytokines TNF- α , IL-1 β and iNOS. There was also a downregulation of NF- κ B activity in joints of rats treated with this inhibitor [92]. Like many of these antiresorptive drugs, IKK- β inhibition may also inhibit immune responses. However, as yet, these side effects have not been reported with regard to the studies in models of RA. The beneficial effects of these inhibitors on inflammation, bone and cartilage loss could indicate that these drugs may be particularly useful for the treatment of RA.

■ Other approaches to treat RA

Vitamin D

Vitamin D, which is essential for the formation of healthy bone, is obtained from dietary sources and the action of sunlight. There are conflicting results with regard to whether vitamin D levels have any effect on the risk of developing RA and whether treatment with vitamin D can slow disease progression. One study found that vitamin D intake was inversely associated with RA onset [155], whereas another study demonstrated that vitamin D deficiency does not increase the risk of RA [156]. Vitamin D may be a potential treatment for RA as it can exert immunomodulatory effects. The oral administration of high-dose vitamin D has been found to be safe and can rapidly correct vitamin D deficiency followed by regular lower doses to maintain adequate levels [157]. In selected patients with RA who are at high risk of vitamin D deficiency, vitamin D replacement may reduce RA disease severity [157]. However, it is still not clear whether vitamin D is effective for treating RA in all patients.

Statins

Statins, also known as 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase inhibitors, are generally used in controlling lipid metabolism; however, recent studies have demonstrated a broader role for these drugs. One of the most commonly used statins in humans is simvastatin, and recent studies in animals and humans have shown promising results in the treatment of RA. In a Th-1 driven CIA model of murine inflammatory arthritis, simvastatin dose dependently suppressed the incidence and severity of CIA development and it also significantly inhibited the progression of RA within 3 days of treatment [158]. Simvastatin was also effective at blocking T-cell/macrophage interactions [159]. More recently, simvastatin was studied in a trial

involving 24 patients with RA and was found to improve the clinical symptoms of RA patients in a short period of time [159]. Treatment with simvastatin significantly decreased the levels of C-reactive protein and rheumatoid factor and it also reduced Th1:Th2 and CD4:CD8 ratios. No adverse effects of simvastatin were observed in this investigation [159]. In a recent study, rats were given an intraperitoneal injection of streptococcal bacteria to induce bone loss. Simvastatin administered at 20 mg/kg/day was found to reduce both early and late joint inflammation, reduce 60% of monocyte/macrophage influx and also to suppress periarticular bone destruction occurring late in the course of disease. It also inhibited the increase in periarticular osteoclasts in the arthritic joints [160]. These studies indicate that simvastatin may have therapeutic benefits for RA treatment, both through the suppression of inflammation and reduced periarticular bone destruction [159].

Physical activity

Other options, aside from drugs, are available to help reduce the progression of RA. Physical exercise is an option chosen by many patients. However, this only tends to have an effect on generalized bone loss associated with RA. A sedentary life style is a risk factor for osteopenia in RA with a relative risk of 1.6 [161], and moderate physical activity was found to reduce the risk of osteopenia by 50% [161].

■ Novel approaches

As our understanding of the mechanisms involved in the regulation of bone metabolism and the RA disease process improves, new approaches for the treatment of bone loss in RA are being developed.

Kinase inhibitors

These small intracellular molecules are new targets for therapeutics to treat RA. These inhibitors are small molecular weight peptides that are less expensive than many current therapies and appear to have the same efficacy as other biological agents with reduced side effects [125]. Kinases are an important class of intracellular molecules involved in signal transduction in inflammation. Binding of extracellular factors to surface receptors results in the activation of cytoplasmic kinases that modulate the activity of transcription factors, thus regulating the expression of genes [125]. There are now small molecular weight orally available inhibitors that target these enzymes. Two different types of kinases that can be targeted – MAPKs (p38,

ERK and JNK) and tyrosine kinases (JAK-3 and Syk) [125]. JAK-3 is a cytoplasmic tyrosine kinase that is activated by cytokines and involved in signaling in cells such as B cells, T cells and natural killer cells [162]. JAK-3 is known to play a significant role in the pathogenesis of RA [133]. A JAK-3 inhibitor (CP-690,550) is in Phase II clinical trials in patients and so far, remission has been achieved in a third of the individuals [125]. SyK is an intracellular protein kinase stimulated by activation of the Fcγ receptors and B-cell receptors. A SyK inhibitor (fostamatinib disodium) has been demonstrated to result in remission in over half the patients included in a RA clinical trial [125]. While the recent data regarding novel kinase inhibitors is encouraging, their direct effects on joint destruction are yet to be determined.

B-cell targets

Depletion of B cells is a new treatment strategy for RA. Rituximab is a chimeric monoclonal antibody targeting the CD20 molecule expressed on developing B cells. Depletion of B cells has been demonstrated to inhibit inflammation by the abolition of B cells as antigen-presenting cells, blocking the synthesis of proinflammatory cytokines such as TNF-α, leading to impaired formation of immune complexes [7]. Although these inhibitors target the inflammation, B cells also express RANKL and may thus play a role in the differentiation of osteoclasts [163]. Therefore, depletion of B cells could contribute to reducing the amount of osteoclast bone resorption.

Wnt pathway

The Wnt pathway is a family of glycoproteins that are involved in the regulation of multiple cellular activities including bone formation and remodeling. Dickkopf-1 (DKK-1) is an inhibitory molecule that regulates the Wnt pathway. DKK-1 is induced by TNF-α and increased levels of DKK-1 have been demonstrated to be associated with bone resorption and decreased levels of bone formation [164]. This Wnt pathway may form an important link between inflammation and bone metabolism and may be an effective target for RA therapeutic agents. Several of the Wnt family members have been demonstrated to modulate the inflammatory response in RA. Wnt7b was found to be expressed in the synovium from patients with RA [165]. In addition, immunohistochemistry revealed that Wnt7b was also present in the articular cartilage, bone and synovium of RA [165]. The cytokines TNF-α, IL-1β and IL-6 were also increased in

RA synovium and Wnt-7b-transfected normal synovial cells [165]. It is believed that the Wnt pathways could potentially promote synovial hyperplasia, inflammation, pannus formation and bone/cartilage destruction [166]. Therefore, the Wnt pathway provides a novel target for treating RA by changing the balance between bone formation and bone resorption.

PAR₂ agonists/antagonists

There has recently been increasing interest in the role that protease activated receptors (PARs), particularly PAR₂, play in mediating chronic inflammation. The PARs are a family of G-protein-coupled seven transmembrane domain receptors that are activated by proteolytic cleavage of the extracellular domain. Four PARs have similar mechanisms of action but have different biological functions and tissue distributions [167]. PAR₂ is found throughout the body, especially in the epithelium, endothelium, fibroblasts, osteoblasts, neutrophils, myocytes, neurons and astrocytes [168]. Expression in osteoblasts has been demonstrated both *in vitro* and *in vivo* [169]. PAR₂ is expressed by a small number of proteases including mast-cell tryptase, factor Xa and trypsin [170].

There is conflicting evidence as to whether PAR₂ activation or inhibition results in reduced osteoclastogenesis. Recent data has indicated that PAR₂ agonists can regulate bone loss and inhibit osteoclast differentiation by reducing the formation of RANKL [170]. In an adjuvant monoarthritis model, a reduction in inflammation and paw swelling was found in PAR₂-deficient mice compared with wild-type mice. However, activation of PAR₂ in the knee joint has demonstrated proinflammatory effects [171]. Injection of a native peptide, SLIGRL0NH₂, or a synthetic PAR₂ agonist, ASKH95, was also demonstrated to result in joint swelling and hyperemia [171], suggesting that PAR₂ antagonists could therefore play a role in inhibiting the inflammation and subsequent joint destruction in RA. Whether PAR₂ agonists or antagonists can be effective in RA treatment remains unclear and further studies are necessary. PAR₂ does remain a potential novel target to treat RA.

Histone deacetylase inhibitors

Enzymes known as histone deacetylases (HDACs) are emerging targets for therapeutic intervention in a variety of diseases [172,173]. These enzymes remove acetyl groups from the histone proteins that condense the chromatin and regulate gene repression [174]. HDAC inhibitors (HDACi) result

in the hyperacetylation of histones, and possibly other proteins, that modify the expression of numerous genes [174]. This can result in upregulation of cell-cycle inhibitors, downregulation of immune stimulators, repression of inflammatory cytokines [175] and suppression of osteoclast bone resorption [176]. In particular, several HDACs are known to regulate the transcription of genes such as *NF-κB* that are crucial for osteoclast development and inflammation [172,173].

There are 11 human isoforms of the zinc-containing HDACs (HDAC 1–11) [177] and a further seven NAD⁺-dependent enzymes (SIRT 1–7) [178]. Class I HDACs include HDAC 1, 2, 3 and 8 enzymes that are found mainly in the nucleus. Class II HDACs include HDAC 4, 5, 6, 7, 9 and 10 and are found in both the nucleus and cytoplasm [179]. Most HDACi research is currently focusing on developing inhibitors that selectively inhibit different HDAC isoforms, since they may have quite different anti- or pro-inflammatory properties. It is still not clear which HDAC class and which HDACs within these classes play a role in suppressing osteoclasts bone resorption.

HDACi have previously been investigated in models of RA and have been demonstrated to reduce bone destruction in both AIA and CIA models [83,176,180]. Two HDACi, suberoylanilide hydroxamic acid and MS-275 decreased bone resorption; however, suberoylanilide hydroxamic acid could not prevent the onset of arthritis while MS-275 displayed dramatic antirheumatic activities. High doses prevented bone erosion and also delayed the onset of arthritis [180]. Two HDACi, phenylbutyrate and trichostatin A, were found to reduce joint swelling, decrease subintimal mononuclear cell infiltration, inhibit synovial hyperplasia and suppress pannus formation and cartilage and bone destruction in a model of adjuvant arthritis [175]. These studies clearly demonstrate the potential of HDACi to suppress the bone resorption in RA. HDACi provide an appropriate therapeutic agent for treating pathological bone loss in RA and could potentially be administered as combination therapy with a DMARD, such as MTX, in which their mechanism of action is to reduce inflammation.

Conclusion

This review has discussed the major mechanisms involved in the osteoclast-mediated bone loss in RA and has given an overview of current and emerging treatments of joint destruction in RA. There is a strong case that antiresorptive treatments are needed in the treatment of RA.

First, antiresorptive therapy in combination with anti-inflammatory therapies can better prevent structural joint damage than treatment with anti-inflammatory therapy alone. Second, early antiresorptive treatments administered at initial diagnosis are likely to prevent the joint damage that may occur before slower acting therapies demonstrate their effects or before an effective anti-inflammatory therapy can be identified for an individual.

Future perspective

Effective targeted treatments for bone resorption by osteoclasts are, and will, become available in the near future. These are likely to become valuable additions for use in conjunction with the

anti-inflammatory therapies currently used to treat RA. Protection of the bone as early as possible in RA will allow rheumatologists time to establish the most effective long-term treatments for individuals with RA, while preserving the joint structure and function.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Executive summary

- Rheumatoid arthritis (RA) is a significant disease that results in the destruction of bone and cartilage.
- Bone loss also occurs around the joint and systemically.

Mechanisms of disease

- Enhanced osteoclast bone resorption is a hallmark feature of RA.
- There is a relationship between the immune system and bone metabolism – osteoimmunology understanding is vital for developing ways to treat RA.
- Imbalance between RANKL and OPG plays a major role in the destruction of RA, and enhanced signaling through the Receptor activator of NF- κ B ligand (RANKL)/ Receptor activator of NF- κ B (RANK) pathway is seen in RA.

Animal models of disease

- Animal models of RA have been useful to gain an understanding of bone loss in RA.
- Live animal micro-computed tomography is a new technique used to give a time-dependent analysis of bone volume loss.

Therapeutic approaches to treat RA

- Conventional treatments, such as DMARDs, inhibit the inflammation but have little direct effect on preserving joint structure.
- Antiresorptive therapies such as bisphosphonates, RANKL inhibitors and other emerging therapies, inhibit enhanced osteoclast bone resorption in RA.

Conclusion

- Antiresorptive therapies in combination with modern anti-inflammatory treatments may better prevent structural joint destruction and disease progression in RA.
- Protection of the bone upon diagnosis of RA will allow rheumatologists time to establish the most effective long-term treatment for individuals with RA while preserving the joint.

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