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Review Article

Bone erosions in rheumatoid arthritis: recent developments in pathogenesis and therapeutic implications

Panagiotis K. Panagopoulos[1], George I. Lambrou[1,2]

[1]Graduate Programme "Metabolic Bone Diseases", National and Kapodistrian University of Athens, Medical School; [2]First Department of Pediatrics, National and Kapodistrian University of Athens, Choremeio Research Laboratory, Athens, Greece

Abstract

Bone erosions develop early in the course of rheumatoid arthritis (RA) and deteriorate progressively, causing joint damage and resulting in impaired functional capacity of patients. During the last years, considerable number of studies has increased our understanding of the pathogenetic mechanisms mediating the development of bone erosions in RA. Increased production of RANKL and other cytokines, dysregulation of innate immune mechanisms, autoantibodies specific to RA and alterations of microRNA expression stimulate differentiation and function of osteoclasts, which are responsible for the development of bone erosions. Besides, increased levels of cytokines, overproduction of antagonists of the canonical Wnt signaling pathway and deficient production of bone morphogenetic proteins result in impaired osteoblast differentiation and function, undermining the capacity of bone erosions to repair. Disease-modifying antirheumatic drugs, synthetic or biological, currently used in the treatment of RA, can halt the progression of bone erosions and may even lead to partial repair, although complete repair is unattainable. Targeting pathogenetic mechanisms participating in the erosive process may add to the therapeutic effect of DMARDs and help in the prevention or repair of bone erosions. However, more studies are still needed to confirm whether such therapeutic strategies are effective.

Keywords: Bone, Bone Erosions, Rheumatoid Arthritis, Pathogenesis

Introduction

Rheumatoid Arthritis (RA) is the most common chronic inflammatory arthritis and is characterised by persistent inflammation of the synovial membrane (synovitis), which may cause progressive damage to the bone and cartilage of the joints. RA presents as a symmetric, peripheral polyarthritis, which, if left untreated, leads gradually to permanent joint damage and physical disability. Bone loss is a hallmark of this disease and occurs in three forms: (a) focal bone erosions located at the joint margins and subchondral bone, (b) periarticular bone loss adjacent to the inflamed joints and (c) systemic bone loss (osteoporosis)[1].

Bone erosions appear early in the course of RA, sometimes within weeks after diagnosis. More than 10% of the patients develop bone erosions within 8 weeks after disease onset, while up to 60% have erosions after 1 year[2]. Bone erosions progress in time and contribute to joint damage in patients with RA, leading to impaired functional capacity[3,4]. Thus, presence of bone erosions predicts a more severe clinical course, rendering them an important outcome measure in RA. Clinical trials with antirheumatic drugs assess their ability to slow down or stop radiographic damage, which includes bone erosions and cartilage destruction. In clinical practice, radiography is used to evaluate joint damage and monitor the efficacy of treatment. During the last years, significant progress has been made on understanding the pathogenetic mechanisms which mediate the formation of bone erosions in RA. This progress creates opportunities for new therapeutic targets in the treatment of this disease, which can be utilized for the prevention of new bone erosions or even the repair of the existing ones.

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Corresponding author: George I. Lambrou, First Department of Pediatrics, Choremeio Research Laboratory, National and Kapodistrian University of Athens, Thivon & Levadeias, 11527, Goudi, Athens, Greece
E-mail: glamprou@med.uoa.gr

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The purpose of this article is to review the literature on the pathogenetic mechanisms leading to the development of bone erosions in RA, as well as the implications on the treatment of this disease.

Osteoclasts are the cells responsible for bone erosions in RA

Chronic inflammation of synovial membrane (synovium) in RA results in hyperplasia and formation of pannus, a thickened, hypertrophied synovial membrane that invades the adjacent cartilage and bone and causes cartilage degradation and bone erosions. Thus, bone erosions in RA are typically located at the interface between pannus and bone at the joint margins and subchondral bone. Osteoclasts are the cells responsible for the development of bone erosions. Using Tartrate-Resistant Acid Phosphatase (TRAP) and calcitonin receptor as markers of osteoclasts, Gravallese et al. (1998) identified these cells at the pannus-bone interface where they form resorption pits leading eventually to bone erosions[5].

Imaging studies have demonstrated that bone marrow edema in Magnetic Resonance Imaging (MRI) often precedes the development of bone erosions in RA[6-8]. Histologic analysis has shown that bone erosions in MRI in RA correspond to a localized destruction of cortical bone adjacent to replacement of bone marrow fat by inflammatory cells. In addition, MRI bone marrow edema is due to infiltration of bone marrow by inflammatory cells, making the term "osteitis" more appropriate than "bone marrow edema"[9]. In another study, histologic examination of bone samples with MRI bone edema in RA identified infiltration of bone marrow by osteoclasts, macrophages, plasma cells, as well as CD8+ T cells and B cells[10]. Therefore, infiltration of bone adjacent to inflamed synovium by inflammatory cells and osteoclasts leads to increased bone resorption and eventually development of bone erosions.

There are a number of pathogenetic mechanisms leading to osteoclast activation and development of bone erosions in RA and they will be addressed in the following sections of this article.

Receptor activator of nuclear factor kappa-B ligand (RANKL) stimulates osteoclasts in RA

Receptor activator of nuclear factor kappa-B ligand (RANKL) is a cytokine belonging to the Tumor Necrosis Factor (TNF) superfamily, which binds to receptor activator of nuclear factor kappa-B (RANK) on osteoclasts and osteoclast precursors and is a key factor for osteoclast differentiation and activation. Osteoprotegerin (OPG) is a soluble decoy receptor of RANKL, which binds to RANKL and inhibits activation of RANK. Inflamed synovium and pannus in RA produce significantly higher levels of RANKL and lower levels of OPG in comparison to healthy synovium[11-13]. The cells responsible for the increased expression of RANKL in the inflamed synovial membrane are Fibroblast-like Synoviocytes (FLS) and T lymphocytes. The increased RANKL/OPG ratio results in increased osteoclast differentiation and activation at the synovium-bone interface and the development of bone erosions in RA[13,14].

The role of cytokines in osteoclast activation in RA

The central role increased cytokine production plays in osteoclastogenesis and bone erosion formation in RA is well known. However, during the last years our knowledge concerning cytokine-induced osteoclastogenesis in RA and implicated signaling pathways is constantly expanding.

Tumor necrosis factor (TNF) is a cytokine playing a principal role in the pathogenesis of RA and it is highly expressed by peripheral blood monocytes and synovial macrophages in patients with RA[15]. TNF stimulates osteoclast differentiation (osteoclastogenesis) both indirectly by enhancing RANK signaling pathway and directly by acting on osteoclast precursors. Firstly, TNF upregulates RANK expression in osteoclast precursors[16], as well as RANKL production by fibroblast-like synoviocytes (FLS)[17]. Moreover, TNF stimulates the expression of nuclear factor of activated T cells (NFATc1) and B lymphocyte-induced maturation protein-1 (Blimp1) in osteoclast precursors. NFATc1 and Blimp1 are transcription factors which are induced by RANKL and enhance ostoclastogenesis[18,19]. On the other hand, TNF acts directly on osteoclast precursors and facilitates their differentiation[20,21]. Furthermore, TNF stimulates the expression of macrophage colony-stimulating factor (M-CSF) by bone marrow stromal cells[22], as well as the expression of c-fms (the receptor for M-CSF) by osteoclast precursors[23]. M-CSF plays an important role in osteoclast differentiation.

Interleukin-1 (IL-1) is another cytokine participating in RA pathogenesis and is produced by peripheral blood monocytes and synovial macrophages in RA synovium[15]. IL-1 stimulates multinucleation and activation of osteoclasts. Besides, IL-1 acts synergistically with TNF in order to promote osteoclast differentiation. TNF enhances IL-1 expression and the latter induces RANKL expression by bone marrow stromal cells[24].

IL-6 also plays part in the pathogenesis of RA and increased osteoclast activity. It is expressed by FLS and synovial macrophages of the RA synovium[25,26] and mediates RANKL induction by TNF and IL-17, since it directly stimulates RANKL expression by FLS[17].

IL-17 is produced by T helper cells type 17 (Th17 cells) and plays an important role in RA pathogenesis and increased osteoclastogenesis. IL-17 stimulates osteoclastogenesis indirectly by enhancing RANK signaling, since it induces RANKL expression on osteoblastic cells[27] as well as RANK expression on osteoclast precursors[28]. IL-17 also enhances osteoclast differentiation and function by inducing prostaglandin-E_2 (PGE_2) expression on osteoblasts[29]. Besides, IL-17 acts directly on osteoclast precursors in order to promote osteoclastogenesis[30]. Furthermore, IL-17 enhances the production of cytokines TNF and IL-1 by macrophages[31] and IL-6 by fibroblasts[32].

IL-15, *IL-33* and *IL-34* also promote osteoclastogenesis in

RA. IL-15 is produced in inflamed synovium in RA, it induces cytokine production by T cells[33] and it enhances RANKL-dependent and T cell-dependent osteoclastogenesis[34,35]. IL-33 is expressed by synovial fibroblasts in RA[36] and stimulates osteoclastogenesis[37]. The expression of IL-33 is induced by TNF and IL-1[36]. IL-34 is produced by FLS in RA and facilitates chemotactic migration of peripheral blood mononuclear cells and RANKL-dependent osteoclast differentiation. The expression of IL-34 by FLS is induced by TNF[38].

On the other hand, other cytokines have an inhibitory effect on osteoclastogenesis. IL-4, IL-10, IL-23, IFN-α, -β, -γ (interferon-α, -β, -γ) and GM-CSF (granulocyte macrophage colony-stimulating factor) are produced by the inflamed synovium in RA[39-44] and inhibit osteoclastogenesis[45-51]. However these cytokines are not able to counteract the osteoclastogenic effect of RANKL, TNF, IL-1, -6, -15, -17, -33 and -34 , resulting in enhanced osteoclast differentiation and activation in RA and development of bone erosions.

Innate immune mechanisms and osteoclast activation in RA

During last years, the role of innate immune mechanisms in increased osteoclastogenesis and bone erosion formation in RA has come into light. These mechanisms include the ITAM signaling pathway and Toll-like receptors.

The immunoreceptor tyrosine-based activation motif (ITAM) signaling pathway

The Immunoreceptor Tyrosine-Based Activation Motif (ITAM) is a common sequence of four amino acids repeated twice in the cytoplasmic domains of transmembrane proteins which serve as signaling adaptors in innate and adaptive immune cells and in osteoclasts. The motif contains two tyrosine residues which are phosphorylated when the associated receptors bind to their ligands. The phosphorylated tyrosine residues form docking sites for tyrosine kinases and other proteins which activate intracellular signaling cascades[52]. The main ITAM-bearing signaling adaptors expressed by osteoclast precursors are DAP12 (DNAX-activating protein 12) and FcRγ (Fc receptor common γ subunit). Receptors associated with DAP12 are TREM2 (triggering receptor expressed on myeloid cells-2), MDL-1 (Myeloid DAP12-associated lectin 1) and Siglec-15 (Sialic acid-binding immunoglobulin-like lectin 15), whereas receptors associated with FcRγ are OSCAR (Osteoclast activating receptor), PIR-A (Paired immunoglobulin-like receptor A) and FcR (Fc receptors). The ITAM signaling pathway in osteoclast precursors acts synergistically with the RANK signaling pathway in order to induce osteoclast differentiation and activation. The activation of ITAM-signaling adaptors leads to an increase of cytosolic concentration of calcium, which is necessary for the activation of NFATc1 - a transcription factor of the RANK pathway[53].

The ITAM signaling pathway is activated in RA. Herman et al. demonstrated that OSCAR was expressed by osteoclasts at the sites of bone erosions in the joints of patients with RA. OSCAR was also expressed by mononuclear cells adjacent to synovial microvessels and by peripheral blood monocytes (PBMCs). The expression of OSCAR by PBMCs in RA patients was significantly higher compared to healthy controls and was correlated to disease activity. Monocytes with higher OSCAR expression were more likely to differentiate into osteoclasts. The expression of OSCAR by monocytes was induced by TNF. Thus, the activation of the ITAM signaling pathway in RA leads to increased osteoclastogenesis[54]. In another study, Crotti et al. found that the percentage of cells expressing OSCAR, FcRγ, DAP12 and TREM2 was significantly higher in active RA synovium compared to healthy synovium. These cells were mostly macrophage-like cells[55].

Toll-like receptors (TLR)

Toll-like receptors play an important role in innate immunity, since they are expressed by innate immune cells and are activated by pathogen-associated molecular patterns. TLRs are also expressed by osteoblasts and osteoclasts and participate in osteoclastogenesis. Activation of TLRs in osteoblasts induces the expression of RANKL and TNF, leading to increased osteoclastogenesis. Activation of TLRs in osteoclast precursors in early stages of differentiation inhibits osteoclastogenesis, whereas in cells having started osteoclastic differentiation, TLR activation enhances osteoclastogenesis. TLRs also promote the survival of mature osteoclasts[56].

The expression of Toll-like Receptor-5 (TLR5) in RA synovial tissue and fluid macrophages and in RA peripheral blood monocytes is significantly higher compared to control monocytes. Besides, the expression of TLR5 correlates with RA disease activity. TLR5 activation in RA monocytes leads to increased TNF expression, while TLR5 expression is enhanced by TNF (positive feedback regulation). Moreover, TLR5 expression in RA macrophages is induced by IL-8 and IL-17[57]. Another study demonstrated that ligation and activation of TLR5 in monocytes from RA patients results in stimulation of RANK expression and increased differentiation into mature osteoclasts. Furthermore, stimulation of TLR5 in a mouse model of RA increased TNF production, joint inflammation, osteoclast infiltration and bone erosions of the inflamed joints. On the other hand, treatment with anti-TLR5 antibody reduced joint inflammation and bone erosions in the RA mouse model[58]. Thus, it appears that increased expression of TLR5 in RA participates to osteoclastogenesis and formation of bone erosions.

TLR4 is another Toll-like receptor whose role in RA pathogenesis and osteoclastogenesis is being investigated. Patients with RA have increased levels of endogenous TLR4 ligands, such as proteins S100A8, S100A9, tenascin-C and HMGB-1 (high mobility group box chromosomal protein 1), in peripheral blood and in synovial fluid[59-63]. Furthermore, stimulation of TLR4 in RA synovial fibroblasts induces RANKL expression by them[64]. Animal models of RA exhibit increased expression of TLR4 in synovial membrane of the inflamed joints, while deletion of TLR4 gene results in reduced joint inflammation, reduced osteoclast infiltration and

reduced bone erosions of the joints[65-67]. In addition, mouse RA models exhibit increased expression of endogenous TLR4 ligands, which enhance osteoclastogenesis via TNF and IL-6. Treatment with anti-TLR4 antibody reduced joint inflammation and osteoclast infiltration of the joints of the RA mouse model[68].

Therefore, apart from TLR5, TLR4 also seems to participate in bone erosion formation in RA. However, more studies are needed to elucidate the role of Toll-like receptors in osteoclastogenesis in RA and the pathogenetic mechanisms that lead to their activation.

Autoantibody-mediated osteoclastogenesis in RA

Anti-citrullinated protein antibodies (ACPA)

Anti-Citrullinated Protein Antibodies (ACPA) are detected in patients with RA and are useful in RA diagnosis, since they are characterised by high specificity. Besides, positive ACPA in RA patients are predictive for the development of severe disease and of bone erosions in the joints[69]. Furthermore ACPA positive healthy individuals, without clinical signs of RA, exhibit bone loss periarticularly. Considering that ACPA positivity precedes clinical onset of the disease by years and that bone erosions may be detected in RA patients within the first few weeks after diagnosis, it may be speculated that osteoclast activation and bone resorption commence before clinical onset of RA and are caused not only by synovitis and cytokines but also by autoantibodies like ACPA[70].

Harre et al. investigated the effect of ACPA on osteoclastogenesis. They demonstrated that osteoclasts and osteoclast precursor cells express enzymes causing protein citrullination (Peptidylarginine Deiminases, PAD) and that citrullination of vimentin in osteoclast precursors is increased during their differentiation. ACPA with specificity to citrullinated vimentin are detected in serum of RA patients and bind to the surface of osteoclast precursors. Harre et al. showed that binding of citrullinated vimentin-specific ACPA to osteoclast precursors stimulates osteoclastogenesis and bone resorptive activity of osteoclasts. They also elucidated the mechanism for this effect; ACPA induce TNF expression by osteoclast precursors, which acts in an autocrine way to enhance osteoclastogenesis. In addition, ACPA induce the expression of RANK and the receptor of M-CSF by osteoclast precursors[71]. The induction of TNF production by monocytes and macrophages caused by ACPA has been demonstrated by another study as well[72].

In a recent study, Krishnamurthy et al. demonstrated that ACPA specific to citrullinated vimentin and enolase promote osteoclastogenesis, whereas ACPA specific to other citrullinated epitopes fail to do so. They also showed that ACPA induce the expression of IL-8 by osteoclast precursor cells, which acts in an autocrine way in order to facilitate osteoclastogenesis. Inhibition of IL-8 with an anti-IL-8 antibody neutralized this effect of ACPA, meaning that IL-8 probably mediates the osteoclastogenic effect of ACPA[73].

Further on, in another report it has been manifested that in ACPA-negative patients higher femoral z-scores were observed, as well as lower osteoporosis prevalence[74]. On the other hand, it has been reported that in ACPA-positive patients lower BMI and disease duration were observed. Finally, lumbar z-score was higher in ACPA–negative patients with a marginal significance, as well as, an inverse correlation between ACPA titer and femoral neck, total femur, and lumbar Z-score were observed[74].

Therefore, increasing evidence indicates a role for ACPA in osteoclastogenesis, bone erosion formation and bone loss in RA. Further studies are needed to show if these findings may have an application in clinical practice.

Autoantibody immune complexes

Besides ACPA, it seems that autoantibody immune complexes (complexes derived from autoantibodies binding to their antigen) also promote osteoclastogenesis in RA. Osteoclast precursor cells and mature osteoclasts express fragment crystallizable (Fc) γ receptors (FcγR) in the cell surface, which recognize and bind to IgG antibodies (immunoglobulin G). Seeling et al. showed that stimulation of FcγRIV on the surface of osteoclast precursors promoted osteoclast differentiation and activity. Moreover, in a mouse model of RA, deletion of the FcγRIV gene specifically from osteoclast precursors resulted in a reduction of both osteoclast infiltration and bone erosions in the inflamed joints[75]. Furthermore, Harre et al. demonstrated that IgG sialylation influences the ability of IgG immune complexes to promote osteoclastogenesis. Desialylated immune complexes stimulated osteoclast differentiation and bone resorption, whereas sialylated immune complexes had not this effect. In addition, by using random IgG, Harre et al. showed that the osteoclastogenic effect of desialylated immune complexes was a common feature of all IgG antibodies, independent of their antigen specificity, because of the stimulation of FcγR receptors. Moreover, patients with RA exhibiting low-sialylated IgG have significantly lower bone volume compared to patients with high levels of IgG sialylation. Besides, pathogenic antibodies like ACPA are less sialylated compared with total IgG and *in vitro* sialylation of ACPA by Harre et al. resulted in a loss of their osteoclastogenic effect. These findings led Harre et al. to examine the effect of the sialic acid precursor N-acetylmannosamine (ManNAc) on a mouse model of RA. Treatment with ManNAc reduced significantly osteoclast infiltration and prevented bone erosions in the joints of the RA mouse model[76]. Thus, it seems that immune complexes contribute to osteoclastogenesis and development of bone erosions in RA, particularly desialylated IgG immune complexes.

microRNAs regulate osteoclastogenesis in RA

microRNAs (miRNAs) are small, single-stranded, non-coding RNAs, consisting of 20-25 nucleotides, which regulate gene expression at post-transcriptional level. miRNAs bind to the 3'-Untranslated Region (3'-UTR) of their target messenger RNA (mRNA) via partial complementarity and they inhibit its translation or cause its degradation. Therefore,

miRNAs inhibit the expression of their target gene at post-transcriptional level. miRNAs are encoded either by separate miRNA genes or by sequences located within the introns of other genes. Multiple miRNAs may regulate the expression of one target gene and each miRNA may regulate the expression of multiple target genes[77]. The expression of 90% of protein-coding genes is regulated by miRNAs. As a result, miRNAs participate in several biological processes, such as cell proliferation, cell differentiation, apoptosis and immune cell lineage commitment. Besides, altered expression of miRNAs is involved in the development of various diseases. During the last years, the involvement of miRNAs in the pathogenesis of RA has drawn notable attention and has been studied broadly[78]. A number of studies have examined particularly the role of miRNAs in the regulation of osteoclastogenesis in RA.

The expression of miRNA-155 (miR-155) is upregulated in synovial membrane macrophages and fibroblasts and in synovial fluid macrophages from patients with RA[79,80]. The target of miR-155 is Src Homology 2-Containing Inositol Phosphatase-1 (SHIP-1), which inhibits the expression of several proinflammatory cytokines. Therefore, upregulation of miR-155 in RA synovial monocytes and macrophages results in downregulation of SHIP-1 and increased expression of TNF, IL-1, IL-6 and IL-8[79]. Indeed, deletion of miR-155 gene in a mouse model of RA (mice with collagen-induced arthritis) prevented joint inflammation and development of bone erosions[79,81]. In another mouse model of RA (K/BxN serum-transfer induced arthritis model) by Bluml et al., deletion of miR-155 reduced significantly osteoclast infiltration and bone erosions of the joints, without affecting joint inflammation. Furthermore, Bluml et al. demonstrated that miR-155 deletion inhibits osteoclast differentiation in osteoclast precursor cell cultures *in vitro*, particularly the late stages of osteoclastogenesis. Thus, Buml et al. concluded that miR-155 plays an important role in late-stage osteoclastogenesis[81].

miR-223 is upregulated in T cells and in synovial membrane from patients with RA[82,83]. The target of miR-223 is Nuclear Factor I-A (NFI-A), which inhibits the expression of the receptor of M-CSF (M-CSFR). Thus, miR-223 downregulates NFI-A in osteoclast precursor cells, resulting in increased expression of M-CSFR and stimulation of osteoclastogenesis. Besides, M-CSF induces the expression of miR-223 in osteoclast precursors via transcription factor PU.1 (positive feedback regulation)[84]. miR-223 is also upregulated in the joints of a mouse model of RA, while inhibition of miR-223 ameliorated joint inflammation, attenuated osteoclastogenesis and reduced bone erosions in the RA mouse model[83].

Another miRNA upregulated in RA is miR-146, which is overexpressed in synovial membrane macrophages and T cells from patients with RA, as well as in peripheral blood monocytes and T cells[85,86]. However, unlike miR-155 and miR-223, miR-146 inhibits osteoclastogenesis, since its target is TNF Receptor-Associated Factor 6 (TRAF6) which participates in RANKL signaling and in osteoclastogenesis[87]. The expression of miR-146 is induced by NF-κB, while its targets TRAF6 and IRAK1 (IL-1 receptor-associated kinase 1) participate in innate immunity, cytokine production and osteoclastogenesis. Thus, it seems that the role of miR-146 in RA involves control of cytokine signaling and osteoclastogenesis through a negative feedback regulation loop[87,88]. Nakasa et al. investigated the potential of utilizing miR-146 for the prevention of bone erosions in RA. They administrated miR-146 in a mouse model of RA, resulting in a reduction of osteoclast infiltration and prevention of bone destruction without amelioration of joint inflammation[87].

miR-124 is another miRNA which was recently studied for its potential of being used in the treatment of RA. One of miR-124 targets is NFATc1, which participates in RANKL signaling and osteoclastogenesis. Administration of miR-124 in a rat model of RA resulted in amelioration of arthritis, downregulation of NFATc1 and RANKL expression, inhibition of osteoclastogenesis, reduction of osteoclast numbers and attenuation of bone destruction[89].

Therefore, alterations in miRNA expression contribute in increased osteoclastogenesis and development of bone erosions in RA joints. Besides, certain miRNAs that inhibit osteoclast differentiation could be used in order to attenuate bone destruction in RA. However, more preclinical studies in this field are needed, in order to address delivery and safety issues, before miRNAs are tested in human patients with RA.

Osteoblast differentiation and function are impaired in RA

Development of bone erosions in RA is attributed not only to enhanced osteoclast differentiation and activation but also to impaired osteoblast differentiation and function. Inhibition of differentiation and function of osteoblasts in RA leads to impaired bone formation and limited capacity of bone erosions to repair. Walsh et al. demonstrated that bone formation was reduced at bone surfaces adjacent to inflamed synovial membrane at sites of bone erosion in a mouse model of RA, compared to bone surfaces distal to the inflamed synovium. In addition, there was a paucity of mature osteoblasts at the sites of bone erosion, whereas there was an abundance of osteoblast progenitor cells. Thus, inflammation impairs osteoblast differentiation and bone formation, leading to failure of bone erosions to repair in RA[90]. Besides, resolution of inflammation in the same mouse model of RA led to an increase in the number of mature osteoblasts populating the sites of bone erosion, resulting in increased bone formation and erosion repair[91].

The pathogenetic mechanisms leading to impaired osteoblast differentiation and function and reduced capacity of the erosions to repair will be addressed in the following sections.

Cytokines inhibit osteoblast differentiation and function in RA

TNF, a key factor in RA pathogenesis, is a potent inhibitor of osteoblast differentiation[92,93]. It promotes degradation of transcription factor Runx2, which plays central role in

osteoblast differentiation[94]. Furthermore, TNF inhibits the production of several bone matrix components by osteoblasts, such as type I collagen and osteocalcin, as well as the production of alkaline phosphatase, which participates in bone matrix mineralization. Thirdly, TNF induces the apoptosis of osteoblasts[95]. Besides, TNF-transgenic mice, a mouse model of RA which overexpress TNF, are characterised by impaired osteoblast differentiation and activity, leading to reduced bone formation and lack of repair in bone erosions[96].

IL-1, another cytokine participating in RA pathogenesis, inhibits osteoblast proliferation and expression of bone matrix components, resulting in reduced bone formation[97]. Furthermore, IL-1 inhibits chemotactic migration of osteoblasts and impairs their recruitment to sites of bone erosions in RA[98].

IL-6 also takes part in RA pathogenesis and has been recently demonstrated to mediate the inhibition of osteoblast differentiation by TNF. Furthermore, IL-6 inhibits bone morphogenetic protein (BMP)-2/7-mediated osteoblast differentiation[99].

The canonical Wnt signaling pathway is inhibited in RA

The canonical Wnt signaling pathway plays a key role in osteoblast differentiation and activity. It is activated by soluble Wnt ligands, which bind to the Frizzled protein receptors and the lipoprotein receptor-related protein (LRP)-5/6 co-receptors. Activation of the canonical Wnt pathway leads to inhibition of Glycogen Synthase Kinase (GSK)-3β and translocation of transcription factor β-catenin to nucleus, where it induces expression of genes controlling osteoblast differentiation. The canonical Wnt pathway is inhibited by soluble antagonists, including secreted Frizzled-Related Proteins (SFRP), Dickkopf (Dkk) proteins and Sclerostin (SOST). SFRPs bind to Wnt ligands and prevent their interaction with the Frizzled-LRP5/6 complex, while Dkk proteins and sclerostin bind to LRP5/6 co-receptors and inhibit Wnt pathway activation[100].

Several studies have investigated the role of Wnt antagonists in osteoblast inhibition and development of bone erosions in RA. Diarra et al. (2007) demonstrated that RA synovial tissue fibroblasts express increased levels of Dickkopf-1 (Dkk-1) and TNF induces Dkk-1 expression. In addition, serum levels of Dkk-1 are increased in RA patients in comparison with healthy population and are correlated with disease activity. Besides, inhibition of Dkk-1 with an anti-Dkk-1 antibody in a mouse model of RA enhanced osteoblast differentiation and function, increased bone formation and prevented development of bone erosions, while it did not influence joint inflammation. Therefore, increased expression of the Wnt signaling antagonist Dkk-1 in RA contributes to development of bone erosions via inhibition of osteoblast differentiation and function[101]. Other studies confirmed increased expression of Dkk-1 by synovial fibroblasts in RA[102] and increased serum levels of Dkk-1 in RA patients[103-105]. Dkk-1 peripheral blood levels correlate with disease activity and are associated with increased risk of developing bone erosions. Treatment with TNF inhibitors and an IL-1 receptor antagonist reduces Dkk-1 levels in RA patients[103-105]. Besides, three single nucleotide polymorphisms (SNPs) of the Dkk-1 gene were associated with bone destruction in a cohort of RA patients, while one Dkk-1 SNP was associated with serum levels of Dkk-1[106]. Furthermore, Dkk-1 enhances TNF-mediated expression of sclerostin by osteoblasts[107], while, unlike TNF, IL-6 suppresses Dkk-1 expression by synovial fibroblasts[108].

Besides Dkk-1, the expression of other Wnt signaling antagonists is upregulated in arthritic joints as well. In a mouse model of RA, in which osteoblast differentiation was impaired at sites of bone erosion, synovial membrane expressed increased levels of Dkk-1, Dkk-3, SFRP-1, SFRP-2 and SFRP-4 in comparison with nonarthritic mice[90]. In the same mouse model of RA, resolution of inflammation resulted in downregulation of the expression of SFRP-1 and SFRP-2 by synovial membrane and upregulation of the Wnt ligand Wnt1Ob, leading to an increase in osteoblast differentiation, bone formation and repair of erosions[91].

Sclerostin is another Wnt signaling antagonist and polymorphisms (SNPs) of its gene have been associated with bone destruction of the joints in patients with RA[106]. In a mouse model of RA (TNF transgenic mice), inhibition of sclerostin with a monoclonal antibody stopped the progression of bone erosions, as well as periarticular and systemic bone loss. Moreover, combination treatment with anti-sclerostin and anti-TNF antibodies led to an increase of periarticular osteoblast numbers and regression of bone erosions[109]. On the other hand, a small study with 40 RA patients did not find significantly different levels of serum sclerostin between RA patients and healthy controls. It also did not find significant correlation between serum sclerostin level and progression of bone destruction in the joints[110]. In addition, inhibition of sclerostin in mice with collagen-induced arthritis (mouse model of RA) was not able to prevent the development of bone erosions or repair them, although it prevented or reversed systemic bone loss[111]. Furthermore, a recent study by Wehmeyer et al. found that sclerostin inhibition in TNF transgenic mice accelerates inflammatory arthritis and join destruction. In another mouse model of RA (K/BxN serum-transfer induced arthritis model), which is independent of TNF signaling, sclerostin inhibition ameliorated disease severity. Wehmeyer et al. concluded that sclerostin may play a protective role in TNF-mediated inflammation[112]. Therefore, more studies are needed on the role of sclerostin in RA pathogenesis and development of bone erosions.

Besides, alterations in the expression of microRNAs (miRNAs) by inflamed synovial tissue influence bone formation in RA and potentially participate in the development of bone erosions. Recent study on a mouse model of RA identified 22 miRNAs whose expression in synovial tissue differed between arthritic and nonarthritic mice and several of which targeted Wnt and BMP signaling pathway. miR-221-3p, which was overexpressed in inflamed synovial tissue, targets Dkk-2, which facilitates osteoblast differentiation and bone matrix mineralization. Moreover, TNF induced the

expression of miR-221-3p by fibroblast-like synoviocytes (FLS) derived from RA patients, as well as the secretion of miR-221-3p by FLS, packaged in extracellular vesicles called exosomes. Therefore, it appears that inflammation in RA synovium upregulates the expression of miR-221-3p by FLS. FLS secrete exosomes containing miR-221-3p, which are taken up by osteoblasts at erosion sites. As a result, Dkk-2 expression in osteoblasts is downregulated, leading to inhibition of osteoblast differentiation and defective bone formation in RA erosions[113].

Thus, increasing number of preclinical and clinical studies indicate a pathogenetic role for the canonical Wnt signaling pathway in RA. It seems that downregulation of this pathway leads to impaired osteoblast differentiation and activity and consequently, to reduced capacity of bone erosions to repair. Preclinical studies show that these findings could have implications in RA treatment, although more studies are required in this direction.

Bone morphogenetic protein (BMP) signaling in RA

Bone Morphogenetic Protein (BMP) signaling and particularly BMP-2, -4, -5, -6 and -7 play an important role in osteoblast differentiation and induce bone formation[114]. Expression of BMP-4 and BMP-5 is significantly decreased in synovial tissue of RA patients compared to that of healthy controls[115] and may be partially responsible for reduced bone formation at sites of bone erosion. In addition, expression of BMP-4, BMP-6 and BMP-7 was found to be downregulated in the joints of a mouse model of RA, whereas expression of BMP receptors type IA and II was upregulated[116]. On the other hand, serum levels of BMP-7 were higher in RA patients in comparison with healthy controls[117].

BMP-3 is a BMP signaling antagonist and its involvement in the erosive process in RA was recently studied on two murine models of RA. Matzelle et al. (2016) found that BMP-3 was highly expressed by osteoblasts at the sites of bone erosion and also TNF induced BMP-3 expression. Thus they proposed that upregulated BMP-3 inhibits osteoblast differentiation and function in RA and contributes to the development of bone erosions[118].

Therefore, dysregulated BMP signaling seems to participate in the inhibition of osteoblast activity and defective bone formation at sites of bone erosions in RA, although further studies are needed in this direction.

Therapeutic implications

Bone erosions progress in time, cause joint damage and impair functional capacity of patients[3,4]. Thus, inhibition of erosive process and repair of bone erosions is an important therapeutic target in RA. Conventional synthetic disease-modifying antirheumatic drugs (csDMARDs), such as methotrexate, sulfasalazine and chloroquine, have demonstrated limited results, since they achieve limited repair of bone erosions in a small proportion (7-10%) of RA patients[119,120]. Thus, attention has been drawn to biological agents (biological DMARDs), whose introduction in RA therapeutics has permitted the achievement of clinical remission or low disease activity. During the last years, it has been investigated whether control of inflammation with biological agents in RA can lead to the repair of bone erosions.

Finzel et al. studied the effect of TNF inhibitors (TNFi) on bone erosions; they used high resolution micro-computed tomography (μCT) in order to assess width and depth of bone erosions in RA patients treated with TNFi versus methotrexate. Bone erosions in patients receiving TNFi showed partial repair and decrease in depth after 1 year of treatment, in contrast to bone erosions in patients receiving methotrexate which deteriorated. Repair was noted only in erosions with evidence of bone apposition (sclerosis). Moreover, deeper erosions were more prone to repair, implying that repair begins from the endosteal lining and the bone marrow[121]. Other studies have also shown that TNFi halt the progression of bone erosions and may achieve partial repair of them[122-124].

Finzel et al. (2013) also investigated the effect of blockade of IL-6 receptor (IL-6R) with a monoclonal antibody on bone erosions in RA patients. IL-6R blockade induced partial repair in erosions exhibiting sclerosis; repair was more prominent in larger erosions[125]. Other studies also showed that blockade of IL-6R stops the progression of bone erosions in RA[126,127]. Furthermore, abatacept, a biological agent that inhibits T cell co-stimulation, has been shown to slow the progression of bone erosions in RA patients[128]. Besides biological agents, targeted synthetic DMARDs, such as JAK inhibitors, have been recently added in the armamentarium of RA therapies. Janus kinases (JAK) participate in pathways transmitting signals of multiple cytokine receptors which take part in RA pathogenesis[129,130]. Tofacitinib inhibits JAK1 and JAK3 and has been shown to arrest the progression of bone erosions in RA patients[129,131]. At the same time, Baricitinib, blocks the JAK1 and JAK2 kinases. Baricitinib reversibly inhibits JAK1 and JAK2 while it is less selective for the JAK3 kinase. In general, JAK inhibitors, act through a signal transduction pathway involving STAT proteins, which conclusively regulates down-stream gene expression in immunological cells[132]. Recently, baricitinib was shown to reduce the progression of bone erosions and structural joint damage in patients with RA[133].

Therefore, biological and synthetic DMARDs control inflammation in RA, achieve clinical remission or low disease activity, halt the erosive process and lead to partial repair of bone erosions. However, these drugs cannot induce full repair and regression of the erosions. A reason for this failure seems to be residual joint inflammation, in spite of clinical remission. In a mouse model of RA mentioned above, inflammation inhibited osteoblast differentiation and function in the sites of bone erosions[90], whereas resolution of inflammation increased the number of mature osteoblasts populating those sites and resulted in new bone formation and erosion repair[91]. Another reason for the possible failure of DMARDs in term of prevention or regression of the erosions might be the presence of concomitant osteoporosis[134].

Besides, bone erosions which progressed in RA patients despite treatment with TNFi were characterized by presence of inflammation (osteitis) in MRI[122]. Thus, administration of osteoanabolics alongside DMARD treatment was considered in order to achieve full erosion repair. However, although coadministration of parathormone (PTH) and TNFi in a mouse model of RA led to full regression of bone erosions[96], coadministration of PTH analogue teriparatide and TNFi in RA patients failed to achieve erosion repair[135,136]. Apart from teriparatide, denosumab is another antiosteoporotic drug which was studied for its effect on bone erosions in RA. Denosumab is a monoclonal antibody targeting RANKL, with anticatabolic and antiosteoclastogenic effects. Several studies have shown that denosumab halts the erosive process in RA[137-139]. Moreover, a recent study found that denosumab led to partial repair of bone erosions in RA patients, since it reduced their width, depth and volume. Moreover denosumab increased bone mineral density in the margin of the erosions[140].

Lately, inhibition of pathogenetic mechanisms participating in the erosive process has been studied in preclinical studies as a novel therapeutic strategy in RA treatment. Blockade of TLR4 with a monoclonal antibody in a mouse model of RA reduced osteoclast infiltration of the joints[68], while blockade of TLR5 inhibited osteoclast differentiation and reduced bone erosions in the same RA mouse model[58]. Moreover, enhancement of the sialylation of IgG immune complexes with the sialic acid precursor N-acetylmannosamine (ManNAc) in a mouse model of RA led to a significant reduction of osteoclast infiltration in the joints and prevented the formation of bone erosions[76]. Besides, inhibition of miR-223 in a RA mouse model attenuated osteoclastogenesis and reduced bone erosions[83], while administration of miR-146 reduced osteoclast infiltration and prevented bone destruction[87]. Similarly, administration of miR-124 downregulated NFATc1 and RANKL expression, inhibited osteoclastogenesis, reduced osteoclast numbers and attenuated bone destruction[89]. However, these are preclinical studies in animal models of RA. Clinical studies are needed in order to determine whether these strategies are effective in the treatment of RA and in the prevention of bone erosions.

Gene therapy in RA

Besides, new innovative therapeutic approaches have emerged for the treatment of RA, including gene therapy and cell based therapy. Several gene targets have been studied for RA gene therapy. In a double-blinded, placebo-controlled, phase 1 study, an adeno-associated virus vector containing a TNF antagonist gene was injected intra-articularly in patients with RA. It was found to be safe and well tolerated, but was not shown to be superior to placebo after 12 weeks of follow-up[141]. In another clinical study, two patients with RA received *ex vivo* gene therapy with the gene of IL-1 receptor antagonist (IL-1Ra). Autologous synovial fibroblasts were transduced with a retrovirus carrying IL-1Ra and then were injected in the arthritic joints of the patients. After 4 weeks, pain and swelling were reduced in arthritic joints, while increased expression of IL-1Ra and reduced expression of IL-1 and matrix-metalloproteinase-3 were measured in the synovium[142]. In a more recent study on an animal model of RA, the IL-1Ra gene was transfected in mesenchymal stem cells (MSCs) which were then encapsulated in alginate-poly-L-lysine (APA) microcapsules. The APA microcapsules containing the IL-1Ra gene modified MSCs were shown to attenuate the arthritis in the rat model of RA[143]. IL-10 is an anti-inflammatory cytokine which has also been studied in RA gene therapy. Nanoparticles containing IL-10 encoding plasmid DNA were injected in a rat model of RA, resulting in a significant reduction of systemic and joint expression of pro-inflammatory cytokines (TNF, IL-1 and IL-6), as well as in attenuation of inflammation and joint damage[144].

Apart from cytokines, calcium channels have also been studied as gene targets in RA. CD4 T cells of patients with RA express increased levels of calcium release-activated calcium (CRAC) channels, resulting in upregulation of Ca^{2+} influx and expression of pro-inflammatory cytokines[145]. Systemic gene silencing of CRACM3 in a RA mouse model, using lentiviral vectors containing short hairpin RNA targeting this specific gene, resulted in significant decrease of cytokine expression (including TNF, IL-2, IL-6, Il-17), a marked attenuation of arthritis severity, as well as a significant reduction on inflammatory infiltration, pannus formation, cartilage destruction and bone erosions[146]. Therefore, gene therapy in RA has shown its effectiveness in several preclinical and some clinical studies and it is a promising approach in RA treatment. However, additional studies are needed in order to attain more information concerning pharmacokinetics and safety issues, before RA gene therapy reaches clinical practice[147].

Cell based therapy in RA

Another novel field in RA therapeutics is cell based therapy; mesenchymal stem cells and dendritic cells have been studied during the last years for their potential in the treatment of RA. Thus, the systemic administration of mesenchymal stem cells (MSCs) in RA patients and in animal models of RA has been shown to have anti-inflammatory and immunomodulatory effects. Indeed, MSC transplantation has been shown to reduce the number of GM-CSF-expressing CD4 T cells[148], as well as the number of T follicular helper cells[149] and CD4 Th1 and Th17 cells[150], which play central role in RA pathogenesis. Besides, MSC administration leads to an increase in the number of regulatory T cells in draining lymph nodes[148]. Therefore, systemic administration of MSCs in animal models of RA attenuates the severity of arthritis, since it reduces synovitis, formation of pannus and destruction of cartilage and bone[148,150]. Besides, MSCs attenuate local bone destruction, periarticular and systemic bone loss in animal RA models, by inhibiting RANKL-induced osteoclastogenesis[151]. Two clinical trials have studied the administration of MSCs in RA patients who have not

responded to standard therapy. The first one enrolled 172 patients with RA who received human umbilical cord MSCs (UC-MSCS) plus DMARDs or DMARDs alone. No serious adverse events were observed and UC-MSCs reduced TNF and IL-6 serum levels, increased the population of regulatory T cells in peripheral blood and induced a significant remission of RA[152]. Recently, a multicentre, randomized, single-blinded (double-blinded for efficacy), placebo-controlled, phase Ib/IIa clinical trial showed the safety of allogenic adipose-derived MSCs in 53 patients with active refractory RA. In addition, it demonstrated a trend for clinical efficacy for the MSCs[153].

Tolerogenic dendritic cells have also been studied as a novel therapeutic approach for RA. Dendritic cells (DCs) play key role in immune system since they present antigens to T cells. Tolerogenic DCs present antigens without adequate costimulation, resulting in T cell anergy, clone deletion, induction of regulatory T cells and ultimately, antigen tolerance. Ren et al. incubated immature DCs with tacrolimus in order to generate tolerogenic DCs and then injected them to a mouse model of RA. Tolerogenic DCs attenuated the development of arthritis and altered the population of CD4 T cells, promoting Th1 and suppressing Th17 differentiation[154]. In another pleclinical study, Zhang et al. injected IL-1O- and TGF-beta-expressing DCs to a RA mouse model and demonstrated that these cells ameliorated arthritis, reduced cartilage and bone erosions, inhibited Th17 differentiation and induced generation of regulatory T cells[155]. Besides, Park et al. found that tolerogenic semimature DCs ameliorate arthritis in a mouse model of RA, by enhancing regulatory T cell population and suppressing Th1/Th17 immunity[156]. Furthermore, a phase 1 clinical trial generated tolerogenic DCs by treating DCs with an NF-κB inhibitor and exposing them to four citrullinated peptide antigens. The DCs were then administered intradermally to 18 RA patients who were HLA-DRB1 and ACPA positive. The immunotherapy was well tolerated, increased regulatory T cell population and reduced disease activity[157]. Recently, another phase 1 clinical trial assessed the safety of intra-articular autologous tolerogenic DCs in patients with RA and inflammatory arthritis. The tolerogenic DCs were incubated with autologous synovial fluid as a source of autoantigens and then were injected arthroscopically to the patients. Intra-articular tolerogenic DC therapy was demonstrated to be safe, although no clinical or immunomodulatory effects in peripheral blood were detected[158].

Therefore, mesenchymal stem cells and tolerogenic dendritic cells have demonstrated promising results in preclinical and in preliminary clinical studies. They have immunomodulatory effects and their administration in early stages of RA could achieve remission. However, larger clinical studies are needed in order to provide more evidence concerning their efficacy and safety.

Conclusion

Bone erosions develop early in the course of RA and are predictive of a worse prognosis. They deteriorate gradually and cause joint damage, resulting in impaired functional capacity and disability. Lately, a considerable number of studies have increased our understanding of the pathogenetic mechanisms participating in the development of bone erosions in RA. Osteoclasts are the responsible cells and multiple factors have been identified to stimulate their differentiation and function. RANKL and other cytokines have been known for a long time to enhance osteoclastogenesis, but the role of other pathways has also been revealed recently. Thus, dysregulation of innate immune mechanisms in RA - including both activation of the ITAM signaling pathway and increased expression of Toll-like receptors in osteoclast precursor cells - results in increased osteoclastogenesis. In addition, anti-citrullinated protein antibodies bind to citrullinated protein epitopes on the cellular membrane of osteoclast precursors and stimulate their differentiation, while autoantibody immune complexes bind to receptors on the same cells and also enhance their differentiation. Furthermore, dysregulation of microRNA expression by osteoclasts and cells of the synovial membrane contributes to increased osteoclastogenesis in RA. Besides to excessive osteoclastogenesis, impaired osteoblast differentiation and function also plays part in bone erosion formation in RA. Inflamed synovial membrane produces increased levels of cytokines and antagonists of the canonical Wnt signaling pathway, which inhibit osteoblast differentiation and function. In addition, lower production of bone morphogenetic proteins impairs osteoblastogenesis.

Increased understanding of the mechanisms contributing to the development of bone erosions in RA has created new therapeutic targets. Currently existing disease-modifying antirheumatic drugs (DMARDs) - either synthetic or biological - control inflammation and disease activity, alleviate symptoms, stop the development of bone erosions and may even partially repair some of them. However, DMARDs never fully repair existing bone erosions. Targeting pathogenetic mechanisms taking part in the erosive process may add to the therapeutic effect of DMARDs and achieve prevention and full repair of bone erosions in RA. There are a small number of preclinical studies in animal models of RA indicating the efficacy of blocking TLR4 and TLR5 in preventing the erosive process, as well as the efficacy of enhancing sialylation of immune complexes, inhibiting miRNAs that facilitate osteoclastogenesis or administrating miRNAs that reduce osteoclastogenesis. Furthermore, novel therapeutic approaches have emerged, including gene therapy and cell based therapy. Nevertheless, more preclinical and clinical studies are needed in order to confirm whether these therapeutic strategies are effective, safe and prevent disease progression and bone erosions. Besides, considering that low bone mineral density at the cortical bone has been associated with bone erosions in patients with RA, an interesting approach would be to treat with agents that not only address inflammation but also improve cortical bone[159].

References

1. Shah A, Clair W. Rheumatoid Arthritis. In: Kasper D, Fauci A, Hauser S, Longo D, Jameson L, Loscalzo J, editors. Harrison's Principles of Internal Medicine. 19th ed: McGraw-Hill Education; 2015. p. 2136-49.
2. Machold KP, Stamm TA, Nell VP, Pflugbeil S, Aletaha D, Steiner G, et al. Very recent onset rheumatoid arthritis: clinical and serological patient characteristics associated with radiographic progression over the first years of disease. Rheumatology (Oxford, England) 2007;46(2):342-9.
3. Odegard S, Landewe R, van der Heijde D, Kvien TK, Mowinckel P, Uhlig T. Association of early radiographic damage with impaired physical function in rheumatoid arthritis: a ten-year, longitudinal observational study in 238 patients. Arthritis and rheumatism 2006; 54(1):68-75.
4. Welsing PM, van Gestel AM, Swinkels HL, Kiemeney LA, van Riel PL. The relationship between disease activity, joint destruction, and functional capacity over the course of rheumatoid arthritis. Arthritis and rheumatism 2001;44(9):2009-17.
5. Gravallese EM, Harada Y, Wang JT, Gorn AH, Thornhill TS, Goldring SR. Identification of cell types responsible for bone resorption in rheumatoid arthritis and juvenile rheumatoid arthritis. The American journal of pathology 1998;152(4):943-51.
6. Haavardsholm EA, Boyesen P, Ostergaard M, Schildvold A, Kvien TK. Magnetic resonance imaging findings in 84 patients with early rheumatoid arthritis: bone marrow oedema predicts erosive progression. Annals of the rheumatic diseases 2008;67(6):794-800.
7. Hetland ML, Ejbjerg B, Horslev-Petersen K, Jacobsen S, Vestergaard A, Jurik AG, et al. MRI bone oedema is the strongest predictor of subsequent radiographic progression in early rheumatoid arthritis. Results from a 2-year randomised controlled trial (CIMESTRA). Annals of the rheumatic diseases 2009;68(3):384-90.
8. McQueen FM, Benton N, Perry D, Crabbe J, Robinson E, Yeoman S, et al. Bone edema scored on magnetic resonance imaging scans of the dominant carpus at presentation predicts radiographic joint damage of the hands and feet six years later in patients with rheumatoid arthritis. Arthritis and rheumatism 2003;48(7):1814-27.
9. Jimenez-Boj E, Nobauer-Huhmann I, Hanslik-Schnabel B, Dorotka R, Wanivenhaus AH, Kainberger F, et al. Bone erosions and bone marrow edema as defined by magnetic resonance imaging reflect true bone marrow inflammation in rheumatoid arthritis. Arthritis and rheumatism 2007;56(4):1118-24.
10. Dalbeth N, Smith T, Gray S, Doyle A, Antill P, Lobo M, et al. Cellular characterisation of magnetic resonance imaging bone oedema in rheumatoid arthritis; implications for pathogenesis of erosive disease. Annals of the rheumatic diseases 2009;68(2):279-82.
11. Crotti TN, Smith MD, Weedon H, Ahern MJ, Findlay DM, Kraan M, et al. Receptor activator NF-kappaB ligand (RANKL) expression in synovial tissue from patients with rheumatoid arthritis, spondyloarthropathy, osteoarthritis, and from normal patients: semiquantitative and quantitative analysis. Annals of the rheumatic diseases 2002;61(12):1047-54.
12. Haynes DR, Barg E, Crotti TN, Holding C, Weedon H, Atkins GJ, et al. Osteoprotegerin expression in synovial tissue from patients with rheumatoid arthritis, spondyloarthropathies and osteoarthritis and normal controls. Rheumatology (Oxford, England) 2003;42(1):123-34.
13. Takayanagi H, Iizuka H, Juji T, Nakagawa T, Yamamoto A, Miyazaki T, et al. Involvement of receptor activator of nuclear factor kappaB ligand/osteoclast differentiation factor in osteoclastogenesis from synoviocytes in rheumatoid arthritis. Arthritis and rheumatism 2000;43(2):259-69.
14. Gravallese EM, Manning C, Tsay A, Naito A, Pan C, Amento E, et al. Synovial tissue in rheumatoid arthritis is a source of osteoclast differentiation factor. Arthritis and rheumatism 2000;43(2):250-8.
15. MacNaul KL, Hutchinson NI, Parsons JN, Bayne EK, Tocci MJ. Analysis of IL-1 and TNF-alpha gene expression in human rheumatoid synoviocytes and normal monocytes by *in situ* hybridization. Journal of immunology (Baltimore, Md : 1950) 1990;145(12):4154-66.
16. Zhang YH, Heulsmann A, Tondravi MM, Mukherjee A, Abu-Amer Y. Tumor necrosis factor-alpha (TNF) stimulates RANKL-induced osteoclastogenesis via coupling of TNF type 1 receptor and RANK signaling pathways. The Journal of biological chemistry 2001;276(1):563-8.
17. Hashizume M, Hayakawa N, Mihara M. IL-6 trans-signalling directly induces RANKL on fibroblast-like synovial cells and is involved in RANKL induction by TNF-alpha and IL-17. Rheumatology (Oxford, England) 2008;47(11):1635-40.
18. Wu L, Guo Q, Yang J, Ni B. Tumor Necrosis Factor Alpha Promotes Osteoclast Formation Via PI3K/Akt Pathway-Mediated Blimp1 Expression Upregulation. Journal of cellular biochemistry 2017;118(6):1308-15.
19. Yarilina A, Xu K, Chen J, Ivashkiv LB. TNF activates calcium-nuclear factor of activated T cells (NFAT) c1 signaling pathways in human macrophages. Proceedings of the National Academy of Sciences of the United States of America 2011;108(4):1573-8.
20. Li P, Schwarz EM, O'Keefe RJ, Ma L, Boyce BF, Xing L. RANK signaling is not required for TNFalpha-mediated increase in CD11(hi) osteoclast precursors but is essential for mature osteoclast formation in TNFalpha-mediated inflammatory arthritis. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 2004;19(2):207-13.
21. Li P, Schwarz EM, O'Keefe RJ, Ma L, Looney RJ, Ritchlin

CT, et al. Systemic tumor necrosis factor alpha mediates an increase in peripheral CD11bhigh osteoclast precursors in tumor necrosis factor alpha-transgenic mice. Arthritis and rheumatism 2004;50(1):265-76.
22. Kitaura H, Zhou P, Kim HJ, Novack DV, Ross FP, Teitelbaum SL. M-CSF mediates TNF-induced inflammatory osteolysis. The Journal of clinical investigation 2005;115(12):3418-27.
23. Yao Z, Li P, Zhang Q, Schwarz EM, Keng P, Arbini A, et al. Tumor necrosis factor-alpha increases circulating osteoclast precursor numbers by promoting their proliferation and differentiation in the bone marrow through up-regulation of c-Fms expression. The Journal of biological chemistry 2006;281(17):11846-55.
24. Wei S, Kitaura H, Zhou P, Ross FP, Teitelbaum SL. IL-1 mediates TNF-induced osteoclastogenesis. The Journal of clinical investigation 2005;115(2):282-90.
25. Field M, Chu C, Feldmann M, Maini RN. Interleukin-6 localisation in the synovial membrane in rheumatoid arthritis. Rheumatology international 1991;11(2):45-50.
26. Okamoto H, Yamamura M, Morita Y, Harada S, Makino H, Ota Z. The synovial expression and serum levels of interleukin-6, interleukin-11, leukemia inhibitory factor, and oncostatin M in rheumatoid arthritis. Arthritis and rheumatism 1997;40(6):1096-105.
27. Sato K, Suematsu A, Okamoto K, Yamaguchi A, Morishita Y, Kadono Y, et al. Th17 functions as an osteoclastogenic helper T cell subset that links T cell activation and bone destruction. The Journal of experimental medicine 2006;203(12):2673-82.
28. Adamopoulos IE, Chao CC, Geissler R, Laface D, Blumenschein W, Iwakura Y, et al. Interleukin-17A upregulates receptor activator of NF-kappaB on osteoclast precursors. Arthritis research & therapy 2010;12(1):R29.
29. Zhang F, Tanaka H, Kawato T, Kitami S, Nakai K, Motohashi M, et al. Interleukin-17A induces cathepsin K and MMP-9 expression in osteoclasts via celecoxib-blocked prostaglandin E2 in osteoblasts. Biochimie 2011;93(2):296-305.
30. Yago T, Nanke Y, Ichikawa N, Kobashigawa T, Mogi M, Kamatani N, et al. IL-17 induces osteoclastogenesis from human monocytes alone in the absence of osteoblasts, which is potently inhibited by anti-TNF-alpha antibody: a novel mechanism of osteoclastogenesis by IL-17. Journal of cellular biochemistry 2009;108(4):947-55.
31. Jovanovic DV, Di Battista JA, Martel-Pelletier J, Jolicoeur FC, He Y, Zhang M, et al. IL-17 stimulates the production and expression of proinflammatory cytokines, IL-beta and TNF-alpha, by human macrophages. Journal of immunology (Baltimore, Md: 1950) 1998;160(7):3513-21.
32. Ogura H, Murakami M, Okuyama Y, Tsuruoka M, Kitabayashi C, Kanamoto M, et al. Interleukin-17 promotes autoimmunity by triggering a positive-feedback loop via interleukin-6 induction. Immunity 2008;29(4):628-36.
33. McInnes IB, Liew FY. Interleukin 15: a proinflammatory role in rheumatoid arthritis synovitis. Immunology today 1998;19(2):75-9.
34. Djaafar S, Pierroz DD, Chicheportiche R, Zheng XX, Ferrari SL, Ferrari-Lacraz S. Inhibition of T cell-dependent and RANKL-dependent osteoclastogenic processes associated with high levels of bone mass in interleukin-15 receptor-deficient mice. Arthritis and rheumatism 2010;62(11):3300-10.
35. Ogata Y, Kukita A, Kukita T, Komine M, Miyahara A, Miyazaki S, et al. A novel role of IL-15 in the development of osteoclasts: inability to replace its activity with IL-2. Journal of immunology (Baltimore, Md : 1950) 1999;162(5):2754-60.
36. Xu D, Jiang HR, Kewin P, Li Y, Mu R, Fraser AR, et al. IL-33 exacerbates antigen-induced arthritis by activating mast cells. Proceedings of the National Academy of Sciences of the United States of America 2008;105(31):10913-8.
37. Mun SH, Ko NY, Kim HS, Kim JW, Kim DK, Kim AR, et al. Interleukin-33 stimulates formation of functional osteoclasts from human CD14(+) monocytes. Cellular and molecular life sciences: CMLS 2010;67(22):3883-92.
38. Hwang SJ, Choi B, Kang SS, Chang JH, Kim YG, Chung YH, et al. Interleukin-34 produced by human fibroblast-like synovial cells in rheumatoid arthritis supports osteoclastogenesis. Arthritis research & therapy 2012;14(1):R14.
39. Alanara T, Karstila K, Moilanen T, Silvennoinen O, Isomaki P. Expression of IL-10 family cytokines in rheumatoid arthritis: elevated levels of IL-19 in the joints. Scandinavian journal of rheumatology 2010; 39(2):118-26.
40. Avci AB, Feist E, Burmester GR. Targeting GM-CSF in rheumatoid arthritis. Clinical and experimental rheumatology 2016;34(4 Suppl 98):39-44.
41. Conigliaro P, Perricone C, Benson RA, Garside P, Brewer JM, Perricone R, et al. The type I IFN system in rheumatoid arthritis. Autoimmunity 2010; 43(3):220-5.
42. Paradowska-Gorycka A, Grzybowska-Kowalczyk A, Wojtecka-Lukasik E, Maslinski S. IL-23 in the pathogenesis of rheumatoid arthritis. Scandinavian journal of immunology 2010;71(3):134-45.
43. Park JK, Han BK, Park JA, Woo YJ, Kim SY, Lee EY, et al. CD70-expressing CD4 T cells produce IFN-gamma and IL-17 in rheumatoid arthritis. Rheumatology (Oxford, England) 2014;53(10):1896-900.
44. Rivas D, Mozo L, Zamorano J, Gayo A, Torre-Alonso JC, Rodriguez A, et al. Upregulated expression of IL-4 receptors and increased levels of IL-4 in rheumatoid arthritis patients. Journal of autoimmunity 1995; 8(4):587-600.
45. Avnet S, Cenni E, Perut F, Granchi D, Brandi ML, Giunti A, et al. Interferon-alpha inhibits *in vitro* osteoclast differentiation and renal cell carcinoma-induced

angiogenesis. International journal of oncology 2007; 30(2):469-76.
46. Cheng J, Liu J, Shi Z, Xu D, Luo S, Siegal GP, et al. Interleukin-4 inhibits RANKL-induced NFATc1 expression via STAT6: a novel mechanism mediating its blockade of osteoclastogenesis. Journal of cellular biochemistry 2011;112(11):3385-92.
47. Evans KE, Fox SW. Interleukin-10 inhibits osteoclastogenesis by reducing NFATc1 expression and preventing its translocation to the nucleus. BMC cell biology 2007;8:4.
48. Hiasa M, Abe M, Nakano A, Oda A, Amou H, Kido S, et al. GM-CSF and IL-4 induce dendritic cell differentiation and disrupt osteoclastogenesis through M-CSF receptor shedding by up-regulation of TNF-alpha converting enzyme (TACE). Blood 2009;114(20):4517-26.
49. Quinn JM, Sims NA, Saleh H, Mirosa D, Thompson K, Bouralexis S, et al. IL-23 inhibits osteoclastogenesis indirectly through lymphocytes and is required for the maintenance of bone mass in mice. Journal of immunology (Baltimore, Md: 1950). 2008; 181(8):5720-9.
50. Takayanagi H, Kim S, Matsuo K, Suzuki H, Suzuki T, Sato K, et al. RANKL maintains bone homeostasis through c-Fos-dependent induction of interferon-beta. Nature 2002;416(6882):744-9.
51. Takayanagi H, Ogasawara K, Hida S, Chiba T, Murata S, Sato K, et al. T-cell-mediated regulation of osteoclastogenesis by signalling cross-talk between RANKL and IFN-gamma. Nature 2000; 408(6812):600-5.
52. Humphrey MB, Lanier LL, Nakamura MC. Role of ITAM-containing adapter proteins and their receptors in the immune system and bone. Immunological reviews 2005;208:50-65.
53. Humphrey MB, Nakamura MC. A Comprehensive Review of Immunoreceptor Regulation of Osteoclasts. Clinical reviews in allergy & immunology 2015.
54. Herman S, Muller RB, Kronke G, Zwerina J, Redlich K, Hueber AJ, et al. Induction of osteoclast-associated receptor, a key osteoclast costimulation molecule, in rheumatoid arthritis. Arthritis and rheumatism 2008;58(10):3041-50.
55. Crotti TN, Dharmapatni AA, Alias E, Zannettino AC, Smith MD, Haynes DR. The immunoreceptor tyrosine-based activation motif (ITAM) -related factors are increased in synovial tissue and vasculature of rheumatoid arthritic joints. Arthritis research & therapy 2012;14(6):R245.
56. Bar-Shavit Z. Taking a toll on the bones: regulation of bone metabolism by innate immune regulators. Autoimmunity 2008;41(3):195-203.
57. Chamberlain ND, Vila OM, Volin MV, Volkov S, Pope RM, Swedler W, et al. TLR5, a novel and unidentified inflammatory mediator in rheumatoid arthritis that correlates with disease activity score and joint TNF-alpha levels. Journal of immunology (Baltimore, Md: 1950) 2012;189(1):475-83.
58. Kim SJ, Chen Z, Chamberlain ND, Essani AB, Volin MV, Amin MA, et al. Ligation of TLR5 promotes myeloid cell infiltration and differentiation into mature osteoclasts in rheumatoid arthritis and experimental arthritis. Journal of immunology (Baltimore, Md: 1950) 2014; 193(8):3902-13.
59. Baillet A, Trocme C, Berthier S, Arlotto M, Grange L, Chenau J, et al. Synovial fluid proteomic fingerprint: S100A8, S100A9 and S100A12 proteins discriminate rheumatoid arthritis from other inflammatory joint diseases. Rheumatology (Oxford, England) 2010; 49(4):671-82.
60. Choi IY, Gerlag DM, Herenius MJ, Thurlings RM, Wijbrandts CA, Foell D, et al. MRP8/14 serum levels as a strong predictor of response to biological treatments in patients with rheumatoid arthritis. Annals of the rheumatic diseases 2015;74(3):499-505.
61. Page TH, Charles PJ, Piccinini AM, Nicolaidou V, Taylor PC, Midwood KS. Raised circulating tenascin-C in rheumatoid arthritis. Arthritis research & therapy 2012;14(6):R260.
62. Park SY, Lee SW, Kim HY, Lee WS, Hong KW, Kim CD. HMGB1 induces angiogenesis in rheumatoid arthritis via HIF-1alpha activation. European journal of immunology 2015;45(4):1216-27.
63. Taniguchi N, Kawahara K, Yone K, Hashiguchi T, Yamakuchi M, Goto M, et al. High mobility group box chromosomal protein 1 plays a role in the pathogenesis of rheumatoid arthritis as a novel cytokine. Arthritis and rheumatism 2003;48(4):971-81.
64. Watanabe T, Takahashi N, Hirabara S, Ishiguro N, Kojima T. Hyaluronan Inhibits Tlr-4-Dependent RANKL Expression in Human Rheumatoid Arthritis Synovial Fibroblasts. PloS one 2016;11(4):e0153142.
65. Abdollahi-Roodsaz S, Joosten LA, Helsen MM, Walgreen B, van Lent PL, van den Bersselaar LA, et al. Shift from toll-like receptor 2 (TLR-2) toward TLR-4 dependency in the erosive stage of chronic streptococcal cell wall arthritis coincident with TLR-4-mediated interleukin-17 production. Arthritis and rheumatism 2008;58(12):3753-64.
66. Abdollahi-Roodsaz S, Joosten LA, Koenders MI, van den Brand BT, van de Loo FA, van den Berg WB. Local interleukin-1-driven joint pathology is dependent on toll-like receptor 4 activation. The American journal of pathology 2009;175(5):2004-13.
67. Pierer M, Wagner U, Rossol M, Ibrahim S. Toll-like receptor 4 is involved in inflammatory and joint destructive pathways in collagen-induced arthritis in DBA1J mice. PloS one 2011;6(8):e23539.
68. Kiyeko GW, Hatterer E, Herren S, Di Ceglie I, van Lent PL, Reith W, et al. Spatiotemporal expression of endogenous TLR4 ligands leads to inflammation and bone erosion in mouse collagen-induced arthritis. European journal of immunology 2016;46(11):2629-38.
69. Jilani AA, Mackworth-Young CG. The role of citrullinated

protein antibodies in predicting erosive disease in rheumatoid arthritis: a systematic literature review and meta-analysis. International journal of rheumatology 2015;2015:728610.
70. Kleyer A, Finzel S, Rech J, Manger B, Krieter M, Faustini F, et al. Bone loss before the clinical onset of rheumatoid arthritis in subjects with anticitrullinated protein antibodies. Annals of the rheumatic diseases 2014;73(5):854-60.
71. Harre U, Georgess D, Bang H, Bozec A, Axmann R, Ossipova E, et al. Induction of osteoclastogenesis and bone loss by human autoantibodies against citrullinated vimentin. The Journal of clinical investigation 2012; 122(5):1791-802.
72. Lu MC, Lai NS, Yu HC, Huang HB, Hsieh SC, Yu CL. Anti-citrullinated protein antibodies bind surface-expressed citrullinated Grp78 on monocyte/macrophages and stimulate tumor necrosis factor alpha production. Arthritis and rheumatism 2010;62(5):1213-23.
73. Krishnamurthy A, Joshua V, Haj Hensvold A, Jin T, Sun M, Vivar N, et al. Identification of a novel chemokine-dependent molecular mechanism underlying rheumatoid arthritis-associated autoantibody-mediated bone loss. Annals of the rheumatic diseases 2016;75(4):721-9.
74. Orsolini G, Caimmi C, Viapiana O, Idolazzi L, Fracassi E, Gatti D, et al. Titer-Dependent Effect of Anti-Citrullinated Protein Antibodies On Systemic Bone Mass in Rheumatoid Arthritis Patients. Calcified tissue international 2017;101(1):17-23.
75. Seeling M, Hillenhoff U, David JP, Schett G, Tuckermann J, Lux A, et al. Inflammatory monocytes and Fcgamma receptor IV on osteoclasts are critical for bone destruction during inflammatory arthritis in mice. Proceedings of the National Academy of Sciences of the United States of America 2013;110(26):10729-34.
76. Harre U, Lang SC, Pfeifle R, Rombouts Y, Fruhbeisser S, Amara K, et al. Glycosylation of immunoglobulin G determines osteoclast differentiation and bone loss. Nature communications 2015;6:6651.
77. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004;116(2):281-97.
78. Chen JQ, Papp G, Szodoray P, Zeher M. The role of microRNAs in the pathogenesis of autoimmune diseases. Autoimmunity reviews 2016;15(12):1171-80.
79. Kurowska-Stolarska M, Alivernini S, Ballantine LE, Asquith DL, Millar NL, Gilchrist DS, et al. MicroRNA-155 as a proinflammatory regulator in clinical and experimental arthritis. Proceedings of the National Academy of Sciences of the United States of America 2011;108(27):11193-8.
80. Stanczyk J, Pedrioli DM, Brentano F, Sanchez-Pernaute O, Kolling C, Gay RE, et al. Altered expression of MicroRNA in synovial fibroblasts and synovial tissue in rheumatoid arthritis. Arthritis and rheumatism 2008;58(4):1001-9.
81. Bluml S, Bonelli M, Niederreiter B, Puchner A, Mayr G, Hayer S, et al. Essential role of microRNA-155 in the pathogenesis of autoimmune arthritis in mice. Arthritis and rheumatism 2011;63(5):1281-8.
82. Fulci V, Scappucci G, Sebastiani GD, Giannitti C, Franceschini D, Meloni F, et al. miR-223 is overexpressed in T-lymphocytes of patients affected by rheumatoid arthritis. Human immunology 2010;71(2):206-11.
83. Li YT, Chen SY, Wang CR, Liu MF, Lin CC, Jou IM, et al. Brief report: amelioration of collagen-induced arthritis in mice by lentivirus-mediated silencing of microRNA-223. Arthritis and rheumatism 2012;64(10):3240-5.
84. Sugatani T, Hruska KA. Impaired micro-RNA pathways diminish osteoclast differentiation and function. The Journal of biological chemistry 2009;284(7):4667-78.
85. Nakasa T, Miyaki S, Okubo A, Hashimoto M, Nishida K, Ochi M, et al. Expression of microRNA-146 in rheumatoid arthritis synovial tissue. Arthritis and rheumatism 2008;58(5):1284-92.
86. Niimoto T, Nakasa T, Ishikawa M, Okuhara A, Izumi B, Deie M, et al. MicroRNA-146a expresses in interleukin-17 producing T cells in rheumatoid arthritis patients. BMC musculoskeletal disorders 2010;11:209.
87. Nakasa T, Shibuya H, Nagata Y, Niimoto T, Ochi M. The inhibitory effect of microRNA-146a expression on bone destruction in collagen-induced arthritis. Arthritis and rheumatism 2011;63(6):1582-90.
88. Taganov KD, Boldin MP, Chang KJ, Baltimore D. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. Proceedings of the National Academy of Sciences of the United States of America 2006;103(33):12481-6.
89. Nakamachi Y, Ohnuma K, Uto K, Noguchi Y, Saegusa J, Kawano S. MicroRNA-124 inhibits the progression of adjuvant-induced arthritis in rats. Annals of the rheumatic diseases 2016;75(3):601-8.
90. Walsh NC, Reinwald S, Manning CA, Condon KW, Iwata K, Burr DB, et al. Osteoblast function is compromised at sites of focal bone erosion in inflammatory arthritis. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research 2009;24(9):1572-85.
91. Matzelle MM, Gallant MA, Condon KW, Walsh NC, Manning CA, Stein GS, et al. Resolution of inflammation induces osteoblast function and regulates the Wnt signaling pathway. Arthritis and rheumatism 2012; 64(5):1540-50.
92. Gilbert L, He X, Farmer P, Boden S, Kozlowski M, Rubin J, et al. Inhibition of osteoblast differentiation by tumor necrosis factor-alpha. Endocrinology 2000; 141(11):3956-64.
93. Huang H, Zhao N, Xu X, Xu Y, Li S, Zhang J, et al. Dose-specific effects of tumor necrosis factor alpha on osteogenic differentiation of mesenchymal stem cells. Cell proliferation 2011;44(5):420-7.
94. Kaneki H, Guo R, Chen D, Yao Z, Schwarz EM, Zhang YE, et al. Tumor necrosis factor promotes Runx2

degradation through up-regulation of Smurf1 and Smurf2 in osteoblasts. The Journal of biological chemistry 2006;281(7):4326-33.
95. Nanes MS. Tumor necrosis factor-alpha: molecular and cellular mechanisms in skeletal pathology. Gene 2003; 321:1-15.
96. Redlich K, Gortz B, Hayer S, Zwerina J, Doerr N, Kostenuik P, et al. Repair of local bone erosions and reversal of systemic bone loss upon therapy with anti-tumor necrosis factor in combination with osteoprotegerin or parathyroid hormone in tumor necrosis factor-mediated arthritis. The American journal of pathology 2004;164(2):543-55.
97. Stashenko P, Dewhirst FE, Rooney ML, Desjardins LA, Heeley JD. Interleukin-1 beta is a potent inhibitor of bone formation *in vitro*. Journal of bone and mineral research: the official journal of the American Society for Bone and Mineral Research 1987;2(6):559-65.
98. Hengartner NE, Fiedler J, Ignatius A, Brenner RE. IL-1beta inhibits human osteoblast migration. Molecular medicine (Cambridge, Mass) 2013;19:36-42.
99. Malysheva K, de Rooij K, Lowik CW, Baeten DL, Rose-John S, Stoika R, et al. Interleukin 6/Wnt interactions in rheumatoid arthritis: interleukin 6 inhibits Wnt signaling in synovial fibroblasts and osteoblasts. Croatian medical journal 2016;57(2):89-98.
100. Clevers H. Wnt/beta-catenin signaling in development and disease. Cell 2006;127(3):469-80.
101. Diarra D, Stolina M, Polzer K, Zwerina J, Ominsky MS, Dwyer D, et al. Dickkopf-1 is a master regulator of joint remodeling. Nature medicine 2007;13(2):156-63.
102. Ray S, Khassawna TE, Sommer U, Thormann U, Wijekoon ND, Lips K, et al. Differences in expression of Wnt antagonist Dkk1 in healthy versus pathological bone samples. Journal of microscopy 2017; 265(1):111-20.
103. Rossini M, Viapiana O, Adami S, Fracassi E, Idolazzi L, Dartizio C, et al. In patients with rheumatoid arthritis, Dickkopf-1 serum levels are correlated with parathyroid hormone, bone erosions and bone mineral density. Clinical and experimental rheumatology 2015; 33(1):77-83.
104. Wang SY, Liu YY, Ye H, Guo JP, Li R, Liu X, et al. Circulating Dickkopf-1 is correlated with bone erosion and inflammation in rheumatoid arthritis. The Journal of rheumatology 2011;38(5):821-7.
105. Seror R, Boudaoud S, Pavy S, Nocturne G, Schaeverbeke T, Saraux A, et al. Increased Dickkopf-1 in Recent-onset Rheumatoid Arthritis is a New Biomarker of Structural Severity. Data from the ESPOIR Cohort. Scientific reports 2016;6:18421.
106. de Rooy DP, Yeremenko NG, Wilson AG, Knevel R, Lindqvist E, Saxne T, et al. Genetic studies on components of the Wnt signalling pathway and the severity of joint destruction in rheumatoid arthritis. Annals of the rheumatic diseases 2013;72(5):769-75.
107. Heiland GR, Zwerina K, Baum W, Kireva T, Distler JH, Grisanti M, et al. Neutralisation of Dkk-1 protects from systemic bone loss during inflammation and reduces sclerostin expression. Annals of the rheumatic diseases 2010;69(12):2152-9.
108. Yeremenko N, Zwerina K, Rigter G, Pots D, Fonseca JE, Zwerina J, et al. Tumor necrosis factor and interleukin-6 differentially regulate Dkk-1 in the inflamed arthritic joint. Arthritis & rheumatology (Hoboken, NJ) 2015; 67(8):2071-5.
109. Chen XX, Baum W, Dwyer D, Stock M, Schwabe K, Ke HZ, et al. Sclerostin inhibition reverses systemic, periarticular and local bone loss in arthritis. Annals of the rheumatic diseases 2013;72(10):1732-6.
110. Mehaney DA, Eissa M, Anwar S, Fakhr El-Din S. Serum Sclerostin Level Among Egyptian Rheumatoid Arthritis Patients: Relation to Disease Activity, Bone Mineral Density and Radiological Grading. Acta reumatologica portuguesa 2015;40(3):268-74.
111. Marenzana M, Vugler A, Moore A, Robinson M. Effect of sclerostin-neutralising antibody on periarticular and systemic bone in a murine model of rheumatoid arthritis: a microCT study. Arthritis research & therapy 2013;15(5):R125.
112. Wehmeyer C, Frank S, Beckmann D, Bottcher M, Cromme C, Konig U, et al. Sclerostin inhibition promotes TNF-dependent inflammatory joint destruction. Science translational medicine 2016;8(330):330ra35.
113. Maeda K, Kobayashi Y, Udagawa N, Uehara S, Ishihara A, Mizoguchi T, et al. Wnt5a-Ror2 signaling between osteoblast-lineage cells and osteoclast precursors enhances osteoclastogenesis. Nature medicine 2012; 18(3):405-12.
114. Chen G, Deng C, Li YP. TGF-beta and BMP signaling in osteoblast differentiation and bone formation. International journal of biological sciences 2012; 8(2):272-88.
115. Bramlage CP, Haupl T, Kaps C, Ungethum U, Krenn V, Pruss A, et al. Decrease in expression of bone morphogenetic proteins 4 and 5 in synovial tissue of patients with osteoarthritis and rheumatoid arthritis. Arthritis research & therapy 2006;8(3):R58.
116. Denninger KC, Litman T, Marstrand T, Moller K, Svensson L, Labuda T, et al. Kinetics of gene expression and bone remodelling in the clinical phase of collagen-induced arthritis. Arthritis research & therapy 2015;17:43.
117. Yuan T-l, Chen J, Tong Y-l, Zhang Y, Liu Y-y, Wei JC-C, et al. Serum Heme Oxygenase-1 and BMP-7 Are Potential Biomarkers for Bone Metabolism in Patients with Rheumatoid Arthritis and Ankylosing Spondylitis. BioMed Research International 2016;2016:7870925.
118. Matzelle MM, Shaw AT, Baum R, Maeda Y, Li J, Karmakar S, et al. Inflammation in arthritis induces expression of BMP3, an inhibitor of bone formation. Scandinavian journal of rheumatology 2016;45(5):379-83.
119. Ideguchi H, Ohno S, Hattori H, Senuma A, Ishigatsubo Y. Bone erosions in rheumatoid arthritis can be repaired through reduction in disease activity with conventional

disease-modifying antirheumatic drugs. Arthritis research & therapy 2006;8(3):R76.
120. van der Linden MP, Boja R, Klarenbeek NB, Huizinga TW, van der Heijde DM, van der Helm-van Mil AH. Repair of joint erosions in rheumatoid arthritis: prevalence and patient characteristics in a large inception cohort. Annals of the rheumatic diseases 2010;69(4):727-9.
121. Finzel S, Rech J, Schmidt S, Engelke K, Englbrecht M, Stach C, et al. Repair of bone erosions in rheumatoid arthritis treated with tumour necrosis factor inhibitors is based on bone apposition at the base of the erosion. Annals of the rheumatic diseases 2011;70(9):1587-93.
122. Dohn UM, Ejbjerg B, Boonen A, Hetland ML, Hansen MS, Knudsen LS, et al. No overall progression and occasional repair of erosions despite persistent inflammation in adalimumab-treated rheumatoid arthritis patients: results from a longitudinal comparative MRI, ultrasonography, CT and radiography study. Annals of the rheumatic diseases 2011;70(2):252-8.
123. Lukas C, van der Heijde D, Fatenajad S, Landewe R. Repair of erosions occurs almost exclusively in damaged joints without swelling. Annals of the rheumatic diseases 2010;69(5):851-5.
124. Moller Dohn U, Boonen A, Hetland ML, Hansen MS, Knudsen LS, Hansen A, et al. Erosive progression is minimal, but erosion healing rare, in patients with rheumatoid arthritis treated with adalimumab. A 1 year investigator-initiated follow-up study using high-resolution computed tomography as the primary outcome measure. Annals of the rheumatic diseases 2009;68(10):1585-90.
125. Finzel S, Rech J, Schmidt S, Engelke K, Englbrecht M, Schett G. Interleukin-6 receptor blockade induces limited repair of bone erosions in rheumatoid arthritis: a micro CT study. Annals of the rheumatic diseases 2013;72(3):396-400.
126. Hashimoto J, Garnero P, van der Heijde D, Miyasaka N, Yamamoto K, Kawai S, et al. Humanized anti-interleukin-6-receptor antibody (tocilizumab) monotherapy is more effective in slowing radiographic progression in patients with rheumatoid arthritis at high baseline risk for structural damage evaluated with levels of biomarkers, radiography, and BMI: data from the SAMURAI study. Modern rheumatology 2011;21(1):10-5.
127. Kremer JM, Blanco R, Brzosko M, Burgos-Vargas R, Halland AM, Vernon E, et al. Tocilizumab inhibits structural joint damage in rheumatoid arthritis patients with inadequate responses to methotrexate: results from the double-blind treatment phase of a randomized placebo-controlled trial of tocilizumab safety and prevention of structural joint damage at one year. Arthritis and rheumatism 2011;63(3):609-21.
128. Kremer JM, Genant HK, Moreland LW, Russell AS, Emery P, Abud-Mendoza C, et al. Effects of abatacept in patients with methotrexate-resistant active rheumatoid arthritis: a randomized trial. Annals of internal medicine 2006;144(12):865-76.
129. Lee EB, Fleischmann R, Hall S, Wilkinson B, Bradley JD, Gruben D, et al. Tofacitinib versus methotrexate in rheumatoid arthritis. The New England journal of medicine 2014;370(25):2377-86.
130. Meyer DM, Jesson MI, Li X, Elrick MM, Funckes-Shippy CL, Warner JD, et al. Anti-inflammatory activity and neutrophil reductions mediated by the JAK1/JAK3 inhibitor, CP-690,550, in rat adjuvant-induced arthritis. Journal of inflammation (London, England) 2010;7:41.
131. Conaghan PG, Østergaard M, Bowes MA, Wu C, Fuerst T, van der Heijde D, et al. Comparing the effects of tofacitinib, methotrexate and the combination, on bone marrow oedema, synovitis and bone erosion in methotrexate-naive, early active rheumatoid arthritis: results of an exploratory randomised MRI study incorporating semiquantitative and quantitative techniques. Annals of the Rheumatic Diseases 2016; 75(6):1024-33.
132. Genovese MC, Kremer J, Zamani O, Ludivico C, Krogulec M, Xie L, et al. Baricitinib in Patients with Refractory Rheumatoid Arthritis. The New England journal of medicine 2016;374(13):1243-52.
133. Dougados M, van der Heijde D, Chen Y-C, Greenwald M, Drescher E, Liu J, et al. Baricitinib in patients with inadequate response or intolerance to conventional synthetic DMARDs: results from the RA-BUILD study. Annals of the Rheumatic Diseases 2017;76(1):88-95.
134. Rossini M, Adami G, Viapiana O, Idolazzi L, Orsolini G, Fassio A, et al. Osteoporosis: an Independent Determinant of Bone Erosions in Rheumatoid Arthritis? Journal of bone and mineral research: the official journal of the American Society for Bone and Mineral Research 2017;32(10):2142-3.
135. Migliore A, Massafra U, Bizzi E, Argento G, Diamanti AP, Germano V, et al. May etanercept and PTH (1-34) association heal erosions in early rheumatoid arthritis? A pilot study. European review for medical and pharmacological sciences 2012;16(3):363-9.
136. Solomon DH, Kay J, Duryea J, Lu B, Bolster MB, Yood RA, et al. Effects of Teriparatide on Joint Erosions in Rheumatoid Arthritis: A Randomized Controlled Trial. Arthritis & rheumatology (Hoboken, NJ) 2017.
137. Cohen SB, Dore RK, Lane NE, Ory PA, Peterfy CG, Sharp JT, et al. Denosumab treatment effects on structural damage, bone mineral density, and bone turnover in rheumatoid arthritis: a twelve-month, multicenter, randomized, double-blind, placebo-controlled, phase II clinical trial. Arthritis and rheumatism 2008; 58(5):1299-309.
138. Deodhar A, Dore RK, Mandel D, Schechtman J, Shergy W, Trapp R, et al. Denosumab-mediated increase in hand bone mineral density associated with decreased progression of bone erosion in rheumatoid arthritis patients. Arthritis care & research 2010;62(4):569-74.
139. Takeuchi T, Tanaka Y, Ishiguro N, Yamanaka H, Yoneda T, Ohira T, et al. Effect of denosumab on Japanese patients with rheumatoid arthritis: a dose–response study of

AMG 162 (Denosumab) in patients with Rheumatoid arthritis on methotrexate to Validate inhibitory effect on bone Erosion (DRIVE) - a 12-month, multicentre, randomised, double-blind, placebo-controlled, phase II clinical trial. Annals of the Rheumatic Diseases. 2016;75(6):983-90.
140. Yue J, Griffith JF, Xiao F, Shi L, Wang D, Shen J, et al. Repair of bone erosion in rheumatoid arthritis by denosumab: A high-resolution peripheral quantitative computed tomography study. Arthritis care & research 2016.
141. Mease PJ, Hobbs K, Chalmers A, El-Gabalawy H, Bookman A, Keystone E, et al. Local delivery of a recombinant adenoassociated vector containing a tumour necrosis factor alpha antagonist gene in inflammatory arthritis: a phase 1 dose-escalation safety and tolerability study. Annals of the rheumatic diseases 2009;68(8):1247-54.
142. Wehling P, Reinecke J, Baltzer AW, Granrath M, Schulitz KP, Schultz C, et al. Clinical responses to gene therapy in joints of two subjects with rheumatoid arthritis. Human gene therapy 2009;20(2):97-101.
143. Hu J, Li H, Chi G, Yang Z, Zhao Y, Liu W, et al. IL-1RA gene-transfected bone marrow-derived mesenchymal stem cells in APA microcapsules could alleviate rheumatoid arthritis. International journal of clinical and experimental medicine 2015;8(1):706-13.
144. Jain S, Tran TH, Amiji M. Macrophage repolarization with targeted alginate nanoparticles containing IL-10 plasmid DNA for the treatment of experimental arthritis. Biomaterials 2015;61:162-77.
145. Liu S, Watanabe S, Shudou M, Kuno M, Miura H, Maeyama K. Upregulation of store-operated Ca(2+) entry in the naive CD4(+) T cells with aberrant cytokine releasing in active rheumatoid arthritis. Immunology and cell biology 2014;92(9):752-60.
146. Liu S, Kiyoi T, Takemasa E, Maeyama K. Systemic lentivirus-mediated delivery of short hairpin RNA targeting calcium release-activated calcium channel 3 as gene therapy for collagen-induced arthritis. Journal of immunology (Baltimore, Md: 1950). 2015; 194(1):76-83.
147. Liu S, Maeyama K. Gene Therapy for Rheumatoid Arthritis. Critical reviews in immunology 2016; 36(2):149-61.
148. Lopez-Santalla M, Mancheno-Corvo P, Menta R, Lopez-Belmonte J, DelaRosa O, Bueren JA, et al. Human Adipose-Derived Mesenchymal Stem Cells Modulate Experimental Autoimmune Arthritis by Modifying Early Adaptive T Cell Responses. Stem cells (Dayton, Ohio) 2015; 33(12):3493-503.
149. Liu R, Li X, Zhang Z, Zhou M, Sun Y, Su D, et al. Allogeneic mesenchymal stem cells inhibited T follicular helper cell generation in rheumatoid arthritis. Scientific reports 2015;5:12777.
150. Gu Y, Shi S. Transplantation of gingiva-derived mesenchymal stem cells ameliorates collagen-induced arthritis. Arthritis research & therapy 2016;18(1):262.
151. Garimella MG, Kour S, Piprode V, Mittal M, Kumar A, Rani L, et al. Adipose-Derived Mesenchymal Stem Cells Prevent Systemic Bone Loss in Collagen-Induced Arthritis. Journal of immunology (Baltimore, Md: 1950) 2015;195(11):5136-48.
152. Wang L, Wang L, Cong X, Liu G, Zhou J, Bai B, et al. Human umbilical cord mesenchymal stem cell therapy for patients with active rheumatoid arthritis: safety and efficacy. Stem cells and development 2013; 22(24):3192-202.
153. Alvaro-Gracia JM, Jover JA, Garcia-Vicuna R, Carreno L, Alonso A, Marsal S, et al. Intravenous administration of expanded allogeneic adipose-derived mesenchymal stem cells in refractory rheumatoid arthritis (Cx611): results of a multicentre, dose escalation, randomised, single-blind, placebo-controlled phase Ib/IIa clinical trial. Annals of the rheumatic diseases 2017;76(1):196-202.
154. Ren Y, Yang Y, Yang J, Xie R, Fan H. Tolerogenic dendritic cells modified by tacrolimus suppress CD4(+) T-cell proliferation and inhibit collagen-induced arthritis in mice. International immunopharmacology 2014; 21(1):247-54.
155. Zhang L, Fu J, Sheng K, Li Y, Song S, Li P, et al. Bone marrow CD11b(+)F4/80(+) dendritic cells ameliorate collagen-induced arthritis through modulating the balance between Treg and Th17. International immunopharmacology 2015;25(1):96-105.
156. Park JE, Jang J, Choi JH, Kang MS, Woo YJ, Seong YR, et al. DC-Based Immunotherapy Combined with Low-Dose Methotrexate Effective in the Treatment of Advanced CIA in Mice. Journal of immunology research 2015;2015:834085.
157. Benham H, Nel HJ, Law SC, Mehdi AM, Street S, Ramnoruth N, et al. Citrullinated peptide dendritic cell immunotherapy in HLA risk genotype-positive rheumatoid arthritis patients. Science translational medicine 2015;7(290):290ra87.
158. Bell GM, Anderson AE, Diboll J, Reece R, Eltherington O, Harry RA, et al. Autologous tolerogenic dendritic cells for rheumatoid and inflammatory arthritis. Annals of the rheumatic diseases 2017;76(1):227-34.
159. Rossini M, Adami G, Viapiana O, Idolazzi L, Gatti D. Denosumab, cortical bone and bone erosions in rheumatoid arthritis. Annals of the rheumatic diseases 2016;75(10):e70.