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Mediators of inflammation and bone remodeling in rheumatic disease

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Abstract

Remodeling of bone is a continuous process that occurs throughout life. Under normal physiologic conditions, bone-resorbing osteoclasts and bone-forming osteoblasts are tightly coupled and regulated to ensure the proper balance, such that there is no net change in bone mass. However, inflammation perturbs normal bone homeostasis. The impact of inflammation on bone is dependent upon the anatomic site affected, cell types, factors and cytokines present in the local microenvironment, and local mechanical forces. Cytokines are central to the pathogenesis of inflammation-induced bone loss and contribute to the uncoupling of osteoclast-mediated bone resorption and osteoblast-mediated bone formation, thereby disrupting normal remodeling. In this review, we will discuss the effects of cytokines on bone in two settings, rheumatoid arthritis (RA) and spondyloarthritis (SpA), a disease category that includes ankylosing spondylitis (AS), psoriatic arthritis (PsA), reactive arthritis, inflammatory bowel disease, and juvenile onset spondyloarthropathy. The outcome for bone in these disease settings is quite different, and an understanding of the pathogenic mechanisms leading to the net impact on bone has been essential in developing new therapeutic approaches to bone health in these diseases.

Keywords

Inflammation; Osteoclasts; Osteoblasts; Rheumatoid arthritis; Spondyloarthritis; Cytokines

1. Introduction

Loss of articular bone is a common feature of chronic, inflammatory rheumatic diseases, including RA and SpA. In RA, pro-inflammatory cytokines expressed within the inflamed synovial tissues induce osteoclastogenesis and inhibit osteoblast differentiation, resulting in focal articular bone erosions. While this also occurs within inflamed joints in SpA, another prominent feature of SpA is the often extensive bone formation characteristically seen in the

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spine and at sites of tendon and ligament insertions into bone, termed “entheses.” The distinct outcomes for bone in these diseases highlights the complex interplay of factors leading to differential regulation of osteoclasts and osteoblasts. These rheumatic diseases are also associated with systemic bone loss, which will not be discussed in this review.

2. Osteoclasts

Osteoclasts are derived from cells of the monocyte/macrophage lineage and are required for the erosion of bone. As monocyte precursor cells differentiate and fuse to become multinucleated osteoclasts, these cells express tartrate-resistant acid phosphatase (TRAP) and calcitonin receptor, the latter of which serves as a definitive marker of bone-resorbing osteoclasts. Osteoclasts utilize a proton pump to acidify the environment deep to the ruffled border and solubilize mineral from bone. In addition, proteolytic enzymes including matrix metalloproteinases (MMPs) and cathepsin K are secreted that degrade the organic bone matrix.

Receptor activator of nuclear factor-kappaB ligand (RANKL) is an essential factor for osteoclast differentiation in the presence of macrophage colony-stimulating factor (M-CSF) [1]. In RA, RANKL is expressed on osteoblasts and synovial fibroblasts, among other cell types, and its expression is upregulated by pro-inflammatory cytokines, including TNF, IL-1, IL-6 and IL-17. RANKL binds to the RANK receptor on osteoclast precursor cells, inducing the differentiation and function of osteoclasts to promote articular bone resorption. Mice deficient in RANKL lack mature osteoclasts and are protected from bone erosion in the K/BxN serum transfer arthritis model [2], demonstrating the essential role of osteoclasts and of RANKL in bone erosion in inflammatory arthritis. Osteoprotegerin (OPG), the soluble decoy receptor for RANKL, is secreted by osteoblasts and other cell types to inhibit osteoclast differentiation. The extent to which osteoclastogenesis occurs is dictated in large part by the balance between RANKL and OPG.

2.1 Innate immune mechanisms regulating osteoclastogenesis

RANKL-RANK interactions ultimately result in the activation of nuclear factor-kappa B (NF- κ B) and activator protein 1 (AP-1), transcription factors that are crucial for the induction of nuclear factor of activated T cells cytoplasmic 1 (NFATc1). NFATc1 is an essential factor for osteoclastogenesis and the induction of NFATc1 requires calcium signals from the immunoreceptor tyrosine-based activation motif (ITAM) pathway. Co-stimulation from innate immune receptors osteoclast-associated immunoglobulin-like receptor (OSCAR) [3] and triggering receptor expressed in myeloid cells 2 (TREM2), as well as their corresponding ITAM adapter proteins Fc receptor gamma subunit (FcR γ) and DNAX-activating protein 12 (DAP12) [4] mediate ITAM signaling.

TREM2 and DAP12 promote osteoclast differentiation. TREM2-deficient osteoclast precursor cells from humans express TRAP but fail to differentiate into mature osteoclasts, lack calcitonin receptor expression, and exhibit impaired bone resorption *in vitro* [5]. DAP12-deficient mice have increased trabecular bone mass as a result of impaired osteoclast activity [6]; furthermore, mice deficient in both DAP12 and FcR γ adapter proteins exhibit severe osteopetrosis and possess osteoclast precursor cells that are incapable of

differentiating into mature osteoclasts *in vitro* despite the addition of exogenous RANKL and M-CSF [3]. Thus, disturbances in ITAMs and their adapter proteins are implicated in the pathogenesis of many bone diseases including RA [7].

2.2 Cytokines affecting osteoclastogenesis

Pro-inflammatory cytokines provide a link between inflammation and bone loss and promote the differentiation of osteoclasts (reviewed in [8]). IL-34 has been shown to induce osteoclastogenesis from murine bone marrow cells and human peripheral blood monocytes in combination with RANKL. The role of IL-34 in promoting osteoclast differentiation and function is demonstrated *in vivo* as exogenous IL-34 administration in mice results in decreased trabecular bone mass [9]. In contrast, cytokines including IL-4, IL-12, IL-18, and IFN- β , inhibit osteoclastogenesis [10-13]. The cytokine IL-33 also inhibits osteoclast differentiation and is expressed in osteoblasts. IL-33 inhibits the expression of RANKL-induced NFATc1 [14] and directs hematopoietic cells away from osteoclast differentiation and towards differentiation to dendritic cells or activated macrophages [15, 16]. Exogenous administration or overexpression of IL-33 in mice results in decreased osteoclast numbers and reduced systemic bone loss [17, 16]. Interferon (IFN)- α and IFN- β also bias myeloid differentiation away from osteoclasts and towards dendritic cells [18]. IL-18 exerts similar effects by inducing expression of GM-CSF from T cells to promote differentiation of dendritic cells [19]. Thus, cytokines present in the local environment play a significant role in determining whether osteoclast differentiation is promoted or inhibited. Additional key cytokines involved in osteoclastogenesis in the context of RA will be highlighted later in this review.

3. Osteoblasts

Bone-forming osteoblasts are derived from mesenchymal precursor cells. Committed osteoblast progenitors express the pro-osteogenic transcription factors Runx2 and Osterix that are required for osteoblast differentiation. As osteoblasts become capable of bone matrix production and mineralization, they initially express alkaline phosphatase and type I collagen, followed by osteocalcin, osteopontin and bone sialoprotein. Some osteoblasts eventually become embedded in the bone matrix to become osteocytes, which act as mechanosensors, sending signals to osteoclasts to promote bone resorption in an unloaded state and to osteoblasts to promote bone formation with mechanical loading. Pathways required for osteoblast differentiation include the bone morphogenetic protein (BMP) and canonical Wntless (Wnt) pathways, and additional factors including cytokines and miRNAs also regulate the differentiation of osteoblasts (Fig. 1).

3.1 The BMP signaling pathway

BMPs belong to the TGF- β superfamily and play important roles in skeletal development and bone homeostasis. Disruption of BMP signaling results in disorders of bone mass. In bone, BMPs are secreted primarily by osteoblasts, chondrocytes, and endothelial cells. The majority of BMP ligands, including BMP-2, -4, -6 and -7 are osteogenic [20]; BMP signaling upregulates the expression of Runx2 and Osterix to promote differentiation of osteoblasts [21]. These BMPs bind membrane-bound BMP receptors, resulting in the

phosphorylation of SMADs 1/5/8 that hetero-oligomerizes with SMAD 4. This complex then translocates to the nucleus to activate transcription of BMP-responsive genes. However, not all BMP ligands are pro-osteogenic. A unique BMP family member, BMP-3, in fact inhibits BMP signaling [22]. Inhibitors of BMP ligands, including noggin and chordin, bind directly to the ligands to prevent receptor binding and BMP signaling.

3.2 The canonical Wnt signaling pathway

The canonical Wnt signaling pathway is also critical in regulating osteoblast differentiation and is activated by secreted Wnt ligands. These ligands bind the Frizzled-lipoprotein receptor related proteins (LRP)5/6 co-receptor complex to inhibit glycogen synthase kinase (GSK)-3 β , preventing it from interacting with cytosolic β -catenin. This allows β -catenin to translocate to the nucleus to activate transcription of genes controlling osteoblast maturation. Crosstalk between BMP and Wnt signaling occurs in the regulation of osteoblast differentiation. For example, pro-osteogenic BMP-2 induces the expression of Wnt ligands Wnt-1 and Wnt-3a in mesenchymal stem cell lines to promote Wnt signaling [23].

Secreted antagonists, including members of the secreted frizzled-related protein (sFRP) and Dickkopf (DKK) families and sclerostin, inhibit the canonical Wnt pathway. sFRPs are soluble decoy receptors that sequester Wnt ligands, preventing them from interacting with the Frizzled-LRP5/6 receptors, while DKKs and sclerostin bind directly to the LRP5/6 co-receptor to suppress Wnt signaling. In these settings, GSK-3 β phosphorylates β -catenin, targeting it for degradation. DKK-1 has also been shown to induce sclerostin to further inhibit Wnt signaling [24]. *In vivo*, mice deficient in sFRP-1 or DKK-1 have increased bone mass [25, 26], while overexpression of either antagonist leads to decreased bone density [27, 28]. Additionally, loss-of-function mutations in sclerostin have been linked to two clinical disorders associated with high bone mass, van Buchem's disease and sclerosteosis [29, 30].

3.3 microRNAs (miRNAs) regulating osteoblast differentiation

More recently, miRNAs have also been identified as playing a role in regulating osteoblast differentiation [31]. miRNAs are small, non-coding RNAs, 20-22 nucleotides in length that regulate gene expression by promoting the degradation of genetic transcripts or preventing protein translation by ribosomes. By targeting existing mRNAs, miRNAs can easily modulate whole programs of gene expression and their related pathways. For example, miR-29b targets Wnt signaling antagonists [32] and increases Runx2 [33] to positively regulate the differentiation of osteoblasts. miR-335 directly inhibits DKK-1 to promote Wnt signaling [34]. A number of miRNAs, including miR-30c, miR-133, miR-135a and miR-204 have been identified as regulating Runx2 expression during osteogenesis and pretreatment with these miRNAs results in a reduction of Runx2 protein and inhibits osteoblast differentiation [35, 36]. miR-145 also inhibits osteoblast differentiation by suppressing the expression of Osterix [37]. These miRNAs therefore may be targets for the regulation of osteoblast function.

4. Rheumatoid arthritis

RA serves as a prototype rheumatic disease in which inflammation leads to loss of bone. In RA, chronic inflammation of the synovial tissue (synovitis) occurs, accompanied by destruction of joint cartilage and bone. Articular bone erosion is a hallmark of RA pro-inflammatory cytokines secreted predominantly by synovial fibroblasts, macrophages, and lymphocytes within the inflamed synovium and pannus promote osteoclastogenesis.

4.1 Cytokines in RA

It is well known that pro-inflammatory cytokines produced by inflamed synovial tissues in RA can affect the differentiation of osteoclasts and osteoblasts with effects on bone, including TNF, IL-1, IL-6 and IL-17. These cytokines induce RANKL expression on osteoblasts and/or synovial fibroblasts, and RANKL is more highly expressed in RA patients with greater disease activity [38-41]. TNF is a key regulatory cytokine in the pathogenesis of RA synovitis and joint destruction. TNF promotes the proliferation of osteoclast precursor cells [42], induces NF- κ B to activate NFATc1 signaling that is central to the maturation of osteoclasts [43] and increases expression of OSCAR to promote osteoclast differentiation [44]. TNF inhibits osteoblast differentiation and bone formation [45] by decreasing Runx2 [46] and osteocalcin expression [47], as well as inducing apoptosis of osteoblasts [48]. IL-1 also impairs mineralizing nodule formation [49] to inhibit osteoblast maturation. In contrast, TGF- β has been shown promote the recruitment of osteoblast precursors [50] and increase osteoblast proliferation at sites of bone damage [51].

TNF and IL-1 are synergistic in promoting bone loss, as TNF induces IL-1 and the IL-1 signaling receptor in stromal cells, while IL-1 in turn mediates TNF-induced osteoclastogenesis both *in vitro* and *in vivo* [52]. Osteoclast cultures containing bone marrow stromal cells from mice lacking the IL-1 receptor, as well as osteoclast cultures treated with an IL-1 receptor antagonist, demonstrate reduced TNF-induced osteoclastogenesis. Furthermore, exogenous administration of TNF significantly increases osteoclast numbers in the calvariae of wild type mice; however, this TNF-induced increase is reduced by roughly 50% in mice deficient in the IL-1 receptor. IL-1 also decreases the apoptotic rate of pre-formed osteoclasts to further promote bone resorption [53].

IL-6 has pleiotropic effects on bone and is another major cytokine involved in the pathogenesis RA. Although IL-6 upregulates RANKL expression, it also inhibits the differentiation of osteoclast precursor cells to mature osteoclasts by disrupting RANK signaling pathways [54]. However, blocking IL-6 function decreases the number of osteoclasts in human TNF transgenic (hTNFtg) mice [55], as well as reduces joint damage in both murine inflammatory arthritis [56] and human RA [57].

Furthermore, IL-6 is involved in the generation of Th17 cells, thereby enhancing IL-17 expression levels [58]. IL-17 contributes to inflammation and bone destruction in RA. In addition to upregulating RANKL expression on osteoblasts and synovial fibroblasts, IL-17 induces secretion of prostaglandin E₂ from osteoblasts to increase osteoclast numbers [59]. Three different *in vivo* models demonstrate amelioration of inflammatory arthritis with IL-17 blockade [60-62]. Furthermore, the direct role of IL-17 on bone was examined by

injecting an IL-17A minicircle DNA construct into mice to overexpress IL-17A *in vivo*, leading to expansion of osteoclast precursors and increased osteoclastogenesis [63]. However, targeting IL-17 or the IL-17 receptor has shown variable improvement in clinical trials of RA disease [64-66]. Nonetheless, there is still potential for IL-17 blockade in treating RA as part of a combination therapy. A recent study demonstrated that blocking both IL-17 and TNF is more effective at decreasing inflammation and joint damage in hTNFtg mice than either antibody alone [67]. Not only were osteoclast numbers decreased with combined blockade, but osteoblast numbers, osteocalcin and bone formation rates were increased.

RA patients have increased serum concentrations of TNF, IL-6, IL-34 and MMP-3 compared with disease-free patients, and levels decrease significantly during treatment with a TNF inhibitor [68]. Expression of the pro-osteoclastogenic cytokine IL-34 is elevated in RA synovium, synovial fluid and synovial fibroblasts and correlates with disease severity [69]. Furthermore, IL-34 mRNA expression levels in RA synovial fibroblasts increase after stimulation with TNF and IL-1, suggesting that this cytokine is downstream of these two pro-inflammatory cytokines [70]. In addition, anti-inflammatory cytokines, including IL-10, are present in RA joints and inhibit joint destruction in murine models of arthritis [71, 72]. However, pro-inflammatory cytokines and other pro-osteoclastogenic factors minimize this protective effect and the net result in RA is bone erosion.

4.2 The challenge of erosion healing in RA

Radiographic progression of bone destruction is associated with a poor prognosis in patients with RA. Protection from bone erosions is therefore an important therapeutic goal. The ability of bone erosions to repair, however, is rare. Even when osteoclast activity is inhibited by therapeutic interventions, only about 10% of RA patients repair erosions and fill in lost bone at erosion sites [73-75]. The presence of inflammation in RA not only promotes osteoclast differentiation and bone destruction, but also directly impairs osteoblast maturation and function. Our laboratory has demonstrated in an animal model of RA that bone formation rates at bone surfaces adjacent to inflammation are significantly lower compared to bone surfaces adjacent to normal, non-inflamed marrow [76]. Furthermore, in the presence of synovial inflammation, immature osteoblasts, but not mature osteoblasts, are present, suggesting that synovial inflammation inhibits local osteoblast differentiation. In the setting of resolution of inflammation, however, mature osteoblasts populate the surfaces of eroded bone, resulting in bone formation and erosion repair [77]. These findings suggest that resolution of inflammation is required for osteoblasts to fully mature, form bone matrix, and repair erosions within the arthritic joint.

4.3 Wnt pathway components in RA

The Wnt signaling pathway plays an important role in bone remodeling in RA. In a seminal study using the hTNFtg mouse model of RA, Diarra *et al.* used a blocking antibody to the Wnt signaling antagonist DKK-1 and demonstrated prevention of focal bone erosion, as well as osteophyte formation and ankylosis at sites of inflammation [78, 79]. Bone histomorphometry revealed increased bone formation rates, as well as an increase in osteoblast number and osteoid deposition, at periosteal surfaces in mice treated with the

neutralizing antibody. Furthermore, TNF induced expression of DKK-1 in RA synovial fibroblasts and DKK-1 levels were shown to be elevated in sera of RA patients compared to healthy controls. These data implicate DKK-1 in inhibiting osteoblast-mediated bone formation in RA. Recently, IL-6 was shown to decrease DKK-1 levels in cultured fibroblast-like synoviocytes isolated from inflamed peripheral joints [80]. Thus, regulation of Wnt signaling may be a therapeutic approach to protection from bone loss in RA.

In addition to DKK-1, the Wnt signaling antagonists DKK-3, sFRP-1, sFRP-2 and sFRP-4 are upregulated in arthritic synovial tissues compared to non-arthritic tissues [77, 76]. Our laboratory has also demonstrated that upon resolution of inflammation in the K/BxN serum transfer arthritis model, synovial expression of sFRP-1 and sFRP-2 are reduced, while expression of Wnt-10b is induced relative to inflamed synovium [77]. This would promote Wnt signaling as inflammation resolves, and allow osteoblasts to populate and repair bone surfaces, as was observed in this study.

4.4 Innate immune mechanisms in inflammation and bone remodeling in RA

ITAMs and their adapter proteins have also been implicated in the pathogenesis of erosions in RA as a result of disturbances in osteoclast differentiation and function. Expression of the ITAM adapter proteins FcR γ and DAP12 that support osteoclast differentiation is upregulated in RA synovial tissue compared to OA and healthy patients [7]. TREM1 is similar to TREM2 in that it also signals through DAP12; however, whereas TREM2 regulates osteoclast differentiation, TREM1 controls inflammation and induces secretion of pro-inflammatory cytokines from polymorphonuclear cells (PMNs) [81]. PMNs isolated from RA synovial fluid and blood express increased levels of TREM1 and DAP12 compared to healthy patients [82]. Furthermore, expression is significantly enhanced in healthy donor PMNs after 24-hour stimulation with TNF, IL-6, or IL-17. Therefore, there may be a role for these proteins in perpetuating the inflammatory environment and promoting osteoclast differentiation in RA.

Like TREM2, sialic-acid-binding immunoglobulin-like lectin 15 (Siglec-15) is involved in regulating osteoclast differentiation in conjunction with adapter protein DAP12. Siglec-15 has recently been described to contribute to periarticular, but not subchondral, bone loss in inflammatory arthritis [83]. Siglec-15-deficient mice induced with antigen-induced arthritis were found to have significantly less periarticular bone loss in the proximal tibia compared to arthritic wild type mice. This protection from periarticular bone loss in arthritic Siglec-15-deficient mice was attributed to impaired osteoclast resorptive function.

TLRs are members of the innate immune system that have also been implicated in osteoclastogenesis [84]. LPS has previously been shown to support the survival of osteoclasts by signaling via TLR4 expressed on bone marrow macrophages and osteoclasts [85] and more recently, TLR5 has been described to activate RANKL and osteoclastogenesis [86]. Activation of TLR5 by its ligand flagellin results in pronounced osteoclast formation and bone resorption *ex vivo* in murine parietal bone cultures [87]. Furthermore, expression of TLR5 is upregulated in RA synovium and synovial fluid compared to healthy controls, and therefore may contribute to bone loss in RA. Together,

these data suggest that targeting ITAMs or TLRs may inhibit inflammation and joint destruction in RA.

4.5 miRNAs in RA

A number of miRNAs have been shown to contribute to the pathogenesis of inflammation in RA, including miR-155 and miR-223. miR-155 is overexpressed in RA synovial fluid and tissue compared with OA, and in macrophages of RA patients compared to those from OA patients [88, 89]. Inhibition of miR-155 in RA synovial macrophages results in decreased TNF expression [88]. Furthermore, mice deficient in miR-155 are protected from collagen-induced arthritis (CIA) and develop less bone erosion despite similar degrees of joint inflammation in the K/BxN serum transfer arthritis model as a result of suppressed osteoclast differentiation compared to wild type mice [90].

Similar to miR-155, miR-223 can affect bone remodeling by positively regulating osteoclast differentiation. Expression of miR-223 is upregulated in the synovial tissue and fluid of RA patients compared with OA patients [91] and this miRNA suppresses nuclear factor 1 A-type, an inhibitor of the M-CSF receptor, to promote osteoclastogenesis [92]. This effect was demonstrated in an *in vivo* setting by silencing of miR-223 in the CIA mouse model, resulting in a reduction in the number of mature osteoclasts, and limiting bone erosion [93]. In contrast, miR-146a suppresses osteoclast differentiation and prevents joint destruction in CIA [94]. Thus, miRNAs may be novel therapeutic targets for the protection from bone erosion in RA.

5. Spondyloarthritis

SpA is a category of chronic inflammatory diseases that includes AS, PsA, reactive arthritis, inflammatory bowel disease, and juvenile onset spondyloarthropathy. Inflammation can affect several anatomic sites in SpA, including peripheral joints, the axial skeleton, and the entheses. Depending upon factors specific to these anatomic sites, including local cytokine production, unique cell types present, and mechanical forces, either bone erosion or bone formation can occur. In SpA, similar to what is seen in RA, inflammatory cell infiltration of synovial tissues, with resulting synovial hyperplasia, angiogenesis, cartilage destruction and bone erosion in diarthrodial joints. In contrast to RA, inflammation at enthesial sites, where ligaments and tendons attach to bone, is a hallmark of SpA and promotes cartilage and bone formation. This bone formation can occur near joints on the periosteal surface, termed “osteophytes;” at entheses, termed “enthesophytes;” or at the annulus fibrosis of intervertebral discs, termed “syndesmophytes.” Sacroiliac joint fusion and spinal ankylosis can ultimately occur, resulting in severe back pain and limited mobility. Pathways that have been implicated in new bone formation in SpA include the IL-23/IL-17/IL-22 axis, and the BMP and Wnt signaling pathways (Fig. 2).

5.1 Cytokines implicated in the pathogenesis of inflammation and bone formation in SpA

The IL-23/IL-17/IL-22 axis has emerged as an important pathway in the pathogenesis of both inflammation and bone formation in SpA. IL-23 augments the differentiation of naïve T cells to T helper 17 (Th17) cells, thereby inducing the secretion of IL-17 and IL-22 by

these cells [95]. Clues to a potential role of IL-23 in AS include elevated levels of IL-17 and IL-23 in the serum of AS patients compared with normal [96], and the association of AS, PsA and inflammatory bowel disease with polymorphisms in the IL-23 receptor [97]. IL-23-expressing cells and IL-17-expressing cells have been observed at increased frequency in subchondral bone from facet joints of AS patients compared to OA or healthy patients [98, 99]. Increased numbers of circulating Th17 cells in AS and PsA have been described [100]. IL-17-expressing CD8⁺ T cells are also significantly increased in number in the synovial fluid in PsA patients compared to the peripheral blood; furthermore, this increase has been shown to correlate with disease activity [101]. Increased serum concentrations of IL-17 and IL-23 in AS patients have also been shown to correlate with disease activity [102]. However, plasma IL-23 levels do not correlate with magnetic resonance imaging findings [103].

Other pro-inflammatory cytokines may play a role in inflammation in SpA. For example, levels of IL-6 are increased in the sacroiliac joints of AS patients [104]. TNF mRNA has also been identified in synovial and enthesial tissues of AS patients, and TGF- β mRNA is present near sites of new bone formation in these samples [105]. In conjunction with IL-6, TGF- β promotes the differentiation of Th17 cells from naïve T cells, thus increasing IL-17 and IL-22 levels. Additionally, elevated levels of OPG have been seen in the plasma of AS patients compared to healthy controls, which would serve to inhibit osteoclast formation and bone resorption [106]. In addition, TGF- β plays a role in the recruitment of mesenchymal precursor cells to the bone microenvironment, which could promote osteoblast differentiation. TGF- β and OPG are two additional factors that may contribute to bone accrual in SpA.

IL-23 has also been shown to play an important role in the pathogenesis of new bone formation in SpA, especially at enthesial sites. An elegant study by Sherlock *et al.* demonstrated that overexpression of IL-23 *in vivo* results in enthesial inflammation concomitant with enthesial new bone formation, despite the absence of synovitis [107]. Specifically, IL-23 promotes inflammation at peripheral and axial enthesial sites by inducing other pro-inflammatory cytokines, including IL-6, IL-17 and IL-22. Systemic expression of IL-23 upregulates BMP-7 mRNA expression in cells obtained from murine Achilles' enthesial tissue and results in periosteal osteoid and new bone formation at the entheses 18 days after IL-23 minicircle administration. In another experiment, systemic expression of IL-22 by minicircle DNA technology revealed that IL-22 also promotes osteoblast function by upregulating gene expression of the pro-osteogenic factors Wnt-3a, Wnt-10b and BMP-4 in murine paws, as well as induces phosphorylation of signal transducer and activator of transcription 3 (pSTAT3) in osteoblast precursor cells *in vitro* to promote their differentiation. Sherlock and colleagues also demonstrated that blocking antibodies to IL-17 or IL-22 reduced inflammation and had synergistic effects when used simultaneously; however IL-17 was shown not to be required for pathogenesis, as mice depleted in Th17 cells by a CD4-specific antibody still developed disease in the presence of IL-23 overexpression. This study establishes IL-23 as central to the pathogenesis of SpA, with IL-22 and IL-17 also contributing. Finally, IL-17 itself has dramatic effects on bone remodeling, as it upregulates RANKL expression on synovial fibroblasts and osteoblasts to

promote osteoclast differentiation and function. Interestingly, IL-23 can also promote osteoclastogenesis, as demonstrated in a co-culture system with osteoblasts [108], indicating further complexity in the pathogenesis of new bone formation in SpA.

5.2 Mechanical stress contributes to new bone formation in SpA

Mechanical stress is another local factor that promotes the generation of new bone formation in SpA. Entheses undergo repetitive mechanical stress as muscles, and the ligaments and tendons that attach them to bone, work in concert to produce movement. In a mouse model of inflammatory arthritis in which entheses are sites of inflammation, mice were protected from hind limb mechanical strain by tail suspension. These mice developed significantly less inflammation and bone formation at the site of the insertion of the Achilles tendon into bone, compared to weight bearing arthritic control mice that do not experience hind limb unloading. These data demonstrate definitively that mechanical stress contributes to both inflammation and new bone formation at enthesial sites, and may even be a precipitating factor [109].

5.3 Role of the BMP pathway in new bone formation in SpA

The BMP signaling pathway is a critical pathway in the regulation of osteoblast differentiation and function. Thus, it is not surprising that this pathway would play a role in bone formation in SpA. Using the spontaneous arthritis model in aging DBA/1 mice in which mice develop ankylosing enthesitis, Lories and colleagues identified BMP-2, -6 and -7 proteins by immunohistochemistry at sites of enthesitis undergoing ossification [110]. Specifically, BMP-2 is visualized in early disease stages, while BMP-7 and BMP-6 are expressed in pre-hypertrophic chondrocytes and hypertrophic chondrocytes respectively. Pre-hypertrophic and hypertrophic chondrocytes are involved in endochondral bone formation, a process in which an initial cartilaginous phase undergoes remodeling and is eventually replaced by bone matrix, forming syndesmophytes. Thus, induction of pro-osteogenic BMPs would promote new bone formation. Furthermore, progression of ankylosis is reduced by overexpression of the BMP inhibitor noggin in the arthritic DBA/1 mice, demonstrating that bone formation is suppressed when the BMP signaling pathway is inhibited.

TGF- β and other pro-osteogenic BMP ligands also contribute to new bone formation in SpA by supporting endochondral ossification [111]. BMP-2 and BMP-4 levels are higher in the serum of AS patients who develop spinal fusion in comparison to AS patients who do not develop spinal fusion, and compared to healthy controls [112]. BMP-2 and BMP-6 are expressed at higher levels in synovial fibroblasts and macrophages from AS synovium compared to non-inflamed synovium [113]. Thus, BMPs contribute to bone formation in AS and are potential targets for the prevention of bone formation in SpA.

5.4 Role of the Wnt signaling pathway in new bone formation in SpA

The Wnt signaling pathway has also been implicated in bone formation in SpA, with much attention having been paid to the Wnt antagonists DKK-1 and sclerostin. Inconsistent data regarding expression levels of DKK-1 in AS patients have been reported, with some studies showing decreased DKK-1 expression in AS patients compared to healthy controls or RA

patients, while other studies have refuted this [114, 78, 115-117]. However, DKK-1 serum levels do not necessarily correlate with functional activity of the DKK-1 protein. Daoussis *et al.* measured DKK-1 protein levels in serum from AS patients by two different ELISA assays [114]. Using a sandwich ELISA, they found that circulating DKK-1 levels are significantly increased compared to healthy controls. However, using a functional ELISA that measures binding of DKK-1 to its receptor LRP6, they showed decreased DKK-1 activity in serum from AS patients, a finding that is consistent with other studies [78, 117]. Additionally, AS patients with increased DKK-1 activity in their serum have less syndesmophyte formation in the spine compared to patients with reduced DKK-1 activity, suggesting that increased DKK-1 levels are protective against new bone formation [115]. Decreases in functional DKK-1 would promote Wnt signaling and promote osteoblast maturation and function, resulting in the bone formation. These findings support the hypothesis put forth by Diarra *et al.* that DKK-1 is a key regulator of the impact of inflammation on bone in the rheumatic diseases.

Expression levels of another Wnt signaling antagonist, sclerostin, have been shown to be inversely correlated with syndesmophyte formation [118]. In comparison with healthy patients, expression levels of sclerostin in both periarticular bone and in serum from AS patients are decreased [118]. Furthermore, expression of both DKK-1 and sclerostin at the level of mRNA is lower in the spine of mice with proteoglycan-induced spondylitis (PGISp) compared with control mice [119]. These data suggest that promotion of Wnt signaling as a result of decreased inhibition by DKK-1 and sclerostin contribute to bone formation in SpA.

6. Conclusion

RA and SpA thus serve as examples of rheumatic disease in which the effects on bone are dramatically different. Cytokines and pathways regulating bone resorption and formation in these diseases are rapidly being identified, providing therapeutic targets to protect from harmful alterations in bone remodeling. Local factors, such as cell types present at specific anatomic sites, as well as mechanical stress, also play a significant role in the outcome of inflammatory diseases on bone. Discovering and obtaining a better understanding of factors that contribute to the pathogenesis of inflammation and bone disturbances will aid in the preservation of bone health in rheumatic disease.

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Fig. 1.

Selected factors involved in the differentiation of osteoclasts and osteoblasts. Membrane-bound and soluble RANKL expressed by synovial fibroblasts and osteoblasts bind to the RANK receptor, located on osteoclast precursor cells, to promote osteoclastogenesis, resulting in mature osteoclasts that erode bone. IL-18 and other factors, including IL-33 and type I interferons, inhibit osteoclast differentiation by diverting the osteoclast precursor cells towards differentiation to dendritic cells. BMP signaling ligands BMP-2, -4, -7, among others, promote the differentiation of osteoblasts from mesenchymal precursor cells. Antagonists of the Wnt signaling pathway, including DKK-1, sFRP family members and sclerostin, inhibit osteoblast differentiation through inhibition of Wnt signaling, while BMP-3 is a negative regulator of the BMP pathway. Many miRNAs can also affect osteoblast differentiation. Among them, miR-335 inhibits DKK-1 to promote Wnt signaling and osteoblast differentiation, and miR-29b is maximally expressed during the mineralization stage of bone formation, and promotes osteogenesis.

Fig. 2.

Major factors involved in new bone formation in SpA. TGF- β , IL-6 and IL-23 promote the differentiation of Th17 cells from naïve T cells. Th17 cells secrete pro-inflammatory cytokines, including IL-17 and IL-22 that generate inflammation at peripheral and axial enthesial sites. IL-23 induces expression of BMP-7 within the enthesis to promote osteoblast differentiation and function. IL-22 induces pro-osteogenic factors to also promote osteoblast differentiation and function. Decreased expression levels of Wnt antagonists DKK-1 and sclerostin in SpA leads to increased Wnt signaling and osteoblast function, resulting in new bone formation. Mechanical stress also promotes inflammation and new bone formation at entheses.