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Abbreviations:

ALP	Alkaline Phosphatase
AP-1	Activator Protein 1
APC	Adenomatous Polyposis Coli
CAFs	Cancer-Associated Fibroblasts
CamKII	Calcium sensitive enzyme calmodulin K II
CD248	Endosialin
CIA	Collagen-Induced Arthritis
CK1	Casein Kinase 1
DKK1	Dickkopf-1
DSH	Dishevelled
FAP1	Fibroblast Activation Protein 1
FGF	Fibroblast Growth Factor
Fz	Frizzled

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G6PI	Glucose-6-Phosphate-Isomerase
GDF-8	Growth/Differentiation Factor 8, myostatin
gp 38	Glycoprotein 38, podoplanin
GSK3β	Glycogen synthase kinase 3β
hTNFtg mice	human Tumour Necrosis Factor transgenic mice
IBD	Inflammatory Bowel Disease
IL	Interleukin
LRP5/6	Low density lipoprotein Receptor-related Protein 5/6
M-CSF	Macrophage-Colony-Stimulating Factor
MMP	Matrix Metalloproteinase
MSC	Mesenchymal Stem Cell
NFAT	Nuclear Factor of Activated T-cells
NF-κB	Nuclear Factor 'kappa-light-chain-enhancer' of activated B-cells
OA	Osteoarthritis
OPG	Osteoprotegerin
PKC	Protein Kinase C
PTH	Parathyroid Hormone
RA	Rheumatoid Arthritis
RA-FLS	RA Fibroblast-Like Synoviocytes
RANK	Receptor-Activator of NF
RANKL	Receptor-Activator of NF-κB
Runx2	Runt-related transcription factor 2
SCID	Severe Combined Immune-Deficient
TGF-β	Transforming Growth Factor beta
Th cells	T-helper cells
TNFα	Tumour Necrosis Factor alpha
TNFΔARE	Tumour Necrosis Factor ΔARE
VCAM-1	Vascular Cell Adhesion Molecule 1
Wnt	Wingless and int-1

Summary

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation, local and systemic bone loss and a lack of compensatory bone repair.

Fibroblast-like synoviocytes (FLS) are the most abundant cells of the stroma and a key population in autoimmune diseases such as RA. An increasing body of evidence suggests that these cells play not only an important role in chronic inflammation and synovial hyperplasia but also impact bone remodelling. Under inflammatory conditions FLS release inflammatory cytokines, regulate bone destruction and formation and communicate with immune cells to control bone homeostasis. Other stromal cells such as osteoblasts and terminally differentiated osteoblasts, termed osteocytes, are also involved in the regulation of bone homeostasis and are dysregulated during inflammation.

This review highlights our current understanding of how stromal cells influence the balance between bone formation and bone destruction. Increasing our understanding of these processes is critical to enable the development of novel therapeutic strategies with which to treat bone loss in RA.

Introduction

Rheumatoid arthritis (RA) is an immune-mediated chronic inflammatory disorder of synovial joints characterised by pain, swelling and progressive joint destruction. The synovium is present in articulated joints and serves to produce and maintain synovial fluid that aids joint lubrication and movement. The synovial membrane is just one to two cells thick in a healthy joint and is formed of macrophages, which remove debris, and synovial fibroblasts that produce hyaluronan and other extracellular matrix components of the synovial fluid. In RA the synovium becomes thickened due to an expansion of synovial fibroblasts as well as an infiltration of immune cells, blood vessels and osteoclasts all of which contribute to the swelling and stiffness characteristic of the disease. Activated RA fibroblast-like synoviocytes (RA-FLS) are the most abundant stromal cell in the inflamed synovium. These cells not only destroy cartilage via matrix metalloproteinase (MMP) secretion but are also able to destroy subchondral bone via regulation of osteoclastogenesis [1-3]. Once activated, RA-FLS maintain their tumour-like, aggressive behaviour even after multiple

passages *in vitro* [4] and to date there is no conclusive molecular explanation for this stable, persistent activation.

Besides FLS the stroma comprises a number of matrix-producing, structural cell types including endothelial cells, pericytes, epithelial cells and osteoblasts [5]. In the last few years several studies have focussed on the influence of stromal cells on bone remodelling. Various factors released by these cells influence the balance between bone-forming osteoblasts and bone-resorbing osteoclasts towards bone loss. This review summarizes the direct and indirect impact of stromal cells on bone resorption in chronic inflammatory disorders.

Bone remodelling under resting conditions

Bone remodelling depends on the tight coupling of bone formation and bone resorption to balance bone mass and adopt bone structure to environmental changes such as mechanical loading (ensure that there is no net bone change). Under normal, resting conditions osteoclasts are required for continuous bone remodelling via communication with osteoblasts. Osteoclasts create resorption lacunae, which in turn activate osteoblasts that fill these lacunae with new bone matrix.

Osteoblasts arise from pluripotent mesenchymal stem cells (MSC). The differentiation of osteoblast progenitor cells into bone matrix-producing osteoblasts requires tight control of essential signals such as parathyroid hormone (PTH) [6] transforming growth factor-β (TGF-β) and fibroblast growth factor (FGF) [7, 8]. Furthermore, essential signalling pathways including the bone morphogenic protein (BMP) pathway and the canonical Wnt/β-catenin pathway regulate the expression of runt-related transcription factor 2 (Runx2) [9], alkaline phosphatase (ALP) [10] and osteoprotegerin (OPG) [11], which are all involved in osteoblast differentiation and metabolism.

Conversely, osteoclasts are multinucleated cells derived from hematopoietic stem cells. Osteoclast development involves an initial differentiation step towards monocytes and macrophages, which is under the control of macrophage-colony-stimulating factor (M-CSF). Following this there is a requirement for the receptor activator of NF-κB (RANK) and the receptor activator of NF-κB ligand (RANKL) [12].

RANKL binds to its receptor RANK on the surface of pre-osteoclasts, stimulating macrophage/monocyte fusion and the development of active, mature osteoclasts [13, 14]. RANKL is produced predominantly by stromal cells, specifically by osteocytes, osteoblasts and fibroblasts, as well as a subset of B cells [15, 16]. The RANKL decoy receptor OPG is also produced by stromal cells and blocks osteoclastogenesis by interacting with RANKL [17]. This is supported by results from mouse models revealing that RANKL deficiency causes osteopetrosis [18] whilst OPG deficient mice are osteoporotic [19]. Importantly, therefore both the main driver of osteoclastogenesis (RANKL) and the major inhibitor (OPG) are produced by stromal cells (osteoblasts and FLS) and the ratio between these two proteins is a key determinant of activation or inhibition of osteoclastogenesis during both normal, healthy bone turnover and in pathological bone destruction during inflammatory disease [17, 20].

Bone remodelling in RA

In addition to the pain, swelling and associated loss of function caused by synovial inflammation, resorption of bone tissue is a classical characteristic of RA. Erosion starts very early in disease, is irreversible and accompanied by permanent functional impairment [21]. This process predominantly occurs at the regions where the articular cartilage, bone and inflamed synovium (pannus) meet with subchondral bone destroyed from the outside inwards by cells within the invading pannus tissue. The predominant bone-degrading cell within the pannus is the osteoclast [22, 23].

In RA, inflammation and bone loss are closely linked processes. In this context, inflammatory cytokines including TNFα, IL-1β, IL-6 and IL-17 are produced by infiltrating macrophages and T-cells. These cytokines induce RA-FLS, osteoblasts and bone marrow stromal cells to express RANKL, thus promoting osteoclastogenesis [24-27]. RA synovial tissues express elevated RANKL mRNA and protein compared to patients with osteoarthritis (OA) or to RA patients with less active disease. This elevated level of RANKL is accompanied by diminished OPG expression [28-30]. Thus, the uneven quantities of OPG and RANKL within the RA microenvironment results in an imbalance in the osteoblast-osteoclast axis leading to an overall bias towards bone resorption.

In addition to RANKL, OPG and cytokines, stromal cells of the inflamed synovial tissue also express other factors, which directly influence osteoclastogenesis. One of these factors is myostatin (also known as GDF-8), which belongs to the TGFβ family and is mainly expressed in skeletal muscle. Deletion of myostatin leads to muscle hypertrophy [31]. In addition to its role as a negative regulator of muscle growth, several studies have revealed a new role for myostatin in bone homeostasis. Genetic deletion or antibody-mediated blockade of myostatin leads to high bone density and volume in a mouse model of osteotomy [32-35]. Recently, Dankbar and colleagues showed for the first time that myostatin is highly expressed in stromal cells of RA synovial tissues and that deficiency or antibody-mediated inhibition leads to an amelioration of arthritis in mouse models of arthritis. Functional *in vitro* studies revealed that myostatin enhances RANKL-mediated osteoclast formation by promoting the fusion of pre-osteoclasts, leading to enhanced bone loss [36].

In addition to encouraging osteoclast activity, inflammatory conditions inhibit the reparative activity of osteoblasts. Gilbert et al. [37, 38] identified that, when TNFα was included in pre-osteoblast cultures, osteoblast differentiation and maturation *in vitro* was arrested. Others have similarly found that osteoblast maturation markers such as collagen type I, alkaline phosphatase and osteocalcin are all reduced in the presence of TNFα [24, 39, 40] , leaving these osteoblasts unable to upregulate matrix mineralisation [41]. Osteoblasts cultured with serum from RA patients on Infliximab therapy show reduced expression of IL-6, a cytokine linked to arthritis-related bone loss [42]. IL-6 binds the IL-6 receptor, an interaction which induces prostaglandin E2 synthesis, in turn reducing the ratio of OPG/RANKL expression by osteoblasts, thereby favouring osteoclastogenesis [43]. In addition, osteoblasts cultured with serum from patients treated with Infliximab show reduced expression of IL-1β [42], known to inhibit bone formation *in vitro* [44] and *in vivo* [45] as well as impaired osteoblast migration [46]. Presumably because of the factors described above, it has been reported that TNF blockade in RA patients corrects bone metabolism imbalance seen in RA [47].

In RA the local microenvironment is profoundly changed due to the influx of immune cells and proliferation of synovial fibroblasts at affected joints. This produces a localised hypoxia and a reduced pH, both of which are capable of influencing the

behaviour of osteoblasts within the joint. Hypoxia inhibits Wnt signalling (discussed in more detail below) in osteoblasts by sequestering β-catenin to inhibit transcriptional activity [48]. By up-regulating Dickkopf-1 (DKK1) [49] low pH causes the down-regulation of alkaline phosphatase synthesis in osteoblasts which prevents mineralisation [50].

Hypoxia and acidosis also increase affect osteoclastogenesis and resorptive capacity. Arnett et al (2003) identified that *in vitro* differentiation of monocytes to osteoclasts (in the presence of M-CSF and RANKL) was four-fold more efficient at 2% oxygen compared to 20% oxygen. Importantly, however, the experimental protocol in this instance in fact subjected the cells to repeated periods of hypoxia and normoxia [51] . The requirement for periods of re-oxygenation was confirmed in another study by Knowles and Athanasou (2009) who demonstrated that repeated hypoxia/reoxygenation cycles, such as those expected to occur during period of inflammation, enhanced osteoclast differentiation, however, when cells were subjected to continued hypoxia at 2% oxygen osteoclastogenesis was in fact dramatically inhibited [52] .In addition the resorption activity of the osteoclasts formed at 2% oxygen was two to fourfold higher than osteoclasts formed at higher oxygen tensions [51, 52] (reviewed in [53]). This combination of increased osteoclast formation and activity combined with a dramatic decrease in osteoblast function in response to hypoxia and low pH combine to drive bone destruction during inflammation.

Cytokine-mediated bone destruction

Pannus tissue contains large numbers of activated macrophages, leukocytes and FLS which release pro-inflammatory cytokines including TNFα, IL-1β, IL-6 and IL-17. TNFα, expressed mainly by monocytes and macrophages but also by T cells, B cells and FLS, plays a pivotal role in the inflamed synovial microenvironment in RA. TNFα is considered to be at the top of the inflammatory cascade, based on observations that TNFα induces the expression of other cytokines (eg. IL-1β, IL-6, IL-8) and that anti-TNFα treatment in RA patients significantly reduces IL-1β release by FLS [54-56]. Moreover, in FLS, TNFα induces the production of adhesion molecules to attract leukocytes into the affected joints [57] as well as matrix metalloproteinases that destroy cartilage [58].

During the last few years the impact of TNFα on bone remodelling has been addressed in several studies. Classically, it has been shown that this pro-inflammatory cytokine increases the number of osteoclast precursors [59] and indirectly activates osteoclastogenesis by triggering RANKL release from lymphocytes [60, 61] and endothelial cells [62] as well as increasing RANKL and M-CSF production by stromal cells [63, 64]. The effect of TNFα on bone erosion has been studied in detail using the hTNFtg [65] and the TNFΔARE [66] mouse models of RA. Both these models overexpress TNFα and result in osteoclast-mediated bone destruction in joints. TNF is known to enhance osteoclast activity directly, by promoting maturation of bone marrow macrophages into mature osteoclasts in the presence of RANK-ligand [60] [67].

Stromal cells promote bone loss via interaction with immune cells

Recent studies have shown that the interaction of RA-FLS with infiltrating immune cells plays a key role in both chronic inflammation and bone destruction. In particular CD4+ helper T cells (Th cells), the prominent T cell subset in the sublining of rheumatoid synovium, express RANKL and also cytokines which have stimulatory, as well as inhibitory, effects on osteoclastogenesis. Th1 cells release IL-4 and IL-10 which blocks osteoclastogenesis, whereas Th17 cells release IL-17, IL-22, RANKL, IL-1, IL-6 and TNFα [60, 67] which activate osteoclastogenesis directly on osteoclast precursors. IL-17 and IL-22 also stimulate RANKL expression in RA FLS to activate osteoclastogenesis, suggesting an indirect role of T-cells in bone loss via crosstalk with RA-FLS [68-71].

The first evidence that activated T-cells play an important role in bone destruction has been shown by Kong and colleagues in 1999 [72]. They could demonstrate that CD4+ T-cells produce a sufficient amount of soluble RANKL to promote osteoclastogenesis which subsequently induces bone loss in a model of adjuvant arthritis.

The importance of Th17 cells and IL-17 in bone destruction is also supported by studies using the collagen-induced arthritis (CIA) mouse model. IL-17A-deficient mice developed a markedly reduced severity of CIA accompanied by less bone erosion and less synovial hyperplasia [73]. Therapeutic treatment with neutralizing anti-IL-

17A antibodies significantly reduces the severity of inflammation and bone erosion in various RA mouse models including CIA [74], antigen-induced arthritis [75] and glucose-6-phosphate isomerase (G6PI)-induced arthritis [76].

IL-17 is considered to be a potential osteoclastic cytokine, because it increases RANKL expression in osteoblasts, RA-FLS, and IL-1 and TNFα expression in synovial macrophages, which activates osteoclastogenesis and subsequently drives bone destruction. In an *in vitro* co-culture model with murine bone marrow cells and osteoblasts, treatment with IL-17 derived from synovial fluid of RA patients results in an increased osteoclastogenesis by an upregulation of prostaglandin E2 in osteoblasts [77]. Higher IL-17A concentrations were found in synovial fluid and sera from RA patients compared to OA patients or healthy controls [78, 79]. Therefore targeting IL-17 is suggested as an attractive therapeutic target in RA. IL-17 blockers have been evaluated and are currently being tested in clinical trials for human RA. However, inhibition of IL-17 did not lead to complete disease remission, so far, and monoclonal antibodies against IL-17 receptor seems to be ineffective in RA [80].

Wnt signalling is critical to bone homeostasis

The Wnt signalling pathway not only controls developmental processes such as skeletal patterning [81, 82], but is also crucial for maintaining bone homeostasis. Three major branches of Wnt signalling exist: the canonical, the Ca2+-dependent non-canonical and the planar cell polarity signalling pathway. Of these, the canonical Wnt/β-catenin pathway is known to be the predominant component that impacts on bone remodelling. In the absence of Wnt, β-catenin forms a destruction complex composed of Axin, Casein Kinase 1 (CK1), Adenomatous Polyposis Coli (APC) and Glycogen synthase kinase 3β (GSK3β). This complex facilitates phosphorylation, ubiquitination and subsequently degradation of β-catenin by the proteasome. Activation of signalling occurs upon binding of Wnt proteins to the Low density lipoprotein Receptor-related Protein 5/6 (LRP5/6) receptors and Frizzled (Fz) co-receptors on the cell surface. Dishevelled (DSH) and the destruction complex is recruited to the cell membrane allowing β-catenin accumulation within the cytoplasm and subsequent translocation into the nucleus where it activates transcription of specific target genes [83].

A strong link between the canonical Wnt/β-catenin pathway and bone homeostasis has been demonstrated by studying mutations of several members of the pathway. Mutations in Wnt proteins such as Wnt3, Wnt3a and Wnt7A/Wnt7a lead to skeletal malformations in humans [84, 85] and in mice [86, 87]. Moreover, loss of function mutations in the human LRP5 gene as well as LRP5 knockout mice are associated with low bone density and skeletal fragility [88, 89], whereas gain of function mutations in the LRP5 gene in humans and in mice lead to an increased bone density [90, 91]. In previous studies it has been shown that the high bone mass caused by LRP5 mutations is associated with decreased binding of the Wnt inhibitors Sclerostin [92, 93] and DKK1 [94]. An explanation for these observed phenotypes could be the influence of Wnt/-catenin signalling on the regulation of the OPG/RANKL ratio. Recent findings have shown that the Wnt/β-catenin pathway in osteoblasts inhibits osteoclastogenesis through downregulation of RANKL expression and upregulation of OPG expression, leading to an altered OPG/RANKL ratio [11, 95]. Therefore, the Wnt/β-catenin pathway not only affects cell differentiation into mature osteoblasts and bone renewal, but also arrests bone degradation by blockade of RANK-RANKL interaction through OPG .

Wnt signalling in RA

The role of Wnt signalling in RA is not yet fully understood. β-catenin expression was found to be elevated in synovial tissues and FLS from RA patients compared to those from OA patients [96]. Several years ago, Sen and colleagues revealed that Wnt1-mediated Wnt/β-catenin signalling is constitutively active in RA-FLS leading to pro-MMP3 secretion and fibronectin expression. However, Wnt1 not only activates the canonical Wnt pathway but also the non-canonical Wnt/Ca2+ (β-catenin and LRP5/6 independent) pathway. Wnt1 and Wnt5a initiate the non-canonical signalling cascade by binding to Fz co-receptor causing intracellular Ca2+ release, activation of the calcium sensitive enzymes calmodulin kinase II (CamKII) and protein kinase C (PKC). These kinases activate several transcription factors such as NFAT, NF-κB and AP-1 [97]. The same authors demonstrated that Wnt5a/Fz-5 non-canonical signalling increased IL-6, IL-8, IL-15 and RANKL release, indicating that the non-canonical Wnt pathway might also be important for RA-FLS activation [98, 99]. These observations suggest that constitutive activation of canonical and/or non-canonical

Wnt signalling in RA-FLS may promote synovial inflammation, pannus formation and bone/cartilage erosion during RA.

Wnt antagonists released by stromal cells under inflammatory conditions control bone remodelling

A number of extracellular Wnt antagonists provide fine-tuning of the Wnt signalling cascade. Secreted glycoproteins such as Sclerostin and DKK1 bind to LRP5 and LRP6 to antagonise canonical Wnt signalling, leading to inhibition of bone formation. Loss of Wnt inhibitor Sclerostin expression results in high bone mass and strength in patients with sclerosteosis [100] and Van Buchem disease [101] as well as in Sclerostin deficient mice [102].

Knowledge that the Wnt/β-catenin pathway regulates bone formation and degradation has sparked tremendous interest in the last decade. In particular the use of anti-Sclerostin antibodies in osteoporosis in which loss of Sclerostin enhances bone mineral density seems to be very promising. Since Wnt signalling is required for bone formation it was assumed that the enhanced production of Wnt-antagonists in the inflamed joints was responsible for the lack of bone repair seen in RA joints, hence, blocking Wnt-antagonists could be a promising approach to re-activate the Wnt pathway and counteract bone destruction. However, in reality the situation *in vivo* is more complicated.

Surprisingly, antibody-mediated blockade of Sclerostin in the hTNFtg mouse model of RA caused an unexpected acceleration of bone erosion. Moreover, loss of Sclerostin in the partially TNF-dependent G6PI mouse model of arthritis had no effect on the progression of RA. Disease severity was ameliorated with loss of Sclerostin in the K/BxN serum transfer model, which is TNF receptor independent. Combined, these data suggest a specific role for Sclerostin in TNFα signalling-induced bone erosions. Sclerostin has a protective function in TNF-dependent but not TNF-independent inflammatory arthritis: the more inflammation is driven by TNF the higher the protective effect of Sclerostin [103]. In line with this data, several publication have shown that inhibition of Sclerostin has either no effect or a destructive effect on cartilage and bone: In the CIA model of RA, Sclerostin inhibition

had no effect on the improvement of focal bone destruction [104] and pharmacological inhibition of Sclerostin in a rat model of osteoarthritis showed no effect on inflammatory cartilage remodelling [105]. Of note, one study reported that increased chondrocyte Sclerostin is chondroprotective in a sheep model of osteoarthritis [106] and Bouzis *et al* found that loss of Sclerostin promotes OA in mice [107]. However, there has been a report that anti-Sclerostin therapy is protective in TNF-driven arthritis [108]. The protective effect was largely seen when arthritic mice were co-treated with blocking TNF antibodies, which is in line with the notion that TNF triggers bone loss. Clearly, there is complexity in the function of Sclerostin as uncovered by the animal models of RA described above and this should be carefully considered when using anti-Sclerostin antibodies in patients with RA or other TNF-dependent immune-mediated inflammatory diseases.

Although, Sclerostin and DKK1 are both Wnt-inhibitors that bind LRP receptors and are upregulated in response to TNFα, they exhibit very different effects on bone under inflammatory conditions compared to non-inflammatory conditions (as seen in most osteoporosis situations). Diarra and co-workers have demonstrated that anti-Dkk1 treatment in arthritic mice is able to reverse the pattern of bone destruction to promote activation of bone repair resulting in new bone and osteophyte formation [109]. To explore the role of DKK1 in RA patients Juarez *et al.* took synovial fibroblasts from treatment-naive patients with undifferentiated inflammatory arthritis of less than 3 months duration. Fibroblasts from patients that would subsequently be diagnosed with RA expressed significantly higher levels of Dkk1 messenger RNA and protein compared to fibroblasts from patients whose arthritis resolved. In co-cultures with lymphocytes, more DKK1 was secreted by RA fibroblasts than by fibroblasts from non-inflamed joints or resolving arthritis and the levels of Dkk1 secretion during co-culture positively correlated with lymphocyte adhesion [110]. Recently, Seror *et al* [111] found increased DKK1 levels in a cohort of early RA patients with enhanced bone destruction. Therefore, together with findings from those of the RA mouse model in which anti-DKK1 antibodies were successfully used to enhance bone formation, blocking DKK1 could provide a new therapeutic target for treating bone loss.

Not all stromal cells are created equal!

Fibroblasts, despite being the most ubiquitous stromal cells in the synovium, have proven difficult to characterize in molecular terms and it is only relatively recently that fibroblast-specific markers have been identified to allow the identification of fibroblast subsets. Clearly, as has been described above, there are differences between the phenotypes of FLS and RA-FLS, however it is becoming apparent that greater complexity exists than simply between disease and healthy synovial fibroblasts. Key fibroblast-specific markers identified so far include Fibroblast Activation Protein 1 (FAP1), Endosialin (CD248), Vascular Cell Adhesion Molecule 1 (VCAM-1) and Podoplanin (GP38). The identification of these markers has allowed us to begin to differentiate between RA-FLS subsets and investigate their function.

In 2016 Croft *et al* assessed the functional differences between two of these disease subsets: Podoplanin+ fibroblasts, which predominate in the RA synovial lining layer, and endosialin+ fibroblasts that are restricted to the sublining. Using a human cartilage and RA-FLS graft in SCID mice they showed that it is the podoplanin+ RA synovial fibroblast subset that is migratory and invasive [112]. A recent publication has also confirmed the assumption that FAP plays a crucial role in inflammatory destructive arthritis. FAP deletion in a mouse model of RA ameliorates cartilage degradation and isolated FLS from these mice show a lower cartilage adhesion capacity. These findings pointing to a previously unknown function of FAP in the attachment of FLS to cartilage during RA [113]. Taken together, these data match with similar findings of podoplanin+ cancer-associated fibroblasts (CAFs) promoting metastasis [114] and FAP+ fibroblasts promoting tumor growth in a mouse xenograft model [115] and suggest that targeting a specific fraction of the stromal cells may be an appropriate therapeutic strategy in inflammation as well as cancer.

The importance of osteocytes in bone remodelling

Other predominant cell types in the synovial joint are the osteoblast and the bone-embedded osteoblast termed the osteocyte. Until relatively recently osteocytes were ignored due to difficulties isolating them from tissue and maintaining their phenotype *ex vivo*. However in the last few years improvements in techniques have allowed researchers to interrogate their function during bone disease. Osteocytes are descendants of matrix-producing osteoblasts. They are embedded in the bone matrix

but are not passive cells in bone behaviour, rather they act on bone remodelling through regulation of both osteoclast and osteoblast activity. It has been reported that osteocytes are able to release RANKL as well as M-CSF to recruit osteoclast progenitors to sites of remodelling, supporting the generation of functional resorbing osteoclasts [116]. It has generally been believed that osteoblasts and stromal cells are the main source of RANKL, however, co-culture studies from Nakashima *et al.* [117] demonstrated that purified osteocytes have a greater capacity to support osteoclastogenesis than osteoblasts and bone marrow stromal cells. Osteocytes not only communicate with osteoclasts but also with osteoblasts through the release of the canonical Wnt/β-catenin Sclerostin, which negatively regulates osteoblast differentiation [118, 119].

Although osteocytes communicate with both osteoblasts and osteoclasts to produce RANKL and Sclerostin the question remains how RANKL and Sclerostin reach the bone surface, from deep within the bone. During their embedding phase, osteocytes form dendritic extensions (40-100 per cell) [120] to build a lacuna-canalicular network, maintaining connections with the bone surface and the vascular space [118]. Recently, Honma and co-workers [121] developed a novel co-culture system using osteoclast precursors together with osteocytes, embedded in a collagen gel. On the basis of this system, they clearly demonstrated that the osteocytic, membrane–bound form of RANKL communicates directly with osteoclast precursors through osteocyte dendritic extensions.

The importance of osteocytes has been reported in many musculoskeletal diseases. Decreased connectivity between the osteocytes occurs in osteoporotic and osteoarthritic bones. Moreover bones taken from patients with osteoporosis display also disorientation of the dendrites [122]. Xiong *et al.* [123] reported that mice with osteocyte-specific RANKL deletion develop postnatal osteopetrosis. Recently it has been reported that osteocytes are associated with bone loss in inflammatory bowel disease in rodents (IBD) [124]. Another study revealed that patients with Crohn´s disease possess increased osteocyte apoptosis and reduced bone mass and bone formation [125].

Currently, the function of osteocytes and the control of osteocytogenesis under inflammatory conditions in RA is not well understood. Recently, Pathak *et al* reported

that *in vitro* stimulation of osteocytes with serum from RA patients results in enhanced osteocyte-to-osteoclast communication. They found that RA serum containing inflammatory cytokines enhances the RANKL/OPG ratio in osteocytes, which subsequently leads to enhanced osteoclastogenesis and bone destruction [126].

However nothing is known so far about changes in connectivity and orientation of osteocytes under inflammatory conditions. It is well known that chronic inflammation is a major risk factor for systemic bone loss leading to osteoporosis. Even in chronic inflammatory diseases such as RA, local bone erosions are typically associated with systemic bone loss. As already discussed above, osteoporosis patients seems to have a decreased connectivity and disorientation of dendritic extensions in their osteocyte network. Therefore, it can be assumed that the osteocyte network in bones of RA patients might be altered, which could influence signalling molecules involved in bone remodelling processes. This is a relatively new area of research, however the influences of inflammatory factors in RA on osteocyte-mediated systemic bone loss has yet to be thoroughly investigated.

Conclusion

Bone loss is a common feature of a variety of musculoskeletal disorders. Under inflammatory conditions such as RA, the main trigger of articular bone erosion is synovitis, including the production of inflammatory cytokines and RANKL, leading to activation of osteoclastogenesis. Both activation of bone destroying osteoclasts and a lack of compensatory bone repair mechanisms contribute to a progressive loss of joint structure in RA patients.

In this review we have explored the role of stromal cells and their influence in bone remodelling. Emerging data obtained have provided evidence that stromal cells are more than just structural cells. Under chronic inflammation they aquire novel features which are important for the development of pathologic processes. Knowledge of stromal cells and their influence in bone formation and bone destruction will facilitate the development of future therapeutic strategies for repair of bone erosion.

Disclosure

The authors have no conflict of interest

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Figure Legend

Fig. 1: The role of FLS in inflammatory bone destruction.

Under healthy conditions there is a balance between bone formation and bone destruction to replace old bone tisssue and to repair bone defects. In RA, more bone is degraded by osteoclasts than created by osteoblasts, shifting the balance towards bone destruction. During inflammation stromal FLS, located in the synovial membrane of the joint space, are able to influence this balance directly or indirecly. FLS release RANKL in response to inflammatory cytokines such as TNFα which subsequently stimulates osteoclastogenesis directly. They also communicate with T cells or release inhibitors of bone formation such as sclerostin and DKK1. In contrast to DKK1, Sclerostin not only blocks osteoblast differentiation but also inhibits specifically TNF-mediated bone destruction, suggesting a protective effect in TNF-mediated bone loss. Other factors released by FLS such as myostatin directly activates bone destruction. Different subsets of FLS, especially gp38+ and FAP+ expressing FLS are highly migratory and invasive and seems to be important for cartilage and bone destruction.