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Non-Canonical (RANKL-Independent) Pathways of Osteoclast Differentiation and Their Role in Musculoskeletal Diseases

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Abstract Osteoclasts are multinucleated cells derived from mononuclear phagocyte precursors (monocytes, macrophages); in the canonical pathway of osteoclastogenesis, these cells fuse and differentiate to form specialised bone-resorbing osteoclasts in the presence of receptor activator for nuclear factor kappa B ligand (RANKL). Non-canonical pathways of osteoclastogenesis have been described in which several cytokines and growth factors are able to substitute for RANKL. These humoral factors can generally be divided into those which, like RANKL, are tumour necrosis family (TNF) superfamily members and those which are not; the former include TNFα *l*ymphotoxin exhibiting *i*nducible expression and competing with herpes simplex virus *g*lycoprotein D for *h*erpesvirus entry mediator, a receptor expressed by *T* lymphocytes (LIGHT), a proliferation inducing ligand (APRIL) and B cell activating factor (BAFF); the latter include transforming growth factor beta (TGF-β), interleukin-6 (IL-6), IL-8, IL-11, nerve growth factor (NGF), insulin-like growth factor-I (IGF-I) and IGF-II. This review summarises the evidence for these RANKL substitutes in inducing osteoclast differentiation from tissue-derived and circulating mononuclear phagocytes. It also assesses the role these factors are likely to play in promoting the pathological bone resorption seen in many inflammatory and neoplastic lesions of bone and joint including rheumatoid arthritis, aseptic implant loosening and primary and secondary tumours of bone.

Keywords Osteoclast · Bone resorption · Cytokine · TNF · LIGHT · RANKL

Introduction

Bone resorption is required for skeletal modelling during bone growth and for bone remodelling throughout life. Bone remodelling involves co-ordinated bone resorption and bone formation which is carried out by osteoclasts and osteoblasts, respectively. Osteoclasts initiate the remodelling process, removing bone which is subsequently replaced by osteoblasts. Osteoclasts are multinucleated cells that are uniquely specialised to carry out bone resorption. Pathological bone resorption occurs when the resorption component of the remodelling process does not accord with physiological demands and is an invariable accompaniment of a wide range of neoplastic and non-neoplastic musculoskeletal conditions [1, 2]. The extent of pathological bone resorption (osteolysis) is a function of the number of osteoclasts and hence the humoral factors that play a role in osteoclast differentiation.

Osteoclasts are specialised multinucleated cells that form by fusion of mononuclear phagocyte osteoclast precursors [3, 4]. Mononuclear osteoclast precursors are derived from the pluripotential haematopoietic stem cell [5]. These cells circulate in the (CD14+) monocyte fraction and differentiate into osteoclasts at or near the bone surface [3, 4]. It has been shown that osteoclast precursors comprise 1–4 % of circulating monocytes and that osteoclasts can be formed from monocyte/macrophage precursors [4, 5]. Osteoclast formation involves a multistep process during which there is loss and

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gain of monocyte/macrophage and osteoclast markers, respectively [6].

Canonical (RANKL-Dependent) Osteoclast Formation

Canonical osteoclast formation involves an interaction between receptor activator for nuclear factor kappa B ligand (RANKL)-expressing bone stromal cells and RANK-expressing mononuclear phagocyte osteoclast precursors [7, 8]. RANKL is a Tumour Necrosis Factor (TNF) receptor ligand family member and is expressed as a membrane-bound protein by osteoblasts, stromal cells and lymphocytes [9]. At the tissue level mRNA has been detected in developing and adult bone, bone marrow and lymphoid tissue (lymph nodes, spleen and thymus) [10]. In bone, RANKL is cleaved into a soluble molecule (sRANKL) by metalloproteinases and secreted mainly (but not exclusively) by osteoblastic stromal cells [7, 8]. The expression of RANKL by osteoblasts/bone stromal cells is upregulated by osteotropic hormones, such as 1,25-dihydroxy vitamin D_3 ($1,25(OH)_2D_3$), parathyroid hormone (PTH), and by locally produced cytokines such as TNFα, Interleukin (IL)-1, and IL-6 [11–13]. RANKL not only promotes the differentiation and fusion of osteoclast precursors cells but also activates the bone resorbing activity of mature osteoclasts [14, 15]. The osteoclastogenic effects of RANKL are mediated through its receptor, RANK (also known as TRANCE-R, and TNFRSF11A), which is a type I membrane protein that associates at the cell surface as a trimer [16]. RANK is expressed by dendritic cells, T cells and osteoclasts. RANK mRNA has been detected in the heart, lung, brain, skeletal muscle, kidney, liver and skin [17].

The importance of the RANKL/RANK system in osteoclast formation is evidenced in RANKL knockout mice which exhibit unopposed bone formation; this results in a form of osteopetrosis that is characterised by the absence of osteoclasts on the bone surface with total bone marrow occlusion and failure of tooth eruption [10, 18, 19]. These mice have domed skulls, shortened limbs and abnormal growth plates and suffer spontaneous fractures due to abnormal mineralisation [19]. The osteoclast precursors in these mice are normal as they are able to differentiate into osteoclasts in vitro with the addition of exogenous RANKL. The osteoblasts, however, are defective as they are unable to support osteoclastogenesis, highlighting the osteoblast as the primary cell source of RANKL [18]. In addition to bone abnormalities, these mice exhibit defective development of the thymus and lymphoid tissue, an indication of the wide-ranging effects of RANKL. RANK-deficient mice also exhibit severe osteopetrosis [16, 20].

Osteoprotegerin (OPG), a member of the TNF receptor superfamily (TNFRSF11B), is a soluble decoy receptor for RANKL which competes with RANK for binding to RANKL and in this way blocks RANKL-induced osteoclastogenesis [14, 21]. OPG is expressed in a variety of tissues including brain, skin, liver, heart and bone as well as haematopoietic and immune cells. The main OPG-producing cells are osteoblasts and bone stromal cells [22]. The OPG knockout mouse develops a severe osteoporosis in which there is loss of trabecular and cortical bone associated with a marked increase in osteoclast formation and resorption [23]. The number of osteoblasts is also increased in response to the increase in osteoclastogenesis. Interestingly, these mice also develop vascular calcification indicating that OPG also plays a role in the prevention of arterial calcification [24].

RANKL/RANK binding initiates activation of a range of signal transduction pathways via interaction of the cytoplasmic tail of RANK with the adaptor proteins TNF-related Factor-1 (TRAF1), TRAF2, TRAF3, TRAF5 and TRAF6 [25]. TRAF6 in particular is important for osteoclast differentiation and function. TRAF6 deficient mice develop severe osteopetrosis with defects in bone resorption and tooth eruption [26]. In contrast, progenitor cells derived from TRAF2 or TRAF5-knockout mice show only mildly reduced osteoclastogenesis [27, 28]. TRAF6 mediates activation of the NFκB and c-src /PI 3-kinase/Akt pathways. PI 3-kinase inhibition in vitro results in impaired osteoclastic bone resorption [29]. Mice deficient in both the p50 and p52 subunits of NFκB [30], in c-src [31] or the Src homology 2-containing inositol-5-phosphatase (SHIP) [32] develop osteopetrosis, suggesting that RANKL-induced activation of NFκB and c-src in osteoclast progenitors is crucial for osteoclast differentiation. Many members of the mitogen-activated protein kinase (MAPK) family are also activated downstream of RANK including the p38-MAPKs, c-Jun N-terminal kinases (JNK1, 2 and 3) and extracellular signal-regulated kinases (ERK1 and ERK2). Bone marrow monocytes derived from JNK1-, but not JNK2-, deficient mice show reduced osteoclastogenesis associated with lack of phosphorylation of the JNK substrate c-Jun [33]. Fos (c-Fos, Fos B, FRA-1, FRA-2) and Jun (c-Jun, JunB, JunD) proteins comprise the homodimeric AP-1 transcription factor. Mice lacking JunB [34] or c-Fos develop osteopetrosis because of an early block of differentiation in the osteoclast lineage [35]. FRA-1 is able to rescue c-Fos functions in bone development [36], suggesting a central role for AP-1 in osteoclastogenesis.

Macrophage colony stimulating factor (M-CSF) is a growth factor which plays a crucial role in the proliferation, differentiation, activation and survival of cells of the mononuclear phagocyte system [37]. M-CSF is synthesised by mesenchymal cells, including fibroblasts and osteoblasts, some epithelial cells, and activated macrophages [38, 39]. M-CSF belongs to the tyrosine kinase receptor family and is encoded by the protooncogene *c-fms* [40]. M-CSF binds to its receptor c-Fms on osteoclast precursors and mature osteoclasts. The

essential role of M-CSF in osteoclast formation has been shown in vivo and in vitro. Osteoblasts/bone stromal cells in osteopetrotic (*op/op*) mice do not produce functionally active M-CSF because of an insertion of an extra thymidine in the coding region of the M-CSF gene [41, 42]. These animals are severely deficient in mature macrophages and osteoclasts. The administration of recombinant human M-CSF corrects the impaired bone resorption in these animals and induces the appearance of resorbing osteoclasts and bone marrow macrophages [41].

Non-Canonical (RANKL-Independent) Osteoclast Formation

Although osteoclast formation induced by RANKL represents the major pathway whereby osteoclastogenesis occurs, a number of other cytokines and growth factors have been shown to be capable of substituting for RANKL to induce osteoclast formation from marrow-derived and circulating osteoclast precursors. These can be divided into humoral factors which, like RANKL, are TNF superfamily members, and those which are not (Table 1). The former include TNFα, APRIL (a proliferation inducing ligand), BAFF (B cell activating factor), and LIGHT (*l*ymphotoxin exhibiting *i*nducible expression and competing with herpes simplex virus *g*lycoprotein D for *h*erpesvirus entry mediator, a receptor expressed by *T* lymphocytes); the latter include transforming growth factor β (TGFβ), the interleukins IL-6, IL-11, IL-8, nerve growth factor (NGF) and IGF-I and II. The existence of these non-canonical pathways implies that there is a degree of redundancy in growth factor signalling for osteoclast formation; in general non-canonical osteoclastogenesis from marrow and monocyte precursors results in the formation of osteoclasts that are smaller and have fewer nuclei than those formed in RANKL-treated cultures; these smaller osteoclasts produce smaller lacunar resorption pits; in LIGHT-treated cultures, the number of resorption pits formed is similar to that seen in RANKL-treated cultures but the pits are not as deep [1, 43, 47] (Table 1; Fig. 1). The significance of non-canonical osteoclastogenesis in physiological bone resorption is uncertain, but it is likely to play a role in pathological bone resorption where high levels of the cytokines and growth factors known to be capable of substituting for RANKL are generally found.

Osteoclast Formation by TNF Superfamily Members

TNFα

TNFα is produced by macrophages, monocytes and T cells. The TNFα transgenic mouse has helped to define the mechanism by which elevated levels of TNFα influence canonical and non-canonical osteoclast formation. In these mice there is a four- to seven-fold increase in the number of osteoclast precursors in the peripheral blood mononuclear cells (PBMC) population and the spleen [64]. In this model, the osteoclastogenic effect was not seen following administration of PTHrP, $1,25(OH)_2D_3$ or IL-1β. The increased number of pre-osteoclasts correlated with detectable levels of TNFα in the serum and the onset of inflammatory arthritis. The addition of a TNFα antagonist inhibited this increase in the pre-osteoclast population. This suggested that TNFα, at elevated concentrations, is able to directly impact osteoclastogenesis by increasing the proliferation of osteoclast precursors. TNFα stimulated the formation of $TRAP^+$ and cathepsin K^+ osteoclasts on the bone surface. Osteoclasts were only observed near the site of administration and there were no radiological signs of resorption. Although some groups have not observed formation of multinucleated cells capable of resorption when TNFα is added to osteoclast precursors without other factors [44], both Azuma et al. and Kudo et al. found that TNFα is able to induce mouse and human osteoclast differentiation and activation independently of RANKL without the addition of other cytokines [45, 65]. The combination of TNFα and IL-1α in cultures was found to synergistically increase osteoclast formation and lacunar resorption in mouse marrow and human monocyte cell cultures [45, 65]. IL-1α, however, did not induce osteoclast formation alone; there is evidence suggesting that IL-1α directly targets osteoclast precursors to promote differentiation but that it requires permissive levels of RANKL to do so; a similar mechanism may operate with regard to TNFα [66, 67].

TNF-α can induce biological reactions via two cell-surface receptors: TNF receptor type 1 (TNFR1) and TNF receptor type 2 (TNFR2). Each receptor mediates different intracellular signals. Analysis of TNFR1- and TNFR2-deficient mice revealed that TNFR1 induces osteoclast differentiation, whilst TNFR2 inhibits osteoclast differentiation [68]. More recently, two independent regulators, A20 [69] and TSG-6 [70], have also been suggested to play a role in the negative loop effects of TNFα, which in part may explain the various temporal effects of TNFα that have been recorded in in vitro/vivo models of osteoclast formation [71–73].

LIGHT and Other TNF Superfamily Members

LIGHT (*l*ymphotoxin exhibiting *i*nducible expression and competing with herpes simplex virus *g*lycoprotein D for *h*erpesvirus entry mediator, a receptor expressed by *T* lymphocytes) is a type II transmembrane protein that is a member of the tumour necrosis factor superfamily (TNFSF14) [74, 75]. LIGHT is primarily expressed by cells that play an immunological role including activated T cells, natural killer cells, spleen cells, immature dendritic cells and macrophages [76–80]. The key biological function of LIGHT is modifying innate and adaptive immune responses via T cell

Table 1 Comparative TRAP+ multinucleated cell and resorption pit formation by soluble RANKL-independent humoral factors

	Soluble Mediator	Number of TRAP-expressing multinucleated cells	Number of lacunar resorption pits	% Area resorption (on dentine slices)	Optimal Conc. (ng/ml)	Reference
Canonical Pathway	Soluble RANKL	√√√	√√√√√	80–100	50–60	[3,7,9,10,15,22]
TNF superfamily	TNF-alpha	√√	√√	20–30	75–100	[27,28,43–46]
	LIGHT	√√	√√√	50–70	25–50	[47–54]
Non-Canonical Pathways	NGF	√	√	1–2	50	[55]
	APRIL	√	√	1–1.5	25–50	[55]
	BAFF	√√	√	2–3	50–100	[55]
Non-TNF superfamily	IGF-I & II	√	√	1–2	30–40	[55–59]
	TGF-beta	√	√	2–5	50–75	[60,61]
	IL-6/IL-11	√	√	2–10	25–50	[62,63]

differentiation, activation and homeostasis [79, 80]. LIGHT has been implicated in graft versus host diseases, rheumatoid arthritis and tumour cell apoptosis [48, 55, 81–83]. LIGHT binds three receptors: herpesvirus entry mediator (HVEM), lymphotoxin β receptor (LTβR) and decoy receptor 3 (DcR3), the cytoplasmic tails of which show structural similarity. The functions of HVEM and LTβR are cell-context specific, but in general HVEM has survival and growth-inducing properties whilst LTβR mediates cell death [84, 85]. DcR3 is a soluble non-signalling receptor that modulates the functions of LIGHT. DcR3 has been shown to induce osteoclastogenesis in one study [86], although the mechanism whereby this occurs is unclear given that DcR3 does not have signalling capabilities.

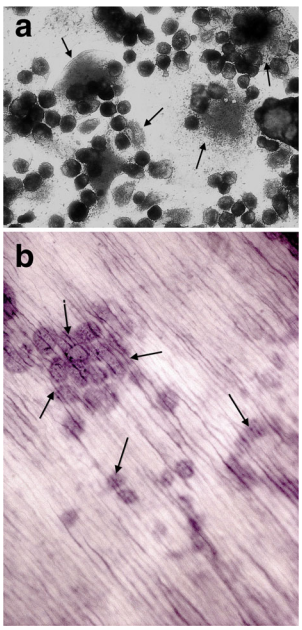

Fig. 1 **a** Small TRAP+ multinucleated cells and **b** correspondingly small resorption pits formed in LIGHT-treated cultures (both at ×400 magnification)

LIGHT is capable of inducing osteoclast formation from both murine RAW264.7 macrophage precursors and human peripheral blood mononuclear cells via a process which is not inhibited by OPG or RANK-Fc [47] (Fig. 1). The effect of LIGHT is not mediated through any of its known receptors (HVEM, lymphotoxin β receptor and DcR3). It was noted that the soluble decoy receptor DcR3 dose-dependently inhibited LIGHT-dependent osteoclast formation [47]. Yang et al. reported that DcR3 is able to stimulate osteoclastogenesis independently of both M-CSF and RANKL in human monocytes, RAW264.7 murine macrophages and rat marrow cells via a mechanism involving secretion of TNFα following reverse signalling to activate ERK and p38 MAPK [86]. LIGHT-mediated osteoclastogenesis was originally shown to be inhibited by a neutralising antibody to TNFRII [47], a receptor LIGHT has not previously been shown to bind [46] and, in common with HVEM and LTβR, is not known to associate with TRAF6.

An analysis of the downstream signalling pathways of LIGHT showed that it activates the Akt, NFκB and JNK pathways in mouse and human monocytes [87]. Activation of these pathways is mediated by TRAFs including TRAF6 which plays an important role in osteoclast differentiation [25]. TRAF6 does not bind to TNFR-II [88] so the precise role of TRAF6 in TNFα and LIGHT-mediated osteoclastogenesis is unclear; TRAF6 also does not associate with HVEM or LTβR. Other TRAFs, however, have been shown to bind to the cytoplasmic tail of TNFR-II and RANK [88]. It has been shown that TRAF2 and TRAF5 are involved in TNFα-mediated osteoclastogenesis [27, 28]. Since the cytoplasmic tail of HVEM and LTβR associates with TRAF2 and/or TRAF5 [88], it is possible that HVEM and LTβR mediate the osteoclastogenic actions of LIGHT via these adapter proteins.

Other members of the TNF superfamily, which have been shown to be capable of inducing osteoclast formation, include APRIL (a proliferation inducing ligand), also known as TRDL1 and TNFSF13, and BAFF (B cell activating factor) [60]. APRIL is not present in normal tissues but is highly expressed by numerous tumour cells [89, 90]. BAFF is expressed by dendritic cells and macrophages and stimulates the proliferation of B lymphocytes and their secretion of immunoglobulin; it is also expressed by tumour cells including B leukaemia cells [91]. Numerous $TRAP^+/VNR^+$ multinucleated cells that express F-actin rings are generated in APRIL and BAFF-treated monocyte cultures, but these osteoclasts produce significantly less resorption than those formed in RANKL-treated cultures [60]. This finding suggests that not all the osteoclast-like cells formed in these cultures are functional.

Compared with LIGHT, APRIL and BAFF are much less efficient in terms of producing mature resorbing osteoclasts from monocytes [60]. The addition of LIGHT to monocyte cultures results in the formation of numerous osteoclasts and extensive lacunar resorption, approximately one-half that seen in RANKL-treated cultures. HVEM, a receptor for LIGHT, shows increased mRNA expression during osteoclastogenesis. The formation of $TRAP^+$ osteoclasts and resorption pits in APRIL/BAFF-treated cultures is similar to those in TNFα-treated cultures. It has recently been shown that TNFα-induced osteoclast formation is markedly increased in mice lacking NF-κB2 (p100) [61]. The precursor protein NF-κB2 acts as a negative regulator of osteoclast formation and TNFα was found to induce a sustained accumulation of NF-κB2 in osteoclast precursors. A similar mechanism of inhibition may control osteoclast resorption induced by APRIL and BAFF.

Osteoclast Formation by Non-TNF Superfamily Members

Transforming Growth Factor β

Transforming growth factor β (TGFβ) is a multifunctional growth factor that is abundant in bone; it is produced by many cells in bone, including osteoblasts, fibroblasts and osteoclasts [92]. TGFβ1 is the most common isoform of TGFβ [62]. There are contradictory reports regarding the specific actions of TGFβ1 in bone [63, 93, 94]. TGFβ1 is a potent chemoattractant for osteoblasts and can stimulate osteoblast proliferation and early stage differentiation [95]. TGFβ1 knockout mice have no osteoblasts and as a result have fragile bones with a low mineral:matrix ratio. Although there are many reports of the inhibitory effect of TGFβ1 on osteoclast differentiation, there are also several which have noted that TGFβ1 is able to induce osteoclastogenesis and resorption [96]. It is probable that these reported differences in TGFβ effects are due to differences in cell populations, the culture methods and TGF β1 concentration employed in various studies. TGFβ1 inhibits osteoclast formation in long-term co-cultures of bone cells at high concentrations due to its action on osteoblasts whereby it suppresses RANKL and increases OPG levels [97]. However, TGFβ1 has been shown to stimulate osteoclast formation from monocytes in the absence of RANKL and other factors known to induce osteoclast formation [96]. Osteoclasts formed in these cultures, like those in TNFα-treated cultures, are small and form small lacunar resorption pits.

Interleukins

IL-6 and IL-11 share many biological properties and are members of the glycoprotein 130 (gp130) cytokine family; these cytokines are produced by osteoblasts, stromal cells, monocytes and macrophages and other cells in bone [98]. They exert their effects through a common signal transducer, i.e. gp130. IL-6 can stimulate osteoclast precursor proliferation, differentiation and activation in a dose-dependent manner, independently of RANKL [99, 100]. Roodman et al. proposed a model to explain the actions of IL-6 [101]. At low concentrations (below 10 ng/ml), IL-6 stimulates osteoclast formation from precursors, but at high concentrations (above 10 ng/ml) IL-6 predominantly stimulates the activation of mature osteoclasts. It has been shown that IL-6 and IL-11 can induce osteoclast formation from human monocytes [100]. This osteoclast formation is inhibited by the addition of a human antibody to the gp130 receptor. Another interleukin that has been reported to be capable of substituting for RANKL is IL-8 [102, 103]; the possible role of IL-23 in inducing osteoclastogenesis is discussed in Section 4.

Nerve Growth Factor

Nerve growth factor (NGF), a member of the neurotrophin family, is expressed in a number of skeletal cell populations and plays a role in bone fracture repair through stimulation of osteoblasts which enhance osteoclast resorption [104–107]. The addition of NGF to monocyte cultures induces the formation of $TRAP^+$ multinucleated cells which produce

approximately five-fold more resorption than APRIL or BAFF [60]. This amount of resorption is comparable to that seen in cultures treated with IL-6 and TGF-β. Sensory and sympathetic neural innervation of the bone and periosteum is well documented [56, 104–108]. Local administration of NGF is known to enhance bone remodelling and is thought to be particularly important in promoting bone formation in fracture healing [57, 104]. Direct neurite-osteoclast cell communication through adrenergic receptors has been shown and it is therefore possible that NGF could act as a neurogenic coupling factor between osteoblasts and osteoclasts [58].

Insulin-Like Growth Factor

Insulin-like growth factor-I/II (IGF-I/II) are non-TNF superfamily growth factors that are known to play a role in bone remodelling. Bone is a major reservoir of IGF-I and IGF-II. IGF-I predominates in rodent bone and IGF-II in human bone [109]. It has been suggested that IGFs may act as coupling factors between osteoblasts and osteoclasts [59, 110, 111]. The effect of IGFs on osteoclasts is not well-defined but promotion of osteoclast formation and activity in vitro has been described [112–114]. Both, IGF I and IGF II, stimulate osteoblast proliferation and matrix synthesis but the effect on osteoclast differentiation is less well characterised [115]. The IGF I knockout mouse clearly exhibits defective osteoblasts and deficient numbers of osteoclasts [116]. In vitro studies have shown that IGF I and IGF II, in the presence of additional growth factors, are able to promote osteoclast differentiation and activation, and it has been shown that IGF I may influence osteoclast formation through regulation of RANKL and RANK expression [117–119]. It has also been shown that both IGF I and IGF II directly support the formation of mature resorbing osteoclasts from human monocyte precursors [60]. Although lacunar resorption in IGF-treated cultures was significantly less than that in RANKL or LIGHT-treated cultures, given the abundance of both IGF I and IGF II in the bone matrix, this mechanism may be significant in physiological bone remodelling. IGFs are stored in the bone matrix and are released upon resorption and can potentially act on nearby osteoblasts and osteoclasts to increase bone remodelling [116, 118]. It has been shown that resorbing osteoclasts express IGF I, IGF II and IGF I receptor mRNA, indicating that osteoclasts are likely to be directly responsive to IGF [119]. An increase in the expression of IGF I mRNA in mature osteoclasts as compared to monocytes has been noted, giving support to the possibility of an autocrine loop.

RANKL-Independent Pathological Bone Resorption

There is now considerable evidence to show that RANKL-independent pathways of osteoclasts formation are likely to play a role in pathological bone resorption associated with several neoplastic and non-neoplastic diseases of bone and joint.

Giant cell tumour of bone (GCTB) is a locally aggressive highly osteolytic bone tumour that contains numerous large, multinucleated osteoclasts. In addition to the osteoclastic giant cell component, there is a significant mononuclear cell population which includes macrophage-like osteoclast precursors and mononuclear stromal cells that are known to express RANKL. Expression of a number of non-canonical osteoclastogenic factors has been noted in GCTB cells including TGFβ, IL-1, IL-6, TNFα, IGF I and II [49, 120–122]. These factors are likely to play a role in the recruitment and formation of the numerous large osteoclasts characteristically seen in this tumour. The expression of mRNA for IGF I and II and the IGF I receptor has been noted in both giant cells and mononuclear stromal cells in GCTB [122]. Mononuclear stromal cells in GCTB are known to exhibit an osteoblast phenotype and it is possible that IGF I and II could represent a therapeutic target which would inhibit replication of these cells and decrease formation of the giant cells in these tumours. Expression of APRIL and BAFF has also been noted in the mononuclear stromal cells and giant cells of GCTB [60, 123].

RANKL-independent osteoclast formation has also been shown to play a role in the osteolysis associated with Ewing sarcoma, metastatic breast carcinoma and melanoma [124–126]. Cultured breast cancer cells produce soluble factors that inhibit RANKL-induced but stimulate non RANKL-induced osteoclast formation from human monocytes. Ewing sarcoma cells express RANKL but, like melanoma cells, also produce a soluble factor that supports osteoclast formation; this is inhibited by an antibody to TNFα. Several RANKL-independent humoral factors are also likely to play a role in the osteolysis associated with myeloma [127]. LIGHT has been shown to increase osteoclast formation in multiple myeloma [128], a condition in which IL-6 and IL-11 have been strongly implicated with regard to osteoclast formation [127]. IL-6 and IL-11 are also thought to play a role in oestrogen deficiency-associated bone loss [129], Paget's disease [129] and Gorham-Stout disease [130]. It has been shown that anti-BAFF treatment can inhibit osteoclastogenesis in a SCID human multiple myeloma model [131]. APRIL is expressed in a number of tumour cell lines and over-expression of APRIL has been linked with tumour progression, in particular in multiple myeloma [89, 132, 133].

Inflammatory conditions of bone and joint are often characterised by the presence of a heavy macrophage infiltrate. This is seen particularly in aseptic loosening of implant components which is associated with the presence in periprosthetic tissues of numerous implant-derived wear particle-containing foreign body macrophages and

periprosthetic osteolysis. It has been shown that two distinct cellular mechanisms of osteoclast formation and bone resorption are likely to operate in aseptic loosening [134]. CD14+ macrophages isolated from the pseudomembrane of loose hip arthroplasties can be induced to form osteoclasts by both RANKL-dependent and TNFα-dependent mechanisms [50, 134]. The addition of an antibody against the p55 receptor subunit of TNF resulted in significant inhibition of osteoclast formation and lacunar resorption. TNFα mediated lipopolysaccharide-stimulated osteoclastogenesis has also been shown to play a role in periodontal disease [51].

Elevated levels of TNFα have been detected in patients with rheumatoid arthritis (RA), and this cytokine is believed to play a central role in the pathogenesis of this disease [52]. High levels of TNFα are known to be present in synovial fluid in many inflammatory joint conditions including RA where there are numerous macrophages as well as lymphoid cells in the synovial membrane. Synovial fluid macrophages in RA are capable of osteoclast formation when incubated with TNFα [53]. It is likely that TNFα (± IL-1)-induced osteoclast formation contributes to the formation of marginal erosions in this condition [54]. Glucocorticoids, which inhibit TNFα-induced osteolysis are of benefit with regard to controlling pathological bone resorption in RA; in contrast, glucocorticoids promote RANKL-dependent osteoclast formation [65]. This finding would argue for two distinct pathways of osteoclast formation, one RANKL-dependent, one RANKL-independent, being operative in conditions of pathological bone resorption.

LIGHT, a potent RANKL-independent osteoclastogenic factor, has been shown in several studies to play a role in RA. An increase in LIGHT levels has been noted in the serum of RA patients [47]. LIGHT is upregulated on B lymphocytes and monocytes in RA and blocking the action of LIGHT reduces the severity of murine collagen-induced arthritis [135, 136]. The formation of osteoclasts from monocytes is significantly decreased by adding a soluble decoy receptor for LIGHT, DcR3, and by blocking antibodies to the p75 component of the TNF receptor [47]. It has been shown that osteoclast formation from monocytes involves interaction with synovial cells [137]. LIGHT promotes RA synovial fibroblast proliferation, survival and activity [138, 139], and cytokine and metalloproteinase expression by synovial macrophages [54]. Polymorphic variants of LIGHT alter binding to HVEM and DcR3 and may influence inflammation and resorption in RA [140]. In recent experiments, we found that LIGHT is highly expressed in the synovial tissue of RA patients and that LIGHT induces RANKL-dependent osteoclast formation from synovial fluid macrophages. BAFF is also associated with autoimmune diseases such as RA, Sjögren's syndrome and systemic lupus erythematosus and may play a role in osteolysis associated with these inflammatory arthropathies [141, 142]. Adamopoulos and co-workers have noted that in RA and crystal arthritis, synovial fibroblasts produce soluble factor(s) that induce osteoclast formation by a RANKL-independent mechanism [143]; osteoclastogenesis was not altered by inhibition of TNF or IL-6. They also showed that mice overexpressing IL-23 show increased osteoclast formation with increased trabecular and cortical bone loss as well as inflammatory joint destruction [144, 145]; this was associated with an increase in marrow myeloid precursors in contrast to IL-23 deficient mice which showed impaired osteoclast differentiation and function. More recently, they have shown that IL-23-induced osteoclastogenesis may be RANKL-independent and that it involves activation of a unique 12 kDa myeloid protein [146]. IL-23 may thus represent another non-TNF superfamily member capable of inducing RANKL-independent osteoclast formation from mononuclear phagocyte precursors.

Conclusion

Although the RANKL/RANK axis undoubtedly accounts for osteoclastogenesis that occurs in physiological bone remodelling, non-canonical (RANKL-independent) pathways of osteoclast formation are likely to play a role in pathological bone resorption associated with neoplastic and non-neoplastic diseases of bone and joint. Although osteoclastogenic humoral factors are not as potent as RANKL in inducing osteoclast formation, these factors are present at relatively high concentration in osteolytic bone and joint disorders which also commonly have a significant CD14+ (pre-osteoclast) macrophage infiltrate. The existence of multiple non-canonical pathways of osteoclast formation has particular implications for the use of therapies targeting RANKL, such as the anti-RANKL antibody, Denosumab™, which has been employed to treat osteoporosis and GCTB. RANKL-independent osteoclastogenesis could represent a mechanism of escape from this therapy. Further investigation into the mechanisms of action of humoral factors that induce RANKL-independent osteoclast formation, particularly with regard to specific osteolytic diseases of bone and joint, will advance our understanding of pathological bone resorption and may lead to the development of new anti-resorptive therapies.

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Compliance with Ethical Standards

Conflict of Interest Afsie Sabokbar, David Mahoney, Francesca Hemingway and Nicholas Athanasou declare that they have no conflict of interest.

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