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OSTEOCLASTS, PRO-INFLAMMATORY CYTOKINES, RANK-L AND BONE REMODELING IN RHEUMATOID ARTHRITIS

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TABLE OF CONTENTS

1. ABSTRACT

Inflammatory joint diseases such as rheumatoid arthritis tend to destroy joint cartilage and bone matrices. Since bone resorption is a common characteristic of rheumatoid arthritis, one of the cell types thought to play a vital role in the destruction of these matrices are the osteoclasts. Osteoclasts and osteoclastogenic factors such as inflammatory cytokines and RANK-L are present within inflamed joints, and osteoclastic bone resorptive activities are also displayed, further suggesting the possibility that osteoclasts are responsible for the joint cartilage and bone matrix damage observed in this joint disease.

2. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, inflammatory joint disease characterized by leukocyte invasion of the synovial lining and hyperplasia of the resident synoviocytes with an ensuing overproduction of cytokines, chemokines, and other inflammatory mediators. Overproduction of these mediators ultimately results in anarchic remodeling of joint structures and destruction of both cartilage and bone. A major cause attributed to this destruction has been the disequilibria of bone remodeling, where the level of bone resorption exceeds the level of bone formation. Maintenance of appropriate regulation of bone mass is controlled by two major cell types: the osteoblast (formation) and the osteoclast (resorption). Osteoclastogensis is a precisely regulated process, and it is now clear that pro-inflammatory cytokines, especially M-CSF, RANK-L, and its interaction with its receptor RANK are necessary and sufficient for promoting differentiation and activation of osteoclast precursors. In this review, we examine the role of the osteoclast in bone remodeling in RA and the role of pro-inflammatory cytokines and the RANK-RANK-L interaction in controlling osteoclasts in RA.

2.1. Osteoclasts: differentiation and activation

2.2. Differentiation

The adult human skeleton undergoes a continuous process of bone remodeling characterized by constant cycles of bone formation and bone resorption. Multinucleated giant cells known as osteoclasts perform the process of bone resorption. These bone resorbing cells, originally described by Alber Kolliker in 1873, are derived from hematopoietic stem cells, specifically, from a fusion of monocyte-macrophage precursors (1-3) (Figure 1). Early in osteoclast development, the osteoclast precursor expresses both monocyte-macrophage markers (non-specific esterase (NSE), MAC-1, and MAC-2) as well as osteoclast markers (calcitonin receptor (CT) and tartrate acid phosphatase (TRP)). As the precursor further

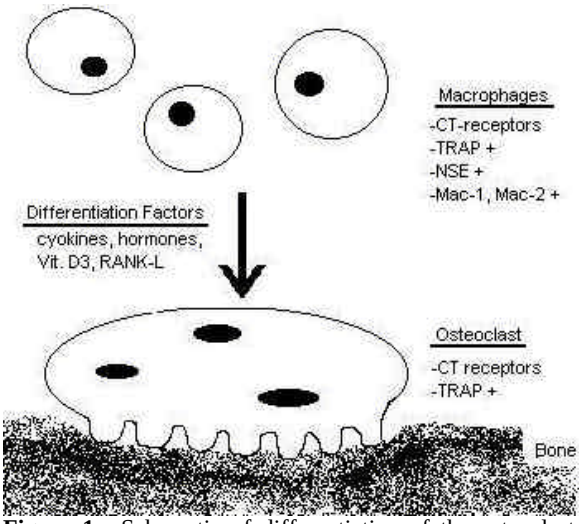

Figure 1. Schematic of differentiation of the osteoclast. Macrophages (osteoclast precursors) differentiate into osteoclasts due to a variety of stimuli.

differentiates into a mature osteoclast it down regulates expression of the monocyte-macrophage markers (4).

2.3. Soluble osteoclast differentiation factors

2.3.1. M-CSF

The differentiation of osteoclasts takes place solely in the close vicinity of mineralized bone, therefore mature osteoclasts do not appear in the circulation (4). Several signals are required for activation of osteoclast differentiation. These osteoclastogenic stimuli are soluble signals, such as cytokines and hormones, and cell-cell mediated interactions, such as RANK/RANK-L. One of the critical soluble factors involved in the development of mature osteoclasts is macrophage-colony stimulating factor (M-CSF). Outside of being a member of a family of growth factors for cells of the mononuclear-phagocyte system, M-CSF can also be considered a pro-inflammatory cytokine (5-8). Evidence suggesting the importance of M-CSF in controlling osteoclastogenesis comes from both *in vitro* and *in vivo* studies. M-CSF addition to cultures containing dentine slices, human PBMC's, and osteoblasts stimulates development of lacunar resorption pits over time. The increase in the number of resorption sites is not observed in the absence of M-CSF, suggesting that M-CSF is necessary for osteoclast differentiation and activation (9). *In vivo* evidence suggesting the importance of MCS-F is demonstrated by the use of MCS-F deficient *op/op* mice in that these mice develop severe osteopetrosis due to a complete lack of osteoclasts (10).

2.3.2. IL-1, -6, -17, and TNF

Although M-CSF is a necessity for osteoclastogenesis, pro-inflammatory cytokines such as interleukin-1, -6, -17, and tumor necrosis factor (TNF) are also involved in regulating this process. IL-1 is a monocyte-macrophage produced pleiotropic cytokine capable of mediating inflammatory, metabolic, physiologic, hematopoietic, and immunological processes (11-14). IL-1 addition to long-term human marrow culture systems increases numbers of osteoclast-like cells (15). When IL-1 is added to osteoclasts that are disaggregated from the long bones of rats, slices of human femur cortical bone, and calvarial cells or osteoblasts, bone erosion and actin ring formation is observed (16, 17). These studies suggest that IL-1 stimulates both the formation of mature osteoclasts, as well as activation of osteoclastic bone resorptive activities. TNF is another monocyte-macrophage produced multifunctional cytokine capable of regulating various cellular reactions (18, 19). As with IL-1, TNF-alpha and TNF-beta also stimulate the formation of osteoclast-like multinucleated cells *in vitro* and co-culturing these cells with osteoblastic cells stimulates bone resorption (15, 17, 20, 21). One mechanism that might explain how IL-1 and TNF regulate osteoclastogenesis is the induction of M-CSF. Several *in vitro* studies have shown that IL-1, TNF-alpha, and TNF-beta are capable of inducing M-CSF and GM-CSF production (22-25). Furthermore, addition of IL-1, TNF-alpha, and TNF-beta to human, non-rheumatoid, synovial cell explant cultures or human articular cartilage chondrocyte monolayers in organ and cell cultures increases M-CSF production (22, 23). Together, these studies suggest that IL-1, TNF-alpha, and TNF-beta stimulate osteoclastogensis in an indirect manner, by first upregulating M-CSF production.

Unlike IL-1 and TNF, IL-17 is a product of T lymphocytes, specifically activated memory $CD4^+$ T cells (26). Interestingly, IL-17 is capable of inducing IL-1beta, -6, -8, and TNF-alpha expression, thus IL-17 may play a role in T cell triggered inflammation by stimulating stromal cells to secrete cytokines and growth factors (27-29). IL-17 stimulation of cultures containing nucleated mouse bone marrow cells and primary osteoblasts isolated from mouse calvaria stimulates osteoclast maturation and activation (30), thereby suggesting IL-17 also functions as an osteoclastogenic stimulus. The effect of yet another macrophage product, IL-6, on osteoclastogensis is not as well defined. This may be due to the fact that this pleiotropic cytokine exhibits both pro- and anti-inflammatory properties *in vitro* and *in vivo* (31-33). Although IL-6 stimulates the formation of osteoclast-like cells from osteoclast precursors *in vitro* (34, 35), other studies show that IL-6 addition to cultures containing monocytes, an osteoblast-like cell line, and $1,25\text{-}(OH)_2D_3$ has no effect on osteoclast formation or lacunar resorption of dentine slices (9). Taken together, this suggests that further work is necessary to determine the exact role of IL-6 in osteoclastogensis.

2.3.3. Hormones

Hormones, such as parathyroid hormone (PTH) and $1,25\text{-}(OH)_2D_3$ are also involved in regulating osteoclastogenesis. PTH is a vital mediator in the maintenance of normal calcium homeostasis due to its stimulatory effects on renal absorption of calcium and osteoclastic bone resorptive activity (21, 36). When Chinese hamster ovarian cells expressing PTH peptides are injected into nude mice, the mice experience hypercalcemia and an increase in osteoclast differentiation. Both increased numbers of committed osteoclast progenitor cells and mature osteoclasts are observed in the calvaria of the nude mice as well (37). $1,25\text{-}(OH)_2D_3$, an active metabolite

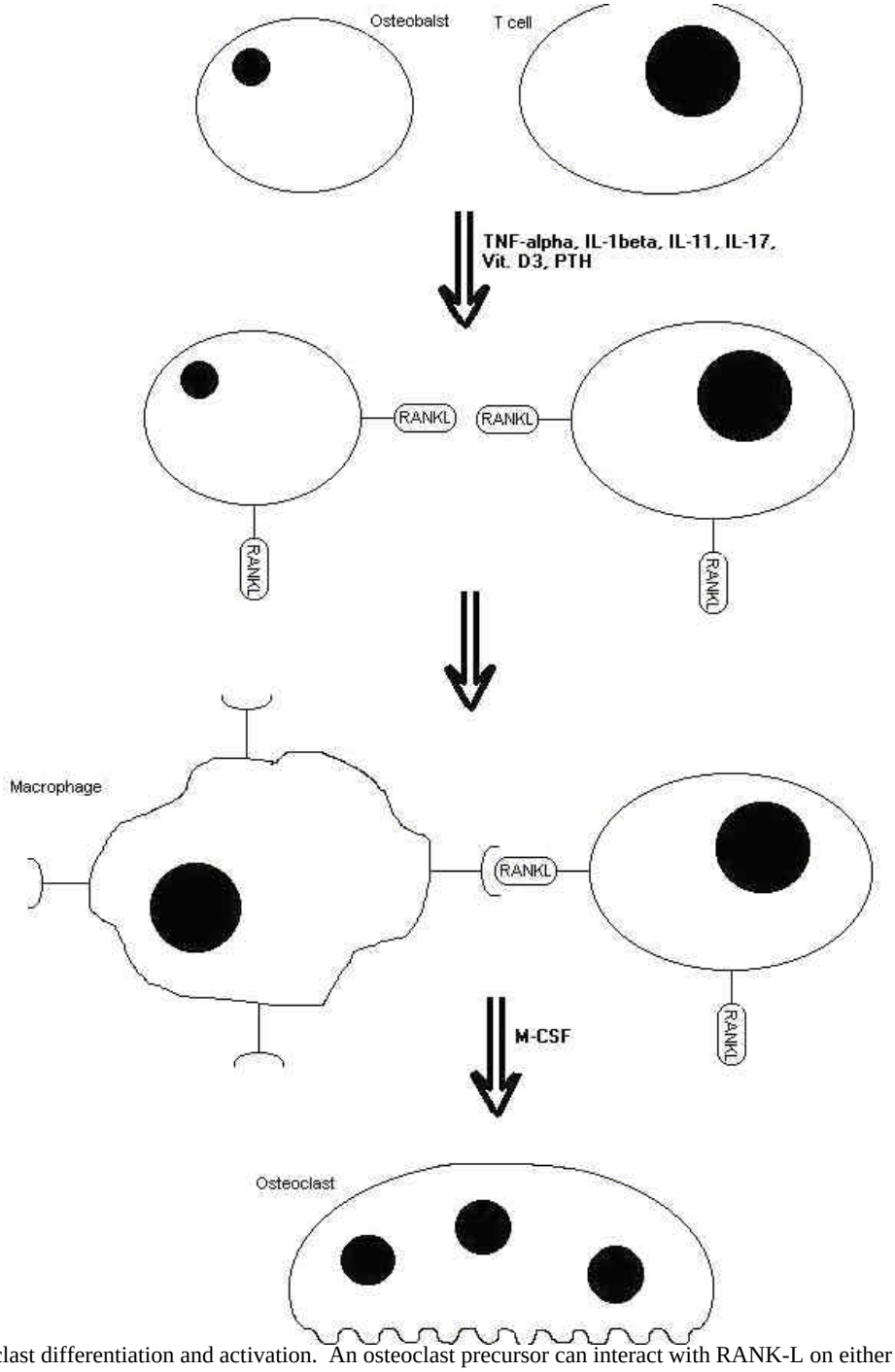

Figure 2. Osteoclast differentiation and activation. An osteoclast precursor can interact with RANK-L on either osteoblasts or T cells and mature into an osteoclast.

of vitamin D_3, is another hormone that acts as a potent stimulator of osteoclast maturation and osteoclastic bone resorption. Addition of 1,25-$(OH)_2D_3$ to cultures containing recombinant human GM-CSF and non-adherent marrow mononuclear cells, enriched for hematopoietic progenitor cells, stimulates osteoclast formation. These studies also demonstrate that 1,25-$(OH)_2D_3$ activates osteoclasts as well, as seen by formation of resorption lacunae on calcified matrices (38).

2.4. Cell-cell contact

2.4.1. Osteoblasts

Although soluble factors are critical for osteoclastogensis, they are insufficient to stimulate osteoclast maturation without additional factors, in the form of direct cell-cell contacts (39-41). One such interaction occurs between osteoclasts and osteoblasts. Osteoblasts, like osteoclasts, play a central role in bone remodeling by controlling bone formation. They are very similar to

fibroblasts, in fact, in culture osteoblasts and fibroblasts are nearly indistinguishable. All genes that are expressed in fibroblasts are also expressed in osteoblasts, however osteoblasts differ in their morphology in that they are surrounded by a mineralized extracellular matrix (42). Although the osteoblast controls bone formation, a separate but equally important function is its involvement in the control of osteoclastogensis. Interestingly, most of the hormones and cytokines that stimulate osteoclast maturation act indirectly through osteoblasts (43). IL-6 receptors are present on the osteoclast, however it is the IL-6 receptor expressing osteoblast that is required for IL-6 induced stimulation of osteoclast activity (44, 45). Similarly, receptors for both PTH and 1,25-$(OH)_2D_3$ are also present on osteoblasts, but absent on osteoclasts (4). Coculture of osteoblasts, mouse splenocytes, and 1,25-$(OH)_2D_3$ stimulates the differentiation of a splenocyte population, possibly splenic macrophages, into osteoclasts (39). Therefore, PTH, 1,25-$(OH)_2D_3$, IL-1, TNF, and TGF-beta may initiate osteoclastogensis by first activating osteoblasts (17, 20, 46, 47).

2.4.2. RANK-L

Osteoblasts are capable of regulating osteoclast activation by secreting the necessary soluble mediators and through surface expression of regulatory molecules, thereby initiating differentiation and activation through direct cell-cell interactions (3, 43, 48). The surface molecule involved in cell-cell contact-induced osteoclastogensis was originally known as the osteoclast differentiation factor (ODF) (Figure 2). In recent years, ODF has been known by several other names: Osteoprotegerin ligand (OPGL), tumor necrosis factor-related activation-induced cytokine (TRANCE), and receptor activation of nuclear factor-kappaB Ligand (RANKL). RANK and RANK-L belong to the tumor necrosis factor receptor (TNFR) and tumor necrosis factor (TNF) superfamily respectively, and TNFR and TNF superfamily members are involved in cellular responses such as proliferation, differentiation, inflammation, and cell survival and death (49, 50). RANK-L was first discovered on activated T cells and RANK on dendritic cells, and therefore first described to play a role in T cell and dendritic cell interactions. The RANK/RANK-L interaction inhibits apoptosis of mouse bone marrow derived and human monocyte derived dendritic cells through upregulation of anti-apoptotic genes such as BcL-x_L. RANK-L also augments the ability of dendritic cells to stimulate naïve T cell proliferation, probably due to the increased number of dendritic cells available for T cell stimulation (51, 52). It was not until later that RANK-L was determined to be the ODF, and play a vital role in osteoclastogensis (53). RANK-L is expressed by osteoblasts and RANK expressed by osteoclasts. Intriguingly, several of the cytokines and hormones that are involved in osteoclast development also regulate RANK-L expression. In bone resorption cultures stimulated with 1,25-$(OH)_2D_3$, the addition of an antibody against ODF (RANK-L) suppresses bone resorption, suggesting the mechanism by which 1,25-$(OH)_2D_3$ stimulates osteoclast function is through a RANK-L dependant manner (54). Furthermore, calvarial osteoblasts cultured with 1,25-$(OH)_2D_3$ exhibit an elevation in RANK-L mRNA expression (55). PTH, IL-1, IL-11, IL-17, and TNF-α are capable of stimulating RANK-L expression as well when cultured with human osteoblasts (30, 55, 56).

The idea that the RANK/RANK-L interaction is the critical cell-contact mediated event for induction of osteoclast development is supported by development of osteopetrosis in RANK-L deficient mice. RANK-L deficient mice lack osteoclasts, which was determined to be due to an inability of osteoblasts to support osteoclastogensis (57). OPG, a soluble RANK-L decoy receptor, addition to ostoclastogenic cultures containing osteoblasts inhibits bone resorption as well (58). Furthermore, soluble RANK-L stimulates osteoclast activation in the presence of other soluble stimuli such as PTH. Intriguingly, the level of bone resorption is equivalent to levels obtained if osteoblasts are used to activate the osteoclasts instead of soluble RANK-L, suggesting that RANK-L expressed by osteoblasts initiates osteoclastic bone resorptive activities (59). Therefore, osteoclast differentiation seems to be dependant on both secreted factors and cell-cell contact. It is also possible that soluble ostoclastogenic factors control cell-cell interactions by regulating the expression of RANK-L.

3. OSTEOCLASTS: MIGRATION, ATTACHEMENT, AND EFFECTOR FUNCTION

3.1. Migration

As noted earlier, circulating monocytes do not differentiate into mature osteoclasts until after they leave the circulation (4). Osteoclast precursors must be found in close proximity to the bone for osteoclast development to occur, therefore the osteoclast precursor must first migrate to the bone. There are a variety of factors that may play a role in this process. When an artificial gradient is created *in vitro*, osteoclasts migrate towards higher concentrations of both M-CSF and MIP-1alpha (60, 61). Interestingly, M-CSF and MIP-1alpha also inhibit the bone resorptive activity of osteoclasts, as seen by a decrease in the number of excavations of bone slices (60, 61). This suggests that M-CSF and MIP-1alpha signals osteoclast precursor migration, but at the same time may also prevent any activation of effector functions that might be deleterious as osteoclast precursors travel through circulation on their way to sites of bone resorption. One receptor that may contribute to osteoclast precursor migration is the adhesion receptor alpha$_v$beta$_3$ (vitronectin receptor). Alpha$_v$beta$_3$ is an integrin that recognizes a variety of extracellular matrix components including vitronectin, denatured type I collagen (> 90% of bone composition), and laminins. The osteoclast precursors must undergo transendothelial migration on their way to the bone (62, 63), and since alpha$_v$beta$_3$ recognizes laminins present in the vascular endothelial basement membrane, it is therefore possible that this receptor is involved in the homing of osteoclast precursors to the bone. Further evidence in support of this hypothesis is the observation that blocking the alpha$_v$beta$_3$ receptor inhibits M-CSF induced migration. In these studies, echistatin, a alpha$_v$beta$_3$ ligand, suppresses

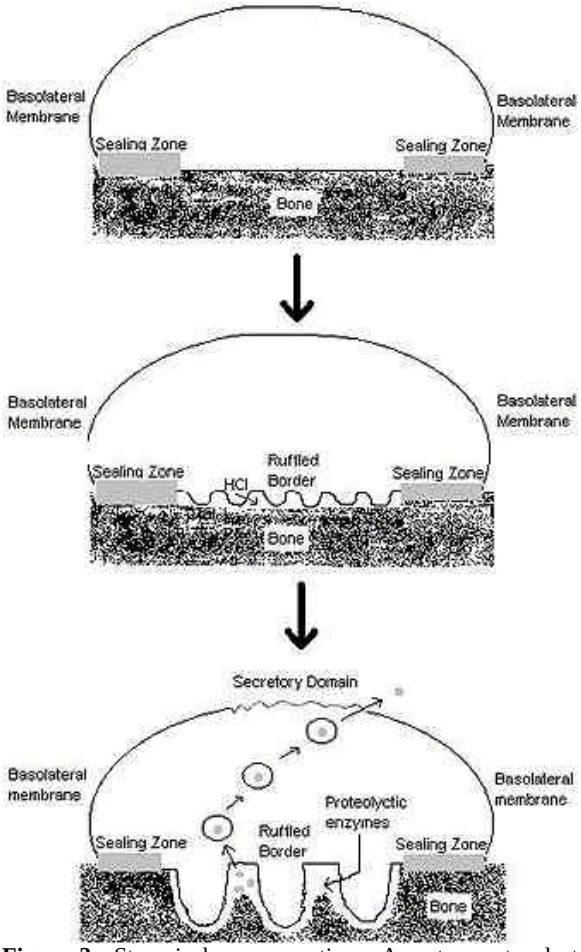

Figure 3. Steps in bone resorption. A mature osteoclast binds to the bone matrix, forming a sealing zone and basolateral membrane. The ruffled border is formed and bone degradation products are exocytosed through the osteoclast, through the secretory domain.

migration of osteoclast like cells in the presence of a M-CSF gradient (64, 65).

3.2. Attachment

Bone resorbing osteoclasts are highly polarized cells that contain a number of specialized membrane domains: a sealing zone, a ruffled border, a secretory domain, and a basolateral membrane (66-68) (Figure 3). For the osteoclast to perform its bone resorptive function, it needs to attach to the bone matrix. Therefore, the first cytoskeletal alteration that osteoclast must undergo is sealing zone formation. The sealing zone allows for the osteoclast to remain in tight association with the bone matrix during bone resorption. The sealing zone also encompasses the resorption lacuna, which is where the osteoclast degradetive functions take place. The resorption lacuna is organized as a secretory ruffled border, which is one of the most essential cytoskeletal changes that occurs within the osteoclast. It is formed by fusion of the intracellular acidic vesicles with the part of the plasma membrane facing the bone (67-69) and the finger-like projections created during ruffled border formation share may features common to the late endosome membranes (70). $Alpha_v beta_3$ is expressed at the ruffled border of the mature osteoclast and may come in contact with type I collagen expressed at the site of bone resorption. Therefore $alpha_v beta_3$ may be involved in attachment to bone matrices as well as migration. Blocking $alpha_v beta_3$ with RGD containing peptides such as echistatin and kirstrin further supports this idea in that bone resorption is inhibited both *in vitro* and *in vivo* (68, 71-73). Since $alpha_v beta_3$ is possibly involved in migration as well as attachment *in vivo*, it remains to be determined whether blocking $alpha_v beta_3$ inhibits osteoclast activity by blocking osteoclast precursor migration, or osteoclast attachment, or both. One critical intracellular signaling component normally present within the ruffled border is $p60^{c\text{-}src}$ kinase. Evidence for this comes from the observation that Src-deficient mice develop osteopetrosis due to a lack of functional osteoclasts, and not to a reduction in osteoclast numbers. In the absence of $p60^{c\text{-}src}$, the ruffled border does not form and osteoclast resorption activity is lost (4, 74-76).

3.3. Effector function

Once the ruffled border is formed, the osteoclast dissolves the crystalline hydroxapatite that covers the organic bone matrix by secreting HCl into the resorption lacunae (67-69). A vacuolar type proton pump ATPase present within the ruffled borders along with chloride channels creates the low pH atmosphere within the resorption lacuna. This pump is also present within the lysosomes and endosomes of several mammalian cells (77-79). As the osteoclast solubilizes the mineral layer, the osteoclast releases several proteolytic enzymes to degrade the organic bone matrix. Two classes of proteolytic enzymes important for this process are lyzozomal cysteine proteinases and matrix metalloproteinases (MMP's). Cathespin K and MMP-9 are two of the proteinases that are released into the resorption lacuna (68, 80-82). The enzymes attack the organic bone matrix and digested products are endocytosed by the osteoclast and transported through the cell via the transcytolytic vesicular pathway. Transport of organic material present within the transcytotic vesicles is further degraded by tartrate-specific acid phosphatase (TRAP) before it is released through the secretory domain (83, 84).

4. OSTEOCLASTS AND RA

4.1 Presence of osteoclasts and RA

In any inflammatory, destructive joint disease, osteoclasts are likely to play a significant role in destruction of the joints. In rheumatoid arthritis (RA), focal bone loss in subchondral bone and joint margins indicates an imbalance in the process of bone remodeling. If osteoclasts are the cell type responsible for joint and bone matrix destruction, then osteoclasts should be present within the diseased tissue. In an animal model of arthritis, collagen type II arthritis, development of inflammation and joint destruction directly correlate with increased numbers of osteoclasts (85). Within the areas of focal bone erosions in RA joints, histologic studies identify cells that represent the osteoclast phenotype. These cells are multinucleated

and express TRAP, cathepsin K, and calcitonin receptor mRNA. Although cathepsin K expression is restricted to the mineralized tissue surfaces, TRAP expression is diffuse within the pannus, remote from bone surfaces (86-90). This suggests that not only are the bone resorbing osteoclasts present on the bone matrix, but osteoclast precursors are also present within RA synovium. Since osteoclasts are derived from monocyte-macrophage precursors, it is possible that the macrophages and macrophage-like synovial cells present within the inflamed joint are TRAP positive osteoclast precursors. In support of this idea, when macrophages isolated from the synovium of RA patients are cultured under the appropriate ostoclastogenic conditions, namely osteoblasts, 1,25-$(OH)_2D_3$, and M-CSF, macrophages differentiate into osteoclasts.

4.2. Soluble osteoclastogenic factor expression and RA

What may be required for osteoclast precursors within the inflamed synovium to develop into mature osteoclasts are the right conditions. As with the presence of osteoclast precursors and osteoclasts, secreted cytokines necessary for maturation and activation of osteoclasts are present in the RA synovium as well. Elevated levels of IL-1alpah and beta, IL-6, TNF-alpha, and M-CSF are all expressed in RA patient synovium (91-100). In addition to the role these cytokines play in inflammation, an additional role may be to stimulate the differentiation and activation of osteoclasts within the inflamed joint (101-107). Evidence for this is supported by the ability to block arthritis development in animal models, or diminish the symptoms of disease in RA patients by blocking TNF-alpha and IL-1beta (105-107). Although blocking IL-beta minimally suppresses inflammation, treatment significantly suppresses joint and cartilage destruction (108). Joosten and colleagues also demonstrate that administration of antibodies to IL-1alpha or IL-1beta prevents bone and cartilage destruction while antibodies to TNF-alpha reduce joint inflammation and edema, but have little effect on prevention of joint destruction (107). If osteoclasts are involved in RA, then blocking M-CSF should be a potent suppressor of arthritis development as well. Unlike IL-1 and TNF, the role of M-CSF in RA is not as clear-cut. Blocking endogenous M-CSF by administering specific antibodies is effective in collagen-induced arthritis but not methylated BSA induced arthritis. Nevertheless, M-CSF deficient *op/op* mice, which lack osteoclasts, are resistant to developing either form of arthritis. Thus, these studies suggest the inability to effectively differentiate osteoclasts may play a critical role in the development of arthritis. However, since inflammation is also effectively blocked, M-CSF may play a dual role in arthritis both contributing to the inflammatory process and induction of osteoclast differentiation.

4..3. Osteoclastic effector function and RA

As with increases in osteoclast precursors and osteoclast numbers, and osteoclastogenic factor expression, osteoclastic bone resorptive activity directly correlates with RA development as well (Table 1). As noted earlier, *op/op* mice lack not only M-CSF but osteoclasts as well. When *op/op* mice are immunized according to the methylated-BSA induced arthritis model, disease is suppressed (109) suggesting not only a role of M-CSF in the induction of arthritis, but also the involvement of osteoclasts and osteoclast function in the development of RA. Further supporting the involvement of osteoclasts, in collagen type II-induced arthritis, development of arthritis correlates with temporal decreases in bone formation and increases in bone loss (85). As mentioned earlier, Src activity is a necessity for osteoclast function in terms of ruffled border formation. When human synoviocytes from RA patients are cultured along with Csk, a Src-kinase inhibitor, the bone resorbing activity of the osteoclasts within the RA synovium is significantly reduced (110). This result can also obtained *in vivo* by injection of an adenovirus containing the Csk gene into rats with adjuvant-induced arthritis. Injection of the Csk gene not only decreases the inflammatory reaction, but also inhibits bone erosion (110). Given that Src is a necessary component of the ruffled border formation, ruffled border and osteoclast function might be compromised in Csk treated cells and Csk treated mice, further suggesting the importance of osteoclast function in the development of RA.

4.4. RANK-L expression and RA

The RANK/RANK-L interaction is vital for the activation of osteoclastic bone resorptive function, therefore a correlation or dependence on RANK/RANK-L expression should be observed in the development of arthritis (Table I). In animal models of arthritis, such as the adjuvant-induced arthritis model and the collagen-induced arthritis model, synovial fibroblasts and inflammatory cells express RANK-L mRNA. Interestingly, in the joints of arthritic mice, osteoclasts are detected at the sites of bone resorption adjacent to RANK-L expressing synovium infiltrating mononuclear cells and chondrocytes (111, 112). Moreover, RANK-L mRNA is highly expressed in synovial tissues of RA patients, but not in normal synovial tissues (113), and immunohistochemical analysis demonstrates the presence of osteoclasts in areas of pannus invasion into the bone, adjacent to sites where RANK-L expression is highest (111, 114). In a serum transfer model of arthritis, the level of inflammation, as measured by clinical signs of arthritis and histopathological scoring, is comparable between wild type and RANK-L deficient mice. However, the degree of bone erosion in the RANK-L deficient mice is significantly reduced compared to wild type mice (115), suggesting differentiation or activation of osteoclast function in arthritis is RANK-L dependant. Additional evidence supporting this concept shows macrophages isolated from the synovium of RA patients have the capacity to differentiate into osteoclasts in the presence of RANK-L and M-CSF (116), suggesting since osteoclast precursors, synovial macrophages, and differentiating factors, cytokines and RANK-L, are present in the RA synovium, osteoclasts will be formed and their bone resorping activities activated.

RANK-L is expressed by both osteoblasts and T cells (51, 52, 57), therefore each of these cell populations may contribute to osteoclast differentiation. As with osteoblasts, RANK-L expressing T cells can support osteoclastogensis *in vitro* (117). Interestingly, in instances of hyperactive T cells, such as is the case in CTLA-4

Table 1. Correlation between expression and disease onset

	Normal synovium	RA Synovium
Osteoclast precursors	present	increased presence
Mature osteoclasts	present	increased presence
Enhanced osteoclastogenic stimuli expression		
soluble (IL-1, -6, -17, TNF, M-CSF)	No	Yes
membrane bound (RANK-L)	No	Yes
Enhanced osteoclast bone resorbing activity	No	Yes

deficient mice, bone loss supported by severe osteoporosis is observed (117). Furthermore, increases in osteoclasts followed by osteoporosis is observed if bone marrow from CTLA-4 deficient mice is transferred into RANK-L deficient mice (117), suggesting that the RANK-L expressing T cell supports osteoclast activation *in vivo.* In antigen-induced arthritis, the osteolysis that develops is T cell dependent. Furthermore, ostelolysis is suppressed by treatment with OPG, suggesting that T cells and RANK-L are playing a critical role in the development of arthritis. Further supporting this idea, in disease states such as RA, T cells not only gain access to the joint, but these $CD4^+$ T cells also dominate the cellular infiltrate within the synovium, providing a source of RANK-L expression that can interact with osteoclast precursors (118). In fact, immunohistochemmical analysis and in-situ hybridization studies of synovial tissues from RA patients demonstrate localized RANK-L expression to T cells within lymphoid aggregates, suggesting not only a role of T cells in initiating inflammation in arthritis, but also control of focal bone erosion.

5. PERSPECTIVE

Enhanced osteoclastogenesis is associated with a variety of bone eroding diseases. In this review, we have discussed the involvement of both osteoclastogenic factors and osteoclasts in the development of rheumatoid arthritis. Based on extensive studies, it has become apparent that both soluble and membrane bound factors are critical for the development and activation of osteoclasts and osteoclast bone resorptive activity. Many of the cytokines involved in the inflammatory events underlying the pathogenesis of arthritis are also involved in osteoclastogenesis. IL-1, -6, -17, TNF, and especially M-CSF all play significant roles in both the development of osteoclast and osteoclast function as well as development of arthritis, suggesting that these events are not mutually exclusive. Based on this evidence, and on the recent findings that RANK-L is not only associated with the development of osteoclasts, but is also linked with the development of arthritis, it is suggestive that understanding the role of the osteoclast in arthritis has clinical implications. For example, cytokine and RANK-L expression may serve as useful makers of osteoclast function. Furthermore, cytokine and RANK-L detected in RA patients may be therapeutically targeted with anti-inflammatory agents, or specific inhibitors. Anti-cytokine therapies have already been initiated with some success, and it is possible that anti-RANK-L or soluble OPG therapies might not be far behind. Hopefully, the study of osteoclastogenic factors and osteoclasts in terms of their relationship to the development of RA will lead to the development of specific immunomodulatory therapies that will suppress joint and cartilage erosion, and therefore, benefit RA patients.

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