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The role of cell–substrate interaction in regulating osteoclast activation: potential implications in targeting bone loss in rheumatoid arthritis

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ABSTRACT

Analysis of tissues retrieved from the bone–pannus interface from patients with rheumatoid arthritis (RA) and studies in animal models of inflammatory arthritis provide strong evidence that osteoclasts, the cells that are essential for physiological bone resorption, are responsible for articular bone destruction in RA. However, current treatments that specifically target osteoclast-mediated bone resorption in RA have not been successful in preventing bone erosions, and new therapeutic strategies are needed. It has been noted that, although osteoclast precursors are present within the bone microenvironment at sites of pathological bone resorption, cells expressing the full morphological and functional properties of mature osteoclasts are restricted to the immediate bone surface and adjacent calcified cartilage. These findings provide evidence that, in addition to requirements for specific cytokines, interaction of osteoclast precursors with these mineralised matrices results in activation of specific signal pathways and the induction of unique gene products that are essential for terminal osteoclast differentiation and activation. These studies are designed to define the gene products and signalling pathways regulated by bone and calcified cartilage, to identify new molecular targets and novel therapeutic approaches for preventing osteoclast-mediated joint destruction in RA and related forms of pathological bone loss.

The development of in vitro cell models for studying osteoclast differentiation and the analysis of human and murine disorders associated with defective osteoclast differentiation and activation have established that osteoclasts are derived from a haematopoietic precursor of myeloid origin.[1 2] Both murine and human bone marrow culture systems have been particularly informative in dissecting the regulatory mechanisms and commitment points in the cytodifferentiation pathway of this bone-resorbing cell. The initial event associated with osteoclast commitment requires interaction with the haematopoietic growth factor, macrophage colony-stimulating factor-1 (M-CSF), which acts via its receptor CSF-1R. At this early stage, the myeloid lineage cell retains its pluri-potential ability to differentiate down the macrophage or osteoclast pathway. Thus, the tissue macrophage and osteoclast share a common haematopoietic origin with other colony-forming unit-macrophage lineage cells, including macrophages that populate the lung (alveolar macrophages), liver (Kupfer cells), synovium (synovial macrophages) and other organs. Osteoclasts also share a common lineage with dendritic cells and macrophage polykaryons (foreign body giant cells) that are involved in immunoregulation and a variety of granulomatous disorders.

After interaction with M-CSF, the further commitment, differentiation and activation of osteoclasts is mediated by a complex network of regulatory factors, including systemic hormones, locally produced cytokines and cell–cell and cell–matrix interactions that are required for transition of the osteoclast precursor into a multinucleated and fully activated cell capable of resorbing the mineral and organic matrix of bone.[1 3–5] A major breakthrough in the understanding of the regulation osteoclast of differentiation came with the identification of RANKL (receptor activator of NF-κB ligand).[6–8] This cytokine is a product of stromal cells that are of osteoblast lineage and it is both necessary and sufficient for osteoclast formation. RANKL is a member of the tumour necrosis factor α family and it mediates its effects via a specific receptor, RANK (receptor activator of NF-κB). Deletion of either RANKL or its receptor leads to a severe form of osteopetrosis,[9 10] providing definitive evidence for the essential role of this ligand-receptor system in controlling osteoclast differentiation and bone resorption.

Although the osteoclast precursor may be derived from both the bone marrow and cells present within the circulation, the assembly of the terminally differentiated osteoclast probably occurs on the bone surface. There is evidence that the initial event associated with osteoclast precursor attachment may involve interaction with cells that line the trabecular bone surface via adhesion molecules such as intercellular adhesion molecule-1 or vascular cell adhesion molecule-1.[11–13] Attachment of the osteoclast precursor then leads to retraction of the lining cells and exposure of the bone surface.[12] These events appear to involve matrix metalloproteinase (MMP) activity that cleaves type I collagen on the bone surface and reveals cryptic binding sites for integrins that are necessary for optimal preosteoclast attachment.[14–16] Evidence supporting a role for MMPs is provided by the studies of Holliday et who used an in vitro model of osteoclast differentiation to show that collagenase inhibition decreases preosteoclast attachment and inhibits osteoclast-mediated bone resorption.[17] Further support implicating proteinase activity in osteoclast-mediated bone resorption is provided by the studies of Zhao et al who showed that bone resorption induced

by parathyroid hormone was markedly impaired in MMP13-resistant mutant mice.[18]

The subsequent steps of osteoclast differentiation that follow attachment are associated with the temporal induction of a genetic programme that is associated with sequential acquisition of the functional machinery that is necessary for multinucleation of the precursor and reorganisation of the cytoskeletal that results in cell surface membrane polarisation and specialisation. The attachment of the precursor involves interaction with cell surface receptors, including the vitronectin receptor $\alpha_v\beta_3$, the collagen/laminin receptor $\alpha_2\beta_1$ and the vitronectin/fibronectin receptor $\alpha_v\beta_1$.[19] During osteoclast differentiation, the $\beta5$ and $\beta3$ integrins are reciprocally regulated, with downregulation of $\beta5$ and upregulation of the $\beta3$ integrin.[20] In our recent studies we have found that $\beta3$ integrin is expressed relatively late in the sequence of osteoclast differentiation, after the induction of osteoclast-associated genes such as tartrate-resistant acid phosphatase (TRAP) and cathepsin K. This would suggest that the initial attachement of the osteoclast precursor does not involve the $\beta3$ integrin, but is mediated via alternate attachment receptors. In support of this speculation, the $\beta3$ integrin is not essential for preosteoclast attachment, since, as we previously reported, the $\beta3$ knockout mouse expresses normal or even slightly increased numbers of osteoclasts on the bone surfaces.[21] The reduced ability of the $\beta3$ knockout mice to resorb bone implies, however, that signalling through this protein is essential for optimal functional osteoclast resorbing activity since the mice develop a mild form of osteopetrosis related to defective bone resorbing activity.

In preliminary studies, we have analysed the genetic profile of osteoclast precursors derived from the $\beta3$ knockout mouse after differentiation on bone surfaces and found that although the prototypical osteoclast-associated genes such as TRAP and cathepsin K are induced during osteoclast differentiation, the pattern of gene expression in the terminally differentiated osteoclast derived from the $\beta3$ knockout mouse differs markedly from wild-type osteoclast precursors. These observations provide evidence that interaction of osteoclast precursors with the bone surface can markedly affect the genetic programme and phenotype of osteoclasts and that the $\beta3$ integrin is among the cell surface recognition molecules that mediate a component of the bone-regulated genetic programme.

Further evidence supporting a role of the bone matrix in regulating osteoclast differentiation and activation is provided by analysis of the tissues from sites of bone resorption in patients with rheumatoid arthritis (RA) and juvenile inflammatory arthritis.[22] [23] We found that, although there were cells within the inflamed synovium that expressed TRAP and low levels of cathepsin K protein or mRNA, cells expressing the full repertoire of osteoclast-associated phenotypic markers were localised exclusively to the immediate bone surfaces. Further evidence supporting the importance of cell–substrate interactions in modulating cell phenotype is provided by comparison of the phenotype of mono- and multinucleated cells associated with wear particles and bone surfaces from patients undergoing revision surgery for peri-implant osteolysis related to prosthetic implant wear debris after total joint replacement.[4] Cells associated with wear particles were strongly CD68 positive, consistent with their classification as macrophages or macrophage polykaryons. We observed that although many of these cells expressed low levels of cathepsin K and TRAP, the levels of expression were markedly reduced compared with mono- and multinucleated cells in resorption lacunae on the bone surfaces. In contrast, we noted that cells expressing two

other osteoclast-associated proteins—namely, the calcitonin receptor and $\beta3$ integrin, were restricted to the bone surface. These findings provided further evidence implicating cell–substrate interactions in regulating terminal osteoclast differentiation and activation.

Based on the differential expression of the calcitonin receptor and $\beta3$ integrin in osteoclasts on or off the bone surface, we speculated that these these two proteins were representative of a family of genes that were components of a larger genetic programme that was dependent upon interaction of the osteoclast with the bone substrate. To further identify the potentially novel osteoclast genes and signal pathways regulated by bone–substrate interactions we developed a model for induction of osteoclast differentiation on discs of devitalised calvarial mouse bone. This model provides a unique experimental approach for analysis of the effects of mineralised bone surfaces on osteoclast phenotype and for identification of specific gene products and signal pathways involved in bone matrix-dependent terminal osteoclast differentiation and activation.

Discs of bone were prepared from mouse calvaria (4 mm) using a leather punch. The samples were devitalised by repeated freeze thawing followed by treatment with collagenase, which markedly enhanced the attachment of osteoclast precursors to the bone surface. Mouse bone marrow macrophages were then seeded onto the bone discs and cells cultured with M-CSF (20 ng/ml) and RANKL (20 ng/ml) for 5–7 days. The incubations were conducted on discs that had been placed in wells in 24 tissue culture trays. The discs occupied only a portion of the surface of the well, providing a system in which osteoclasts formed on either plastic or bone within the same well could be compared.

After osteoclast induction, cells adherent to the tissue culture plastic or on the bone were fixed with 4% paraformaldehyde for histological examination. RNA was isolated from either the plastic- or bone-adherent populations in replicate cultures to enable comparison of the transcriptional profiles in osteoclast precursors differentiated on bone or tissue culture plastic. RNA prepared from the cultured cells was reverse transcribed and cDNA analysed by oligonucleotide array expression profiling on Affymetrix Mouse Genome 430 2.0 GeneChips. After microarray normalisation and gene comparison, hierarchical clustering was performed on the subset of genes showing the greatest amount of variation using the dChip 1.3 program.

In our preliminary analyses we identified clusters of genes that were specifically up- or downregulated by adhesion to the bone or plastic. We chose to focus initial attention on characterisation of the expression pattern and functional role of genes with the largest fold induction and for which antibody and other reagents are available. This approach ensured that we could test bone matrix-dependent expression at the protein level by western blot and in vivo by immunohistochemistry. Importantly, the availability of suitable reagents also permitted validation of the genes as suitable targets for inhibiting osteoclast-mediated bone resorption in animal models. The authenticity of the gene profiles as markers of osteoclast differentiation and activation were provisionally validated by examination of tissues from patients with pathological osteoclast-mediated bone resorption associated with RA or peri-implant osteolysis.

We are in the process of more rigorously characterising the bone-regulated genes with the goal of identifying new molecular targets for inhibiting osteoclast-mediated bone loss in RA and related disorders of pathological bone loss.

CONCLUSION

Our findings provide evidence that, in addition to requirements for specific cytokines, interaction of osteoclast precursors with these mineralised matrices results in activation of specific signal pathways and the induction of unique gene products that are essential for terminal osteoclast differentiation and activation. Our preliminary studies have defined specific gene products and signalling pathways regulated by bone and calcified cartilage. These studies will permit the identification of new molecular targets and novel therapeutic approaches for preventing osteoclast-mediated joint destruction in RA and related forms of pathological bone loss.

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