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Cells in focus

Clastic cells: Mineralized tissue resorption in health and disease

Victor E. Arana-Chavez*, Vivian Bradaschia-Correa

Laboratory of Mineralized Tissue Biology, Department of Cell and Developmental Biology, Institute of Biomedical Sciences, University of São Paulo, 05508-900 São Paulo, SP, Brazil

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ABSTRACT

Clastic cells are responsible for mineralized tissue resorption. Bone resorbing cells are called osteoclasts; however, they are able to resorb mineralized dental tissues or calcified cartilage and then they are called odontoclasts and chondroclasts, respectively. They derive from mononuclear precursors of the monocyte–macrophage lineage from hemopoietic tissue, reach target mineralized tissues and degrade them under many different physiologic or pathologic stimuli. Clastic cells play a key role in calcium homeostasis, and participate in skeletal growth, tooth movement, and other physiological and pathological events. They interact tightly with forming cells in bone and dental hard tissues; their unbalance may result in disturbed resorptive activity thus, causing local or systemic diseases.

Cell facts

- Clastic cells may resorb all mineralized tissues in the body.
- Clastic cells derive from the monocyte–macrophage lineage.
- Clastic cells present different stages of activity depending on stimulus.

1. Introduction

Clastic cells are responsible for the resorption of mineralized matrix of hard tissues. Although firstly described as osteoclasts, they are able to resorb mineralized dental tissues and calcified cartilage, where they are called odontoclasts and chondroclasts, respectively. In that sense, although osteoclasts are the best studied mineralized tissue resorbing cells, odontoclasts and chondroclasts belong to the same cell type and therefore in this article they are called clastic cells.

Clastic cells form when mononuclear precursors derived from a monocyte–macrophage cell lineage are attracted to certain mineralized surfaces and subsequently fuse and adhere onto them for exerting their resorbing activity. These cells present different functional stages, remaining latent or active (Lerner, 2000). The activity of these cells can be observed in both physiological and pathological phenomena throughout life.

Functional clastic cells are responsible for degradation of calcified extracellular matrix composed of organic molecules and hydroxyapatite. This process is mainly required in bone turnover and growth, spontaneous and induced (orthodontic) tooth movement, tooth eruption, and bone fracture healing, as well as in pathological conditions such as osteoporosis, osteoarthritis, and bone metastasis. In addition, they are responsible for daily control of calcium homeostasis. Clastic cells also resorb the primary teeth for shedding before the permanent teeth erupt into the oral cavity.

2. Cell origin and activation

Pluripotent hemopoietic stem cells rise into myeloid stem cells, which are capable of proliferating and differentiating into the leukocyte family of blood cells, i.e., megakaryocytes, granulocytes, monocyte–macrophages and also into clastic cells (Lerner, 2000). The earliest precursor of clastic cells identifiable from hemopoietic tissue is the granulocyte–macrophage colony-forming cell (CFU-GM) (Roodman, 2006). The recruitment of clastic cell precursors involves complex interactions between osteoblasts, stromal bone marrow cells and hemopoietic cells. Although cellular and molecular phenomena that occur in clastic cell formation are currently well known, some gaps in the exact sequence of events involved in the whole process remain unsolved.

The precursors of clastic cells are responsive to growth factors secreted by several types of mesenchymal cells, depending on the biological process, condition and localization. For exam-

* Corresponding author. Tel.: +55 11 3091 7308; fax: +55 11 3091 7402.
E-mail address: vearana@usp.br (V.E. Arana-Chavez).

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ple, interleukin-3 (IL-3) and macrophage-colony stimulating factor (M-CSF) are secreted by stromal cells of bone marrow and bind to receptors in membrane of the postmitotic precursors cells, which proliferate and differentiate into committed pre-osteoclasts (Roodman, 2006). Clastic cells become multinucleated by fusion of numerous committed mononuclear precursors. There are evidences that some proteins and glycoconjugates of the plasma membrane are involved in this process. Although molecules such as e-cadherin, macrophage fusion receptor, ADAM9, CD98, P2X7 receptor, CD9 and alpha (2,6)-linked-sialic acid had been described as components of cell–cell fusion process in osteoclast differentiation, their exact functions in the process are not completely understood (Takahata et al., 2007).

The activation of fused clastic cells is regulated by activation of the receptor activator of NF-kB (RANK), expressed in plasma membrane of precursors and fused clastic cells; its ligand (RANKL), a soluble protein secreted by osteoblasts and their precursors in bone, binds to RANK and stimulates differentiation and signaling pathways in clastic cell precursors. RANKL expression is induced by $1\alpha,25(OH)_2D3$, parathyroid hormone, prostaglandins and M-CSF (Yasuda et al., 1998). The antagonist of RANKL is osteoprotegerin (OPG), a soluble protein that is also secreted by osteoblasts. Binding of OPG to RANKL inhibits the genesis of clastic cells, thus preventing the RANKL linkage to RANK (Tanaka et al., 2003). Fig. 1A illustrates the mechanisms of osteoclastogenesis.

After differentiation and activation, rearrangement of cytoskeleton of clastic cells and therefore their attachment to bone mineralized matrix is regulated by the intracellular c-Src protein; loss of c-Src results in latent clastic cells (Miyazaki-Sanjay et al., 2004). In addition, gap junctions established between clastic cell and osteoblasts/stromal cells appear to play an essential role in the genesis of clastic cells by the establishment of a pathway for cellular crosstalk and diffusion of messenger molecules that interact with signaling pathway downstream RANKL in clastic cell differentiation (Matemba et al., 2006). Peripheral inervation and intrinsic neurotransmitter-like signaling in the bone microenvironment are able to influence the differentiation and activity of osteoblasts and clastic cells and the regulation of the bone remodeling cycle. Neurotransmitters and neuromodulators have been also considered to regulate bone remodeling by mechanism attributed to the excitatory amino acid glutamate (Spencer et al., 2007).

The concept of osteoimmunology has recently emerged due to the intimate relationship between immune system and the skeleton. As RANKL is also expressed by activated T cells, it is likely that this molecule is important in both the skeletal and immune systems. The bone loss in autoimmune arthritis occurs due to a defective control of bone metabolism by the immune system, where T-cell mediated regulation of osteoclast formation takes place through a signaling crosstalk between RANKL and interferon γ (IFNγ). These findings contribute to understanding the pathogenesis and developing new therapeutic strategies for diseases affecting both skeletal and immune systems (Takayanagi, 2005).

Clastic cells also resorb dental hard tissues; in these situations they are named odontoclasts. Recruitment of precursors as well as the structure and function of clastic cells follow the characteristics discussed above. A particularity is that clastic cells resorbing dentine from roots of primary (deciduous) teeth before shedding are frequently mononuclear (Domon et al., 2001). In orthodontic movement, alveolar bone and dental root resorption are mediated by inflammatory reaction. Periodontal ligament is compressed by the orthodontic force applied that yields a region of hyalinization in which aseptic necrosis takes place. After macrophages remove the hyalinized area, the adjacent alveolar bone is resorbed by recruited and activated clastic cells. Although only alveolar bone should be resorbed, the resorption of root surface is an undesirable side effect that frequently occurs during orthodontic treatment depending on type and/or magnitude of applied force (Casa et al., 2006).

3. Cell structure and function

Once clastic cell precursors are fused and activated, polarization events take place by rearrangement of the cytoskeleton and formation of the sealing zone. The activated clastic cell present four different specialized membrane domains: the sealing zone and ruffled border that face the bone matrix, the basolateral domain and the functional secretory domain, which are not in contact with the bone matrix but with the extracellular fluid and other cells (Mulari et al., 2003). Fig. 1B illustrates the osteoclast structure and function.

As they differentiate into multinucleated cells, the sealing zone that encircles the plasma membrane develops unique form of matrix adhesions known as podosomes. Ultrastructuraly, this portion of the cell does not present organelles, and its clear aspect originated the denomination “clear zone” (Fig. 2A and B). These structures consist of an actin “ring” enriched in $\alpha v\beta 3$ integrin and plaque proteins, classically found in focal adhesions, including vinculin, paxillin, talin, tensin and actin-associated proteins such as α-actinin, fimbrin, gelsolin, cortactin and dynamin. The $\alpha v\beta 3$ integrin binds to the RGD sequence of noncollagenous bone matrix proteins such as vitronectin, osteopontin, and bone sialoprotein (Luxemburg et al., 2006).

The ruffled border formed by the fusion of intracellular acidic compartments to the bone-facing plasma membrane at the early phase of the resorption cycle is responsible for the resorptive activity of clastic cells. Endosomal vesicles fuse to the plasma membrane and then release substances that degrade inorganic and organic matrix components. The membrane fusion takes place at the peripheral ruffled border, while uptake of degraded matrix occurs at its central portion.

The dissolution of hydroxyapatite crystals occurs due to acidification in the resorption lacuna. The low pH is created by a vacuolar type proton pump ATPase (V-ATPase) and chloride channels in the ruffled border. The V-ATPase originates from cytosolic compartments to the ruffled plasma membrane of osteoclasts as they activate to resorb bone (Holliday et al., 2005). The protons released by the proton pump are generated by induction of carbonic anhydrase II. Regulation and balancing of intracellular pH during acid secretion is maintained by a HCO_3^-/Cl^- exchanger located at the basolateral membrane. This structure transports the excess of intracellular HCO_3^- ions outside the cells thus, preventing intracellular alkalinization, while importing chloride ions inside the cell for secretion to the resorption lacuna (Mulari et al., 2003).

Once the mineral is dissolved, the organic phase is exposed to proteolytic enzymes synthesized and secreted by the clastic cell into the resorbing lacuna. This process involves participation of different enzymes, e.g., cysteine proteinases, matrix metalloproteinases (MMPs), serine proteinases and tartrate-resistant acid phosphatase (TRAP) (Delaissé et al., 2003). The degradation products are uptaken by the clastic cell in the resorption site, and vesicles containing organic and inorganic material are transported to the functional secretory domain at the plasma membrane. This mechanism allows these cells to maintain resorptive activity throughout their active life-span without accumulation of degradation products in the Howship’s lacuna (Mulari et al., 2003).

4. Associated pathologies

After the completion of skeletal growth, bone health is maintained by the coupled processes of bone resorption and bone formation, together called bone remodeling. At least one million of

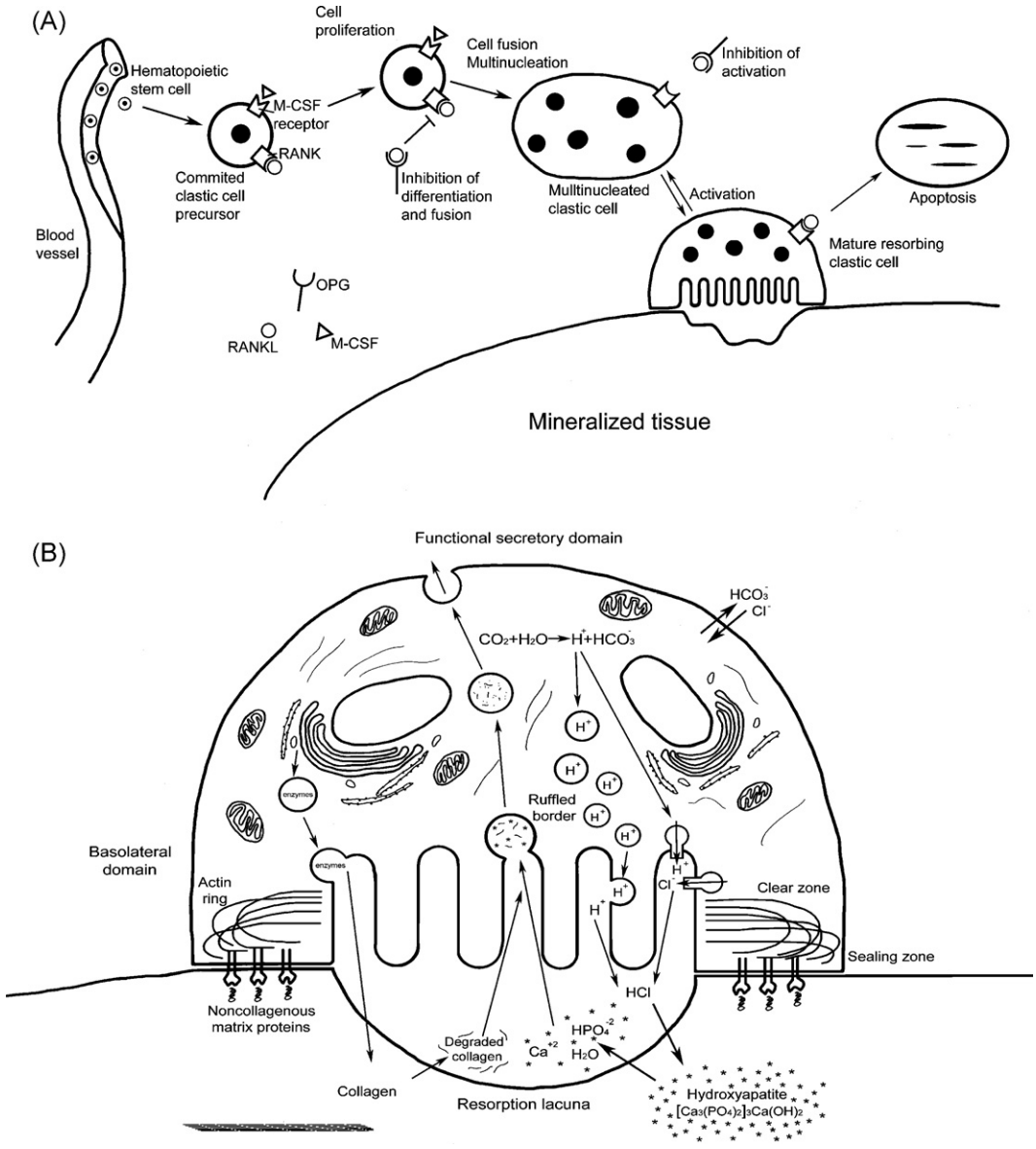

Fig. 1. (A) A schematic drawing showing the recruitment and activation mechanisms of clastic cells. Hematopoietic precursors are attracted from blood circulation into a resorbing area. RANKL and M-CSF bind to RANK and M-CSF receptor becoming committed clastic precursors, which proliferate and fuse into multinucleated cells. Activation of the multinucleated cell occurs in response to RANKL, reaching the state of a mature resorbing clastic cell. Differentiation, fusion and activation can be inhibited by OPG-RANKL binding. (B) A diagram of an active clastic cell apposed to a mineralized tissue. Protons are supplied by hydration of CO_2, which are pumped to the resorption lacuna. The HCO_3^- produced during CO_2 hydration is exchanged for chloride ions at the basolateral cell domain, which are transferred to the resorption lacuna through a chloride channel. HCl decreases the pH thus, dissolving the hydroxyapatite, while enzymes exocytosed degrade the organic matrix components. Degradation products are then endocytosed at the ruffled border membrane and transported to the functional secretory domain.

microscopic remodeling foci are present at any one time in the adult skeleton (Boyce and Xing, 2007). Old or damaged bone is removed and replaced by healthy bone. In young adults these processes are balanced, and skeletal renewal occurs without significant change in bone mass. However, various diseases, drugs, and metabolic abnormalities adversely affect bone health, culminating in skeletal disorders mainly due to defects in osteoclast function or formation. The mainly known defects are lack of acid secretion, proteinase deficiency and disturbs in OPG and RANK signaling (Helfrich, 2003).

Osteopetrosis is characterized by a general increase in bone mass, associated with the presence of normal or increased number of osteoclasts. Other clinical features are bone sclerosis (resulting in pathological fractures), renal tubular acidosis, cerebral calcifications and cranial nerve compressions. Histologically, osteoclasts remain as unpolarized cells, which therefore have no ruffled border and do not secrete acids. The disturbances of these cells can occur due to genetic defect in vacuolar ATPase, deficiency in carbonic anhydrase II, and deletion of signaling molecules such as M-CSF and components of NFkB signaling pathway. As a result, osteopetrotic bone exhibits an unremodeled appearance (Helfrich, 2003).

The middle-age Paget's disease of bone (PDB) occasion enlargement and softening of bone due to a mutation in RANK that is responsible for the osteoclast hyperactivity (Helfrich, 2003). Another type, the juvenile PDB, is associated to deletion or mutation of the gene encoding OPG, and results in severe systemic high-turnover osteoporosis. Some components of ubiquitylation during RANK-signaling pathway in osteoclasts are mutated, resulting in increased sensitivity of these cells to the cytokines above mentioned (Layfield and Shaw, 2007). Clinically, long bones and extremities, pelvis and skull are affected most, becoming curved;

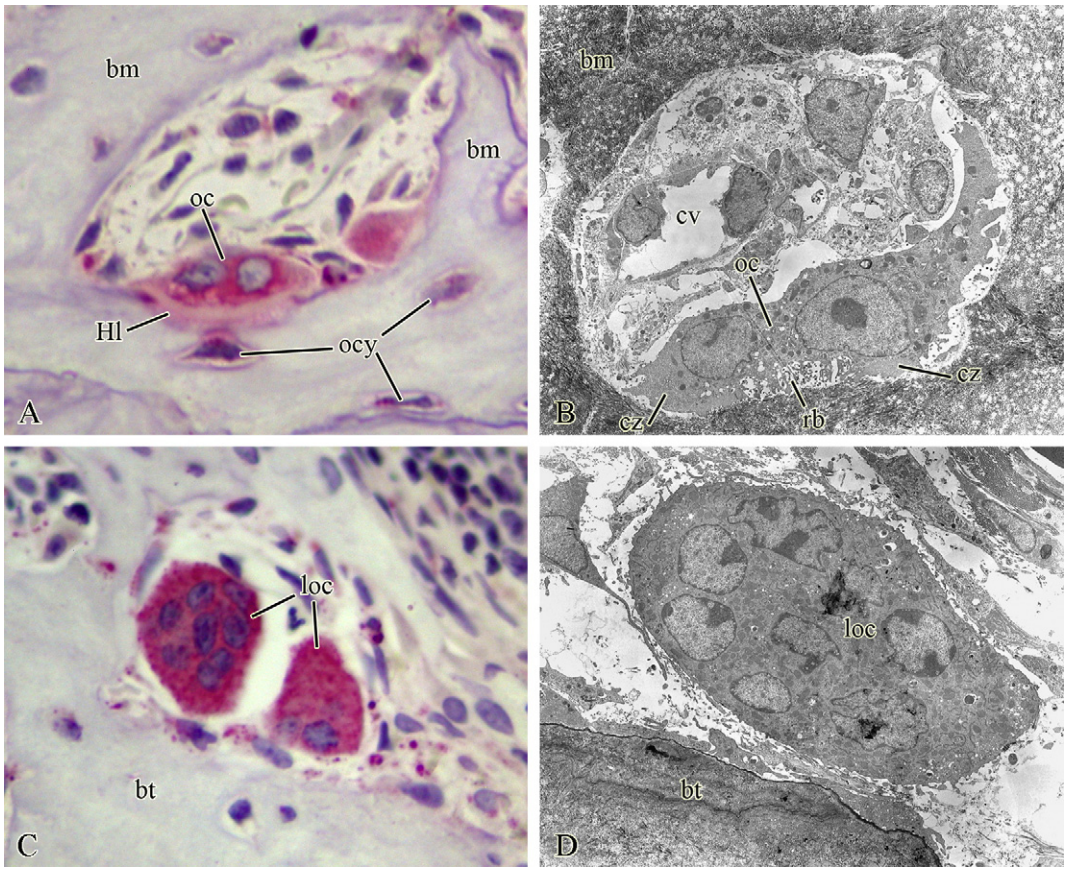

Fig. 2. A. Light micrograph showing an active TRAP-positive clastic cell apposed to the bone matrix (bm), thus being an osteoclast (oc). The resorbing bone surface that is called Howship's lacuna (Hl) is almost reaching an osteocyte (osteoblast buried in the bone mineralized matrix) (ocy). Hematoxylin counterstaining: 250×. B: Transmission electron micrograph of an active osteoclast (oc) inside a Haversian canal displaying typical clear zone (cz) and ruffled border (rb). cv, capillary vessel: 2100×. C. Light micrograph illustrating two round latent TRAP-positive osteoclasts (loc), next to a bone trabecula (bt). Hematoxylin counterstaining: 200×. D. Transmission electron micrograph of a latent osteoclast (loc) loose in the bone marrow space. Note several nuclei with condensed chromatin and the absence of clear zone and ruffled border: 2300×.

bone pain, deafness and pathological fractures are important symptoms.

In osteoporosis, the main bone disorder nowadays, bone resorption is higher than formation yielding skeletal fragility that increases fracture risk. Clinically, it is characterized by a decrease in bone mineral density. Antiresorptive drugs as bisphosphonates effectively prevent bone loss in both postmenopausal women without osteoporosis and preserve or improve bone mass and substantially reduce fracture risk in postmenopausal women and men with osteoporosis. In fact, they may interfere at fusion of clastic cell precursors or later on activation and function of formed clastic cells. Alendronate, for example, inactivate the fused clastic cells (Bradaschia-Correa et al., 2007), which are unable to bind to mineralized surfaces thus, remaining latent (Fig. 2C and D).

Besides the bone pathologies above, abnormal osteoclast activity also plays a role in bone metastasis of tumors. Indeed, oncogenic cells producing osteolytic or mixed osteolytic/osteoblastic lesions in metastic bone diseases have been shown to secrete soluble RANKL (Michigami et al., 2001).

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