

COMMENTARY

The osteoclast clear zone is a specialized cell-extracellular matrix adhesion structure

H. Kalervo Väänänen^{1,*} and Mike Horton²

¹Department of Anatomy and Biocenter, University of Oulu, 90220 Oulu, Finland

²Department of Medicine, University College London Medical School, Thorn Institute, The Middlesex Hospital, London W1N 8AA, UK

*Author for correspondence

INTRODUCTION

Bone turnover depends upon the balance of bone formation and bone resorption, which is carried out by two separate cell types, osteoblasts and osteoclasts (for review see e.g. Rifkin and Gay, 1992; Väänänen, 1993). Osteoblasts are specialized bone forming cells, which synthesize bone matrix, regulate its mineralization and finally differentiate to mature bone cells, osteocytes. The only cells which have been shown so far to be able to destroy mineralized bone under physiological conditions are osteoclasts. They are a unique cell type with several highly specialized features. These multinucleated cells form by fusion of mononuclear precursors of haemopoietic origin and show remarkable changes in their phenotype during different phases of the process of bone resorption.

THE RESORBING OSTEOCLAST IS A HIGHLY POLARIZED CELL

During activation towards bone resorption osteoclasts undergo rapid and dramatic changes in cellular polarization. Thus, a resting cell creates at least two new membrane domains: the sealing zone, which is the specialized cell-extracellular matrix adhesion structure and the ruffled border, the actual resorbing organ which faces the resorption lacuna (Fig. 1). Degradation of bone matrix in the resorption lacuna is facilitated by a low pH and secretion of a range of matrix degrading proteinases (Delaisse and Vaes, 1992; Hill et al., 1994). The low extracellular pH is generated via an active secretion of protons by V-type proton pumps in the ruffled border membrane and in intracellular vacuoles (Blair et al., 1989; Väänänen et al., 1990; Sundquist et al., 1990).

The formation of the 'proton-impermeable' resorption lacuna by tight sealing of the surrounding plasma membrane to the bone matrix is necessary to create conditions where bone resorption can take place. This tight sealing of plasma membrane to the bone surface is visualized at the ultrastructural level as the clear zone of osteoclasts. In the clear zone area the plasma membrane of the osteoclast follows the mineralized matrix surface very closely (the gap is <10 nm; see

Holtrop and King, 1977; Zhou et al., 1993; Pierce, 1989) suggesting that it forms the functional sealing zone and the diffusion barrier between the resorption lacuna and extracellular fluid. A question that has elicited considerable interest among bone biologists concerns the nature of the molecular interactions between the plasma membrane of the osteoclast and the bone surface during the different phases of resorption cycle. Further, it is not known if these molecular interactions are based on the same molecules throughout the whole resorption cycle whilst the morphological appearance of cell-matrix interaction seems to be different.

ORGANIZATION OF THE CYTOSKELETON VARIES DURING THE RESORPTION CYCLE

The change in the polarization of the osteoclast during activation is driven by profound re-organization of the actin cytoskeleton (Aubin, 1992; Kanehisa et al., 1990; Teti et al., 1991; Lakkakorpi et al., 1991; Turksen et al., 1988; Zamboni-Zallone, 1989; Marchisio et al., 1984). When the osteoclast is 'transformed' from the non-resorbing to the resorbing stage, three clearly distinct organizations of F-actin and five patterns of vinculin distributions are observed (Fig. 2). In the first stages of the resorption cycle actin and vinculin are distributed throughout the developing punctate structures ('podosomes') on the bone matrix interphase. Similar structures are seen when cells are plated on an artificial surface such as glass or plastic (Lakkakorpi et al., 1991). Towards the resorption stage these podosome-like structures coalesce to the specific area of the osteoclast, actin and vinculin staining dissociate from each other and actin forms a dense continuous band around the future resorptive area and the podosome type dot-like appearance is lost. This accumulation of actin filaments roughly corresponds to the morphological clear zone and is delineated by a double ring of vinculin and talin. These changes in the cytoskeletal organization presumably also reflect changes in the pattern of cell-extracellular matrix interactions.

Key words: osteoclast, clear zone, integrin, tight junction

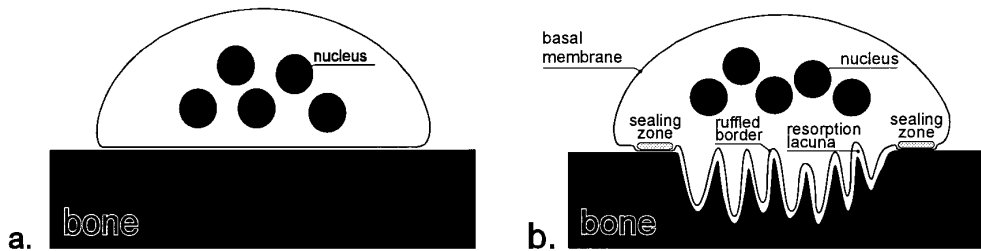


Fig. 1. Schematic drawing of an osteoclast. Osteoclasts undergo profound changes in polarization during activation from the non-resorbing (non-polarized) (a) to the resorbing (polarized) stage (b). In the resorbing osteoclast a specialized ruffled border membrane is separated from a basal membrane by a sealing zone, which mediates tight attachment of the osteoclast to the bone matrix.

CELL-MATRIX INTERACTIONS OF THE OSTEOCLAST

Osteoclasts express three integrin extracellular matrix receptors: $\alpha_v\beta_3$, the classical vitronectin receptor; $\alpha_2\beta_1$, a collagen receptor; and $\alpha_5\beta_1$ (Nesbitt et al., 1993; Helfrich et al., 1992; M. H. Helfrich et al., unpublished). Extensive analysis in a variety of species has clearly demonstrated a role for $\alpha_v\beta_3$ in adhesion to a variety of RGD-containing proteins, and involvement of β_1 integrins (most probably $\alpha_2\beta_1$) in the recognition of collagen (Horton and Rodan, 1995). Interruption of integrin-mediated adhesion results in inhibition of bone resorption, including induction of hypocalcemia in vivo (Fisher et al., 1993; Crippes et al., 1994; Yamamoto et al., 1994). From these data it is clear that some aspects of the interaction of osteoclasts with bone matrix are integrin-dependent, whether at the stage of initial cell attachment, migration on bone surfaces, or during the formation or dissolution of the tight seal prior to or succeeding resorption.

An analysis of the spatial requirements for integrin-mediated adhesion, when compared with the size of the gap between the osteoclast membrane and bone, suggests that this could not be mediated by integrins. Rotary EM shadowing of purified $\alpha_5\beta_1$ fibronectin receptor and the platelet fibrinogen receptor,

gpIIbIIIa (Nermut et al., 1988; Weisel et al., 1992) shows an extended structure (20–22 nm long) with a globular N-terminal head region (12–15 nm diameter) that interacts with the ligand. If one considers three well known integrin-mediated interactions - platelet aggregation, leucocyte interaction and fibroblast adhesion - then it is clear this structure can be accommodated. Platelets aggregate by the bridging of their integrin receptors (gpIIbIIIa), once activated, by fibrinogen. Electron microscopic data (Weisel et al., 1992) shows that isolated gpIIbIIIa molecules interact at right angles with both ends of extended, rod-like fibrinogen molecules; these measurements support a distance between adjacent platelet membranes of 43–47 nm. Leucocyte β_2 integrins bind to the first, N-terminal, Ig domain of ICAM-1 (Staunton et al., 1990) which is thought to lie at an angle from the 3 domains adjacent to the cell membrane. This implies an intercellular membrane distance in the range of 27 to 42 nm (a minimum distance for the smallest ICAM-2 variant with 2 Ig domains and greatest for VCAM-1 with 7 Ig domains) (see Barclay et al., 1993) that fits observations on cell-cell contacts in leucocytes (Dustin et al., 1992). Similarly, fibroblast adhesion to a fibronectin-coated surface results in the formation of integrin and F-actin enriched focal adhesions, the structures that mediate cell-matrix adhesion;

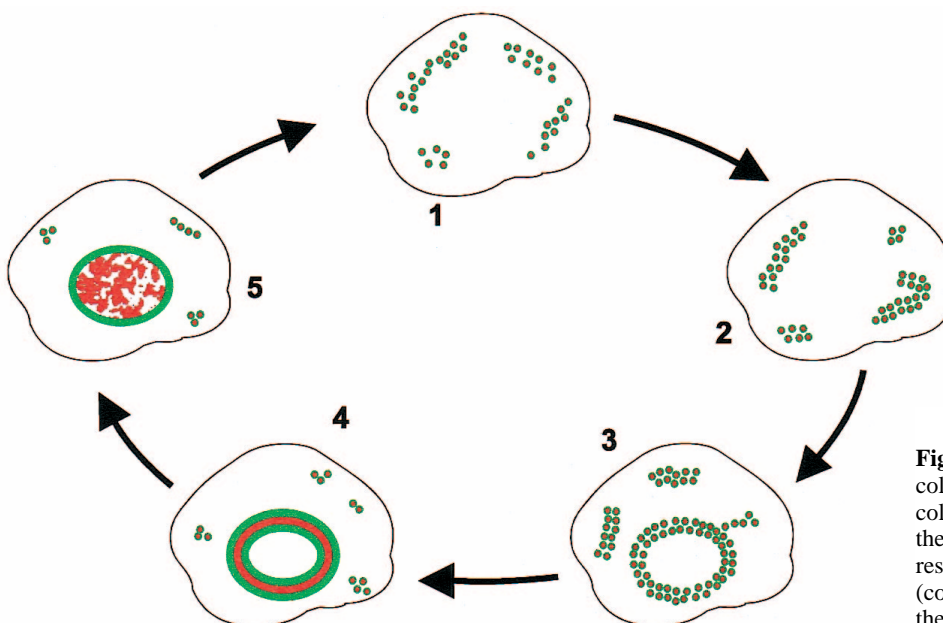


Fig. 2. Five different patterns of vinculin (green color) and three different patterns of actin (red color) staining can be seen in osteoclasts during the resorption cycle. Stages 1 to 3 represent pre-resorptive events, stage 4 is a resorbing cell (corresponds to Fig. 1b) and stage 5 represents the post-resorptive stage.

these are 'dark' when observed by interference reflection microscopy implying a membrane to surface distance of about 25 nm (Opas and Kalnins, 1984). These situations contrast with that of the resorbing osteoclast; here, a membrane to matrix distance of only a few nanometers is observed in the clear zone of osteoclasts in established resorption (Holtrop and King, 1977; Zhou et al., 1993; Pierce, 1989), clearly too small for an integrin-mediated event.

The foregoing argument when taken with the presence of dense F-actin bundles leading to the tight junction implies a receptor mediated mechanism and strongly suggests that some other molecular interactions are needed to create observed tight sealing between the osteoclast cell membrane and extracellular matrix of bone. This specialized functional prerequisite, taken with our own unsuccessful efforts over the years to visualize integrins in the sealing zone, lead us to hypothesize that a specific molecular form of cell membrane-extracellular matrix interaction is needed to create this ion impermeable cell-extracellular matrix structure. We briefly discuss the data which has led us to this hypothesis.

CLEAR ZONE IS A SPECIALIZED FORM OF CELL-MATRIX INTERACTION

During initial adhesion and migration on the bone surface there is a co-localization of vinculin and $\alpha_v\beta_3$ integrin suggesting that the attachment of osteoclasts to the bone surface at this stage of the resorption cycle is dependent on vitronectin receptor containing focal adhesions. The inhibition of bone resorption by RGD-peptides has been used as an argument that $\alpha_v\beta_3$ integrin also mediates the attachment of osteoclast to the bone surface in the clear zone area (Hultenby et al., 1993; Reinholt et al., 1990). This idea has also been supported by some immunoelectron microscopic data from the distribution of $\alpha_v\beta_3$ integrin and some of its potential extracellular ligands (Reinholt et al., 1990). However, our own data on the distribution of vitronectin and β_1 integrin receptors (Lakkakorpi et al., 1991, 1993) indicates that it is not polarized in resorbing osteoclasts but can be seen both in basal membrane as well as in ruffled border membrane. Moreover, it appears to be absent from the clear zone or actin ring facing membrane area (Lakkakorpi et al., 1991, 1993). This conclusion is based both on immunofluorescence staining from cultured osteoclasts as well as on the use of light and electron microscopic immunohistochemistry from bone tissue. These observations strongly suggest that the actin cytoskeleton is not connected to extracellular matrix through vinculin/talin and integrin complex in the area of the clear zone. Hence some other, so far uncharacterized, molecules must mediate the interaction of actin to the extracellular anchor in the sealing zone.

It is also worth noting that inhibition of bone resorption in vitro and in vivo by RGD-peptides can readily be explained by means other than via direct interaction of vitronectin receptor at the sealing zone membrane (Horton and Rodan, 1995; Sato et al., 1990; King et al., 1994). This is in accordance with the observations which indicate that RGD-containing peptides induce drastic changes in the cytoskeleton of the osteoclasts inducing cell membrane retraction (Lakkakorpi et al., 1991; Horton et al., 1991, 1993) at the resting and migration stages whether on bone or on glass where clear zones are not formed.

These shape changes eventually lead to the inhibition of bone resorption. Also, osteoclasts cultured on decalcified bone or collagen (our unpublished observations) do not form typical actin rings and clear zones. This favors the idea that tight sealing is actually formed between plasma membrane and bone mineral and/or mineral associated proteins which are liberated from bone matrix during decalcification. It is also known that osteoclasts are unable to degrade mineral devoid osteoid in vivo (Chambers and Fuller, 1985).

The lack of association of vinculin and talin with actin in the central area of the sealing zone resembles zonula adherens type cell-cell contacts. Polarization of resorbing osteoclasts and the fact that functional tight junction type sealing is needed to create the acidic environment into resorption lacuna fits with the idea that the sealing zone is functionally equivalent to the epithelial tight junction type cell-cell adhesion complex. If this similarity is also true at a structural and molecular level, or if some unique osteoclast-specific mechanism is utilized, remains to be seen and surely will be a focus of future research for bone biologists.

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