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Static and dynamic osteogenesis

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Summary

Two subsequent different types of bone formation, we respectively named *static osteogenesis* (SO) and *dynamic osteogenesis* (DO), were observed in intramembranous ossification centers of newborn rabbits and chick embryos as well as during bone repair. In all cases the onset of intramembranous ossification is characterized by the appearance, around the vessels, of pluristratified cords of unexpectedly stationary osteoblasts that transform into osteocytes in the same site where they differentiated, whence the name of *static osteogenesis* (SO). Soon after, typical monostratified laminae of well known movable osteoblasts differentiate along the surface of the bony trabeculae laid down by SO and thicken them by DO. No significant structural and ultrastructural differences were found between stationary and movable osteoblasts, all being polarized secretory cells joined by gap junctions. However, unlike in typical movable osteoblast laminae, stationary osteoblasts inside the cords are irregularly arranged, variously polarized, and transform into osteocytes clustered within confluent lacunae. Briefly SO seems to be devoted to building the first trabecular bony framework having, with respect to the subsequent bone apposition by typical movable osteoblasts, the same supporting function as calcified trabeculae in endochondral ossification. SO-bone is a bad quality woven-bone, whereas DO-bone generally is a lamellar-bone and thus mechanically more resistant. The relevance of this fact in bone repair and clinical practice will be discussed.

Key words

Intramembranous ossification; Static osteogenesis; Dynamic osteogenesis; Osteoblasts; Osteocytes

Introduction

According to the classical view, bone matrix deposition should only depends on the secretory activity of monostratified osteoblastic laminae, whose elements are synchronized by side-to-side gap junctions (Jeansonne et al. 1979; Palumbo et al. 1990a,b) and are all polarized towards the same direction, i.e. the osteogenic surface. It is also generally admitted that, as osteoid seam secretion proceeds, the osteoblastic laminae move away from the osteogenic surface and the osteoblasts selected to transform into osteocytes remain entrapped within the pre-osseous matrix by widening their secretory territory (Marotti et al. 1992). In previous reports (Marotti et al. 1999, Ferretti et al. 2002) we referred to this type of bone formation, which involves osteoblast movement, as *dynamic osteogenesis* (DO) to distinguish it from a disregarded type of bone deposition occurring at the onset of intramembranous ossification. We named the latter *static osteogenesis* (SO) because it is performed by immobile stationary osteoblasts that transform into osteocytes at the same site where they differentiated. In the

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present paper, an additional documentation of this new process will be provided not only in normal bone histogenesis but also during bone repair.

Materials and Methods

The present structural and ultrastructural study was carried out on the intramembranous perichondral center of ossification surrounding the mid-shaft level of various long bones of new-born rabbits and White Leghorn chick embryos aged 8-16 days. Additionally the process of bone repair was analyzed by light and transmission electron microscopes inside transcortical holes (4,5 mm diameter) drilled at the mid-shaft level of the 3rd metacarpal bone in adult horses. All specimens were fixed for 2 h with 4% paraformaldehyde in 0.13 M phosphate buffer pH 7.4, postfixed for 1 h with 1% osmium tetroxide in 0.13 M phosphate buffer pH 7.4, dehydrated in graded ethanol, embedded in epoxy resin (Durcupan ACM), and sectioned with a diamond knife mounted on an Ultracut-Reichert Microtome. The perichondral centers of ossification were cross-sectioned perpendicular to the longitudinal axis of the shaft. Thin sections (1 μm) were stained with toluidine blue and examined by an Axiophot-Zeiss light microscope (LM). Ultrathin sections (70-80 nm) were mounted on Formvar- and carbon-coated copper grids, stained with 1% uranyl acetate and lead citrate, and examined by a Zeiss EM109 transmission electron microscope (TEM).

Results

In all intramembranous ossification centers studied, the onset of osteogenesis is morphologically recognized by the appearance, at about midway between adjacent blood capillaries, of variously shaped (cuboidal, polygonal, globoid), plump osteoblasts, with a highly developed rough endoplasmic reticulum and a large Golgi apparatus. These osteoblasts never form typical monostratified osteogenic laminae; they are irregularly arranged in cords of 2-3 cell layers and each osteoblast is connected to the adjacent cells by gap junctions and appears to be polarized in a different, often opposite, direction with respect to them (Fig 1A). Additionally, these osteoblasts are *stationary* since they directly transform into osteocytes at the same site where they differentiated: they secrete all around their cell cord a pre-osseous matrix that soon undergoes mineralization. The osteocytes to which they give origin are irregularly grouped inside confluent lacunae and display a globoid cell body; also, they radiate very short cytoplasmic processes, which are connected by means of simple contacts and gap junctions (Fig.1B).

As this process of *static osteogenesis* (SO) is in progress, at the periphery of the ossification center the compaction of the trabecular spaces formed first (the so-called *primary Haversian spaces*) takes place by *dynamic osteogenesis* (DO). Typical osteogenic laminae, made up of movable osteoblasts all polarized in the same direction, differentiate along the surface of the trabeculae previously laid down by stationary osteoblasts (Fig.1C). These movable osteoblastic laminae deposit layers of bone by DO, that thicken the SO-trabeculae and/or fill the primary Haversian spaces with primary Haversian systems.

Much the same sequence of events takes place during the repair of transcortical holes experimentally drilled at the mid-shaft level of the 3rd metacarpal bone in adult horses. As generally occurs during bone healing, after the hematoma and inflammatory stages, all the holes are filled with a highly cellular and vascularized fibrous tissue. Afterward, cords of plump stationary osteoblasts differentiate in between the blood capillaries and give origin to a trabecular bony framework laid down by SO. Soon after, typical laminae of movable osteoblasts differentiate along the surface of this SO-trabeculae and thicken them by DO.

It must be noted that SO-bone is made up of highly porous woven bone containing numerous osteocyte lacunae, whereas DO-bone generally is a lamellar bone.

Discussion

The present paper provides a further demonstration of the existence of two mechanisms of bone formation, i.e., *static* (SO) and *dynamic osteogenesis* (DO), sequentially occurring during intramembranous ossification under both normal bone histogenesis and bone repair. The former process is performed by stationary osteoblasts and allows the formation of a trabecular bony framework, enclosing blood vessels. These appear to be essential for the subsequent bone apposition by typical movable osteoblasts. In fact, these trabeculae have the same supporting function as those made up of calcified cartilage in endochondral ossification. It also appears from our findings that SO is mainly devoted to the expansion of the ossification center and consequently to increasing bone size, whereas DO is mainly involved in bone compaction or, at least, in thickening the primitive trabeculae.

No substantial differences were found in structure and ultrastructure between stationary and movable osteoblasts. Both display an ill-defined euchromatic nucleus and a highly developed organelle machinery, characteristically ordered as in polarized secretory cells. This means that stationary osteoblasts also secrete pre-osseous matrix from one cell surface (i.e. secretory territory) only, and not all around them. The differences between the two types of osteoblasts concern their arrangement and polarization: stationary osteoblasts are irregularly arranged in cords of 2-3 layers of cells, and each cell is polarized in a different direction with respect to the adjacent ones. In contrast, movable osteoblasts form monostratified laminae and are all polarized in the same direction. In other words, while mobile osteoblastic laminae share the same osteogenic surface, stationary osteoblastic cords have different osteogenic surfaces, thus allowing each stationary osteoblast to be surrounded completely by bone matrix. This means that in SO the osteoblasts become osteocytes by a mechanism of "self-burial", whereas in DO the osteoblasts selected to transform into osteocytes are embedded within the bone by the secretory activity of the adjacent movable osteoblasts (Marotti et al. 1992). This fact explains why clusters of osteocytes within "lacunae confluentes" can only form during static osteogenesis.

We believe that different factors and signals should be involved in the two types of osteogenesis: SO seems to depend on inductive stimuli (cytokines like endothelin 1 or growth factors like PDGF, EDGF, etc.), rather than mechanical signals, since osteocytes (which behave as bone mechanosensors, as is now generally admitted) are not present at its inception; whereas DO appears to be driven by mechanical strain sensed

by osteocytes contained in SO-trabeculae. Another intriguing problem is that SO-bone is a poor quality bone because of its woven texture and high microporosity, due to the many osteocyte lacunae it contains, whereas DO-bone generally is a lamellar bone, mechanically much more resistant. Therefore it becomes crucial in clinical practice to know how long SO goes on before DO starts, to establish when a poor quality bone is reinforced with a bone actually capable of resisting mechanical loading.

References

Jeansonne B.G., Feafin F.F., McMinn R.W., Scoemaker R.L., Rehm V.S. (1979) Cell-to-cell comunication of osteoblasts. J. Dent. Res. 58:1415-1423.

Marotti G., Ferretti M., Muglia M.A., Palumbo C., Palazzini S. (1992) A quantitative evaluation of osteoblast-osteocyte relationships on growing endosteal surface of rabbit tibiae. Bone 13: 363-368.

Marotti G., Ferretti C., Palumbo C., Benincasa M. (1999) Static and dynamic bone formation and the mechanism of collagen fiber orientation. Bone 25: 156.

Ferretti M., Palumbo C., Contri M., Marotti G. (2002) Static and dynamic osteogenesis: two different types of bone formation. Anat. Embryol. 206: 21-29.

Palumbo C., Palazzini S., Zaffe D., Marotti G. (1990a) Osteocyte differentiation in the tibia of newborn rabbit: an ultrastructural study of the formation of cytoplasmic processes. Acta Anat. 137: 350-358.

Palumbo C., Palazzini S., Marotti G. (1990b) Morphological study of intercellular junctions during osteocyte differentiation. Bone 11: 401-406.

Figures

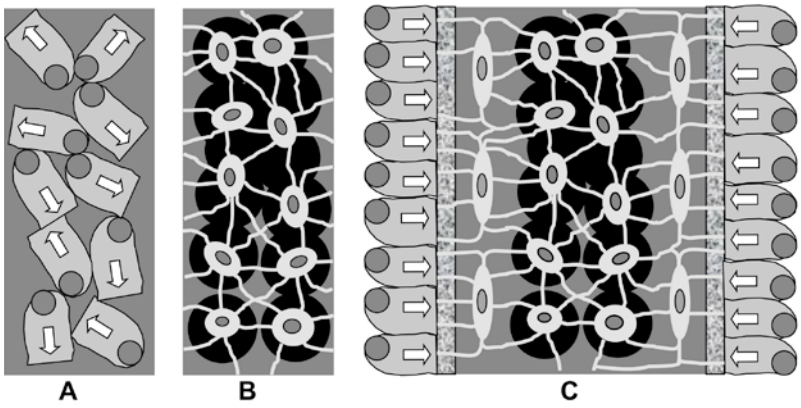

Fig. 1 – Schematic drawing showing *static osteogenesis* (SO) and *dynamic osteogenesis* (DO) sequentially occurring during intramembranous ossification. **A**) A cord of *stationary osteoblasts*, differentiating in preosseous mesenchymal blastema, transforms (**B**) into osteocytes at the same site where the cells had differentiated, thus forming an SO trabecula. (**C**) On both sides of this trabecula, typical laminae of *movable osteoblasts* increase its thickness by DO. The arrows inside the osteoblasts indicate their polarization. Note that all cells are always in contact to each other.