〔ORIGINAL〕

Appearance and distribution of osteoclast precursors and the morphological change during mouse mandibular osteogenesis

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Abstract

The appearance and distribution of osteoclast precursors and the morphological change of the precursors were examined during mouse mandibular osteogenesis, using enzyme histochemistry of tartrate-resistant acid phosphatase (TRAPase). Osteogenic tissue was not observed in the prospective region of the mandibles at embryonic day 12 (E12), but TRAPase-positive cells often existed in the region. Immature osteoblasts were seen as a population in E13 mandibles, and a thin layer of bone matrix had been formed at the central part of the osteogenic region in E14 mandibles. A number of TRAPase-positive osteoclast precursors were tandemly localized along the region. At these stages, the TRAPase-positive cells were oval and round in the vicinity of blood vessels, but at an earlier stage of the osteogenesis, the positive cells extended long processes towards the interspace between the osteoblasts. The present results demonstrated the morphology characteristic of the TRAPase-positive osteoclast precursors during osteoclast differentiation, suggesting the possibility that there is a cell-cell interaction between the osteoclast precursors and the osteoblasts in vivo.

Key words : Osteoclast precursor, Differentiation, TRAPase, Enzyme histochemistry, Mandibular osteogenesis

Introduction

In osteoclastogenesis, it is established that osteoclasts are derived from hematopoietic stem cells[1-3]. In craniofacial osteogenesis, the osteoclast precursors appear to be located in the restricted anlage before the onset of osteogenesis. Culture experiments using cell populations isolated from mouse embryonic day 14 (E14) calvaria indicated that the calvaria tissue contains osteoclast precursors at this stage of mouse embryogenesis[4]. We also reported that tartrate-

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resistant acid phosphatase (TRAPase)-positive mononuclear cells, presumably osteoclast precursors, were observed in mouse E11 mandibles in vivo[5]. Thus, early osteogenesis in the intramembranous ossification of the craniofacial region may provide a useful model for examining the differentiation of osteoclasts during osteogenesis. However, although TRAPase is a marker enzyme for osteoclasts and the precursors in normally developing bone tissues[6-8], it is not known whether the TRAPase-positive cells in our model are osteoclast precursors. The morphological change of the TRAPase-positive cells in mouse osteogenesis remains unknown.

In the present study, we examined the appearance of TRAPase-positive cells in mouse E12-14 mandibles by enzyme histochemistry and light microscopy, and demonstrated that the TRAPase-positive cells are osteoclast precursors that differentiate into multinucleated osteoclasts. In addition, the morphology characteristic of the precursors is described.

Materials and Methods

Mandibles from ddY strain E12-14 mice were used in this study. The presence of a vaginal plug after mating during a 3-hr period (9 a.m. to 12 p.m.) was considered day 0. The mandibles were fixed in 4% paraformaldehyde buffered with 0.1M sodium cacodylate (pH 7.4) overnight at 4°C. The fixed samples were dehydrated in a graded series of ethanol after rinsing with the same buffer, then cleared with xylene and embedded in paraffin. The paraffin-embedded tissues were stored at −20°C until use, to preserve the activity of TRAPase. Serial sections were cut at 5μm in thickness on a frontal plane. For the detection of TRAPase activity, the deparaffinized sections were incubated in the reaction medium described in our previous report[9]. Briefly, the reaction medium contained 50ml of 0.1M acetate buffer (pH 5.2), 5mg naphthol AS-MX phosphate (Sigma. St. Louis, MO), 30mg fast red violet LB salt (Sigma), and 50mM L(+)-tartaric acid (Wako, Osaka). For the estimation of the enzyme reaction, the sections were incubated in a pre-warmed chamber for 30min at 37°C and were not counterstained. The observation by light microscopy, unless stated otherwise, was performed in the first molar region of the mandible.

Results

In the mouse E12 mandibles, the tongue had already formed and dental epithelium started growing towards mesenchymal tissue (Fig.1). However, osteogenic tissue was not yet observed at this stage, and a few TRAPase-positive cells often existed between the undifferentiated mesenchymal cells in the prospective region of mandibular osteogenesis (Fig.2). The positive cells were oval or round in shape and showed faint TRAPase activity.

In the E13 mandibles, the mesenchymal cells differentiated into immature osteoblasts in the prospective region of mandibular osteogenesis, and were observed as a population of osteogenic tissue without bone matrix (Figs.3 and 4). At this stage, TRAPase-positive cells appeared in

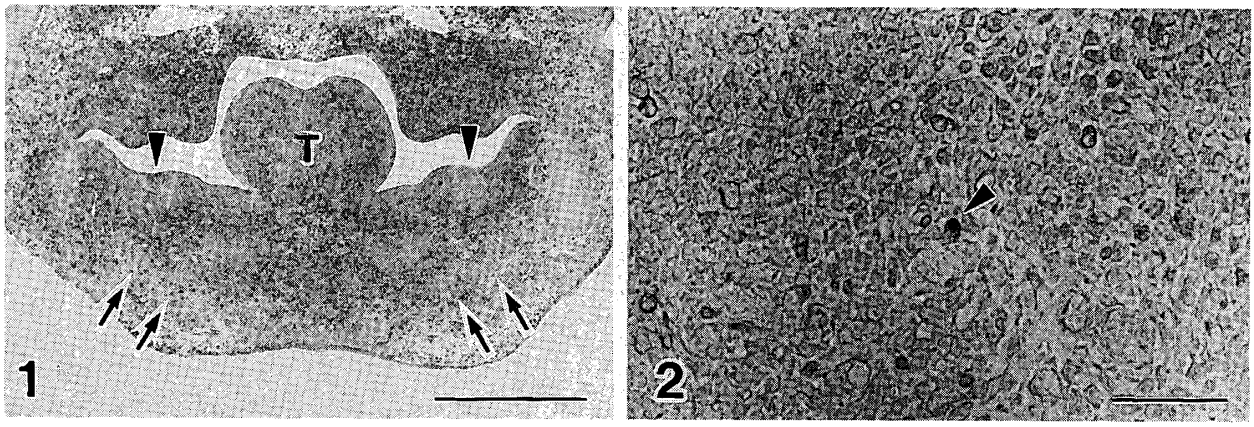

Fig.1 A frontal section of an E12 mandible. Tooth morphogenesis has started by growth of the dental epithelium (arrowhead) into adjacent mesenchyme. Arrows indicate the prospective region of mandibular osteogenesis. T, tongue. Bar : 500μm.

Fig.2 High magnification of the prospective region of E12 mandibular osteogenesis. Arrowhead indicates TRAPase-positive cells with faint activity. Bar : 50μm.

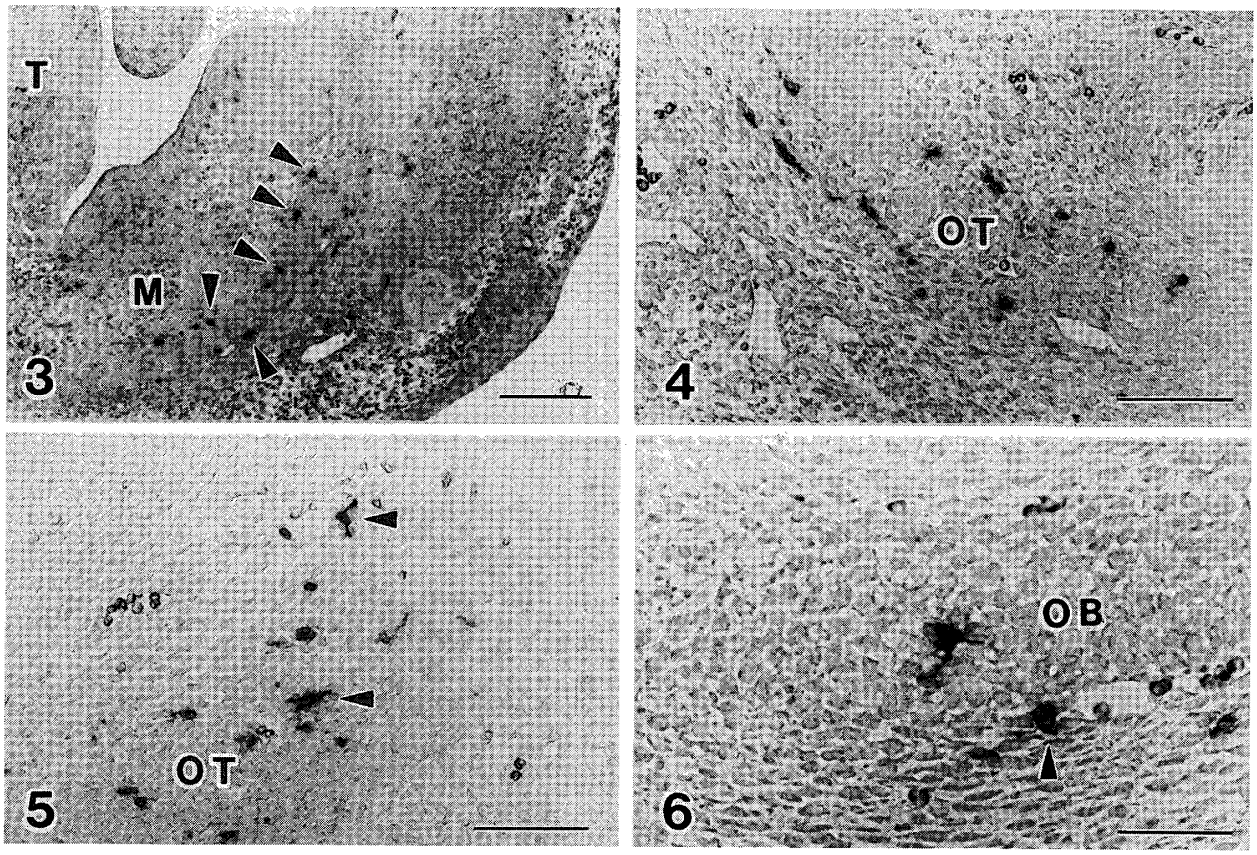

Fig.3 Left part of an E13 mandible. Note the appearance of TRAPase-positive osteoclast precursors (arrowheads) in the restricted region of mandibular osteogenesis. M, Meckel's cartilage, T, tongue. Bar : 200μm.

Fig.4 Alignment of TRAPase-positive osteoclast precursors in an E13 mandible. OT, osteogenic tissue. Bar : 100μm.

Fig.5 Scattered TRAPase-positive osteoclast precursors in the region of diastema. Note short processes in some precursors (arrowheads). OT, osteogenic tissue. Bar : 100μm.

Fig.6 High magnification of a TRAPase-positive precursor with long process. An oval positive cell is seen in the vicinity of a blood vessel (arrowhead). OB, osteoblasts. Bar : 50μm.

the restricted region of mandibular osteogenesis (Fig.3), and the positive cells with intense enzyme activity were aligned surrounding the osteogenic tissue (Fig.4). Most of the positive cells exhibited an oval or spindle profile, and some showed short processes. In the region of diastema where is a toothless space between the incisor and molar, the TRAPase-positive cells with short processes were dispersed between the immature osteoblasts (Fig.5). The long processes of the positive cells sometimes extended towards the interspace of the osteoblasts, and the positive cells, oval in appearance, were localized in the vicinity of blood vessels (Fig. 6).

In the E14 mandibles, the mandibular osteogenesis widely expanded towards the regions of Meckel's cartilage and first molar tooth organ, and a number of TRAPase-positive cells were located in the region of mandibular osteogenesis (Fig.7). A thin layer of bone matrix had been formed at the central part of the osteogenic region (Fig.8). In this region, the TRAPase-positive cells were seen along the layer of osteoblasts, not bone matrix. Osteoclasts with

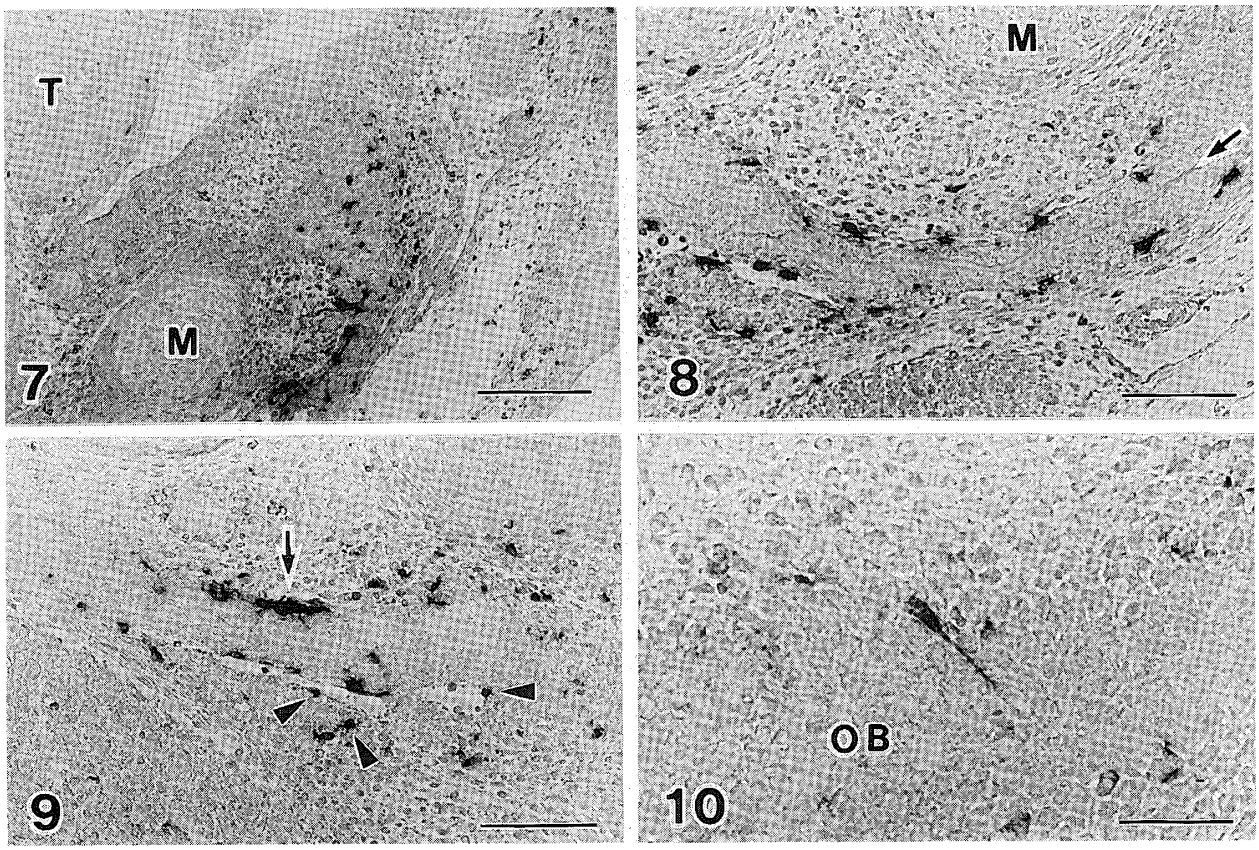

Fig.7 Left part of an E14 mandible. A number of TRAPase-positive cells ara recognized in the restricted region of the osteogenesis. M, Meckel's cartilage, T, tongue. Bar : 200μm.

Fig.8 Advanced osteogenesis with a thin layer of bone matrix (arrow) in an E14 mandible. Note the localization of TRAPase-positive cells surrounding the region of mandibular osteogenesis. M, Meckel's cartilage. Bar : 50μm.

Fig.9 Occurrence of osteoclast (arrow) with intense TRAPase activity in an E14 mandible. Oval and round cells with TRAPase activity (arrowheads) are observed in the vicinity of a blood vessel. Bar : 100μm.

Fig.10 A TRAPase-positive osteoclast precursor with a long cellular process. The process extends towards the interspace between osteoblasts (OB). Bar : 100μm.

intense TRAPase activity were frequently observed at this stage (Fig.9). A number of the positive cells were localized in the vicinity of blood vessels that were seen adjacent to the osteogenic region (Figs.8 and 9). In the more rostral region of the E14 mandibles, the TRAPase-positive cells extended long cellular processes into the interspace between the osteoblasts (Fig.10).

Discussion

TRAPase-positive cells appeared in the prospective region of the osteogenesis in E12 mandibles, and the positive cells were localized in the restricted region of osteogenesis in E13 mandibles. The TRAPase-positive and multinucleated osteoclasts were seen in the vicinity of bone matrix in E14 mandibles. Although TRAPase was used as a marker for osteoclasts and the precursors[6-8] in the present study, some recent studies have demonstrated that TRAPase is not always a specific marker for the identification of multinucleated osteoclasts in mouse bone marrow culture[10,11]. They suggested that not all TRAPase-positive multinucleated cells in a cell culture are osteoclasts, and may in part be macrophages. In addition, certain conditions induce the TRAPase activity of bone marrow macrophages[12], monocytes[13] and reticular cells[14]. Therefore, phagocytic cells appear to express TRAPase activity in certain pathological conditions and in vitro cell cultures. However, the results of the present in vivo study indicate that the TRAPase-positive cells are osteoclasts and the precursors in normally developing bone tissues.

The present findings demonstrated that E12 mandibles contained TRAPase-positive osteoclast precursors. A similar result has been demonstrated in culture experiments using bone cell populations isolated from mouse E14 calvaria before the start of cranial osteogenesis[4]. The appearance of osteoclast precursors in the mandibular anlage at a stage earlier than that in calvaria may be associated with the first initiation of osteogenesis in the skeletal system[15]. Since bone marrow was not observed at these stages in the present study, the early appearance of osteoclast precursors may be explained by the ontogeny of the hematopoietic system in mice. Multipotential stem cells have been demonstrated in the mouse yolk sac[16]. In addition, a recent study demonstrated that primitive macrophages first appear in the blood islands of mouse yolk sac at E9 and differentiate into fetal macrophages at E10[17], suggesting an early occurrence of mononuclear phagocytic cells during mouse embryogenesis. Moreover, the yolk sac is open to the fetal circulation at E9[17,18], and the circulation of yolk sac has been completely established at E10[19]. Since osteoclasts are derived from hematopoietic stem cells[1-3], the ontogeny of the hematopoietic system in the mouse embryo and the circulation of osteoclast progenitors or precursors in embryonic blood vessels may be involved in the first initiation of mandibular osteogenesis in the skeletal system.

The TRAPase-positive cells were oval or round in the E12 mandibles, and the round positive cells appeared in the vicinity of blood vessels in E13 and 14 mandibles (Figs.6 and 9). However,

the positive cells exhibited the morphology characteristic of osteoclast precursors with long processes in the immature region of E13 and E14 mandibular osteogenesis (Figs.6 and 10). The morphological changes of the osteoclast precursors may be associated with osteoblast differentiation. Recent studies of co-cultures of osteoblastic stromal cells and spleen or bone marrow cells demonstrated that the osteoblastic cells induced the expression of osteoclast phenotypes and the multinucleation of osteoclast-like cells[20-23]. The in vitro experiments suggested a cell-cell interaction between osteoblasts and osteoclast precursors. In addition, macrophage-colony stimulating factor (M-CSF) appears to be essential for osteoclast differentiation in osteopetrotic (*op/op*) mice that fail to produce a functional M-CSF molecule[24-28]. Moreover, in the present study, the TRAPase-positive osteoclast precursors with long processes appeared in the restricted region of mandibular osteogenesis during the morphogenesis. This may indicate osteoclastic chemotaxis against M-CSF during osteogenesis[29]. In addition, the chemotaxis of M-CSF secreted by differentiated osteoblasts may be responsible for the early appearance of osteoclast precursors and the subsequent changes of the cell morphology in the restricted region of mandibles. Although further examinations are necessary to test this hypothesis, mandibular osteogenesis would provide a useful model to examine cell-cell interactions between the TRAPase-positive osteoclast precursors and osteoblasts in vivo.

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