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Microdontia: A Specific Tooth Anomaly in Osteopetrotic (*op/op*) Mice

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ABSTRACT

Osteopetrotic (*op/op*) mice have a severe deficiency of osteoclasts. Osteopetrosis is always accompanied by the failure or/and delay of tooth eruption. The purposes of the present study were to describe the relationship of upper third molar formation and TRAP-positive cells in the environment of macrophage colony-stimulating factor (M-CSF) absence. Ten-day-old normal and *op/op* mice showed no detectable difference in the shape of third molar follicles. However, in the *op/op* mice it became obscured by the proliferation of neighboring bone trabeculae. The number of tartrate-resistant acid phosphatase (TRAP) positive cells in the normal mice approached a maximum of 15-day-old and then gradually decreased up to 30-day-old, although the numbers were substantially different for all ages. The *op/op* mice, meanwhile, presented no osteoclasts until 15-day-old and the number increased significantly from 20 to 30-day-old. Throughout the experimental period, of 10 to 30 days postnatal, the alveolar ridge covering the tooth crown remained unresolved in the *op/op* mice. The third molars were erupted into the oral cavity before 30-day-old normal mice, and the crowns, roots and periodontal ligaments appeared well-developed. We consider that the primary cause of the microdont in the mutant mouse was a deficiency of osteoclasts, with attendant lack of bone remodeling.

INTRODUCTION

Osteopetrosis is an inherited metabolic disease, exhibiting an excessive accumulation of bone as a result of qualitative and/or quantitative osteoclast defects[1]. This bone disease has been found in animals including humans. The osteopetrotic (*op/op*) mutation in the mouse was first found in the Jackson Laboratory in 1970. Thus, the mutation of *op/op* mice were reported by Marks and Lane[2]. In the *op/op* mice, both the number and size of osteoclasts are much smaller than in the normal mice[2]. Accordingly, osteopetrosis is usually also known that to be accompanied by the failure or/and delay of tooth eruption. It is assumed that abnormal dental development is due to reduced bone resorption that causes blocking of the eruption pathway, and distortion and ankylosis of the roots under development[3-5]. Osteopetrotic mutants are characterized by systemic bone sclerosis, deformation of the skull and jaw, and failure of tooth eruption due to the defect in bone resorption[6-10].

Generally, the upper third molars exhibits development and eruption last among all the teeth. Furthermore, the *op/op* mice can be judged only 10 days after birth. The tooth formation was observed third molar of the *op/op* mouse was convenient for these reasons.

The purpose of the present study was to examine the sequence of upper third molar formation under an environment with an abscence of M-CSF.

MATERIALS AND METHODS

Animals

Osteopetrotic (*op/op*) mice and control littermate (normal) mice were obtained from B6C3F1-*a/a-op/+* breeding pairs (Jackson Laboratory, Bar Harbor, USA). Mice were kept in metal cages (22×32×11 cm) with autoclaved wood chips for bedding in an animal room (temperature; 24±2°C, relative humidity; 50±5%). Newborn male mice were weaned in 15-20 days. Homozygous recessive *op/op* mice were identified by

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failure of tooth eruption and a characteristic domed skull 10 days after birth. The *op/op* mice were fed a granulated diet, and normal mice were fed a solid diet. Five *op/op* mutants and five normal mice were sacrificed for histological examination at the agees of 10, 15, 20 and 30 days.

Light microscopic observation

The upper jaws removed from the skulls were fixed with 4% formaldehyde for 12 hrs at 4°C, decalcified in 5% ethylenediamine tetraacetic acid (EDTA, pH. 7.4), for one week, embedded in paraffin, and cut into frontal sections of 7 μm thickness. Alternative sections were stained with hematoxylin and eosin (HE). Sections from 10, 15, 20 and 30-day-old *op/op* and normal mice were stained with tartrate-resistant acid phosphatase (TRAP), staining for which is generally regarded as a cytochemical marker for osteoclasts, and counter-stained with hematoxylin. Moreover, the upper jaws removed from the skulls were fixed with 4% formaldehyde for 12 hrs at 4°C, and 100 μm bucco-ligual ground sections were made for 30-day-old normal mice. The sections were examined under a light microscope (BH; Olympus, Tokyo, Japan). Each tooth was measured for three spots (TS, ES and DS) on both the labial and lingual aspects, according to the method designed by Ooe[11] (Fig. 1). The number of TRAP-positive cells of the median portion of whole teeth was enumerated for two groups. All data were subjected to statistical analysis with a the Student's *t*-test.

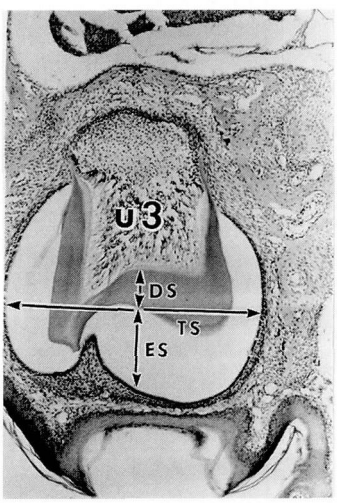

Fig. 1 Light microphotograph of the mid-belly portion of upper third molar of the normal mouse. U3, upper; 3, molar; TS, tooth space; ES, enamel space; DS, dentin space.

RESULTS

In 10-day-old normal mice, TRAP-positive cells appeared in the bone surfaces (Figs. 2A, arrowheads, and 7). TRAP-positive cytochemistry revealed no enzyme activity in the upper jaw of the 10-day-old *op/op* mice (Figs. 2C and 7). However, the third molar follicle of mice appeared to become obscured by the proliferation of neighboring bone trabeculae in the *op/op* mice (Fig. 2C). In the 10-day-old normal and *op/op* mice, no major changes were observed in the sizes of teeth (Figs. 2A, C and 4).

Throughout the experiment, in 15-day-old normal mice, the osteoclast number reached its maximum value (Figs. 2B and 7). TRAP-positive cytochemistry revealed no enzyme activity in the upper jaw of the 15-day-old *op/op* mice (Figs. 2D and 7). The upper third molars were larger in 15-day-old normal mice than in *op/op* mice (Figs. 2B, D and 4). In normal and *op/op* mice, no major changes were observed in the dentin spaces (Figs. 2B, D and 6). However, the enamel spaces of normal mice were larger than in the *op/op* mice (Figs. 2B, D and 5).

The number of TRAP-positive cells decreased from 15 to 20-day-old in the normal mice (Figs. 2B, 3A and 7). A few TRAP-positive cells were found on the bone surface in the *op/op* mice at 20-day-old (Figs. 3C and 7). The upper third molars were larger in 20-day-old normal mice than in *op/op* mice (Figs. 3A, C and 4). The enamel and dentin space of normal mice was larger than in the *op/op* mice (Figs, 3A, C, 5 and 6). In 20-day-old normal mice, the space between the bony crypt and tooth germ became progressively wider and formation of the periodontal ligament was evident as cells and fibers having an oblique orientation (Fig. 3A). No sign of eruption was observed in *op/op* mice (Fig. 3C).

The number of TRAP-positive cells decreased from 20 to 30-day-old in the normal mice (Figs. 3A, B and 7). A few TRAP-positive cells were found on the bone surface in the 30-day-old *op/op* mice (Figs. 3D and 7). The dentin was larger in the 30-day-old normal mice than in the *op/op* mice (Figs. 3B, D and 6). In the 20 and 30-day-old *op/op* mice, no major changes were observed in the size of teeth (Figs. 3C, D and 4). The third molars emerged into the oral cavity by 30-day-old normal mice,

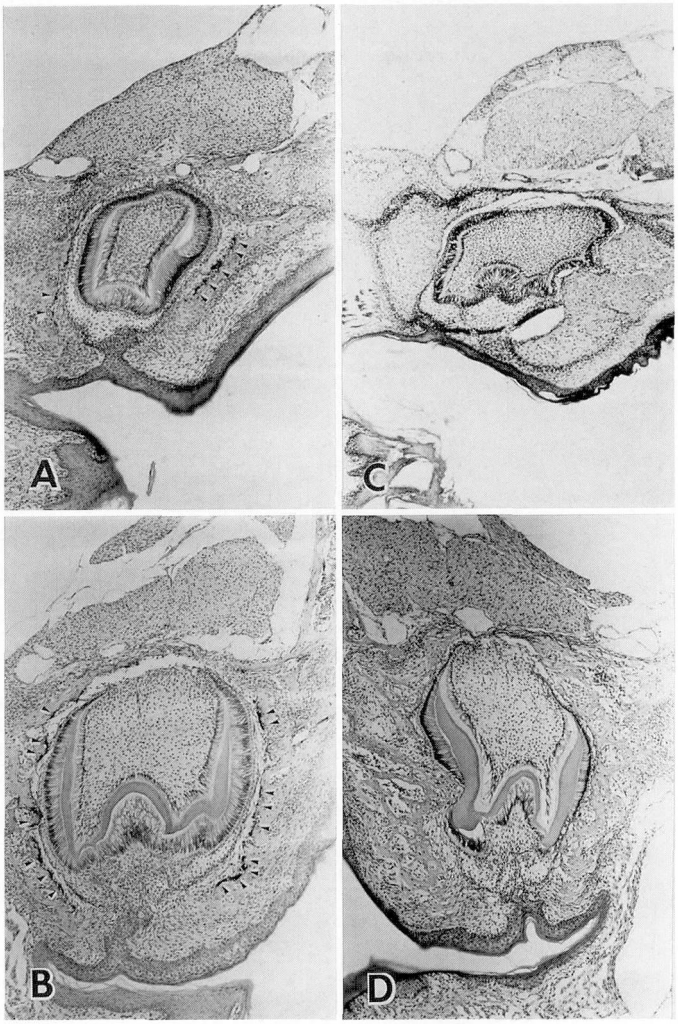

Fig. 2 Light microphotographs of upper third molars. 10-day-old normal mouse (A) and *op/op* mice (C). 15-day-old normal mice (B) and *op/op* mice (D). ×100. TRAP-positive cells (arrowheads) are observed on the surface of trabecular bone. Each micrograph represents a group of maxillodentals from five mice.

and their roots and periodontal ligaments appeared well-developed (Fig. 3B). No sign of eruption was observed in 30-day-old *op/op* mice (Fig. 3D).

DISCUSSION

Recent studies have revealed that the deficiency of osteoclasts, monocytes, and macrophages in *op/op* mice essentially results from a defect in the production of functional macrophage colony-stimulating factor (M-CSF)[12,13]. Osteopetrotic mutants are characterized by systemic bone sclerosis, deformation of the skull and jaw, and failure of tooth eruption due to defects in bone resorption[6,8-10]. Moreover, this present study revealed subnormal growth, dwarfed teeth and a developing root in *op/op* mice. *Op/op* mice suffer from a severe deficiency of osteoclasts due to an autosomal recessive inactivating mutation in the M-CSF gene, resulting in the absence of M-CSF[14]. We measured the upper third molar tooth crown, enamel and dentin size until 30-day-old normal and *op/op* mice. In the 10-day-old normal and *op/op* mice, no major changes were observed the size of the tooth-germ. At 15 and 20-day-old, the third molar of the *op/op* mice was very small in comparison with the normal mice. The cause

Fig. 3 Light microphotographs of upper third molars. 20-day-old normal mouse (A) and *op/op* mice (C). 30-day-old normal mice (B) and *op/op* mice (D). ×100. TRAP-positive cells (arrowheads) are observed on the surface of trabecular bone. Each micrograph represents a group of maxillodental from five mice.

of this difference is related to the number of osteoclast. TRAP activity detected around the tooth germ is presumably involved in creating the space for formation of the tooth. However, it is evident that the epithelial sheath embedded in osteopetrotic bone trabeculae retains the ability to form roots, because TRAP-positive osteoclasts and resorption lacunae were localized on the crypt wall directly facing the tooth germ as well as on the endosteal surface. The third molars suffered from appreciable undevelopment of growing tooth crowns and roots caused by the invasion of osteopetrotic bone trabeculae in *op/op* mice, while the tooth crowns of the earlier-developing third molars in normal mice appeared fairly normal in size. It is known that the dentin thickens gradually after tooth eruption. Generally, the enamel does not grow after tooth eruption. These observations are consistent with the finding of previous studies[11].

The mechanism of the dwarfed teeth in *op/op* mice is principally a deficiency of resorption in the bone surface surrounding the tooth germ. Thus, the dwarfed teeth in *op/op* mice are most likely secondary to the bone resorption abnormalities.

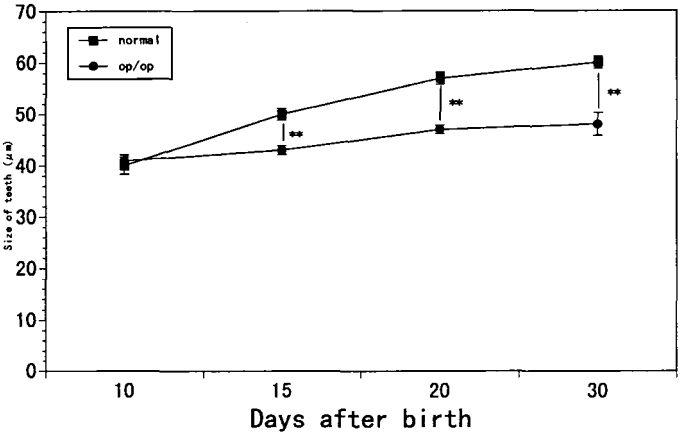

Fig. 4 Changes in size of upper third molars in normal and *op/op* mice. The results represent the mean±standard deviation (SD) of five mice. **p<0.01

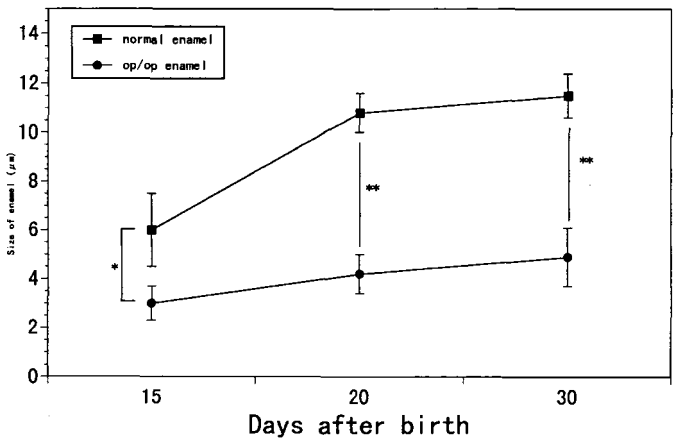

Fig. 5 Changes in size of enamel upper third molars in normal and *op/op* mice. The results represent the mean±standard deviation (SD) of five mice. *p< 0.05, **p<0.01

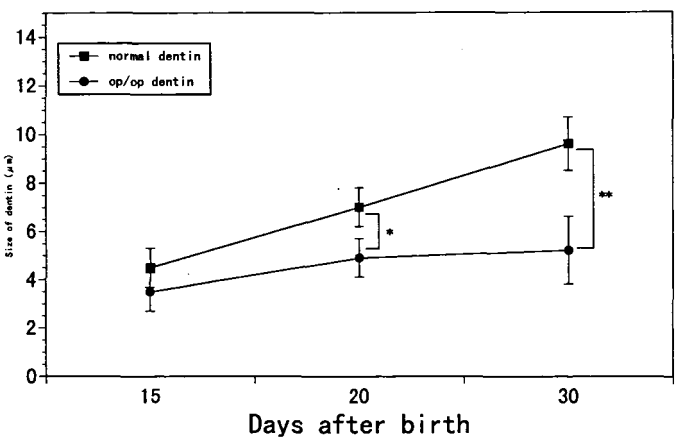

Fig. 6 Changes in size of dentin upper third molars in normal and *op/op* mice. The results represent the mean±standard deviation (SD) of five mice. *p< 0.05, **p<0.01

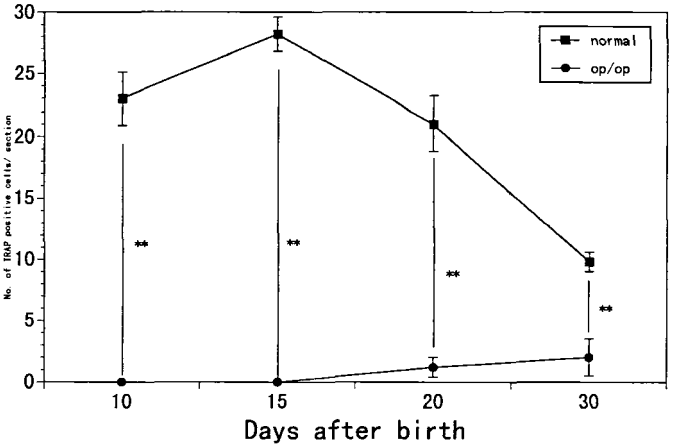

Fig. 7 The number of TRAP-positive cells on the surface of bone trabeculae for the normal and osteopetrotic mice. The results represent the shows mean±standard deviation (SD) of five mice. $^{**}p<0.01$

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