〔ORIGINAL〕

Localization of anti-monocyte/macrophage antibody-positive cells in periodontal tissue of rat maxillary molars after orthodontic tooth movement

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Abstract

To examine the localization of monoclonal anti-monocyte/macrophage (ED1) and macrophage (ED2) antibody-positive cells in periodontium, rat maxillary molar teeth were moved by insertion of band materials. The orthodontic tooth movement was elicited for 5 days, and paraffin-embedded maxillary teeth were stained by fluorescent immunocytochemistry and observed using a confocal laser scanning microscope. The localization of ED1-positive mononuclear cells in the experimental teeth was little different from that in the controls. While ED2-positive mononuclear cells were located throughout the periodontium on the distal side of controls, the number of positive cells decreased on the pressure side of the treated teeth. The present study suggested that most of the immunoreactive mononuclear cells on the distal side of controls are macrophages, while the positive cells on the pressure side of the experimental teeth are osteoclast precursors and a small number of macrophages.

Key words : Monoclonal antibody, Macrophage, Osteoclast, Confocal laser scanning microscope

Introduction

The role of osteoclasts and odontoclasts is important in the remodeling processes of alveolar bone and cementum during orthodontic and physiological tooth movement. Osteoclasts appear to be derived from monocyte/macrophage cell lineages[1,2], and recently, monoclonal antibodies which recognize a broad spectrum of monocyte/macrophage cell lineages have been used in

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studies of the diffrentiation of osteoclasts and the detection of leukocyte subsets in mice and rats[3-5]. In monoclonal antibodies for rats, the immunoreactivity of ED1 antibody has been recorded in monocytes, free and fixed macrophages, multinucleated osteoclasts, and dendritic cells[6-8] and another antibody (ED2) is able to detect a membrane antigen of tissue macrophages[6]. Macrophages as well as osteoclasts play an important role in the remodeling of periodontal ligament through phagocytosis, but the changes in appearance and distribution during the tooth movement are little known.

To examine the distribution of monocyte/macrophage antibody-positive cells after orthodontic tooth movement, rat maxillary molars were treated by insertion of bands and the immunoreaction of ED1 and ED2 antibodies were detected by confocal laser scanning microscopy (CLSM).

Materials and Methods

Tissue preparation

The study used Wistar, male seven-week-old rats (150±10g). Orthodontic tooth movement was elicited by insertion of 0.13mm bands between the maxillary right (experimental side) first and second molars for 5 days[9]. The animals were anaesthetized by intraperitoneal injection of sodium pentbarbital (2.5mg/100g body weight), and the maxillae were dissected and fixed in 2% paraformaldehyde and 2% glutaraldehyde mixture in 0.1M cacodylate buffer (pH7.2) for 24 hr at 4°C. The samples were not perfused by the fixative to be able to examine the appearance of the antibody-positive cells in the blood vessels of the periodontal ligament. The fixed samples were decalcified in 5% EDTA (pH7.2) for 28 days at 4°C and embedded in paraffin. Horizontal 5μm thick sections of the maxillary molars together with alveolar bone were prepared and stored at -20°C until use. The left molars were used as controls.

Immunocytochemistry of ED1 and ED2 antibodies

Immunocytochemical staining was performed on deparaffinized sections using the indirect fluorescein isothiocyanate (FITC) labeling procedure modified after Whiteland et al.[5]. Briefly, the deparaffinized and rehydrated sections were digested with 0.0025% trypsin and 0.1% $CaCl_2$ in 0.05M Tris-HCl buffer (pH7.8) for 5 min at 37°C. After rinsing in phosphate-buffered saline (PBS), the sections were incubated with 1.5% normal rabbit serum in PBS for 30 min. After further rinsing in PBS, incubation was performed with mouse anti-rat monocyte/macrophage (ED1, 1 : 100) and macrophage (ED2, 1 : 500) antibodies (Serotec, Oxford, England) in PBS containing 0.1% bovine serum albumin (BSA) overnight in a humid chamber at 4°C. The sections were then incubated with FITC-conjugated rabbit anti-mouse IgG (whole molecule, Cappel™ Organon Teknika Corp., West Chester, PA, USA) antibody in 0.1% BSA in PBS for 60 min at room temperature. To detect the fluorescent reaction of ED1 and ED2, the sections were mounted in glycerin mountant containing 1% *p*-pheneylenediamine. Spleen was used as

a positive control for the immunocytochemical procedure.

CLSM observation

The periodontal tissue of maxillary second and third molars were observed under an FITC filter set using CLSM (Leica, TCS 4D, Cambridge, England) equipped with a differential interfering device. Both fluorescent and differential interfering images were superposed by Photoshop™ software (Adobe Systems Incorporated, CA, USA). The fluorescence of the antibodies was presented as red.

Results

Immunofluorescence of ED1 and ED2 antibodies were intensely detected in mononuclear and multinuclear cells of rat periodontium using decalcified, paraffin-embedded sections and CLSM. Macrophages in the spleen also showed fluorescence of the antibodies (data not shown).

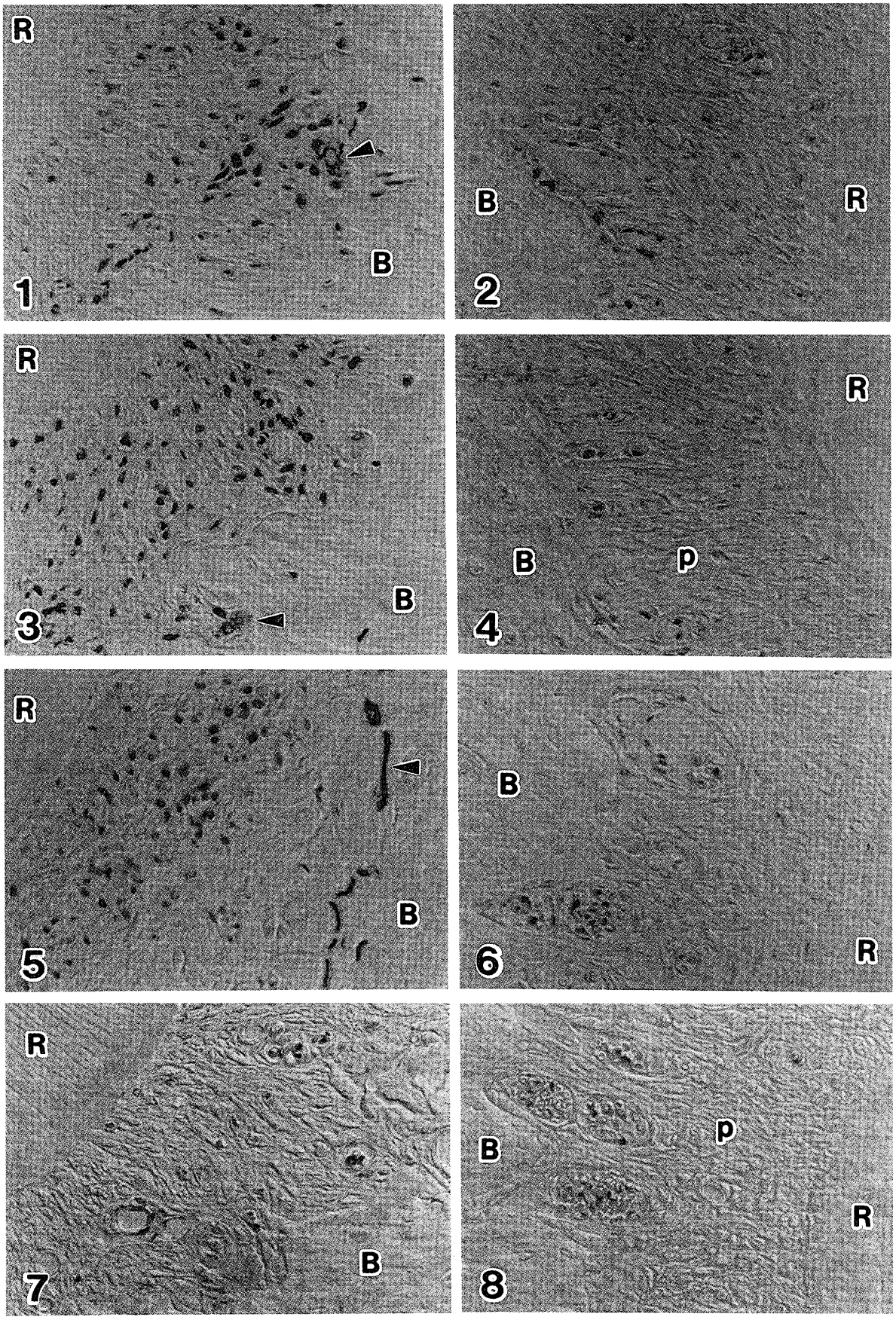
Distribution of ED1- and ED2-positive cells in the controls

On the distal side in the controls, the immunoreactive cells exhibiting various intensities of ED1 fluorescence were observed throughout the periodontium (Fig. 1). Relatively strong fluorescence appeared in the mononuclear cells surronding the small sized blood vessels in the middle region of the periodontal ligament. In addition, the cytoplasm of multinuclear osteoclasts also showed faint fluorescence. On the proximal side (Fig. 2), there were blood vessels in the region adjacent to the surface of the alveolar bone, and the predominant localization of the mononuclear positive cells was around the vessels. The mononuclear cells on the cementum of dental roots showed faint fluorescence.

The ED2-positive cells, as well as the ED1-positive cells, were located in the periodontal ligament on the distal side (Fig. 3), and the intense fluorescence of ED2 antibodies appeared in the mononuclear cells that were dispersed throughout the tissue. These cells were also seen around the small blood vessels. Faint fluorescence was often found in the multinuclear osteoclasts attached on the alveolar bone. On the proximal side, the distribution of ED2-positive cells was little different from that of the ED1-positive cells (Fig. 4).

Distribution of ED1- and ED2-positive cells in the treated teeth

On the pressure side of the treated teeth, the multinuclear osteoclasts increased in number on the surface of the alveolar bone, and almost all of the osteoclasts exhibited very intense fluorescence of ED1 antibodies in the ruffled borders (Fig. 5). Fluorescence was also observed in odontoclasts attached on the root cementum, but mononuclear positive cells distributed in the same manner as in the controls. On the tension side of the teeth, the blood vessels that were located adjacent to the surface of the bone were much wider in diameter, and a number of blood cells appeared in the lumen of the vessels (Fig. 6). The ED1 antibody fluorescence was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detected in some of the cells.

The appearance of ED2-positive cells on the pressure side of the treated teeth was different from that in the controls (Fig. 7). The number of ED2-positive cells decreased in the periodontal ligament and a few positive cells were found mainly surrounding the blood capillaries. On the tension side, the positive cells were seen only in some cells within the blood vessels in the region of the bone surface (Fig. 8).

Discussion

After the 5 day orthodontic treatment, the localization of ED1-positive mononuclear cells in the experimental teeth was little different from that in the control teeth. The appearance of ED2-positive mononuclear cells on the distal side of the controls and the pressure side of treated teeth was very different. While the positive cells were dispersed throughout the periodontium on the distal side of the controls, the number of ED2-positive cells decreased on the pressure side of treated teeth. As the rat molars moved physiologically towards the distal direction[10], the present results may reflect differences between a gentle remodeling of periodontium during the physiological movement and the rapid resorption of bone matrix by a number of osteoclasts in the experimental teeth.

The monoclonal ED1 antibody recognizes monocytes, macrophages, and dendritic cells[6]. The present study did not clarify which type of cell the antibody detected. However, comparing the appearance of ED1-positive cells with that of ED2-positive cells in the distal side of controls, most of the ED1-positive cells in the distal side of controls appear to be macrophages, as the ED2 antibody does not recognize monocytes and dendritic cells[6]. Then, although the localization of ED1-positive cells in the distal side of controls was very similar to that on the pressure side of experimental teeth, not all the positive cells in the pressure side of experimental teeth can be macrophages. As osteoclast numbers on the pressure side increased by the orthodontic treatment and as osteoclasts appear to be derived from monocyte/macrophage cell lineages[1,2], it seems quite possible that the ED1 antibodies also detect the osteoclast precursor cells which were recruited on the pressure side of the experimental teeth by the orthodontic

Figs. 1-4 CLSM images of fluorescent immunoreactivity in periodontium of control teeth. B, alveolar bone; P, periodontal ligament; R, dental root. Field of width 250 μm.
Fig. 1. Anti-monocyte/macrophage ED1 antibodies on the distal side. A faint fluorescence appears in the cytoplasm of a multinuclear osteoclast (arrowhead). Fig. 2. ED1 antibodies from the poximal side. Fig. 3. Anti-macrophage ED2 antibodies on the distal side. Mononuclear positive cells are dispersed throughout the periodontium. A faint fluorescence appears in the cytoplasm of a multinuclear osteoclast (arrowhead). Fig. 4. ED2 antibodies on the proximal side.

Figs. 5-8 CLSM images of experimental teeth after 5 days of orthodontic treatment. B, alveolar bone; P, periodontal ligament; R, dental root. Field of width 250 μm.
Fig. 5. ED1 antibodies on the pressure side. Positively stained mononuclear cells distribute throughout the periodontium. The ruffled border of osteoclasts (arrowhead) shows intense fluorescence. Fig. 6. ED1 antibodies on the tension side. Fig. 7. ED2 antibodies on the pressure side. Note the lower number of mononuclear positive cells in the periodontal ligament. Fig. 8. ED2 antibodies on the tension side.

treatment. This may be supported by our observation that ED2-positive cells decreased on the pressure side of experimental teeth where a large number of multinucleated osteoclasts appeared.

Osteoclasts and the precursor cells showed immunoreactivity to ED1 antibodies[7], but it is not known if there is antigen against ED2 antibodies in the cells. In the present study, a faint immunofluorescence of ED2 antibodies was found in a small number of osteoclasts using fluorescent immunocytochemistry and CLSM. The immunocytochemistry used here indicate that monoclonal ED2 antibodies may recognize a cell type which belongs to an osteoclast cell lineage, in addition to recognizing osteoclasts.

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