Changes in Location and Number of Tartrate-Resistant Acid Phosphatase (TRAP)-Positive Cells During the Development of Type II Collagen-Induced Arthritis in DBA/1J Mice

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Abstract: The authors investigated changes in the location and number of osteoclasts and their precursors during the development of articular lesions in type II collagen-induced arthritis in mice using tartrate-resistant acid phosphatase (TRAP) staining. The limb joints were examined at 6 to 15 weeks after the second immunization. The number of TRAP-positive cells increased as the articular lesions progressed. TRAP-positive macrophage-like cells were found in the hyperplastic synovial tissue and bone marrow stroma in the early stage. In the advanced stage, in addition to many TRAP-positive osteoclasts on the bone surface, TRAP-positive macrophage-like cells were observed in the pannus apart from the bone surface in the pannus-joint junctions. The above mentioned TRAP-positive macrophage-like cells are considered to be osteoclast precursors.

Key words: osteoclast, TRAP staining, type II collagen-induced arthritis in mice

Rheumatoid arthritis (RA) is one of the important autoimmune diseases. Although the pathogenesis of RA has not yet been clarified, one of the most important lesions of RA is destruction of the articular cartilage and marginal and subchondral bone in the affected joints [3]. In a severe case of RA, marked destruction of joints with juxtaarticular osteoporosis due to abnormal osteoclastic bone resorption is often observed [6, 15]. Therefore, the role of osteoclasts in the destruction of joints of RA patients has recently been attracting a great deal of attention.

Type II collagen-induced arthritis (CIA) in mice which have chronic proliferative synovitis similar to that detected in RA patients is now widely used as a RA model animal [16, 18]. The authors recently reported many similarities between CIA and RA in the dynamics of extracellular matrix components, plasma proteins and adhesion molecules in articular lesions, and also suggested that CIA might also be a useful model to clarify the mechanism of joint destruction in RA [18]. In this study, as the first step, the authors examined changes in the location and number of tar-
trate-resistant acid phosphatase (TRAP)-positive cells during the development of articular lesions in CIA. TRAP activity is a well-known marker of osteoclasts and their precursors [1].

Twenty-eight 6-week-old male DBA/1J mice (Charles River Japan Co., Kanagawa) were used. The animals were kept under controlled conditions (temperature, 23 ± 2°C, relative humidity, 55 ± 5%) in an isolator caging system (Niki Shoji Co., Tokyo) and fed pellets (MF, Oriental Yeast Co. Ltd., Tokyo) and water ad libitum.

Bovine type II collagen (CII) was dissolved in a 0.05 M acetic acid solution (Collagen Research Center, Tokyo) and the concentration was adjusted to 2 mg/ml. Equal volumes of the CII solution and complete Freund's adjuvant (Difco, Detroit, MI, USA) were emulsified and used as an immunogen.

At 7 weeks old, 20 mice (experimental group) were intradermally immunized with 0.1 ml of the above-mentioned emulsion at the root of the tail. The remaining 8 mice were injected with an emulsion of equal volumes of complete Freund's adjuvant and 0.05 M acetic acid solution and served as controls. Three weeks later, the second immunization was performed in the same way.

Five mice with CIA and two control mice were sacrificed by exanguination under ether anesthesia at 6, 9, 12 and 15 weeks after the second immunization. The limb joints were fixed in 4% periodate-lysine-paraformaldehyde (PLP) for 8 hr at 4°C and then decalcified with 10% EDTA 2K-glycerol solution for 3 weeks at −5°C [8]. After decalcification, the samples were washed with phosphate buffered saline (PBS) containing glycerol, and processed and embedded in paraffin by AmEx methods [14]. Four μm-paraffin sections were subjected to both hematoxylin and eosin (HE) staining and tartrate-resistant acid phosphatase (TRAP) staining for the detection of TRAP activity. The TRAP activity was detected according to the procedure with naphthol AS-BI phosphate (Sigma Chemical Co., St. Louis, MO, USA) containing 100 mM L(+)-tartaric acid as substrate and hexazotized pararosaniline (Sigma Chemical Co.) as coupler [2]. The sections were counterstained with hematoxylin.

Clinical onset of arthritis was first detected around 5 weeks after the second immunization. Although the time of onset varied among individuals, the number of affected joints in each mouse increased with time. In the affected joints, erythema and swelling persisted for a long time, and finally they disappeared, leaving dyskinesia due to ankylosis.

Although the degree and developmental stage of articular lesions varied somewhat among individuals as well as among joints in each mouse, the common sequence of changes in the location and number of TRAP-positive cells was as follows. The number of TRAP-positive cells increased with the development of articular lesions. TRAP-positive cells were observed in both hyperplastic synovial tissue and bone marrow stroma in the early stage when a prominent proliferation of synovial and bone marrow stromal cells was seen, and they were also observed in pannus-joint junctions in the advanced stage when severe destruction of marginal and subchondral bones was seen. On the other hand, in the joints of control mice, a small number of large multinucleated TRAP-positive cells, i.e., osteoclasts, were seen beneath the growth plate and on the bone surface within the bone marrow cavity.

Interestingly, hyperplastic changes were observed not only in the synovial tissue (Fig. 1a) but also in the bone marrow cavity (Fig. 2a) in the early stage. Round- or polygonal-shaped macrophage-like cells positive for TRAP were found in the proliferative synovial tissue (Fig. 1b) and in the proliferative foci of spindle-shaped bone marrow stromal cells (Fig. 2b).

In the advanced stage of CIA, pannus invaded the bone marrow cavity (Fig. 3a) from the articular cartilage as well as from the periosteum. On the bone surface, there were many large multinucleated TRAP-positive cells, i.e., osteoclasts (Fig. 3b), suggesting that an active bone resorption occurred. In addition, in the invaded pannus apart from the bone surface, there were also many TRAP-positive macrophage-like cells (Fig. 3b) which were similar to those seen in the proliferative synovial tissue and bone marrow stroma, so that it was clarified in the present study that bone destruction in CIA was brought about by both pannus invasion and osteoclastic bone resorption.

Osteoclasts have generally been considered to originate in hematopoietic stem cells. Recent studies have clearly indicated that peripheral monocytes and tissue macrophages also have the potency to differentiate into osteoclast-like cells having a bone resorption function under specific in vitro condition [5, 10–12, 19, 22]. Bone marrow stromal cells are known to produce some cytokines related to the differentiation of osteoclasts.
Fig. 1. Hyperplastic synovial tissue of knee joint in mice with CIA. Proliferation of macrophage-like cells in the synovial lining layer and fibroblast-like cells in the synovial sublining layer (a). A small number of TRAP-positive cells are seen in the border between the two layers (b). (a) HE staining, x 150, (b) TRAP staining, x 270.

Fig. 2. Bone marrow cavity of knee joint in mice with CIA. Proliferation of spindle-shaped stromal cells (a). Round-, polygonal- and spindle-shaped TRAP-positive cells are seen intermingled with stromal cells. (a) HE staining, x 220, (b) TRAP staining, x 180.

Fig. 3. Pannus-joint junction of carpal joint in mice with CIA. Pannus has invaded the bone marrow cavity (a). Many TRAP-positive cells are seen on the destroyed bone surface and in the pannus apart from the bone surface (b). (a) HE staining, x 70, (b) TRAP staining, x 140.
Macrophage colony stimulating factor (M-CSF) is considered to play an important role in the induction of osteoclasts, and M-CSF is known to be produced by osteoblasts under physiological conditions [17]. Moreover, Fujikawa et al. revealed that synovial macrophages were capable of differentiating into osteoclast-like cells which had the ability of bone resorption when co-cultured with rat osteoblast-like cells (UMR106) and added with 1, 25-dihydroxy vitamin D₃ and recombinant human M-CSF (rhM-CSF) in this system [4]. In addition, IL-6 is also known to play an important role in the differentiation and activation of osteoclasts [21], proliferation of synovial fibroblast-like cells [7], and induction of infiltration of inflammatory cells by activation of endothelial cells [13]. In addition, it has been reported that bone marrow stromal cells migrate into the joint cavity through the canal and contribute to synovial cell proliferation [9] and that synovial fibroblast-like cells induce differentiation of macrophage-like cells into osteoclasts in vitro [20]. From these reports and the above mentioned findings in the present study, TRAP-positive macrophage-like cells found in the proliferative synovial tissue, bone marrow stroma and pannus in CIA seem to be osteoclast precursors.

Besides the above mentioned large multinucleated osteoclasts and macrophage-like osteoclast precursor cells, there were also spindle-shaped TRAP-positive cells in the proliferative synovial tissue (Fig. 1b), bone marrow stroma (Fig. 2b) and pannus (Fig. 3b). The nature of these cells is still obscure, and further studies should be done to clarify this. In the near future further studies will be carried out to obtain more detailed data on osteoclast precursors and factors related to osteoclast differentiation in mice with CIA.

References