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Original Article

T cell and periosteum cooperation in osteoclastogenesis induced by lipopolysaccharide injection in transplanted mouse tibia

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Abstract *Background/purpose:* We previously reported that injedctions of lipopolysaccharide (LPS) into the gingiva of mice induce inflammatory bone resorption that actively involved T cells. Receptor activator of NF-κB ligand (RANKL), which is an essential factor for osteoclastogenesis, was reportedly produced by osteoblasts, fibroblasts, and T cells in vitro; however, it has not been established which cells affect osteoclastogenesis in vivo. Here we determined the roles of T cells and the periosteum on osteoclastogenesis in LPS-induced inflammatory bone resorption.

Materials and methods: Thirty-five BALB/c (wild-type: WT) and 10 BALB/c-nu/nu (nude: Nu) mice congenitally lacking T cells were used. Using inbred WT mice, tibias were transplanted with and without the periostea [(+) and (−), respectively, n = 15 per group] into the dorsal subcutaneous connective tissue of WT or Nu mice. Each group received four injections around the transplanted site: experimental groups were injected with LPS, and control groups were injected with phosphate-buffered saline. Isolated tissues were prepared for histopathological observation of the transplanted bone surface.

Results: Many infiltrating inflammatory cells were present near the surface of the tibias in the LPS-injected groups. Only the WT (+) LPS group showed osteoclasts. The number of mononuclear preosteoclasts and RANKL-positive cells was highest in the WT (+) LPS group, and there were no significant differences among the other three groups.

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Conclusion: T cells and the periosteum are closely involved in osteoclastogenesis in inflammatory bone resorption in vivo.

Introduction

Periodontitis is accompanied by attachment loss and alveolar bone resorption. We previously established an inflammatory bone resorption model using injections of lipopolysaccharide (LPS), which is the structural component of the cell wall of gram negative bacteria, into the gingiva of mice.[1] Using this model, we elucidated that osteoclasts appeared much later in nude (Nu) mice congenitally lacking T cells than in wild-type (WT) mice. Furthermore, osteoclastogenesis occurred earlier when T cells were reconstituted in Nu mice. Thus, T cells actively promote inflammatory bone resorption. Osteoclastogenesis requires receptor activator of NF-κB ligand (RANKL), which is a membrane-bound protein belonging to the tumor necrosis factor superfamily, as an essential factor.[2,3] While there have been authors who have reported that T cells produce soluble RANKL and promote osteoclastogenesis,[4,5] others have reported that RANKL produced by T cells has no effect on osteoclastogenesis.[6] The functional mechanism of T cells accelerating osteoclastogenesis has not been clarified in vivo. Osteoblasts existing on the bone surface in the periosteum have been reported to induce osteoclastogenesis by producing RANKL not only in physiological bone remodeling but also under inflammatory conditions.[7,8] In addition, connective tissue fibroblasts have been reported to induce osteoclastogenesis by expressing RANKL on the cell surface.[9] However, it is not known which cells affect osteoclastogenesis in vivo and whether T cells affect osteoblasts and/or fibroblasts in vivo.

Therefore, the present study examined the effects of T cells and the periosteum on osteoclastogenesis in LPS-induced inflammatory bone resorption. We transplanted inbred tibias with or without periostea into the dorsal subcutaneous connective tissue of WT and Nu mice, induced inflammatory bone resorption on the bone surface using LPS injections around the transplanted bone, and analyzed the histopathology. The results showed that multinuclear osteoclasts can be detected when both T cells and the periosteum were present but not when only the periosteum or T cells were present.

Materials and methods

Mice

Thirty-five 8-week-old male BALB/c (WT) and 10 BALB/c-nu/nu (Nu) mice were purchased from Nippon CLEA (Tokyo, Japan). The mice were maintained under specific pathogen-free conditions in Biomedical Research Center, Center for Frontier Life Sciences, Nagasaki University. The experimental procedures followed the ARRIVE guidelines, the National Institutes of Health guide for the care and use of Laboratory animals, as well as the Guidelines for Animal Experimentation of Nagasaki University. The experimental protocol was approved by the Local Institutional Animal Care and Use Committee of Nagasaki University.

Bone transplantation

The information of animal experimental schedule (date), groups, and sample size(n) was as follows (Fig. 1A). Both tibias of 15 WT mice were used for bone transplantation as follows: mice were sacrificed under ether anesthesia, and tibias were carefully removed. Fifteen tibias were used with the periosteum (+), and the other 15 tibias had the periosteum removed (−). The muscles around the (+) tibia were removed using scissors, and the periosteum was left intact. The muscle and the periosteum around the (−) tibia were completely removed using a scalpel. All processes were performed under aseptic conditions.

For bone transplantation, mice were intraperitoneally injected with combined anesthesia of medetomidine hydrochloride (0.75 mg/kg), midazolam (4 mg/kg), and butorphanol tartrate (5 mg/kg).[10] The dorsal skin of the WT mice was shaved prior to transplantation. After administering anesthesia, a 1-cm-long incision was made using scissors on the dorsal skin of all mice. Through the incision, subcutaneous connective tissue was detached from the fascia using a stripper. Thereafter, a (+) tibia was transplanted using tweezers to subcutaneous connective tissue in 10 WT mice and five Nu mice. In the remaining five WT and Nu mice, a (−) tibia was transplanted using the same method. There were four groups of mice: WT (+), WT (−), Nu (+), and Nu (−). Finally, the incisions were closed and sutured using nylon thread.

Five micrograms of *Escherichia coli* LPS (*E. coli* O111: B4; Sigma−Aldrich Corp., St Louis, MO, USA) dissolved in 3 μL of phosphate-buffered saline (PBS) was prepared for injection. LPS injections were administered as previously described.[1] Five mice from the each of the WT (+), WT (−), Nu (+), and Nu (−) groups received four injections of LPS at the area where the tibia was transplanted at 48-h intervals under ether anesthesia, thus creating the WT (+) LPS, WT (−) LPS, Nu(+) LPS, and Nu(−) LPS groups. The remaining 10 WT mice were injected with 3 μL of PBS as controls, forming the WT (+) PBS and WT (−) PBS groups. The PBS injections had not induced inflammatory cell infiltration and changed bone surface condition in the WT (+) PBS and WT (−) PBS groups. For this reason, we did not prepare the Nu (+) PBS and Nu (−) PBS groups. Four injections were used because our previous study indicated the appearance of tartrate-resistant acid phosphatase (TRAP)-positive

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