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Protective effect of p38 MAPK inhibitor on wear debris-induced inflammatory osteolysis through downregulating RANK/RANKL in a mouse model

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ABSTRACT. Aseptic loosening associated with wear particle-induced inflammation is a major cause of joint implant failure. Recent studies have demonstrated the therapeutic effects of p38 mitogen-activated protein kinase (MAPK)-based therapies on chronic inflammatory diseases. The purpose of this study was to investigate whether SB203580, a p38 MAPK inhibitor, inhibits wear debris-induced inflammatory osteolysis in mice through downregulation of receptor activator of nuclear factor kβ (RANK)/RANK ligand (RANKL). We used a murine osteolysis model to study the effect of SB203580 on RANKL/RANK signaling and titanium particle-induced osteolysis *in vivo*. Pouch membranes

with intact bone implants were analyzed using histological analysis and transmission electron microscopy, and the levels of RANK and RANKL protein and mRNA were evaluated by immunohistological staining and real-time reverse transcriptase-polymerase chain reaction. SB203580 had less of an effect on RANK and RANKL expression under wear debris-induced conditions. The number of TRAP-positive cells was remarkably reduced in Ti-particle-induced pouch tissues. These effects were confirmed through the transmission electron microscopy results. These results suggest that p38 MAPK-based therapies are beneficial in preventing aseptic loosening associated with total joint replacement by modulating RANK-RANKL signaling.

Key words: Titanium particles; p38 mitogen-activated protein kinase; Inflammatory; Osteolysis; Receptor activators of nuclear factor-kβ

INTRODUCTION

Total joint replacement (TJA) is an effective operation for treating end-stage degenerative arthritis, post-traumatic arthritis, and other types of disabling arthritis (Koulouvaris et al., 2008; Chiu et al., 2010). Despite improvements in prosthesis biomaterials, implant design, and surgical techniques, aseptic loosening secondary to prosthetic osteolysis is a major complication of total joint arthroplasty (Bozic et al., 2009). Osteolysis is initiated by aseptic local inflammation associated with wear debris particles, leading to osteoclastic periprosthetic tissue destruction and bone resorption (Harris, 2001); however, the precise mechanism underlying the development of osteolysis in such cases remains unclear. A recent approach has focused on understanding and manipulating wear debris-induced inflammatory osteolysis at the molecular level by pharmacological intervention (Purdue et al., 2007). Previous studies found that phagocytic macrophages in the tissue surrounding the prosthetic site engulf small wear particles, resulting in high levels of cytokine secretion, osteoclastogenesis, activation, and subsequent bone resorption (Purdue et al., 2007; Minoda et al., 2009). Moreover, small wear particles stimulate fibroblasts, macrophages, and foreign body giant cells in the interfacial membranes, resulting in the secretion of high levels of proinflammatory cytokines and other factors, such as interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)-α, metalloproteinases (MMPs), and prostaglandin (PG)E_2. These factors directly and/or indirectly stimulate osteoclastogenesis and ostoclastic bone resorption (Shanbhag et al., 1995; Merkel et al., 1999).

Studies reported that periprosthetic osteolysis is initiated by activation of the receptor activator of nuclear factor-kβ (RANK) and nuclear factor-kβ ligand (RANKL) signaling pathway (Clohisy et al., 2003, 2004; Ren et al., 2006a). Binding of RANK, a membrane-bound factor expressed on the surface of macrophages, to its ligand RANKL promotes the differentiation of cells into mature and functional osteoclasts (Yasuda et al., 1998; Holding et al., 2006; Valles et al., 2008). Therefore, the activated RANK/RANKL receptor complex is an important factor in osteoclastogenesis, leading to bone resorption near the prosthetic site (Purdue et al., 2007).

Several studies have shown that p38 mitogen-activated protein kinase (MAPK), an intracellular signal-transducing molecule, is responsible for regulating a number of inflammatory responses, such as the high expression of pro-inflammatory cytokines, leukocyte adhe-

sion, and chemotaxis (New and Han, 1998). p38 MAPK activation may play an important role in inflammatory osteolysis (Rakshit et al., 2006). These studies have shown that wear particles initiate an increase in RANK/RANKL gene expression in a murine osteolysis model (Yoshiji et al., 2000). However, the relationship between p38 MAPK and RANK/RANKL biological signaling during inflammatory osteoclastogenesis remains unclear. In the current study, we showed that treatment with SB203580, a p38 MAPK inhibitor, effectively alleviated wear debris-induced inflammatory osteolysis (Chen et al., 2012). p38 MAPK signaling may be actively involved in the process of wear debris-induced inflammatory osteolysis. The purpose of this study was to test the hypothesis that amelioration of wear debris-induced inflammatory osteolysis by treatment with p38 MAPK inhibitor occurs through the downregulation of RANK/RANKL in a murine osteolysis model.

MATERIAL AND METHODS

Titanium particles

Commercially prepared titanium (Ti) particles (diameter <20 μm) were obtained from Alfa Aesar (Ward Hill, MA, USA). Scanning electron microscope (Hitachi FESEM S-4800; Hitachi; Kyoto, Japan) analysis was used to confirm particle size and morphological features (Figure 1). The particles were sterilized by heating at 180°C for 6 h, followed by treatment with 70% ethanol for 48 h to remove bound endotoxin. The particles were then washed 3 times in sterile phosphate-buffered saline (PBS), suspended in sterile PBS at a concentration of 5% (w/v), and stored at 4°C until use. A Limulus assay kit was used to determine the endotoxin level of the particles in accordance with the manufacturer instructions.

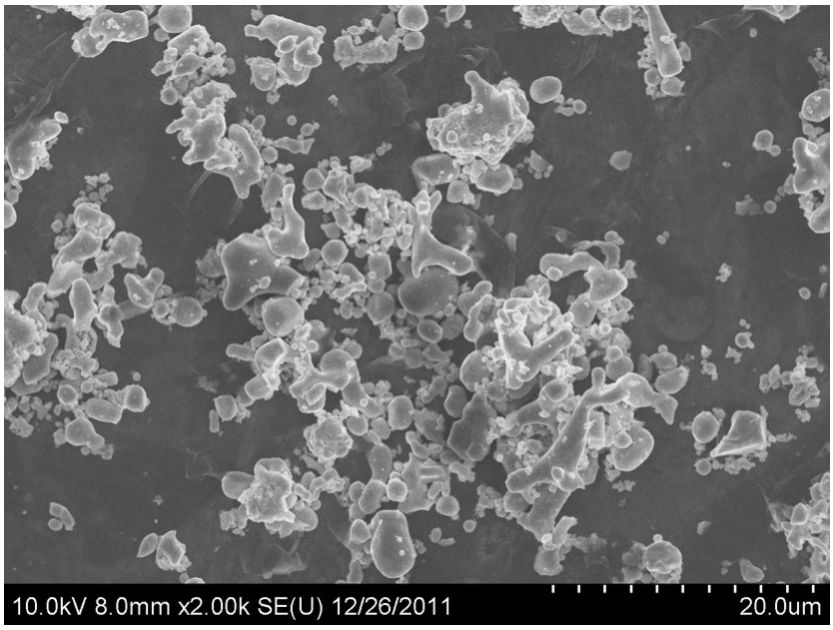

Figure 1. Scanning election microscopy appearance of titanium particles (magnification 2000X).

Experimental animals

All animal procedures were conducted in accordance with the principles and procedures approved by the Institutional Animal Care and Use Committee of Shanghai Jiaotong University in China. Female BALB/c mice, aged 8-10 weeks, were obtained from the Shanghai Laboratory Animal Centre (Chinese Academy of Sciences). All mice weighed 20-24 g at the beginning of the study. A total of 45 mice were randomized into 3 groups of 15 mice each: control group, Ti group, and Ti + p38 MAPK inhibitor group.

Murine osteolysis model and drug treatment

Previously described techniques were used to establish a model of debris-induced osteolysis with bone-implanted inflammatory air pouches (Ren et al., 2006a). To establish the air pouches, a 2 x 2-cm section of dorsal skin was shaved and sterilized, and 2 mL sterile air was injected subcutaneously. To maintain the pouch, 0.5 mL sterile air was injected into the pouch each day for 6 days. Mice were then anesthetized by intraperitoneal injection of 50 mg/kg pentobarbital and a 0.5-cm incision was made overlying the pouch; a section of the calvarial bone (approximately 0.4 x 0.25 cm) from a genetically identical donor mouse was inserted into the pouch. Pouch layers and the skin incision were closed using 4-0 Prolene sutures. On the following day, the pouches were injected with 0.3 mL suspension containing 5% (w/v) Ti or PBS alone (control). SB203580 was then injected intraperitoneally at a dose of 0.1 mg/kg daily (Ti + p38 MAPK inhibitor group), while the control and Ti group mice received an intraperitoneal injection of 0.1 mL sterile PBS. Injections were continued daily for 14 days, after which the pouch membranes containing implanted bone were harvested for histological and molecular analyses.

Gene expression analysis for RANK and RANKL

Total RNA was extracted from pouch tissue using TRIzol Reagent (Invitrogen; Carlsbad, CA, USA) according to manufacturer instructions, and cDNA was synthesized from total RNA using the SYBR Prime Script™ reverse transcription-polymerase chain reaction (RT-PCR) kit (Takara Bio Inc.; Shiga, Japan). The following primers were used in this study: RANK, 5'-CTGCCTCTGGGAACGTGACT-3' (forward) and 5'-GCGAGGTCTGGCTGACATAC-3' (reverse); RANKL, 5'-CAGCATCGCTCTGTTCCTGTA-3' (forward) and 5'-CTGCGTTTTC ATGGAGTCTCA-3' (reverse). The housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was used as an internal control; the sequences used for *GAPDH* were as follows: 5'-CCAATGTGTCCGTCGTGGAT-3' (forward) and 5'-TGCTGTTGAAGTCGCAGGAG-3' (reverse).

Quantitative real-time RT-PCR was performed to quantify the relative mRNA levels of RANK and RANKL using the SYBR Prime Script™ RT-PCR kit and an ABI7500 real-time thermal cycler (Applied Biosystems; Foster City, CA, USA) according to manufacturer instructions. Gene-specific primers for RANK and RANKL were designed using the Primer 5.0 software (Premier Biosoft International; Palo Alto, CA, USA). The cycling program involved preliminary denaturation at 95°C for 2 min, followed by 40 cycles of denaturation at 95°C for 15 s, and annealing at 60°C for 1 min. To determine the relative level of gene expression, the comparative threshold cycle (C_T) method was used. Gene activity in the control (PBS) group

was arbitrarily assigned as 1 to serve as a reference. The expression of the target gene in the experimental groups represents the fold-difference in expression relative to the reference gene expression.

Immunohistological staining for RANK and RANKL

Tissue sections from air pouches were embedded in paraffin and stored until use. Tissue sections were then deparaffinized in xylene and rehydrated in ethanol. The rinsed sections were immersed in antigen-retrieval buffer containing ethelynediaminetetraacetic acid (EDTA) in a 97°C water bath for 30 min. Samples were then allowed to cool to room temperature before pre-incubation for 10 min with 0.3% (v/v) hydrogen peroxide in methanol to suppress the activity of endogenous peroxidases. Sections were blocked with 1.5% normal goat serum for 30 min. Subsequently, tissue sections were incubated with mouse anti-RANK and mouse anti-RANKL primary antibodies (Abcam; Cambridge, MA, USA) at 2 μg/mL overnight in a moisturized chamber at 4°C. After extensive rinsing, the sections were incubated for 30 min with a biotin-conjugated secondary antibody (Sigma-Aldrich; St. Louis, MO, USA) at 37°C. Streptavidin-horseradish peroxidase conjugate (SA-HRP) was applied to sections for 30 min at 37°C and then 3,3'-diaminobenzidine tetrahydrochloride (DAB) was added for color development with counterstaining using hematoxylin. Digital photomicrographs were obtained and analyzed using the Image-Pro image analysis software, version 6.0 (Media Cybernetics, Inc.; Bethesda, MD, USA). Reproducibility of multiple sections from the same site was checked through blind analysis of duplicates.

Histological evaluation and image analysis

Tissue samples were fixed in 4% polyoxymethylene, pH 7.4, for 24 h. After decalcification in 10% EDTA, tissue specimens were processed for dehydration in graded alcohols, cleared in dimethyl benzene, and embedded in paraffin. Six micrometer-thick tissue sections were stained with hematoxylin and eosin (H&E) to evaluate pouch membrane inflammation and implant bone erosion. Stained sections were examined under a light microscope (Olympus DP70; Olympus Optical Co., Tokyo, Japan) and digital photomicrographs were captured and analyzed using a computerized image analysis system with the Image-Pro Plus software. Pouch membrane thickness and the total number of infiltrated cells were measured using digital image analysis. Four sections per specimen were analyzed in a blinded manner. Pouch membrane thickness was measured at 6 points on each section. The total number of cells (cells/mm^2) was based on the nucleus count and was analyzed as described previously (Wooley et al., 2002).

Evaluation of osteoclastogenesis

Histochemical tartrate-resistant acid phosphatase (TRAP; Sigma-Aldrich) activity was used to identify the osteoclast-like cells in the pouch tissue (Suzuki et al., 1998). The tissue sections were incubated and stained for TRAP expression according to manufacturer instructions. The presence of dark purple-staining granules in the cytoplasm, as observed by light microscopy, was the specific criterion for TRAP-positive cells. Positive TRAP localization was quantified by conducting a pixel area count and reported as a percentage of the total implanted bone area in the pouch tissue with the Image-Pro Plus software, version 6.0.

Determination of osteolysis by transmission electron microscopy (TEM)

Tissue samples were fixed for 1 h in Karnovsky solution (4% paraformaldehyde, 2.5% glutaraldehyde) and rinsed in 0.2 M sodium cacodylate buffer, pH 7.4. After decalcification in 10% EDTA, samples were post-fixed for 1 h in osmium tetroxide, diluted in 0.2 M sodium cacodylate buffer, dehydrated in a graded ethanol series, and left overnight in a 1:1 mixture of absolute ethanol and Epon. On the following day, the samples were embedded in Epon Araldite and incubated at 60°C for 1 day. Semithin sections were cut perpendicularly to the sample layers using a diamond knife. Since it was not possible to obtain sections through the Ti particles and bones, we observed the overall structural organization of the implanted bone and the pouches of neighboring particles. Sections were mounted on glass slides, stained with methylene blue (Azur III), and examined under a light microscope for orientation. Ultra-thin sections were prepared, collected on copper grids, and stained with 5% uranyl acetate in water for 4 min and with lead citrate for 2 min. The sections were then examined under a transmission electron microscope (Hitachi H-7650; Hitachi; Kyoto, Japan).

Statistical analysis

Statistical analysis among groups was performed with the SPSS statistical package (version 11.0, SPSS, Inc.; Chicago, IL, USA). Values are reported as mean and standard deviation (SD). Differences between groups in the mean values of variables of experimental parameters were analyzed using analysis of variance. A P value of less than 0.05 was considered to be statistically significant.

RESULTS

Animal health

The mice in this experiment tolerated both the operative procedures and the p38 MAPK inhibitor treatments well. No mice were excluded from this study because of weight loss, pouch infection, or drug toxicity as determined by clinical observation and morphological analysis.

Gene expression analysis of RANK and RANKL in murine pouch tissues

mRNA expression of RANK and RANKL in the pouch tissues was determined by real-time RT-PCR. As shown in Figure 2, Ti particle stimulation significantly increased the expression of both RANK and RANKL gene transcripts compared with the PBS control ($P < 0.05$). By contrast, treatment with the p38 MAPK inhibitor significantly reduced the level of RANK and RANKL gene transcripts ($P < 0.05$).

Immunohistochemical staining of RANK and RANKL in murine pouch tissues

Immunohistochemical staining, as depicted in Figure 3, revealed an increase in the percentages of RANK- and RANKL-positive cells in Ti particle-containing calvaria-implanted pouches compared with the percentages for the PBS controls ($P < 0.05$). By contrast, SB203580

treatment reduced the percentage of RANK- and RANKL-positive cells. RANKL staining was predominantly observed at the interface between the air pouch membrane and the implanted calvaria bones, and was reduced by p38 MAPK inhibitor treatment. RANKL stimulates osteoclastogenesis through its membrane receptor RANK by RANK/RANKL signaling; thus, RANK/RANKL signaling appears to be critical for osteoclastogenesis (Dougall et al., 1999). These data showed that p38 MAPK inhibition reduced RANK and RANKL expression in Ti particle-stimulated calvaria-implanted pouches via the p38 MAPK signal transduction pathway.

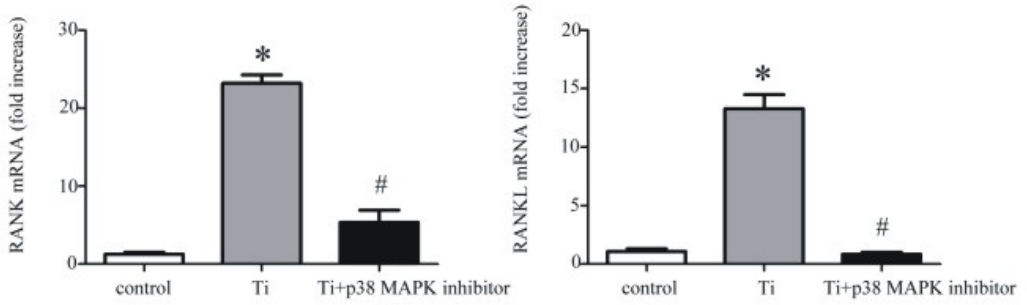

Figure 2. Real-time RT-PCR analysis of the RANK and RANKL expression in a murine pouch with calvaria implantation. (**A**) Control, (**B**) Ti, and (**C**) Ti + p38 MAPK inhibitor groups. Increased expression of RANK and RANKL was observed in Ti particle-stimulated calvaria-implanted pouches compared to that in PBS controls. This effect was significantly reduced by p38 MAPK inhibitor treatment as determined by analysis of variance. *$P < 0.05$, #$P < 0.05$ compared with the control group.

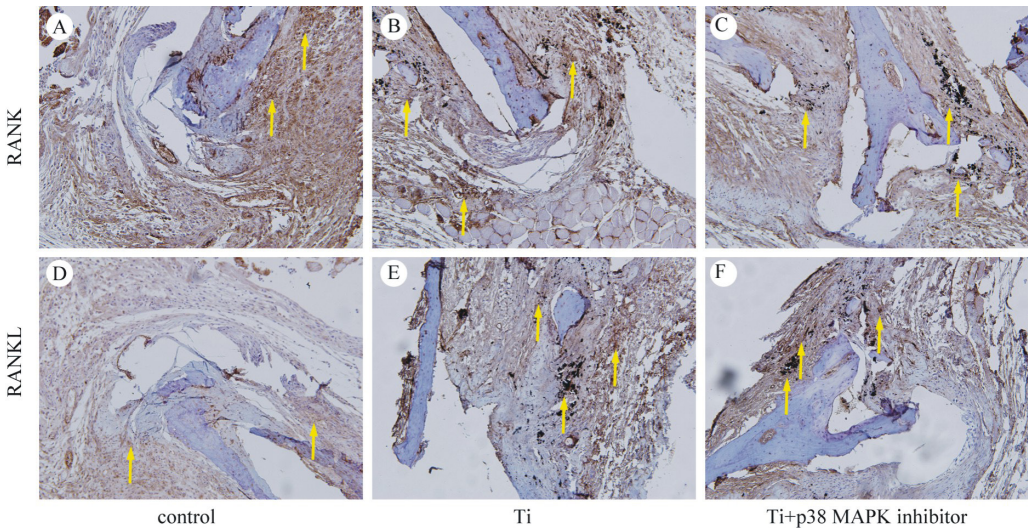

Figure 3. Immunohistochemical detection of RANK and RANKL in the murine osteolysis model RANK expression in pouches with calvaria implantation in the control (**A**), Ti (**B**), and Ti+ p38 MAPK inhibitor groups (**C**). RANKL expression in the control (**D**), Ti (**E**), and Ti + p38 MAPK inhibitor groups (**F**). Ti particles stimulated an increase in RANK and RANKL immunohistochemical-positive cells, respectively, while p38 MAPK inhibitor reduced these responses (original magnification, 200X).

Analysis of implanted bone morphological features

Air pouch membrane proliferation and cellular infiltration increased with biomaterial particle-stimulated tissue inflammation (Ren et al., 2003, 2006b). As shown in Figure 4, bone morphological features remained nearly entirely intact in the PBS controls, while bone erosion markedly increased in pouches containing Ti particles, and erosion pits were observed in the bone-membrane interface region. p38 MAPK inhibitor treatment significantly suppressed these effects in Ti particle-stimulated bone-implanted pouches. Membrane thickness and frequent occurrence of infiltrated cells in the Ti particle-stimulated calvaria-implanted pouches were significantly higher than those in PBS controls ($P < 0.05$). However, these effects were significantly decreased by p38 MAPK inhibitor treatment ($P < 0.05$).

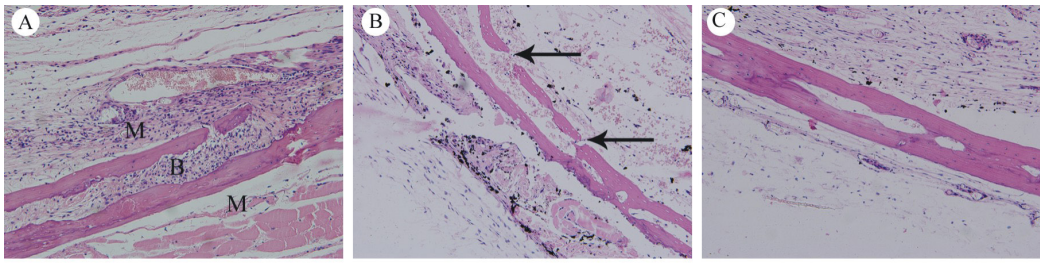

Figure 4. Morphological appearance of pouches of bone-implanted after staining with hematoxylin and eosin. Air pouch membrane proliferation and cellular infiltration increased with Ti stimulation in tissues. (**A**) Pouches with calvaria implantation in the control group. (**B**) Pouches with calvaria implantation in the Ti group. The arrows indicate bone erosion pits on the bone surface. (**C**) Pouches with calvaria implantation in the Ti+ p38 MAPK inhibitor group. B indicates the implanted bone and M indicates the pouch membrane (original magnification, 200X).

Therapeutic effects of p38 MAPK inhibitor on Ti particle-induced osteoclastogenesis

TRAP-stained photomicrographs were used to detect whether downregulation of RANK and RANKL by p38 MAPK inhibitor treatment decreased the number of osteoclasts in calvaria-implanted pouches. As shown in Figure 5, discrete foci of dark purple TRAP-positive cells were observed at the interface between implanted calvaria and pouch membranes in pouches without Ti particle stimulation. Bone morphological features remained intact and no osteoclastic bone resorption was observed. Intense TRAP staining was observed in Ti particle-stimulated calvaria-implanted pouches, and TRAP-positive cells extended into adjoining areas. Regions in which TRAP-positive cells localized were often pitted, suggesting active osteoclastic bone resorption. p38 MAPK inhibitor treatment significantly reduced the number of TRAP-positive cells and bone resorption lacunae were remarkably reduced.

Determination of osteolysis by TEM

TEM allows visualization of the ultrastructural organization of cells in implanted bones and pouches near the Ti particles. TEM observations showed osteoclasts surrounded

by an extracellular matrix composed of densely packed collagen cut in either transverse or longitudinal sections. The unmineralized osteoid matrix and the mineralized bone matrix were separated by a mineralized front. At higher magnification, needle-shaped crystals close to the mineralization front, corresponding to bone nucleation sites, were observed. In addition, mineralized foci were found to be dispersed in the collagenous matrix (Figure 6A-C).

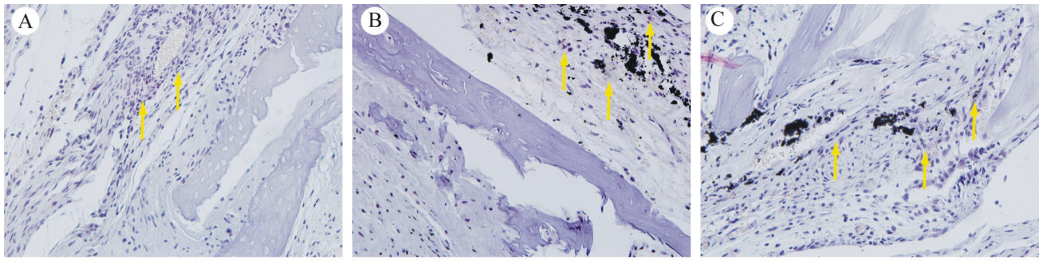

Figure 5. Representative TRAP staining in a murine pouch with calvaria implantation. (**A**) A calvaria-containing pouch with PBS injection. (**B**) A bone pouch with Ti particle stimulation. Extensive dark purple staining was observed with deposits of osteoclast-like cells along the pouch membranes. (**C**) A bone pouch for Ti particles + p38 MAPK inhibitor treatment (original magnification, 400X).

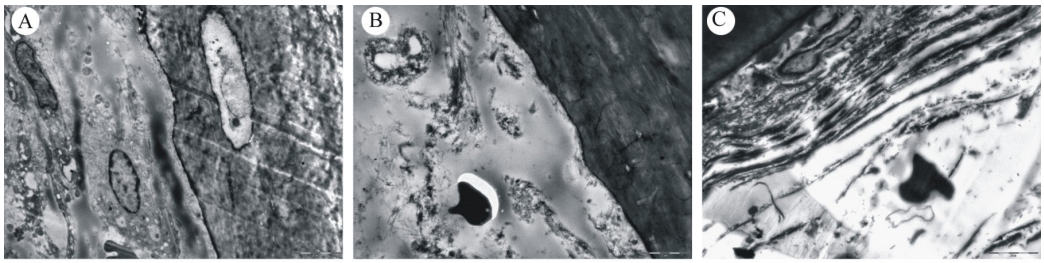

Figure 6. Transmission electron microscopy (TEM) appearance of cells in implanted bones and pouches around the Ti particles (magnification, 10,000X). (**A**) Control, (**B**) Ti, and (**C**) Ti + p38 MAPK inhibitor group.

DISCUSSION

This study showed that the wear debris-induced inflammatory osteolysis in the murine model was effectively regulated by the p38 MAPK signal transduction pathway. Stimulation of inflammatory factors by Ti particles in pouch tissue was markedly inhibited by p38 MAPK inhibitor treatment. Expression of RANK and RANKL at the mRNA and protein levels was remarkably increased following stimulation with Ti particles and was subsequently reduced by p38 MAPK inhibitor treatment. These findings indicate that wear debris-induced inflammatory osteolysis is significantly ameliorated by p38 MAPK inhibitor treatment and that this occurs through downregulation of RANK and RANKL in a murine osteolysis model.

Inflammatory osteolysis is the most important factor limiting the longevity of total joint arthroplasty. Although the precise mechanism is unknown, wear debris-stimulated per-

sistent inflammation, osteoclastogenesis, and osteoclastic bone resorption are crucial events surrounding prosthetic osteolysis. Inflammatory osteolysis may result from the biological reaction to wear debris from implant materials such as metal, polyethylene, ceramics, and polymethylmethacrylates (Purdue et al., 2007), and may be related to particle size, particle amount, and duration of implantation. Wear particle size is associated with the implant materials used, which are typically produced from Ti and are less than 3 μm in size (Doorn et al., 1996). Small wear particles are easily phagocytosed but cannot be digested. Particle size and phagocytosis are associated with adverse biological reactions, such as macrophage activation. Macrophages in the tissues around the implant site engulf wear particles, resulting in activated cells that secrete high levels of inflammatory and potentially osteolytic cytokines, including TNF-α and interleukin (IL)-1β, both of which promote osteoclastogenesis and eventually initiate the osteolytic process (Zhou et al., 2010). In a phagocyte-depletion murine model, TNF-α and IL-1β expression significantly decreased, as did the number of TRAP-positive cells in pouch tissues (Ren et al., 2008). Osteoclasts play a direct and central role in wear debris-induced bone resorption (Taki et al., 2007). Previous studies have demonstrated that TNF-α and IL-1β are important mediators of osteoclastogenesis and are targets for therapeutic agents intended to treat and prevent wear debris-induced bone resorption.

Inflammatory osteolysis is a relatively frequent and incapacitating complication of rheumatoid arthritis and multiple other inflammation-associated bone diseases. It is thought to operate through a common pathway of accelerated osteoclast recruitment and activation under the control of cytokines produced in the inflammatory environment (Wei and Siegal, 2008).

Osteoclasts are multinucleated cells formed through the fusion of mononuclear precursors of the monocyte/macrophage lineage and are influenced by the specific osteoclastogenic cytokine RANKL and macrophage colony-stimulating factor (M-CSF) (Teitelbaum, 2000). RANKL, a member of the TNF superfamily, is a membrane-bound homotrimeric protein found on the surface of mesenchymal cells of osteoblast lineage that exerts its effects by recognizing its receptor, RANK, on marrow macrophages, thereby activating them to assume the osteoclast phenotype (Teitelbaum, 2000). Production of RANKL is enhanced by osteoclast-stimulating agents such as the parathyroid hormone (Lee and Lorenzo, 1999). Because RANKL is the critical cytokine stimulating osteoclast differentiation, analyzing the intracellular signals that follow RANK activation is of considerable interest. Known signaling events arising from ligation of RANK are similar to those of other members of the TNF receptor superfamily. RANKL activates NF-kβ signaling, the PI3K/Akt axis, and the 3 MAPK pathways, including the extracellular signal-regulated kinase (ERK), c-Jun NH_2-terminal kinase, and p38 pathways (Wei et al., 2001, 2002). While the Akt signaling pathway is crucial for the survival of precursor and mature osteoclasts, the 2 events in RANK signaling critical for osteoclast differentiation are activation of both the NF-kB complex and transcription factor AP-1, a dimer composed of the c-Fos and c-Jun proteins, as demonstrated by gene-targeting studies (Franzoso et al., 1997; Teitelbaum and Ross, 2003).

Notably, p38 MAPK plays a crucial role in mediating osteoclastogenesis under inflammatory conditions. Activation of p38 MAPK occurs within synoviocytes embedded in both the synovial lining layer and in juxtaposition to endothelial cells and is induced chiefly by proinflammatory cytokines such as TNF-α and IL-1 (Schett et al. 2000). p38 MAPK, most notably its p38a isoform, is activated mainly within cells involved in the inflammatory process (Herlaar and Brown, 1999). Activation of p38 induces the synthesis of key inflammatory

mediators such as TNF-α, IL-1, IL-6, IL-8, and cyclooxygenase-2, either via direct activation of gene transcription or via mRNA stabilization (Winzen et al., 1999; Paccani et al., 2002). In addition, p38 mediates the synthesis of other compounds involved in inflammation, including chemokines and adhesion molecules, the metalloproteinases responsible for cartilage breakdown, and prostaglandins (Berenbaum et al., 2003). In the context of bone erosion, p38 mediates TNF-α-induced IL-1 upregulation in stromal cells and the subsequent IL-1-induced RANKL production. However, in macrophages, the synthesis of IL-1 functional receptor by TNF-α, IL-1, or RANKL is mediated via p38 (Wei et al., 2005).

The p38 MAPK pathway was recently reported to play a critical role in wear debris-induced inflammatory osteolysis. p38 MAPK activation increased in inflammatory osteolysis, indicating that p38 MAPK is important for regulating the inflammatory process in wear debris-induced inflammatory osteolysis.

In this study, morphological analysis revealed that SB203580, a p38 MAPK inhibitor, reduced cellular infiltration and pouch membrane thickness by stimulating inflammation in murine bone-implanted pouches. Moreover, TRAP-positive cells at the interface between the implanted calvaria and the Ti particles-stimulated pouch membrane were markedly higher than in the PBS group. Treatment with a p38 MAPK inhibitor effectively decreased the number of TRAP-positive cells in pouches with Ti particle-induced inflammatory osteolysis.

Gene transcripts of RANK and RANKL from osteoclasts and the concentrations of RANK and RANKL proteins in the implanted calvaria and the pouch membrane significantly increased in Ti particle-induced inflammatory osteolysis in a mouse model of osteolysis. However, p38 MAPK inhibitor treatment remarkably reduced these effects.

Wear debris-induced inflammatory osteolysis is associated with high levels of expression of many inflammatory enzymes, inflammatory cytokines, and chemokines, which may increase aseptic loosening. In this study, we observed high levels of expression of RANK and RANKL in wear debris-induced bone-implanted pouches, which was decreased by SB203580 treatment. Accordingly, SB203580 may inhibit aseptic loosening by downregulating RANK/RANKL in mouse osteolysis, a process that may involve the p38 MAPK pathway.

Recent studies involving wear particle stimulation have demonstrated that p38 MAPK inhibitor treatment markedly decreases RANK/RANKL expression. Inflammation and osteoclastogenesis are recognized as hallmarks of bone loss associated with aseptic loosening. Several studies have demonstrated that the expression of RANK/RANKL on wear particle stimulation in bone-implanted pouch tissues remarkably increased, with a close relationship between the level of RANK/RANKL and pouch tissue inflammation (Roux and Orcel, 2000). Activation of the RANK/RANKL pathway may play a prominent role in aseptic loosening due to bone resorption. However, the mechanism regulating the expression of RANK and RANKL remains unclear. In addition, numerous effects on osteoclast physiology, such as recruitment, differentiation, and activation, have been shown to occur through the p38 MAPK pathway. RANK/RANKL and p38 MAPK exert their functions through multiple signal transduction pathways that have not been completely characterized. These data indicate that p38 MAPK signaling plays a critical regulatory role in osteoclastogenesis through RANK/RANKL signaling and may be important in the pathogenesis of aseptic loosening.

In this study, we found that p38 MAPK is associated with the regulation of RANK/RANKL expression and exerts a regulatory effect on the development of wear debris-induced inflammatory osteolysis.

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