WEAR DEBRIS PARTICLES PROMOTE BONE MARROW STROMAL CELLS TO DIFFERENTIATE INTO OSTEOCLAST: A MECHANISM OF ORTHOPAEDIC PARTICLES RELATED OSTEOLYSIS

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INTRODUCTION
The particulate wear debris generated from arthroplasty bearing surfaces that leads to implant failure results from regional chronic inflammation and osteolysis. The regional interaction of cellular components with the particulate of wear debris is a critical parameter in the pathology of aseptic loosening. Numerous studies support the concept that osteoclastogenesisc is responsible for the osteolysis. Since the particles commonly accumulate in periprosthetic tissues and in the local bone marrow, how the wear debris particles directly affect the differentiation of osteoclastogenesis from osteoprogenitor cells (bone marrow stromal cells) remains largely unknown. Although it is clear that the inflammatory mediators highly associate with osteolysis. The particulate wear debris generated from arthroplasty bearing surfaces that leads to implant failure results from regional chronic inflammation and osteolysis. The regional interaction of cellular components with the particulate of wear debris being a critical parameter in the pathogenesis of aseptic loosening. Numerous studies support the concept that osteoclastogenesis is responsible for the osteolysis. Since the particles commonly accumulate in periprosthetic tissues and in the local bone marrow, how the wear debris particles directly affect the differentiation of osteoclastogenesis from osteoprogenitor cells (bone marrow stromal cells) remains largely unknown. Although it is clear that the inflammatory mediators highly associate with osteoclastogenesis, the osteoclastogenesis may play a role in the pathology of osteolysis that may be responsible for the osteolysis. Our study hypothesis was that wear debris (metallic and non-metallic particles) may stimulate BMSC differentiation into osteoclasts rather than osteoblasts. To test the hypothesis, STRO-1 positive BMSC were cultured with Ti and lipopolysaccharide (LPS) treated Ti particles. To monitor the osteoclastogenesis, the mRNA and protein levels of tartrate-resistant acid phosphatase (TRAP) were determined by dynamic RT-PCR and protein immunoblotting studies. The numbers of TRAP-positive cells from BMSC cultures were also estimated from the particle-stimulated and non-treated control cells after immunocytochemistry studies. The ability of the TRAP-positive cells to resorb bone was confirmed by a dentine pit formation assay. The results from this study indicate that Ti particles can directly interact and promote BMSC differentiation to osteoclast. Without particle exposure, BMSC cultures were negative to TRAP stain (thus there was no osteoclast differentiation?). When compared to the cultures treated with Ti and LPS-Ti, the numbers of TRAP+ cells were increased in LPS-Ti treated cultures. When using mouse BMSC primary cultures, we also demonstrated that the immunoresponse of the BMSC to LPS-Ti was dependent on their TLR4 expression. Decrease of the numbers of TRAP-positive cells in LPS-Ti treated cultures. The results of this study supported our hypothesis that BMSC plays a role in the osteolysis, and suggests that one mechanism of osteolysis may be the biological stimulatory effects of particles and endotoxin contaminated particles on osteoclast differentiation.

MATERIALS AND METHODS
Bone marrow stromal cell isolation and cell culture. Mice were sacrificed, and bone marrow was flushed with culture medium from femoral marrow using minor modified method described by Abu-Amer et al. (1). CD68 and CD34-positive cells were depleted from marrow cells by negative selection using MACS goat anti-rat IgG microbeads (Miltenyi Biotec, Auburn, CA). RBC were lysed with NH4Cl (0.727%) and Tris-HCl (0.017%) at pH 7.2 at room temperature for 5 min. A total of 1 x 10^7 cells/ml were resuspended in MEM with 2% FBS. PBS dissolved LPS were mixed with Ti for 4 hours and washed with PBS 3 times before being added to the cell cultures. Detection of TLRs expression. RT-PCR was used to detect the message of TLR and TRAP. Total cellular RNA from monocytic cultures treated with or without particles for various time were determined. Primers were used as follows: For TRAP, cathepsin K and TLR-4, forward primers 3'CTTTCTACCGTCTGACCTTC5', 5'CTGTTTTCTTGAGCCAGGAC5' and reverse primers 5'GCTGTTTCTTGAGCCAGGAC5', 5'TCCACCTCGAGTTAAGGTT-3', and 5'-GCCACCTGGAAACTCCG-3' respectively. Immunocytochemistry and immunoblotting assays were used to determine the TRAP and TLR protein expression. Anti-human TRAP and TLR4, monoclonal antibodies were used to localize the positive cells and detect protein. Anti-sense & LPS treatment. TLR4 anti-sense sequences were designed based on information from the Genebank database. The oligonucleotides were synthesized by Biognostik. Cell cultures were treated with chimeric and scrambled control ODN (0-10 ug/ml) for 18 hours prior to addition of particles. TRAP positive cell determination. After immunocytochemistry staining, the number of TRAP positive cells from each well were determined using a light microscopy from cell cultures that had or had not been treated with particle exposure.

RESULTS
1. The purity of BMSC magnetic-microbeads was estimated with FACS assay. The histograms revealed that the STRO-1 positive cells were CD34 negative (Figure 1). The results indicated that BMSC were able to directly interact with Ti particles and differentiate into osteoclasts. 3. Using western blot, we also demonstrated that BMSC expressed TLR4 (Figure 3). These results supported the assertion that that LPS-Ti strongly enhances BMSC osteoclastogenesis. This result also implied that TLR4 may play a role in the osteoclast differentiation. Suppression of TLR4 expression in the TLR4 antisense treated BMSC cultures decreased the TRAP expression duration.?? LPS-Ti treatment.

DISCUSSION
Particulate wear debris has been shown to increase osteoclastogenesis from either bone marrow or peripheral blood monocytes. How the particles directly interact with BMSC and/or influence BMSC differentiation has not been fully documented. Our results provide evidence suggesting that the particles are able to induce BMSC osteoclastogenesis. When the particles were contaminated with endotoxin, the particle’s osteoclastogenic stimulatory function became even stronger as a result TLR4 expression. These results indicate that osteoclasts can be derived from BMSC on the inner surface of bone cavities after chronic Ti exposure. The location of newly formed osteoclasts may play a critical role on the osteolysis after prosthetic procedure.

REFERENCES