ORTHOPAEDIC WEAR DEBRIS PARTICLES EXPOSURE INDUCES BONE OSTEOCLAST DIFFERENTIATION FROM MARROW STROMAL CELLS

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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. Many studies support the concept that osteoclastic bone resorption is responsible for the osteolysis. Since the particles commonly surround periprosthetic tissues in the local bone marrow, the wear debris particles may directly affect the differentiation of osteoclastgenesis from osteoprogenitor cells (e. g. bone marrow stromal cells). Osteogenesitic bone marrow stromal cells (BMSC) usually locate in a thin layer of reticular connective tissue attached to the inner surface of the bone cavities. The hypothesis of this study was that wear debris particles (metallic and non-metallic particles) may stimulate BMSC to differentiate into osteoclasts rather than osteoblasts. To test the hypothesis, STRO-1 positive BMSC were cultured with Ti and lipopolysaccaride (LPS) coated Ti particles. To monitor the osteoclastgenesis, the mRNA and protein levels of tartrate-resistant acid phosphatase (TRAP) were determined by dynamic RT-PCR and protein immunochemistry studies. The numbers of TRAP-positive cells from BMSC cultures were also estimated from the particle and non-particle exposed cultures after immunocytochemistry stains. The ability of the TRAP-positive cells to resorbe bone tissue 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. After cultures treated with Ti and LPS-Ti, the numbers of $TRAP^{+}$ cells were increased significantly in LPS-Ti treated cultures. We also demonstrated that BMSC response to LPS-Ti dependent s on cell TLR4 expression. Decrease of the BMSC TLR4 expression using antisense oligodeoxynucleotide (ODN) significantly reduced the numbers of TRAP-positive cells in LPS-Ti treated cultures. The results of this study supported our hypothesis that particles interact with BMSC plays a role in the osteolysis.

MATERIALS AND METHODS

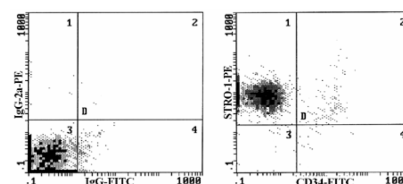
Bone marrow stromal cell isolation and cell culture. Mice were sacrificed, and bone marrow cells were flushed with culture medium from femoral marrow using minor modified method described by Abu-Amer et al. (1). CD68 and CD34-positive cells were depleted from marrow cells by negative selection using MACS goat anti-rat IgG microbeads (Miltenyi Biotec, Auburn, CA). RBC were lysed with NH_4Cl (0.727%) and Tris-HCl (0.017%) at pH 7.2 at room temperature for 5 min. A total of 1 x 10^8 cells/ml were resuspended in MEM with 2% FBS. The cells were then incubated with anti-mouse STRO-1 Ab (2) (10 µg Ab for 3x10^6 cell) for 15 min at 18°C. After incubation, the cells were washed with same medium and resuspended in 1ml of MEM. BMSC were purified magnetic beads loaded column. STRO-1 positive cells were confirmed by FACS. _Treatment of the monocytic cultures with Ti debris particulates:_ commercial available Ti debris particulate and LPS (Sigma-Aldrich), were used. Ti-particulate suspension medium was freshly prepared 1 hour prior added to the cultures. 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 TRAP and TLR. Total cellular RNA from monocytic cultures treated with or without particles for various times were determined. 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.

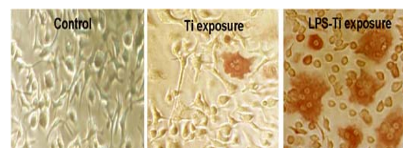
Antisense & LPS treatment: TLR4 antisense sequences were designed based on information from the GeneBank database. Biognostik synthesized the oligonucleotides. Cell cultures were treated with chimeric and scrambled control ODN (0-10 ug/ml) for 18 hours prior to addition of particles.

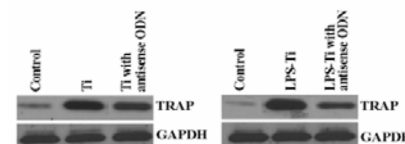
TRAP positive cell determination After immunocytochemistry staining, the numbers of TRAP positive cells from each well were determined using a light microscopy from cell cultures that had or had not been treated with particle exposure.

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 the isolated BMSC double magnetic-microbeads did not contain any mature monocytic lymphocytes or hematopoietic stem cells. **2.** Immunostaining and microscopy showed that multinuclear cells were positively interacted with TRAP antibody after the cells were exposed to Ti and LPS-Ti particles (Figure 2). 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 osteoclastgenesis. This result also implied that TLR4 may play a role in the BMSC 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 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 induceBMSC osteoclastogenesis. Whenhe particles were contaminated with endotoxin, the particle's osteoclastogenetic stimulatory function become 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

1. Abu-Amer, Y., J. Erdmann, et al. *J. Biol. Chem.* 2000 *275:27307.*
2. Gronthos S, Graves SE, et al. Blood. 1994 84:4164