SOLUBLE FACTORS FROM RABBIT SYNOVIAL CELLS IN RESPONSE TO ALUMINA PARTICLES STIMULATE OSTEOCLAST-MEDIATED BONE RESORPTION

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Relevance to Musculoskeletal condition:
Polyethylene debris induce osteolysis which leads to aseptic loosening of total joint prostheses. Recently, new articulation such as alumina on alumina in total joint arthroplasty has been developed. We investigate whether alumina particles stimulate osteoclastic bone resorption using *in vitro* bone resorption assay that we developed.

Introduction:
It has been suggested that interleukin-1 (IL-1) and interleukin-6 (IL-6) released from macrophages and fibroblasts stimulated by polyethylene particles generated from total joint prostheses play a major role in osteoclastic bone resorption around failed implants. Therefore, new articulation without polyethylene such as alumina on alumina has been developed in total joint arthroplasties. Before the clinical use, we should evaluate the characteristics of cell-alumina particles interaction because any type of articulation could generate wear debris. In this study, we investigate the effects of alumina particles (Al) on the production of bone resorbing cytokines from the cells as well as osteoclast-mediated bone resorption, and compare those results with polyethylene (PE) and hydroxyapatite (HA) particles.

Materials and Methods:

Cytokine Assays
Rabbit synovial cells (Hig cells) were suspended at a concentration of 1 x 10^5/ml in 10% FCS, and cultured with 0.1 mg/ml of HA, Al, and PE particles (mean size of each particle; 3 ± 2 microns) for three days. HA and Al particles were made by milling and provided by Kyocera (Kyoto, Japan). PE particles were spherical high density polyethylene and provided by Sumitomo Seika (Tokyo, Japan). The levels of IL-1 and IL-6 in the conditioned medium (CM) were determined by bioassay. IL-1 activities in CM from Hig cells were determined using a growth inhibition assay with a human melanoma A375 cell line as previously described (1). IL-6 activity in CM was measured by the ^{3}H-thymidine incorporation of an IL-6 dependent cell line (B45-3).

Osteoclast-Mediated Bone Resorption Assay
Osteoclast-mediated bone resorption assay was performed by previously reported method (2). Briefly, unfractionated bone cells were prepared from femora of a 10-day-old rabbit. 1.5 x 10^5 bone cells + α-MEM supplemented with 5% FBS was added to each well containing a dentin slice of a 96-well plate, and the cultures were incubated for 2 hours. The conditioned medium exposed to each particle was added to each well at the concentrations of 0.1 mg/ml, and cultured for 24 hours. Pits formed on the slices were stained with acid hematoxylin, and resorbed areas were determined under a microscope by counting the number of mesh squares covering pits to evaluate osteoclast bone resorption. In addition, we examined the effects of 1, 25 $(OH)_2D_3$ (VD) on bone resorption as a positive control.

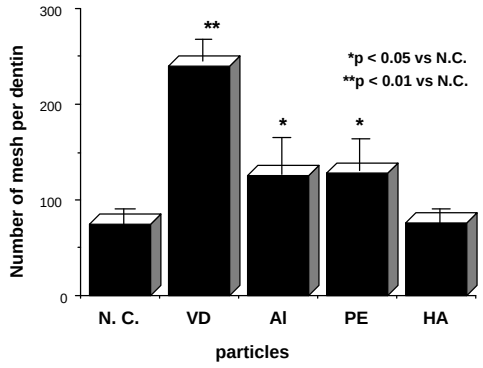
Results
IL-1 and IL-6 activities in CM from Hig cells stimulated by wear particles
HA, Al, and PE particles significantly stimulated IL-1 and IL-6 production from a rabbit synovial cell line (Hig cell). The levels of IL-6 in CM from Hig cells stimulated by Al and PE particles were significantly higher than that in CM from Hig cells stimulated by HA particles although the levels of IL-1 in CM from Hig cells stimulated by HA particles tended to be higher than that in CM from Hig cells stimulated by Al and PE particles (Table 1).

	IL-1 (pg/ml)	IL-6 (ng/ml)
N.C.	104 ± 15	undetectable
Al	185 ± 23*	15 ± 4**
PE	183 ± 18*	12 ± 3**
HA	420 ± 53*	7 ± 2*

Table 1. The IL-1 and IL-6 activity in the conditioned medium from Hig cells stimulated by Al, PE, and HA particles. N.C.: no particles
*p < 0.05 vs N.C., **p < 0.01 vs HA.

Osteoclast-mediated bone resorption
According to the osteoclast-mediated bone resorption assay, the resorbed area on a dentin slice significantly increased when the CM from Hig cells stimulated by Al and PE particles was added to rabbit unfractionated bone cells (P<0.05), whereas CM from Hig cells stimulated by HA particles did not activate osteoclastic bone resorption on a dentin slice (Figure 1).

Fig. 1 Osteoclastic bone resorption assay



Discussion:
The results of this study have a clinical implication that wear debris from alumina on alumina or alumina on polyethylene may induce periprosthetic bone resorption through the activation of osteoclasts by soluble factors such as IL-1 and IL-6 produced from synovial membranes around prostheses. Although IL-6 seems to be more important for osteoclastic bone resorption when compared to IL-1 in the present study, it is still unknown which cytokine has a regulatory effect on the activation of osteoclasts. In addition, it has yet to be determined whether differential effects of each particle on cytokine production and osteoclastic bone resorption are due to the difference of chemical composition or physical properties of each particle.

Conclusion:
Alumina particles, as well as polyethylene particles stimulated IL-1 and IL-6 production from rabbit synovial cells. Furthermore, supernatants from those cells cultured with each particle activated rabbit osteoclastic bone resorption.

References:
(1) Nakai, S. et al., Biochem Biophys Res Commun 1988, 154, 1189-1196.
(2) Kim, K.J. et al., J. Biomed. Mater. Res., 1996, 32, 3-9.

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