ENHANCED NON-OSTEOCLASTIC OSTEOLYTIC POTENTIAL OF MONOCYTES/MACROPHAGES AFTER PARTICLE STIMULATION

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INTRODUCTION:
Activated monocytes/macrophages have been reported to be involved in osteoclast formation, maturation and activation in the process of periprosthetic osteolysis in aseptic loose hip joints. Previous *in vivo* observations showed demineralized and fragile bone surfaces surrounded by numerous cathepsin K-positive monocytes/macrophages kept in contact with acidic interface, in which osteoclasts were scare. These data suggest that monocytes/macrophages adjacent to fragile periprosthetic bone have a direct potential of bone resorption. We hypothesized that periprosthetic monocytes/macrophages have a potential, not only to accelerate inflammation and osteoclast-mediated periprosthetic osteolysis, but also to resorb periprosthetic bone directly. This study was designed to evaluate non-osteoclastic osteolytic potential of monocytes/macrophages derived from bone marrow.

METHODS:

1. **Culture of macrophages derived from bone marrow;**
   Bone marrow cells were collected from Wistar rats. The bone marrow cell suspension was filtered by synthetic fiber membrane and cultured under the condition of humidified 95% air admixed 5% CO2 for 72 hours at 37℃ in the media of consisting 90% DMEM, 10% fetal bovine serum and with 10ng/ml M-CSF. Adherent cells were collected by exposure to trypsin-EDTA solution. Phagocytic activity and reactivity to CD68, cathepsin K, tartrate resistant acid phosphatase (TRAP) were examined in the adherent cell fraction (5x10^6 cells/ml).

2. **Reverse transcriptase polymerase chain reaction (RT-PCR) and quantitative real-time PCR analysis;**
Titanium particles (0.15%) were added to cultured palates. Total RNA was isolated after 0.5, 1, 3, 6, 12 hours of exposure and reverse transcription was performed. Total RNA was converted into cDNA and enzymatic amplification of the specific cDNA sequences was performed by Light Cycler system (Rosch, Germany). Quantitative analysis was performed with the use of Light Cycler Software. mRNA of cathepsin K, IL-1 beta, IL-6, TNF-alpha, M-CSF and RANKL were amplified, and GAPDH was used as control. Statistical analysis was performed by Fisher’s PLSD test.

3. **Resorption assay;**
The adherent cell fraction (1x10^6 cells/ml) was harvested into dentin slice and carbonated calcium phosphate plate (OCT, USA). After one hour cultivation, supernatant containing non-adherent cells was removed, and cultivation continued up to 3 weeks in the presence of 0.01% titanium particles. Resorption pits were densitometrically verified in the time course.

RESULTS:

1. **Cellular phenotypes;**
   Adherent cells revealed phagocytotic activity of carbon and titanium particles (carbon: 99.8% up to 72 hours, titanium: 99.9% up to 12 hours) (Fig 1). Immunoreactivity to CD68 and cathepsin K was found and maintained well up to 72 hours (CD68: 99.9%, cathepsin K: 94%, Fig 2). No marked multinucleation and TRAP reactivity were not found.

2. **Quantitative real-time PCR;**
   Cathepsin K, IL-1 beta, IL-6, TNF-alpha, M-CSF and GAPDH were detected in all samples, but not RANKL. Each melting curve analysis showed only single peak, which revealed the accurate PCR performance without nonspecific products. mRNA levels of cathepsin K, IL-1 beta, IL-6, TNF-alpha, and M-CSF standardized by GAPDH, were significantly higher after titanium particles stimulation (p < 0.05) (Fig 3). Expression of RANKL was not detected even after the stimulation.

3. **Analyses of resorption pits;**
   Dentine slice assay showed slight resorption after particle stimulation. However, resorption area assessed by carbonated calcium phosphate plate was significantly enhanced after particle stimulation (Fig 4, 5). The cells during the assay revealed no marked multinucleation and reactivity to TRAP.

DISCUSSION:

Harvested bone marrow cells expressed marked phagocytic activity and abundant monocyte/macrophage specific marker CD68 with purity of over 99%, but not TRAP characteristic to osteoclast and without marked multinucleation. The cells also revealed phenotype of not only osteolytic cytokines, also cathepsin K, which is active at acidic circumstances and can degrade extracellular matrices. The mRNA levels were enhanced by particle stimulation. Taken together with resorption capacity of carbonated calcium phosphate, monocytes/macrophages in periprosthetic tissue not only play a critical role in activation of osteoclastic pathway via osteolytic cytokines, but also have a direct osteolytic potential to attack mineralized tissue, thus enhancing osteolysis around implants. The data implicated importance of biotribological approach not only to osteoclast but also to monocytes/macrophages as target cells to be modulated.