THE ROLE OF ENDOTOXIN ON PRO-INFLAMMATORY CYTOKINE mRNA EXPRESSION IN TITANIUM STIMULATED HUMAN MONOCYTES

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Introduction
Phagocytosis of wear particles by perimplant macrophages results in cytokine release, differentiation and activation of osteoclasts and osteolysis. Endotoxin is a bacterial cell wall component and has been found in revision surgery. In-vitro and in-vivo evidence suggests that particles with adherent endotoxin may have increased biological activity and increase monocyte/macrophage pro-inflammatory cytokine release, osteoclast differentiation and osteolysis versus endotoxin free particles.

The aim of this study was to determine the role of endotoxin in modulating pro-inflammatory cytokine messenger ribonucleic acid (mRNA) expression of human monocytes when stimulated with titanium particles using relative quantitative real-time polymerase chain reaction (rqRT-PCR)

Materials and Methods
Human peripheral blood mononuclear cells from 3 subjects were isolated from venous blood by sucrose gradient and were plated in 8 well chamber slides. After 2 hours incubation, cells were washed with phosphate buffered saline (PBS) leaving 5x10^6 adherent monocytes in each well.

Three types of titanium were prepared according to protocols previously described: commercially pure titanium (cpTi, Johnson Matthey, USA), endotoxin stripped particles and endotoxin stripped particles with adherent endotoxin (LPS) added back. Endotoxin contamination on the particles was assessed using a High Sensitivity Limulus Amebocyte Lysate Assay previously described and confirmed endotoxin levels of 450, 0 and 140 Eu/ml for the cpTi, endotoxin stripped particles and endotoxin stripped particles with adherent endotoxin (LPS) added back respectively.

The monocytes were stimulated using titanium particles at concentrations of 8.3, 83 and 830 particles per cell for 3 hours. After stimulation, cells were lysed and homogenized using QiaShredder columns (Qiagen Ltd. Crawley, UK) and total RNA was extracted using the RNeasy mini kit (Qiagen). mRNA was transcribed using Moloney Murine Leukemia Virus Reverse Transcriptase, RNase H Minus (Promega, Southampton, UK).

Cytokine mRNA levels for Interleukin-1α (IL-1α), Interleukin-1β (IL-1β), Interleukin-6 (IL-6) and Tumour Necrosis Factor α (TNFα) were assessed using rqRT-PCR which was performed on an ABI Prism 7900HT sequence detection system and results were analysed using ABI PRISM SDS version 2.1 software.

Statistical analysis was performed using the Kruskal Wallis test with SPSS, version 11.5 (Chicago, IL).

Results
Stimulation of human monocytes with cpTi demonstrated a significant dose dependent increase in TNFα, IL-1α, IL-1β and, IL-6. (Kruskal-Wallis p=0.01, p=0.017, p=0.001 and p=0.013 respectively, figure 1). IL-18 mRNA levels were not increased (P>0.05). Data not shown. The expression of mRNA following stimulation with the highest dose of titanium particles was similar to that following LPS stimulation.

Endotoxin-free cpTi particles did not elicit any increase in mRNA expression above base line levels (P > 0.05, all cytokines, figure 2).

This lack of response was rescued in endotoxin-stripped particles with LPS added back (figure 3). Particle dose dependent increases in cytokine mRNA levels were observed for TNFα, IL-1α, IL-1β and, IL-6 mRNA but not IL-18 (p=0.01, p=0.01, p=0.01, p=0.05 and p=0.05 respectively).

Discussion
Our results show that adherent endotoxin plays a major role in modulating particle induced pro-inflammatory cytokine mRNA expression in human monocytes in-vitro. A possible mechanism for the role of particles is to present endotoxin to inflammatory cells rather than induce inflammation themselves. Further study is required in evaluating the role of adherent endotoxin on wear debris generated in-vivo from functioning hip replacements.

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References

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