INTRODUCTION: Wear particles induce aseptic loosening by increasing production of bone resorptive cytokines that in turn, stimulate osteoclast differentiation [1]. Both commercially pure titanium (cpTi) particles as well as cpTi and Ti-6Al-4V implant surfaces contain large amounts of endotoxin [2]. We have previously reported that removal of >99.9% of adherent endotoxin eliminates the ability of the cpTi particles to stimulate cytokine production, osteoclast differentiation, and bone resorption [3]. These studies suggest that adherent endotoxin is involved in wear particle-induced osteolysis. The current study was designed to address the following limitations of our previous experiments: although the endotoxin removal procedure does not affect the size or shape of the particles [2], it may affect their physico-chemical properties and/or may remove molecules other than endotoxin; murine cells may not respond similarly to human cells; and the in vitro experiments may not reflect in vivo processes.

METHODS: >99.9% of the adherent endotoxin was removed from cpTi particles (Johnson Matthey, 75%±6.5μm) without affecting their size or shape [2]. Adherent endotoxin was added back to these “endotoxin-free” particles by incubation with 1.5μg/ml lipopolysaccharide (LPS, Sigma) followed by washing 10x with PBS to remove any unbound LPS. Murine marrow cells [1, 3] or human peripheral blood monocytes [4] were cultured with or without particles and conditioned media collected as described [1, 3]. IL-1β, IL-6 and TNF-α levels were determined by ELISA and the ability of the conditioned media to stimulate osteoclast differentiation was determined in co-cultures of murine spleen cells and C1MC−/- mesenchymal support cells [1, 3]. The in vivo effects of the cpTi particles were determined using a quantitative version [5] of the murine calvarial model of particle-induced osteolysis [6]. All data are presented as means ± SEM (n=6, except Fig. 2A where n=3). Statistical analyses were by ANOVA.

RESULTS: To determine whether the results of the endotoxin removal experiments are due to altered physico-chemical properties and/or removal of molecules other than endotoxin, we performed two types of experiments. First, we found that adding back LPS to the “endotoxin-free” particles restores their ability to stimulate osteoclast differentiation (p<0.0001, Fig. 1). Second, we performed studies that do not rely on removal of the adherent endotoxin. Thus, we compared responses to cpTi particles by marrow cells from LPS hyporesponsive C3H/HeJ mice [7] with those from normoresponsive C3H/HeJ mice. As seen in vitro, the “endotoxin-free” cpTi particles induce significantly less osteolysis in vivo than the cpTi particles with adherent endotoxin (p<0.0001, Fig. 3).

To determine whether human cells respond similarly to murine marrow cells, we studied human peripheral blood monocytes. cpTi particles with adherent endotoxin stimulate human peripheral blood monocytes to produce conditioned media that potently stimulate osteoclast differentiation (p<0.0001, Fig. 3). However, we have seen with murine marrow cells, the “endotoxin-free” cpTi particles have no detectable effect on the human monocytes (Fig. 3). To determine whether the in vitro experiments reflect in vivo processes, we used a quantitative version [4] of the murine calvarial model of particle-induced osteolysis [5]. As seen in vitro, the “endotoxin-free” cpTi particles induce significantly less osteolysis in vivo than the cpTi particles with adherent endotoxin (p<0.0001, Fig. 4).

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