NUMBER OF PHAGOCYTOSED PARTICLES (DOSE) MORE THAN PARTICLE SIZE OR SURFACE AREA AFFECT THP-1 MONOCYTE REACTIVITY

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INTRODUCTION: A wide size range of particulate debris is produced from both traditional and alternative bearing surfaces of total joint replacements. While alternative bearing surfaces may dramatically reduce the amount of gravimetric wear, the degree to which less bioreactivity will result from these newer designs will depend on what type of debris is produced. If greater amounts of smaller particles are produced by alternative bearing TJAs, and these smaller particles are more bioreactive, then presumably the benefits of less wear may be partially offset. The relative importance of debris larger than 5 microns remains incompletely characterized. Will large particles produce similar levels of cytokine responses as small particles in macrophages/monocytes? We hypothesized that large-size debris can be phagocytosed and that the amount of activation (cytokine release) will be greater than that of smaller particles on an equal number basis. To test our hypothesis, we assessed how many particles (large vs small) are typically phagocytosed by human macrophages/monocytes and then tested these different size particles with a human macrophage cell line to determine the relative amounts of cytokine production.

MATERIALS AND METHODS: Healthy subjects (n=6) were randomly selected. Monocytes were collected and plated on 2 wells. The plates were then washed out after 48 hours leaving monocytes that adhered to the plate. 2 sizes spherical smooth Co-Cr-Mo particles were used (1micron and 10microns Dia). One well was treated with a saturating amount of Co-Cr-Mo particles of 10 microns in size. The second well was exposed to a saturating amount of 1 micron sized particles. After 24 hours, macrophages were washed to remove unphagocytosed particles. Using a microscope, 50 monocytes were visualized and the number of particles phagocytosed were counted and recorded. Using a THP-1 macrophage cell line, 25,000 cells were plated on a 48-well. Cells were then treated with 4 {beta}-Phorbol 12-myristate 13-acetate(TPA) for 48h to cause the monocytes to adhere and mature on the 48-well plate. Cells were then treated with 10 microns particles in ratios of 1:1. Cells were also treated with 1 microns sized particles in increasing ratios of 1:1 up to 1:7. Supernatants were then collected at timepoints 1, 2, 4, 6 hours and assayed for tnf-alpha production. Elisas were then performed to determine TNF-alpha production.

RESULTS: *Subject phagocytosibility:* The average number of particles phagocytosed of the 10 micron size was 1.39, the range was between 1-3. In monocytes challenged with 1uM sized particles the average number of particles phagocytosed was 4.6 with a range between 1-8. In all subjects, was either 4 for 5 1uM sized particles.

THP-1 Monocyte responses: THP-1 monocytes treated with 1 micron particles show an initial increase in TNF-alpha followed by a gradual decline to below control levels at 6 hours (Fig 1). There was a general increase in response to particle challenge with maximal stimulation at 5 particles per cell (6-fold increase in TNF-alpha). TNF-alpha production appeared to be an early cytokine with maximal release at 1 hour at all challenge concentrations. Monocytes treated with greater than 5 particles per cell showed less than control levels of cytokine production. A comparison of 1 and 10 micron particles at a concentration of 1 particles per cell (the approximate maximal level of phagocytosis demonstrated for primary human macrophages/monocytes demonstrated a 4-fold increase in cytokine production during in the first hour (Fig. 2). Monocytes treated with only 1 small particle showed very little increased production in cytokine.

DISCUSSION: The greater amounts of TNF-alpha released by THP-1 human monocytes challenged with 10 micron particles when compared to 1 micron particles supports our original hypothesis. The challenge ratio of 1 particle per cell was the approximate maximum number of large (10 micron) particles able to be phagocytosed. Thus a comparison of large vs small particle reactivity was limited to a ratio of 1 particle per cell (Fig 2). At this level of particle burden the larger particles demonstrated double the response of the small particles on an equal number basis. However, this response was expected due to the 100x greater surface area of the 10 micron particles (1,257um^2/particle) when compared to the small 1micron particle (12.6um^2/particle), where there was a 100X increase biologically available particle surface area. This 100x increase in surface area caused a 2 fold increase in TNF-alpha production. However, a 5x increase in surface area (5 x 1micron particles in Fig 1), demonstrated a nearly 6x response in TNF-alpha or 2x that of the larger particles. That is a 5x increase in surface area using more numbers of 1 micron particles produced more TNF-alpha than a 100x increase in surface area of an equal number of 10 micron particles. Thus it seems that surface area is not the most important factor leading to bioreactivity. The number of phagocytosable particles seems to be a more critical determinant of wear debris reactivity. A significant aspect of these findings is that large phagocytosable debris can be a source of inflammation. More importantly however, is that a single large 10 micron particle is much less reactive (approx 1/500) than 1,000 small 1 micron particles of equivalent mass. Thus, our results support the contention that alternative bearing surfaces, which produce less mass loss may provoke a greater response if the debris consists of greater particle numbers. Also critical, is not only how many particles are produced, but how widely they are distributed among macrophages. That is, if the particles are diffusely distributed in peri-implant region such that not more than 5 particles per macrophage are phagocytosed, then the relative proportion of cytokine response is approximately directly proportional to the number of particles. Small particles appear to impair cytokine production as the numbers become too high. Whether this is a toxic or down regulated response is the subject of current efforts. Previous contentions of macrophage related reactivity being only dependent on particle size are not supported by this study. Rather, it is the number of phagocytosable wear debris particles that were shown to more powerfully affect cytokine release and inflammation. Whether this dependence breaks down at a critically small particle size (e.g. 1 micron vs 0.1 micron) is not known and also is the subject of further study. Ultimately, this study should be expanded to primary monocytes in order to better establish the human monocyte THP-1 cell line as an effective method for evaluating responses to particulate debris.

ACKNOWLEDGMENTS:

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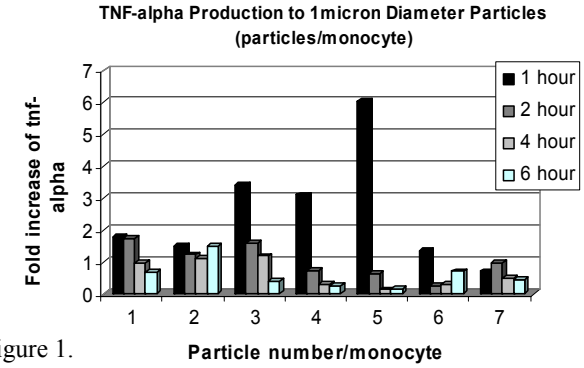

Figure 1.

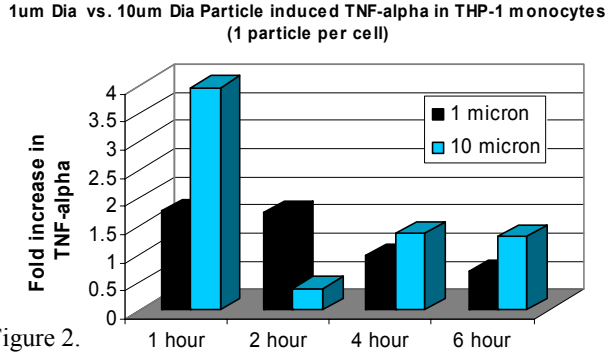

Figure 2.