TNF ALPHA PRODUCTION IN PATIENTS AWAITING TOTAL HIP REPLACEMENT. SPECIFIC MONONUCLEAR PHAGOCYTIC ACTIVITY IN RESPONSE TO POLYETHYLENE AND LPS

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Introduction: UHMWPE wear debris is now known to be a major cause of periprosthetic osteolysis and the long-term failure of total joint replacements. The mechanism by which this process occurs is thought to be macrophage-mediated. Macrophages stimulated with polyethylene particles have been shown to produce a number of inflammatory mediators with osteolytic potential [1,2]. In particular TNFα, has been demonstrated to be a potent mediator of osteolysis in addition to other yet uncharacterised factors [3]. The aim of this study was to compare the *in vitro* response of mononuclear phagocytes from patients undergoing total hip arthroplasty to challenge with polyethylene particles or stimulation with lipopolysaccharide (LPS). Production of TNFα was used as the measure of cell activation.

Methods: Peripheral blood was taken from 2 healthy donors and 16 patients admitted to hospital to undergo total hip arthroplasty. Human mononuclear phagocytes were isolated from the blood samples by density centrifugation and selective adherence to glass coverslips at a density of 10^5 mononuclear phagocytes/cm^2. Commercially available polyethylene particles (Ceridust® 3615; Hoechst, Germany) were sequentially filtered to obtain clinically relevant and biologically active particles (between 0.1 and 0.6μm in diameter) as established by previous studies [2, 4]. Cells plus particles (particle volume to cell number ratio of 100μm^3 per cell) or cells plus LPS (2μg/ml) were then co-cultured together in supplemented RPMI-1640 culture medium for 24 hours by the "inversion technique" [3]. A negative control of cells plus culture medium only was included (n = 3). Culture supernatants were then harvested and the concentration of TNFα quantified using a modified double antibody sandwich ELISA technique (OD at 490nm). The number of viable cells attached to each glass coverslip was measured using the ATPLite™-M assay (Packard BioScience B.V., Groningen, The Netherlands) . The mean specific activity was then calculated (concentration of TNFα in ng/ml divided by the result of the ATP assay in counts per second/10^6). Results were analysed by computation of the product-moment correlation coefficient.

Results: The levels of TNFα obtained after stimulation with PE particles and LPS in both the patient and control groups are shown in figure 1. The mean specific activity (production of TNFα) of mononuclear phagocytes stimulated by LPS varied from 0.097 to 0.208 in the control group and from 0.03 to 17.693 in the patient group. After stimulation with polyethylene, the mean specific activity (TNFα production) ranged from 0.043 to 0.059 in the control group and from 0 to 1.1 in the patients group.

When considering all the subjects in the study, no correlation was found between the response of their cells to polyethylene particles and LPS stimulation in neither the control nor the patients group. However four subjects cells (represented by triangles in figure 1) gave a much higher response to LPS than the rest and when these where excluded the correlation between the response to LPS and PE particles was significant with an R^2 value of 0.9076 as shown in Figure 2.

Discussion: Polyethylene particles and LPS activate mononuclear phagocytes by two different mechanisms. Whilst LPS activates these cells simply through the binding of specific cell surface receptors, polyethylene particles interact with cells via multiple surface adsorbed proteins (e.g. complement, C3b, vitronectin) and must additionally be actively phagocytosed for the macrophages to become stimulated to manufacture and secrete cytokines. Despite these different mechanisms of cell activation, the patient group with 'normal' or low response to LPS had a significant correlation in their response to LPS and particle stimulation. Why a small number of subjects had a much higher response to LPS without a proportional response to PE particles is not known but could be due to an increased expression of LPS receptors or genetic polymorphism. A greater than tenfold difference in the patient response to particles will be of clinical importance in their susceptibility to loosening through osteolysis.

Figure 1 Production of TNFα by mononuclear phagocytes (Mean specific activity)

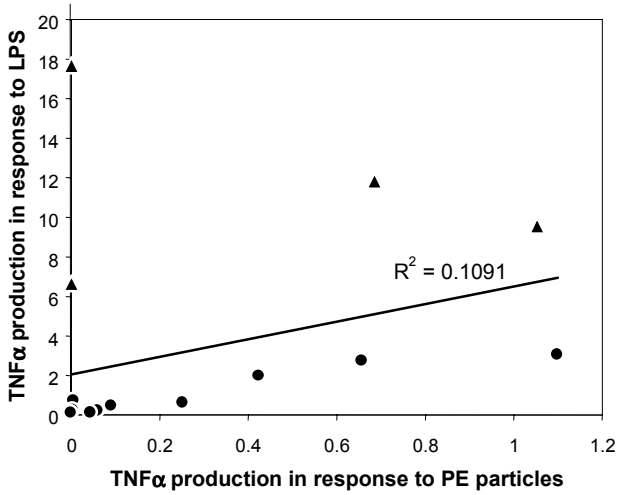
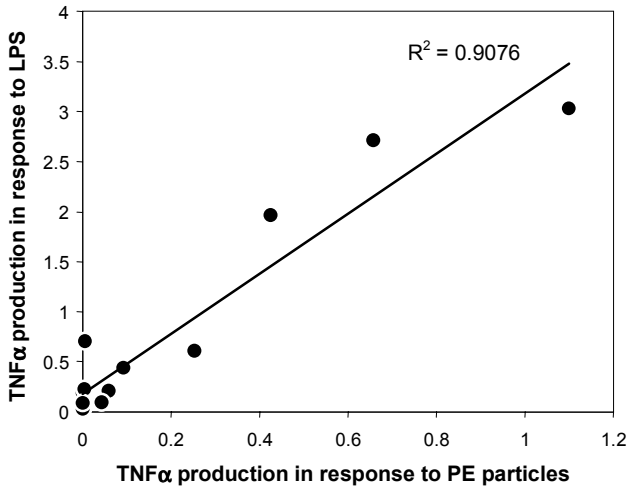


Figure 2 Production of TNFα by mononuclear phagocytes, excluding individuals with extreme values for LPS stimulation (Mean specific activity)



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