JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 12 (2001) 249–258

Comparison of the response of three human monocytic cell lines to challenge with polyethylene particles of known size and dose

J. B. MATTHEWS[1*], T. R. GREEN[1], M. H. STONE[2], B. M. WROBLEWSKI[3], J. FISHER[4], E. INGHAM[1]
[1]*Division of Microbiology, The University of Leeds, Woodhouse Lane, Leeds, LS2 9JT, UK*
[2]*Department of Orthopaedic Surgery, The General Infirmary at Leeds, Leeds, UK*
[3]*Centre for Hip Surgery, Wrightington Hospital, Wigan, UK*
[4]*Department of Mechanical Engineering, The University of Leeds, Leeds, LS2 9JT, UK*

The response of three human monocytic cell lines (Monomac-1, U937 and THP-1) to challenge with polyethylene particles of known size and dose was evaluated. Particles with a mean size of 0.21, 0.49, 4.3, 7.2, and 88 μm were co-cultured with the cells for 24 hours prior to the assessment of cell viability and production of the pro-inflammatory cytokines, IL-1β, IL-6 and TNFα. Additionally, GM-CSF and prostaglandin E_2 were measured in culture supernatants from particle stimulated U937 cells. All particle fractions were evaluated at particle volume (μm^3) to cell number ratios of 100 : 1, 10 : 1, 1 : 1 and 0.1 : 1. None of the test fractions had any effect on cell viability. Only the response of the U937 cell line was demonstrated to be comparable to that of primary macrophages as determined in a previous study. Furthermore only particle volume to cell number ratios of 10 : 1 or greater consistently stimulated significantly enhanced levels of cytokine secretion with particles within the phagocytosable size range (0.1 to 10 μm) being the most biologically active. No response was observed when U937 macrophages were stimulated with the largest (88 μm) particles at any of the volume ratios tested in this study. These results suggest that the size and volume of polyethylene particles are critical factors in macrophage activation. In addition, the U937 cell line has been shown to be a suitable model for the *in vitro* study of macrophage-particle interactions.

1. Introduction

UHMWPE wear particles play a critical role in the periprosthetic osteolysis and loosening of total joint replacements, which ultimately results in their long-term failure. This process is now commonly believed to be mediated by tissue resident macrophages [1]. Evidence for this has come from a number of explant and histological studies which have identified a granulomatous tissue rich in macrophages and giant cells in association with both intra- and extracellular particulate wear debris [2, 3]. Additionally, elevated levels of the osteolytic cytokines IL-1β [4, 5], TNFα [5], IL-6 [5, 6] and GM-CSF [7] have been demonstrated in the pseudomembranes from patients with failed prostheses along with other mediators of bone lysis including ecosanoids, e.g. PGE_2 and proteolytic enzymes, e.g. collagenase [4, 8]. Macrophage activation is believed to be influenced by a number of factors, including – composition, number, size and shape of particles plus the volume of debris [9–12]. The generation of wear particles at the articulating surfaces results in debris with considerable variation in size and morphology. Very few large particles (up to several millimeters) are produced [1, 13] and these are believed to be relatively benign, eliciting a giant cell granulomatous reaction. However, many more small particles ($< 10\,\mu m$) are generated and it is this fraction of the total wear volume which is thought to be the most biologically active [1], initiating a more osteolytic response. Furthermore, analysis of debris retrieved from the tissues surrounding failed prostheses has determined that the vast majority of particles are in fact sub-micrometre in size [14–16]. In order to elucidate the mechanisms by which prosthetic loosening occurs, interest has focused on studying cell-particle interactions *in vitro* and these studies have demonstrated that particles will stimulate macrophages to produce mediators of bone lysis [9, 11, 17–19]. However, many *in vitro* studies have utilized commercially available particles, which differ widely from clinically generated debris in terms of their uniform shape and size distributions. In

*Author to whom all correspondence should be sent.

particular with polyethylene debris, it has proved difficult to obtain and use particles in the sub-micrometer size range, the most common size range found *in vivo*. Furthermore, various dosing regimens (e.g. wt %, vol %, number of particles) have been used which disregard the importance of the relative size or density of particles of different materials. Dosing by mass, volume or, surface area to cell number ratios [9] enables more accurate and clinically relevant comparisons to be made between particles of different composition or size. In addition, a number of model cell lines have been used in studies, e.g. murine J774 [18, 20], P388D$_1$ [9, 11] or IC-21 [11]. However, the response of these transformed cells has never been fully validated in comparison to primary macrophages or monocytes. Furthermore, the origin of the cells used to study the biological response to implant materials is also potentially an important factor in determining the nature of the interaction between cells and particles. Factors such as cell maturity or the stage of differentiation, or the species or donor tissue from which the cells were derived, are all likely to affect the reaction. Indeed it has been noted previously that various macrophage populations may respond differently to the same micro-environmental signal [11]. All of these factors, therefore, make the interpretation and comparison of results extremely difficult. To date, few studies have evaluated the response to polyethylene debris *in vitro* [17, 18, 21]. To some degree, this probably reflects the lack of availability of particulate polyethylene of clinically relevant morphology, size and mass distribution and the difficulty of working with this material *in vitro* owing to its comparatively low density. A number of studies have simply added polyethylene to culture wells without any means of ensuring that cells and particles are cultured in the same plane. Other authors have, however, employed a number of methods to overcome this problem, e.g. culture of cells on the underside of tissue culture well inserts and inversion over floating particles [18] or immobilization of particles, at the base of culture wells, in gelatin or agarose [22].

The aim of this study was to use an optimized polyethylene particle delivery system [24] to evaluate the response of three human monocytic cell lines to challenge with polyethylene particles of known size distributions at various doses. Three human cell lines of monocytic lineage (Monomac-1, THP-1 and U937) were, therefore, chosen for evaluation. Each population of cells represented different stages in the monocyte-macrophage differentiation pathway. The ultimate goal was to compare the response of these human cells with that of primary murine macrophages in the same model [24], in order to identify a suitable model cell line for future *in vitro* studies of cell-particle interactions.

2. Materials and methods

2.1. Preparation and characterization of particle fractions

Ceridust® 3615 (Hoechst, Germany), a low molecular weight polyethylene powder of fine particle mesh, was resuspended in RPMI-1640 (Gibco Life Technologies Ltd, Paisley, Renfrewshire, Scotland) plus 0.125% (v/v) 7X detergent (ICN Biomedicals Ltd, Thame, Oxfordshire, UK). Following sonication for 1 h, particles were sequentially filtered through 10, 1.0, 0.6, 0.4, 0.2 and 0.1 μm Cyclopore® polycarbonate membrane filters (Whatman International Ltd, Maidstone, Kent, UK) and the mass of particle fractions determined. Particle fractions on the 10, 1.0, 0.4 and 0.1 μm membranes were retained for cell testing. The mean particle size of each individual test fraction was determined by scanning electron microscopy (SEM) (Jeol JSM T20; Jeol, Tokyo, Japan) and image analysis (Microscale TC Image Analysis System, Digithurst, UK) to be: 7.2 ± 3.15 (range = 2.17–15.25), 4.3 ± 1.89 (range = 1.31–10.94), 0.49 ± 0.11 (range = 0.32–0.82) and 0.21 ± 0.07 (range = 0.08–0.47) μm (mean ± SD) for the debris on the 10, 1.0, 0.4 and 0.1 μm membranes respectively. Additionally, the cellular response to pure GUR 1120 UHMWPE resin particles (Hoechst, Germany) was evaluated (88 ± 29 μm: mean particle size ± SD). These particles were used to represent the largest shards of debris found in clinical specimens. In order that the low-density polyethylene particles could be cultured in the same plane as the cells, an agarose gel technique was employed [21, 24]. Fractionated Ceridust® 3615 polyethylene particle preparations or the pure UHMWPE resin were therefore resuspended in RPMI-1640 at the appropriate density. Each particle suspension was then mixed with a 1% (w/v) agarose (ultra pure, low melting point; Gibco Life Technologies Ltd, Paisley, Renfrewshire, Scotland) solution (in RPMI-1640) at a ratio of 2 vol. : 1 vol. The particle gels were then dispensed into wells of 48-well plates (Corning Costar UK Ltd, High Wycombe, Bucks UK) at 200 μl, well^{-1} and centrifuged at 800 g to result in a superficial layer of polyethylene particles.

2.2. Co-culture of cells and particle fractions

All particle fractions were evaluated at particle volume (μm^3) : cell number ratios of 100 : 1, 10 : 1, 1 : 1 and 0.1 : 1, and tests were performed in triplicate. Three human monocytic cell lines; U937, THP-1 (European Collection of Animal Cell Cultures, Porton Down, Salisbury, UK) and Monomac-1 (DSMZ, Braunschweig, Germany) were seeded at 3×10^5 cells · well^{-1}. Cells were co-cultured with the particles in supplemented RPMI-1640 culture medium (1 ml · well^{-1}) for 24 h in an atmosphere of 5% (v/v) CO_2 in air at 37 °C prior to the harvest of culture supernatants for cytokine quantification and subsequent determination of cell viability (MTT assay). Negative (cells cultured without particles) and positive (cells stimulated with 20 ng · ml^{-1} lipopolysaccharide (Sigma-Aldrich Company Ltd, Poole, Dorset, UK) controls were also included.

2.3. Assessment of cell viability by the MTT assay

Following removal of the culture supernatant for cytokine analysis, wells were replenished with fresh culture medium and cell viability was determined using the MTT assay. To each well was added 100 μl MTT (5 mg · ml^{-1}; Sigma-Aldrich Company Ltd, Poole,

Dorset, UK) and plates were subsequently incubated for 4 h. Following incubation, 0.5 ml medium was carefully removed from each well and replaced with 0.5 ml 10% (w/v) sodium dodecyl sulfate in 0.1 N HCl (Sigma-Aldrich Company Ltd, Poole, Dorset, UK). Plates were then incubated for a further 18 h after which time 200 µl of supernatant from each well was transferred to a 96-well flat-bottomed plate and the optical density (OD) read spectrophotometrically (Dynatech MR7000) at 570 nm.

2.4. Measurement of cytokines by ELISA

Culture supernatants from the three cell lines were assayed for the pro-inflammatory cytokines, IL-1β, IL-6 and TNFα by enzyme linked immuno-sorbent assay (ELISA). Additionally, GM-CSF and PGE_2 were quantified, in supernatants from the U937 cell line, by ELISA and competitive enzymatic inhibition assay (EIA) respectively. IL-1β was assayed using paired monoclonal antibodies (Endogen, Woburn, MA, USA); IL-6 and TNFα were assayed using a modified double antibody sandwich technique with antibodies prepared and purified in-house [23]. Prior to use, antibody specificity was verified by determining cross-reactivity against a panel of recombinant human cytokines (i.e. IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-6 (anti-TNFα antibodies only), IL-7, IL-8, G-CSF, GM-CSF, TGFβ, INF-γ, TNFα (anti-IL-6 antibodies only)) – data not shown. No cross-reactivity was observed with any of the test molecules. Conversely, the antibodies demonstrated a good affinity with their respective standard (i.e. TNFα and IL-6) as determined by the development of color in the ELISA. (All recombinant human cytokine standards were supplied by NIBSC, Potters Bar, UK.) An avidin-biotin-HRP detection system was used for measurement of all three pro-inflammatory cytokines. GM-CSF was assayed using Predicta® ELISA kits (Genzyme Diagnostics, West Malling, UK) and PGE_2 was determined using EIA kits (R & D Systems Europe Ltd, Abingdon, UK).

2.5. Cytokine quantification and statistical analysis of data

Cytokine secretion was expressed as the specific activity (ng · cytokine/OD @ 570 nm) which relates cytokine production to the number of viable cells present. Levels of cytokine in particle-stimulated cultures were compared with negative controls (cells cultured without particles) by one way analysis of variance (ANOVA) and calculation of the minimum significant difference using the T-method at the $p < 0.01$ level.

3. Results

Cell viability was unaffected by any of the particle treatments. The Monomac-1 cell line did not respond to particle stimulation, as assessed by secretion of the pro-inflammatory cytokines evaluated, at any of the particle : cell number ratios evaluated (data not shown).

All results for the U937 and THP-1 cell lines are expressed as the mean specific activity ± 95% confidence limits in the figures.

3.1. Production of IL-1β by U937 cells stimulated with polyethylene particles

Fig. 1 shows the production of IL-1β by U937 cells stimulated with LPS (positive control) and particles at particle volume to cell number ratios of 0.1 : 1 (Fig. 1a), 1 : 1 (Fig. 1b), 10 : 1 (Fig. 1c) and 100 : 1 (Fig. 1d). U937 cells secreted low levels of IL-1β (mean value of 0.32; negative control: 0) in response to stimulation with LPS. However, particles with a mean size of 0.21 and 0.49 µm stimulated U937 cells to produce significantly higher levels of IL-1β than the negative control at a ratio of 100 : 1. The 0.49 µm particles were, however, ten-fold more potent in stimulating cytokine production compared with the 0.21 µm particles (mean values of 7.07 and 0.70 respectively; Fig. 1d). At a ratio of 10 : 1 only the 0.49 µm particles significantly stimulated IL-1β production above the negative control value (mean value of 0.71; Fig. 1c). The 4.2, 7.2 and the 88 µm particle fractions failed to stimulate IL-1β production above the negative control value at any of the particle volume to cell number ratios tested (Fig. 1).

3.2. Production of IL-6 by U937 cells stimulated with polyethylene particles

Fig. 2 shows the production of IL-6 by U937 cells stimulated with LPS (positive control) and particles at particle volume to cell number ratios of 0.1 : 1 (Fig. 2a), 1 : 1 (Fig. 2b), 10 : 1 (Fig. 2c) and 100 : 1 (Fig. 2d). U937 cells secreted a significant amount of IL-6 (mean value of 1.64; negative control: 0) in response to stimulation with LPS. Particles with a mean size of 0.49, 4.3 and 7.2 µm stimulated U937 cells to produce significantly higher levels of IL-6 than the negative control at a ratio of 100 : 1. The mean values were 1.43, 1.81 and 1.12 respectively (Fig. 2d). Additionally, the 4.3 and the 7.2 µm particles stimulated significant cytokine secretion at ratios of 1 : 1 and 10 : 1 respectively (mean values of 0.83 and 1.0; Fig. 2b and c). The 0.21 and the 88 µm particles failed to stimulate IL-6 production above the negative control value at any of the particle volume to cell number ratios tested (Fig. 2).

3.3. Production of TNFα by U937 cells stimulated with polyethylene particles

Fig. 3 shows the production of TNFα by U937 cells stimulated with LPS (positive control) and particles at particle volume to cell number ratios of 0.1 : 1 (Fig. 3a), 1 : 1 (Fig. 3b), 10 : 1 (Fig. 3c) and 100 : 1 (Fig. 3d). Stimulation of U937 cells with LPS resulted in the secretion of a significant level of TNFα (mean value of 0.43; negative control: 0). Particles with a mean size of 0.21, 0.49 and 4.3 µm stimulated U937 cells to produce significantly higher levels of TNFα than the negative control at a ratio of 100 : 1. The mean values were 0.09, 0.26 and 0.26 respectively (Fig. 3d). At a ratio of 10 : 1 the 0.21, 0.49 and the 7.2 µm particles stimulated significant levels of cytokine secretion above the

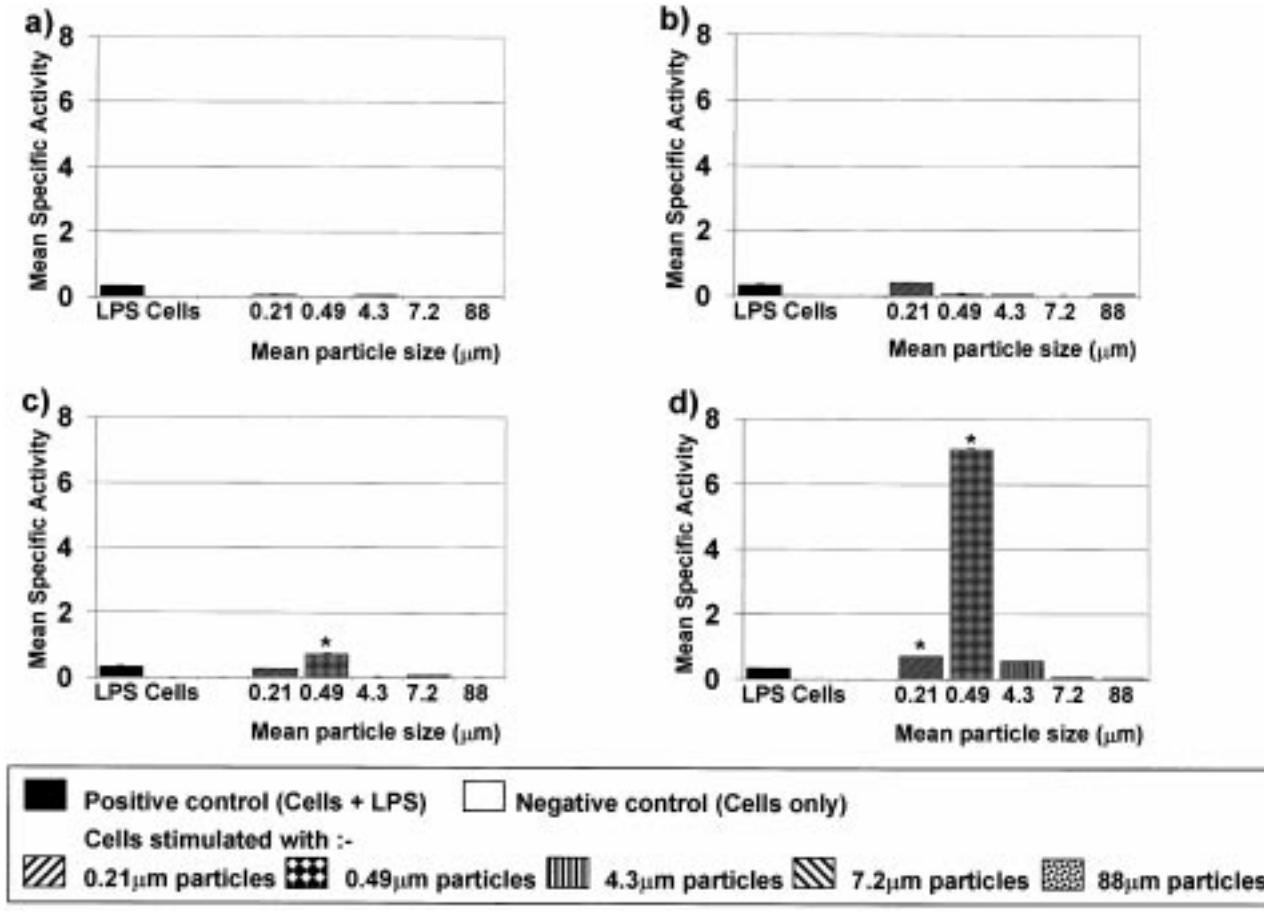

Figure 1 Secretion of IL-1β by U937 cells in response to polyethylene particles at a ratio of (a) 0.1 : 1, (b) 1 : 1, (c) 10 : 1 and (d) 100 : 1. Results are expressed as the mean $(n = 3) \pm 95\%$ confidence limits; *significantly greater than the negative control ($p < 0.01$).

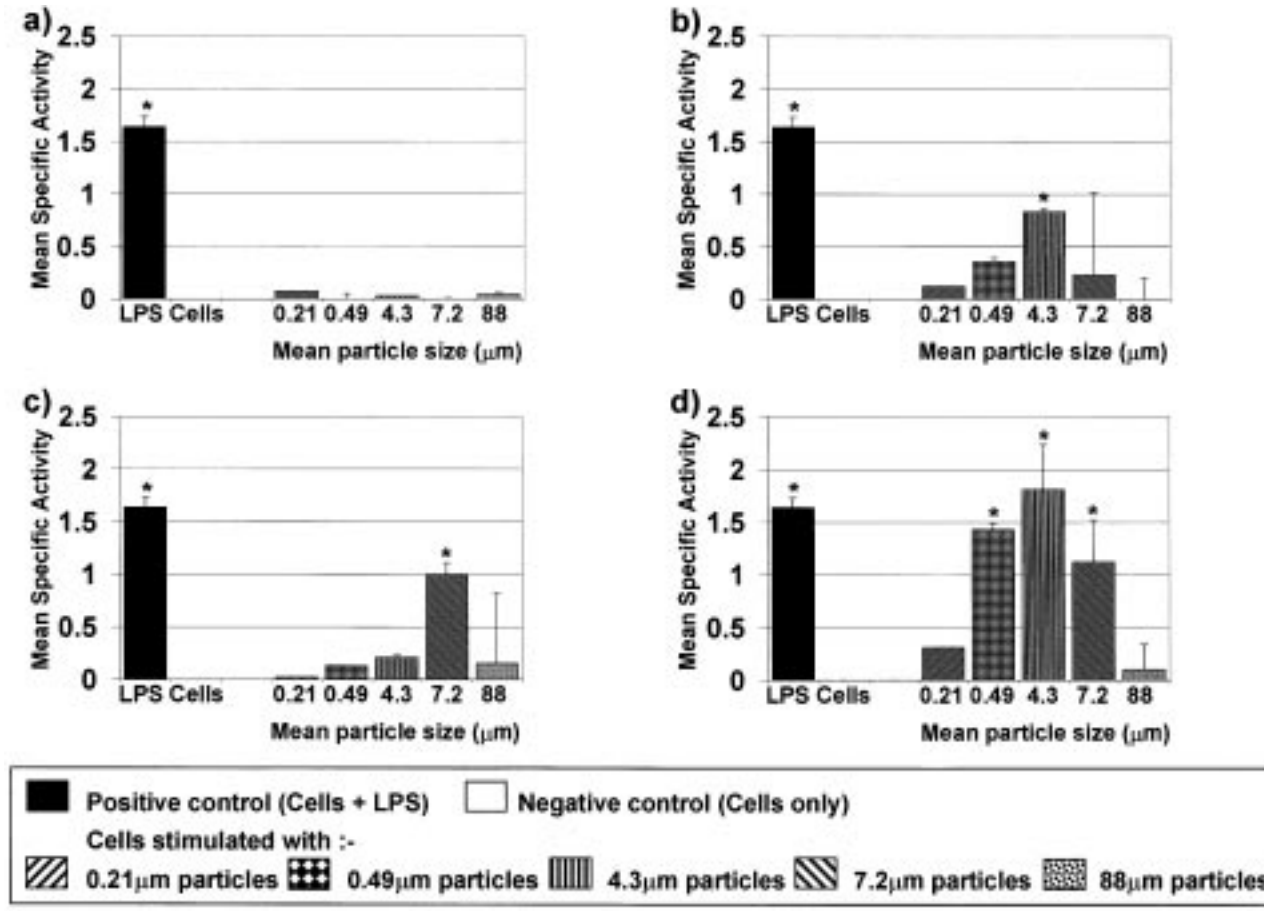

Figure 2 Secretion of IL-6 by U937 cells in response to polyethylene particles at a ratio of (a) 0.1 : 1, (b) 1 : 1, (c) 10 : 1 and (d) 100 : 1. Results are expressed as the mean $(n = 3) \pm 95\%$ confidence limits; *significantly greater than the negative control ($p < 0.01$).

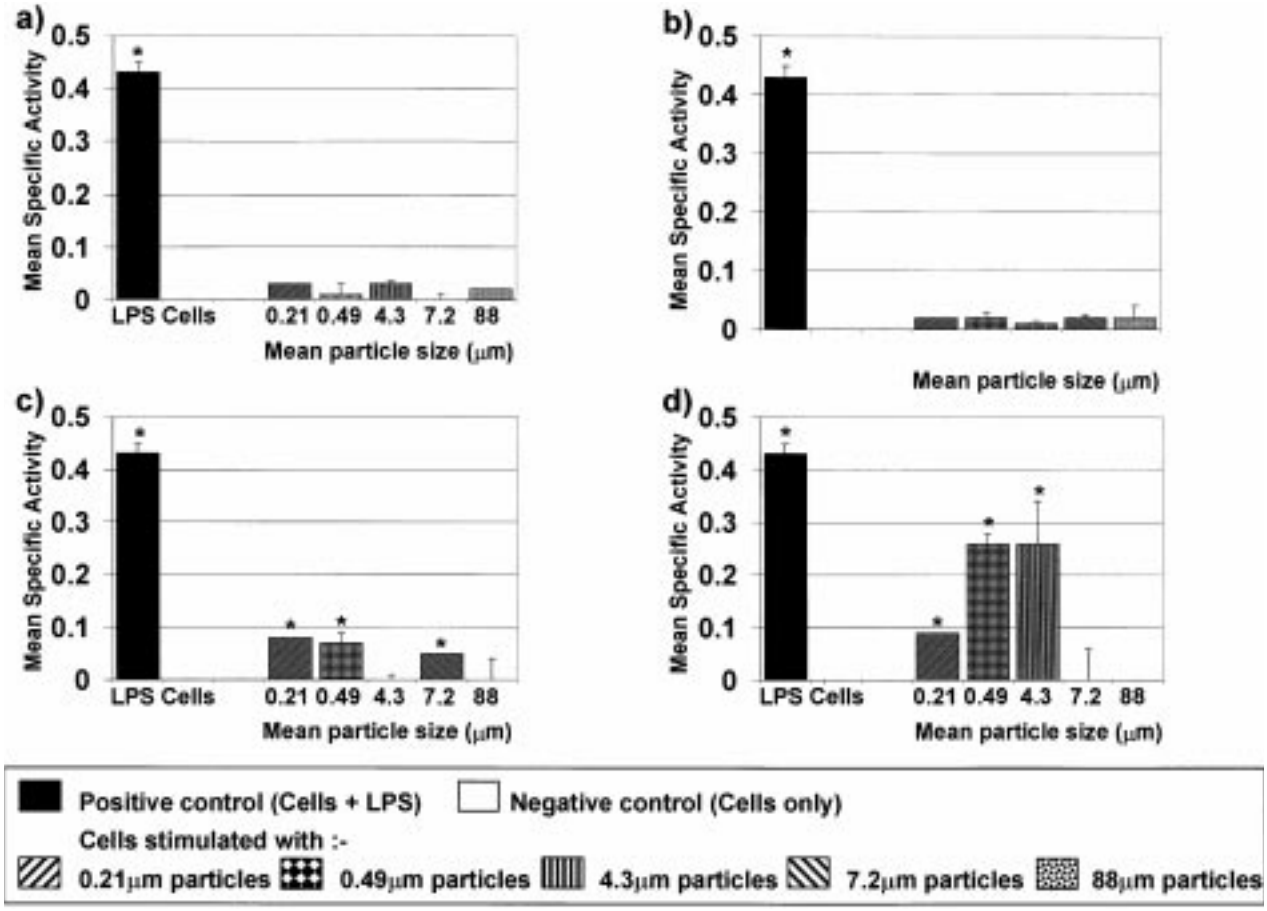

Figure 3 Secretion of TNFα by U937 cells in response to polyethylene particles at a ratio of (a) 0.1 : 1, (b) 1 : 1, (c) 10 : 1 and (d) 100 : 1. Results are expressed as the mean ($n = 3$) ± 95% confidence limits; *significantly greater than the negative control ($p < 0.01$).

negative control value (mean values of 0.08, 0.07 and 0.05 respectively; Fig. 3c). Only the 88 μm particle fraction failed to stimulate TNFα production above the negative control value at any of the particle volume to cell number ratios tested (Fig. 3).

3.4. Production of GM-CSF by U937 cells stimulated with polyethylene particles

Fig. 4 shows the production of GM-CSF by U937 cells stimulated with LPS (positive control) and particles at particle volume to cell number ratios of 0.1 : 1 (Fig. 4a), 1 : 1 (Fig. 4b), 10 : 1 (Fig. 4c) and 100 : 1 (Fig. 4d). U937 cells secreted a significant amount of GM-CSF in response to LPS (mean value of 1.3; negative control: 0.02). Particles with a mean size of 0.49 and 4.3 μm stimulated U937 cells to produce significantly higher levels of GM-CSF than the negative control at a ratio of 100 : 1. The mean values were 0.87 and 1.07 respectively; negative control: 0.02 (Fig. 4d). The 0.21, 7.2 and the 88 μm particle fractions failed to stimulate GM-CSF production above the negative control value at any of the particle volume to cell number ratios tested (Fig. 4).

3.5. Production of PGE_2 by U937 cells stimulated with polyethylene particles

Fig. 5 shows the production of PGE_2 by U937 cells stimulated with LPS (positive control) and particles at particle volume to cell number ratios of 0.1 : 1 (Fig. 5a), 1 : 1 (Fig. 5b), 10 : 1 (Fig. 5c) and 100 : 1 (Fig. 5d). Although not reaching significance, U937 cell secreted high levels of PGE_2 in response to LPS stimulation (mean value of 5.5; negative control: 0.4). Furthermore only particles with a mean size of 4.3 μm stimulated U937 cells to produce significantly higher levels of PGE_2 than the negative control at a ratio of 100 : 1. The mean value was 6.53 ($p < 0.05$); Fig. 5d. Although not reaching significance, however, the 0.49 μm particle fraction also stimulated the production of high levels of PGE_2 at a ratio of 100 : 1 (mean value of 4.8). The 0.21, 7.2 and the 88 μm particle fractions failed to stimulate PGE_2 production above the negative control value at any of the particle volume to cell number ratios tested (Fig. 5).

3.6. Production of IL-1β by THP-1 cells stimulated with polyethylene particles

Fig. 6 shows the production of IL-1β by THP-1 cells stimulated with LPS (positive control) and particles at particle volume to cell number ratios of 0.1 : 1 (Fig. 6a), 1 : 1 (Fig. 6b), 10 : 1 (Fig. 6c) and 100 : 1 (Fig. 6d). THP-1 cells secreted significantly high levels of IL-1β (mean value of 0.26; negative control: 0) in response to stimulation with LPS. Only particles with a mean size of 0.49 μm stimulated THP-1 cells to secrete significantly more IL-1β than the negative control at a ratio of 100 : 1 (mean value of 0.20; Fig. 6d). The 0.21 μm and the 0.49 μm fractions also gave significantly elevated

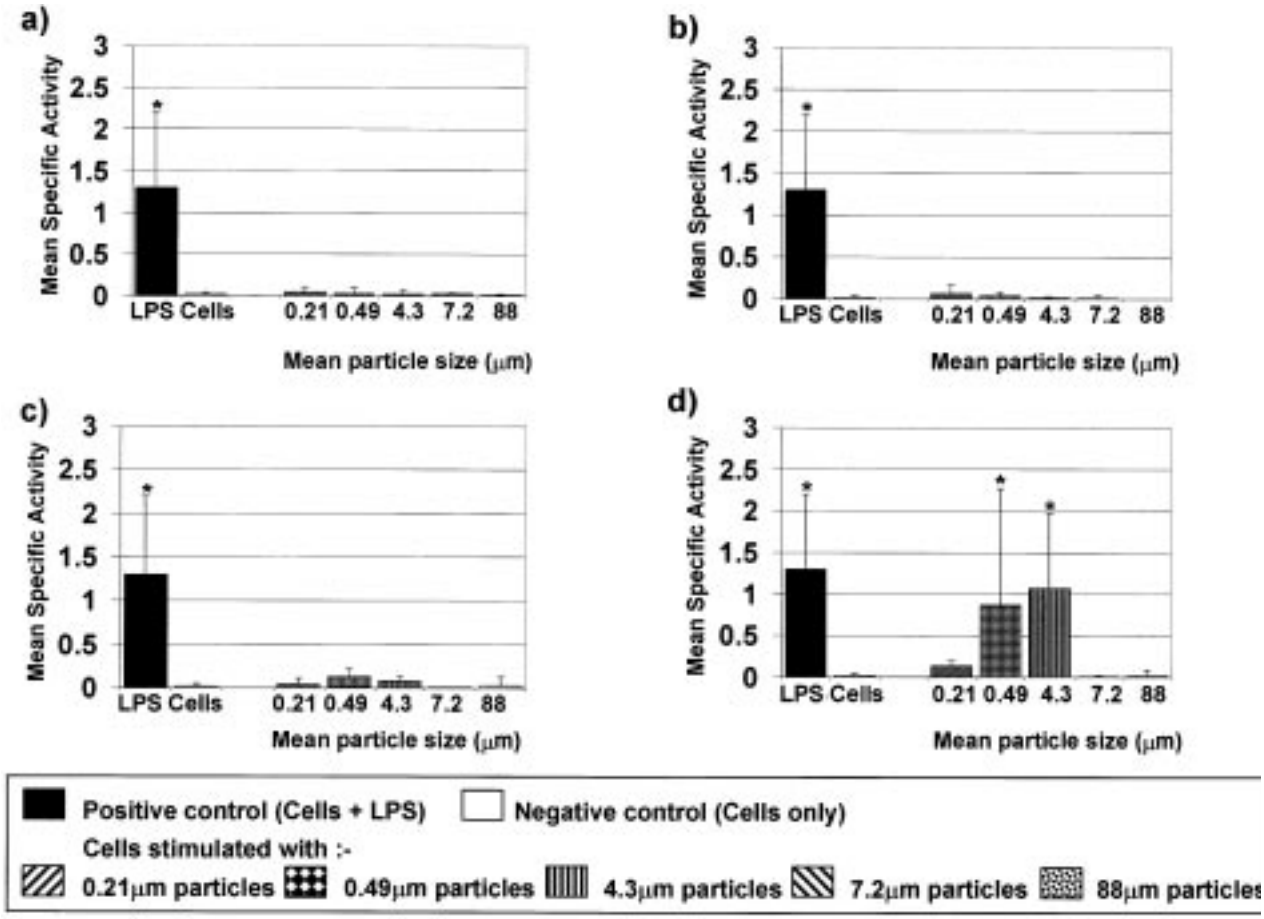

Figure 4 Secretion of GM-CSF by U937 cells in response to polyethylene particles at a ratio of (a) 0.1 : 1, (b) 1 : 1, (c) 10 : 1 and (d) 100 : 1. Results are expressed as the mean $(n = 3) \pm 95\%$ confidence limits; *significantly greater than the negative control ($p < 0.01$).

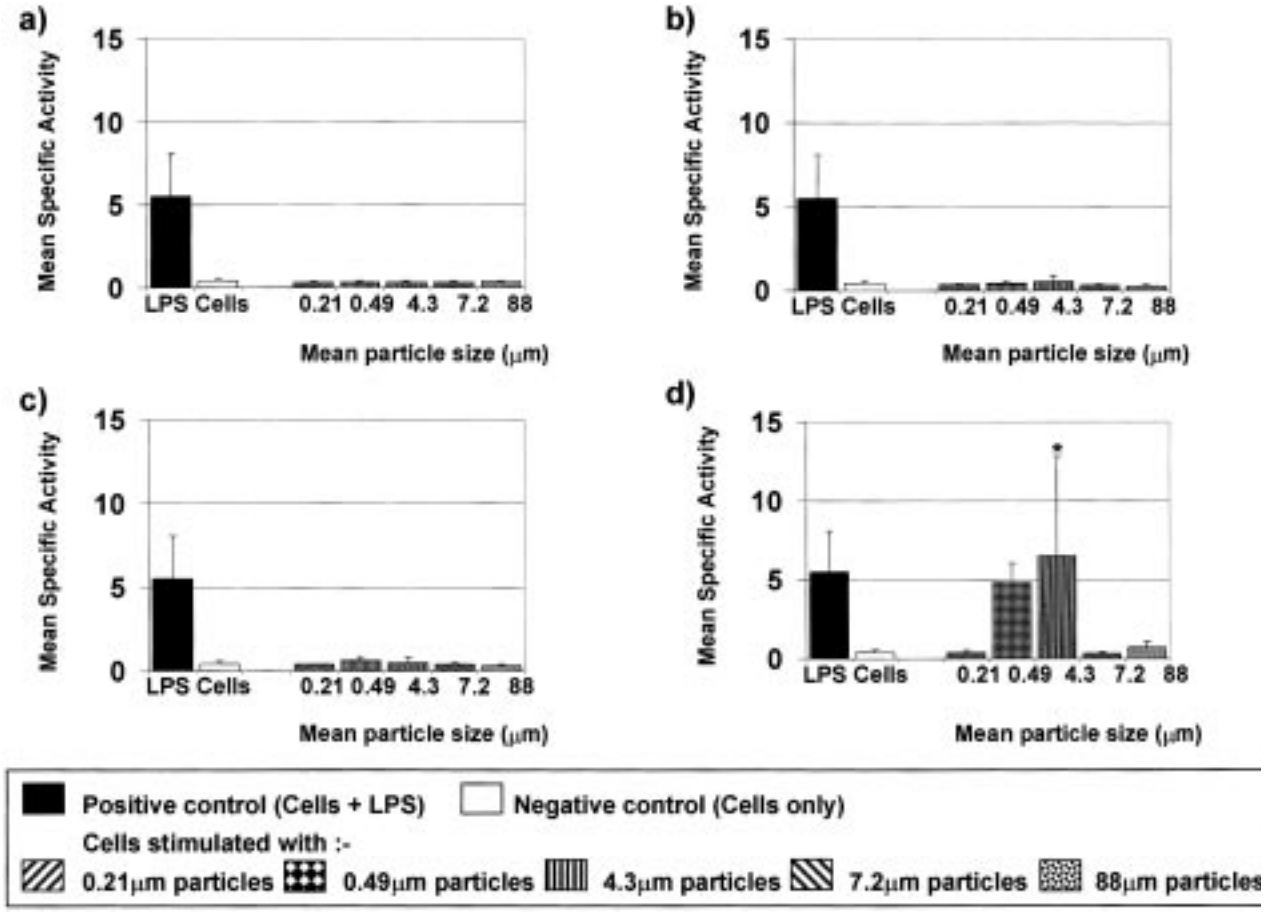

Figure 5 Secretion of PGE_2 by U937 cells in response to polyethylene particles at a ratio of (a) 0.1 : 1, (b) 1 : 1, (c) 10 : 1 and (d) 100 : 1. Results are expressed as the mean $(n = 3) \pm 95\%$ confidence limits; *significantly greater than the negative control ($p < 0.05$).

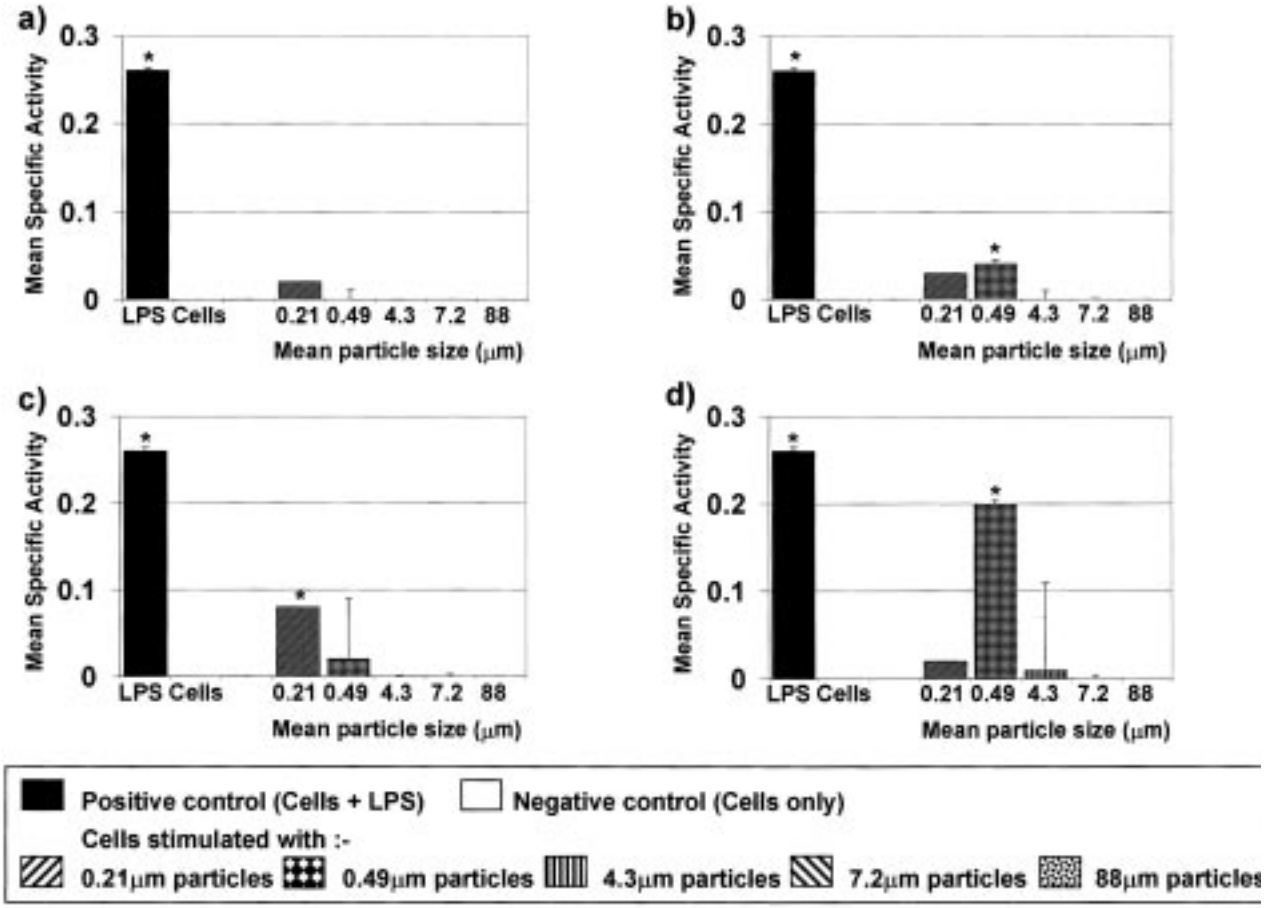

Figure 6 Secretion of IL-1β by THP-1 cells in response to polyethylene particles at a ratio of (a) 0.1 : 1, (b) 1 : 1, (c) 10 : 1 and (d) 100 : 1. Results are expressed as the mean ($n = 3$) ± 95% confidence limits; *significantly greater than the negative control ($p < 0.01$).

cytokine production at ratios of 10 : 1 (Fig. 6c) and 1 : 1 (Fig. 6b) respectively, however, the levels produced were far less than those induced by the 0.49 μm particles at a ratio of 100 : 1 (mean values of 0.08 and 0.04 respectively). The 4.3, 7.2 and the 88 μm particles failed to stimulate significant IL-1β production at any of the particle volume to cell number ratios evaluated (Fig. 6).

3.7. Production of IL-6 by THP-1 cells stimulated with polyethylene particles

Fig. 7 shows the production of IL-6 by THP-1 cells stimulated with LPS (positive control) and particles at particle volume to cell number ratios of 0.1 : 1 (Fig. 7a), 1 : 1 (Fig. 7b), 10 : 1 (Fig. 7c) and 100 : 1 (Fig. 7d). High levels of IL-6 were produced by THP-1 cells in response to LPS (mean value of 0.82; negative control: 0.01). However, the production of IL-6 by THP-1 cells in response to particle stimulation was very irregular. At a ratio of 100 : 1, particles with a mean size of 0.21 and 0.49 μm stimulated significantly elevated secretion of IL-6 (mean values of 0.09 and 0.04 respectively; Fig. 7d). However, at ratios of 10 : 1; 1 : 1 and 0.1 : 1 particles with mean sizes of 0.49 and 88 μm (Fig. 7c); 4.3 μm (Fig. 7b); and 0.21 μm (Fig. 7a) stimulated significant levels of IL-6 secretion (mean values of 0.15 and 0.20; 0.12 and 0.09 respectively). The 4.3 and the 7.2 μm particle fractions failed to stimulate significant IL-6 production at any of the particle volume to cell number ratios evaluated (Fig. 7).

3.8. Production of TNFα by THP-1 cells stimulated with polyethylene particles

Fig. 8 shows the production of TNFα by THP-1 cells stimulated with LPS (positive control) and particles at particle volume to cell number ratios of 0.1 : 1 (Fig. 8a), 1 : 1 (Fig. 8b), 10 : 1 (Fig. 8c) and 100 : 1 (Fig. 8d). THP-1 cells secreted very high levels of TNFα in response to LPS stimulation (mean value of 2.06; negative control: 0). Particles with a mean size of 0.21, 0.49 and 4.3 μm stimulated THP-1 cells to produce significantly higher levels of TNFα than the negative control at a ratio of 100 : 1. The mean values were 0.13, 0.75 and 0.19 respectively; Fig. 8d). At a ratio of 10 : 1 only the 0.49 μm fraction stimulated significant cytokine production above the negative control value (mean value of 0.08; Fig. 8c). The 7.2 and the 88 μm particle fractions failed to stimulate TNFα production above the negative control value at any of the particle volume to cell number ratios tested (Fig. 8).

4. Discussion

Most importantly, this study has clearly demonstrated that both the size and volume of polyethylene particles are critical factors in the process of macrophage activation. Particles within the phagocytosable size range (0.1–10 μm) were observed to be the most biologically active in terms of their ability to stimulate the secretion of various pro-inflammatory cytokines (IL-1β, TNFα, IL-6) and other osteolytic mediators (GM-CSF, PGE_2). Furthermore, with the exception of IL-6

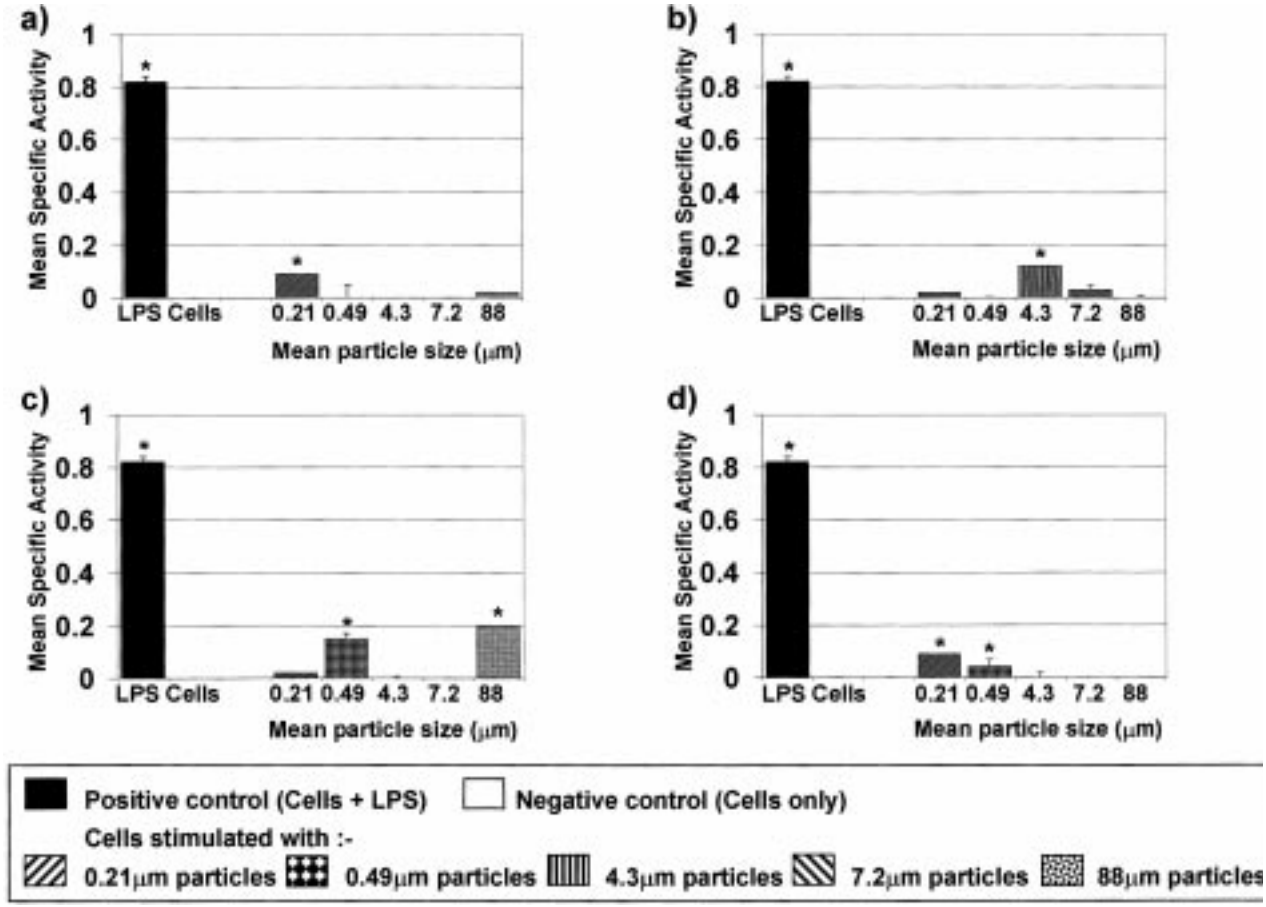

Figure 7 Secretion of IL-6 by THP-1 cells in response to polyethylene particles at a ratio of (a) 0.1 : 1, (b) 1 : 1, (c) 10 : 1 and (d) 100 : 1. Results are expressed as the mean $(n = 3) \pm 95\%$ confidence limits; *significantly greater than the negative control ($p < 0.01$).

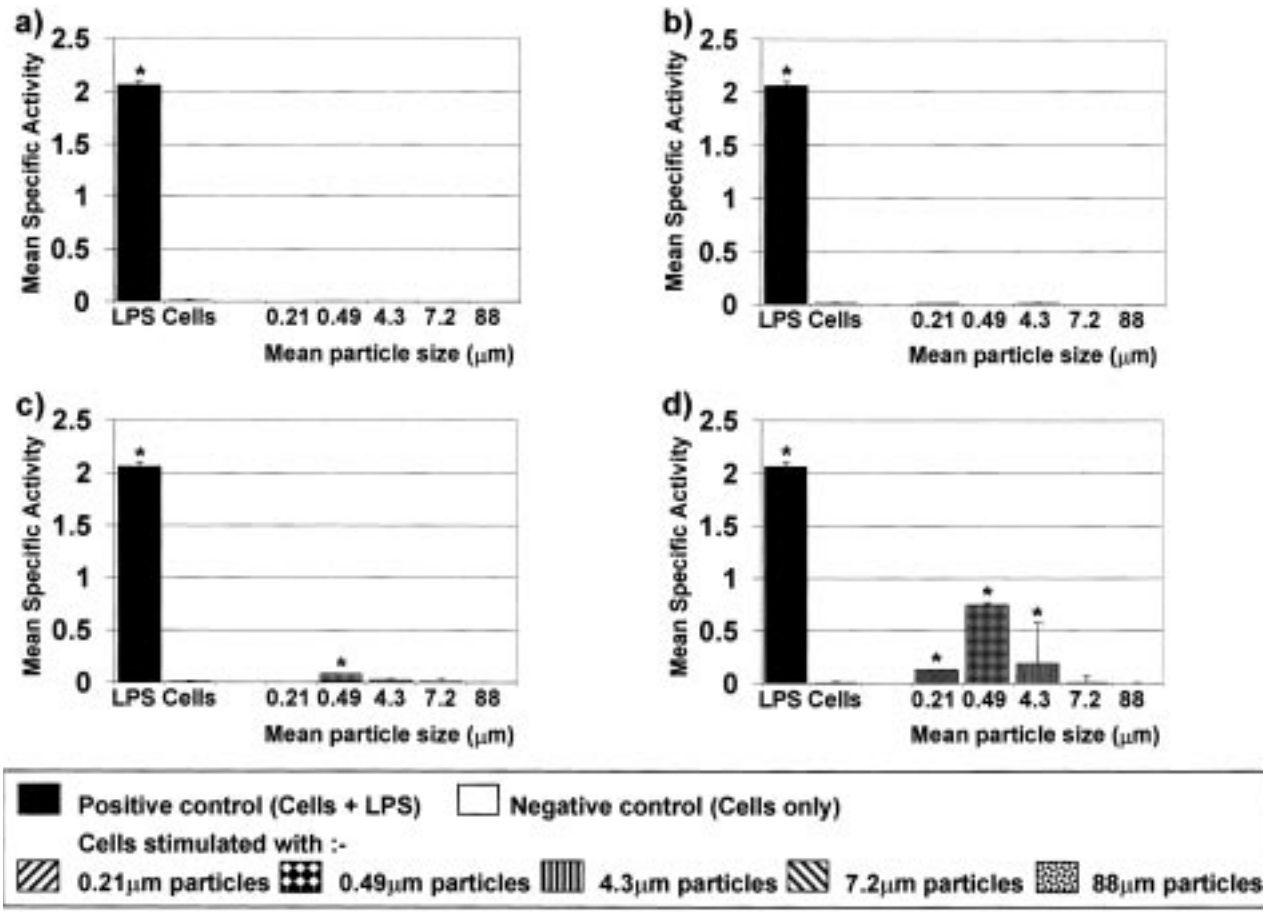

Figure 8 Secretion of TNFα by THP-1 cells in response to polyethylene particles at a ratio of (a) 0.1 : 1, (b) 1 : 1, (c) 10 : 1 and (d) 100 : 1. Results are expressed as the mean $(n = 3) \pm 95\%$ confidence limits; *significantly greater than the negative control ($p < 0.01$).

production by the THP-1 cell line (particle volume to cell number ratio of 10 : 1), no reaction to the larger (88 μm) particles was observed at any of the particle volume ratios tested.

Whilst the THP-1 cell line responded to particle volume to cell number ratios of as little as 0.1 μm^3 per cell, only ratios of 10 : 1 or greater were found to stimulate significant levels of cytokine secretion by the U937 cells. This finding compared favorably with previous studies performed at Leeds, in which the UHMWPE wear debris and cellular content of the tissues surrounding aseptically loosened ''Charnley'' hip prostheses were evaluated. Tissue samples, isolated during revision surgery, were digested and the debris collected. Following gravimetric, SEM and image analysis, it was calculated that there were approximately 10^{10} particles, or, 1 mg debris per gram of tissue [15]. This equated to 1 mm^3 debris per cm^3 of tissue. At particle volume to cell number ratios of 10 and 100 μm^3 per cell, therefore, this would be sufficient to activate 10^7 to 10^8 macrophages. Further histological evaluation of these explant tissues showed that there were around. 1.6×10^7 macrophages present per cm^3 tissue (unpublished data). This equated to a ratio of approximately 60 μm^3 debris per cell, i.e. well within the experimental biologically active range.

The use of a transformed cell line for studies of cell-particle interactions would provide a number of benefits over the use of primary human cells (reproducibility, economy of cost and time, practical simplicity and availability) providing that their response profile had first been validated. Previous studies have identified the danger of assuming that the response of different cell populations, either primary or transformed, will be comparable [11], however, many investigators have used transformed cell lines apparently without initial optimization [9, 18, 20]. Three human cell lines of monocytic lineage (Monomac-1, THP-1 and U937) were, therefore, chosen for evaluation as potential model systems, each population representing different stages in the monocyte-macrophage differentiation pathway. In this study, the Monomac-1 cell line failed to synthesize osteolytic mediators in response to challenge with polyethylene particles. Furthermore the response of the THP-1 cells was shown to be very irregular. Whilst THP-1 cells were unresponsive to challenge with the 7.2 μm particle fraction they responded both to the very large particles (88 μm) at a ratio of 10 μm^3 per cell and to the other particle sizes at concentrations as low as 0.1 μm^3 particles per cell. Only the response of the U937 cell line was comparable to that of primary macrophages, as determined in a previous study by Green *et al*. [24]. This study evaluated the reaction of primary murine peritoneal macrophages to the same polyethylene particles *in vitro*. Similarly, this earlier study demonstrated that the most biologically active particles were within the phagocytosable size range (0.3–10 μm) as determined by their ability to stimulate the secretion of a number of osteolytic mediators. Both larger (88 μm) and smaller (0.21 μm) particles stimulated cells to secrete considerably less cytokine at all volumes tested. Furthermore, in agreement with the current work, only particle volume to cell number ratios of 10 : 1 or greater initiated a response. Although the lower threshold of reactive particle size was slightly reduced for the U937 cells (a response to the 0.21 μm particle fraction was observed), the most biologically active particles were still within the phagocytosable size range.

In terms of the concentration of cytokine secreted by cells activated by either LPS or particle stimulation, the response of the THP-1 and U937 cell lines differed greatly. Production of TNFα by the THP-1 cell line was far higher than by the U937 cells. Conversely, U937 macrophages were able to secrete greater levels of both IL-6 and IL-1β. When THP-1 cells were challenged with either polyethylene particles or LPS, cytokines were secreted in the following order of decreasing magnitude: TNFα > IL-6 ≥ IL-1β. However, U937 cells secreted PGE_2 in the highest concentrations followed by IL-6, GM-CSF and TNFα. The U937 cells also produced a very high level of IL-1β, comparable to the level of PGE_2, in response to particle stimulation, however, only low levels were secreted in response to LPS. Such differences in the capacity of the cell lines evaluated in this study, to secrete different cytokines, represents biological variation. Genetic heterogeneity within the human population results in individuals who differ both in their sensitivity to, e.g. LPS and also in their ability to synthesize and secrete different molecules. Just as the magnitude and profile of the response of primary cells from individual donors would vary, so too does the response of individual cell lines since each was derived from a different person.

In addition to the source of the cells, the stage of differentiation is a further factor in determining their reactivity to phagocytic stimulation. The U937 cell line represents a later stage in the monocyte/macrophage differentiation pathway, than either the Monomac-1 or the THP-1 cell lines. Whilst the U937 line retains many monocytic characteristics, it is in fact derived from the pleural effusion of an acute histiocytic lymphoma. Conversely, the THP-1 and the Monomac-1 lines are derived from the peripheral blood of individuals suffering from acute monocytic leukaemia and, therefore, are more immature phenotypically. As the macrophages which line the tissue-implant interface (i.e. those cells which respond to particles *in vivo*) are mature cells in terms of their state of differentiation, it is not surprising that the histiocytic cell line gives the most comparable response. The differential response of the three cell lines evaluated in the current study, therefore, clearly demonstrates the caution which should be excised in choosing transformed cell lines as model systems. Both the Monomac-1 and the THP-1 cell lines were demonstrated to be unsuitable for studying cell-particle interactions *in vitro*. Only the U937 cell line has been shown to be a suitable model for future *in vitro* studies of cell-particle interactions.

Future studies will address the reaction of the U937 cell line to real ''tribologically generated'' wear debris produced from the different types of UHMWPE used in artificial joints. Our recent work has shown considerable differences in particle mass and number distributions, as a function of size, for different types of polyethylene [25]. Importantly, this study would indicate that this

difference in particle morphology may well influence the biological reactivity of the different types of debris.

Acknowledgments

The authors would like to thank the Arthritis Research Campaign for their generous financial support. Particles were supplied by Hoechst Germany through the Brite Euram Project (Number BE7928).

No benefits in any form have been received, or will be received from a commercial party related either directly or indirectly to the subject of this article.

References

1. M. JASTY and E. SMITH, *Current Opinion in Rheumatology* **4** (1992) 204.
2. H. G. WILLERT and M. SEMLITSCH, *J. Biomed. Mater. Res.* **11** (1977) 157.
3. L. MINTZ, A. K. TSAO, C. R. MCCRAE, S. D. STULBERG and T. WRIGHT, *Clin. Orthopaed. & Rel. Res.* **273** (1991) 215.
4. L. D. DORR, R. BLOEBAUM, J. EMMANUAL and R. MELDRUM, *ibid. & Rel. Res.* **261** (1990) 82.
5. D. C. AYERS, J. D. CALORE, S. TAFFET, A. WANG and D. G. MURRAY in Proceedings of the 39th Annual Meeting of the Orthopaedic Research Society, San Francisco, California, February 1993, p. 290.
6. S. A. PARRY, C. J. SUTHERLAND, G. MILLER, J. GILDEN and M. E. JOYCE in Proceedings of the 41st Annual Meeting of the Orthopaedic Research Society, Orlando, Florida, February 1995, p. 216.
7. N. AL SAFFAR, H. A. KWHAJA, P. A. REVELL and Y. KADOYA, *Anatomic Pathology* **105** (1996) 628.
8. S. R. GOLDRING, A. L. SCHILLER, M. ROELKE, C. M. ROURKE, D. A. O'NEILL and W. H. HARRIS, *J. Bone Joint Surg.* **65-A** (1983) 575.
9. A. S. SHANBHAG, J. J. JACOBS, J. BLACK, G. O. GALANTE, and T. T. GLANT, *J. Biomed. Mat. Res.* **28** (1994) 81.
10. I. C. CLARKE and P. CAMPBELL in Proceedings of an international seminar held on the occasion of the 25th anniversary of the Strathclyde Bioengineering Unit, Glasgow, September, *Progress in Bioengineering* **18** (1988) 104.
11. T. GLANT and J. JACOBS, *J. Orthopaed. Res.* **12** (1994) 720.
12. H. GELB, H. R. SCHUMACHER, J. CUCKLER and D. G. BAKER, *ibid.* **12** (1994) 83.
13. J. L. HAILEY, E. INGHAM, M. STONE, B. M. WROBLEWSKI and J. FISHER, *Proceedings of the Institution of Mechanical Engineers* **210** (1996) 3.
14. P. CAMPBELL, S. MA, B. YEOM, H. MCKELLOP, T. P. SCHMALZRIED and H. C. AMSTUTZ, *J. Biomed. Mat. Res.* **29** (1995) 127.
15. J. L. TIPPER, E. INGHAM, J. L. HAILEY, A. A. BESONG, M. STONE, B. M. WROBLEWSKI and J. FISHER in Proceedings of the 43rd Annual Meeting of the Orthopaedic Research Society, San Francisco, California, February 1997 p. 355.
16. T. P. SCHMALZRIED, M. JASTY, A. ROSENBERG and W.H. HARRIS, *J. Appl. Biomater.* **5** (1994) 185.
17. J. CHIBA, W. MALONEY, M. HORIKOSHI, L. MCINTYRE, and H. RUBASH in Proceedings of the 39th Annual Meeting of the Orthopaedic Research Society, San Francisco, California, February 1993, p. 270.
18. S. M. HOROWITZ and J. B. GONZALES, *J. Orthopaed. Res.* **15** (1997) 50.
19. D. W. MURRAY and N. RUSHTON *J. Bone Joint Surg.* **72-B** (1990) 988.
20. S. M. ALGAN, M. PURDON, and S. M. HOROWITZ, *J. Orthopaed. Res.* **14** (1996) 30.
21. A. S. SHANBHAG, J. BLACK, J. J. JACOBS, G. O. GALANTE and T. T. GLANT in Proceedings of the 40th Annual Meeting of the Orthopaedic Research Society, New Orleans, Louisiana, February 1994, p. 849.
22. Y. HARADA, V. A. DOPPALAPUDI, A. A. WILLIS, W. JASTY, W. H. HARRIS and S. R. GOLDRING Research Society, New Orleans, Louisiana, *ibid.* p. 842.
23. A. VOLLER, D. E. BIDWELL and A. BARTLETT, in ''The Enzyme Linked Immunosorbent Assay (ELISA)'' (Pub. 1979), p. 14.
24. T. R. GREEN, M. STONE, B. M. WROBLEWSKI, J. FISHER, and E. INGHAM, *Biomaterials* **19** (1998) 2297.
25. A. A. BESONG, J. L. TIPPER, E. INGHAM, M. H. STONE and J. FISHER *J. Bone Joint Surg.* **80-B** (1998) 340.

Received 27 August 1998
and accepted 21 June 2000