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Increased Cytokine Secretion in Patients with Failed Implants Compared with Patients with Primary Implants

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All total joint replacements generate wear debris; yet, some implant prostheses fail while others survive despite the presence of ultrahigh molecular weight polyethylene particulate. It was hypothesized that patients with failed hip implants who have osteolysis will secrete higher inflammatory cytokines than patients receiving total joint replacements. Our study evaluated the peripheral blood monocyte response to varying polyethylene particle volume ratios through cytokine quantification in two patient populations: patients having revision surgery for failed total hip replacements (failed implant group) and patients having primary total hip surgery for osteoarthritis of the hip (primary implant group). We observed elevation of all three proinflammatory cytokines tested (interleukin-6, interleukin-1, and tumor necrosis factor-α) in response to polyethylene particulate challenge when compared with the controls in both patient groups. The population with failed implants also had a higher absolute cytokine response to polyethylene exposure compared with the control patients having primary implants. These findings suggest that patients with failed implants have a greater inflammatory cytokine response to polyethylene than seen in patients with primary implants.

Aseptic loosening of total hip replacements (THR) is the major cause of failure of this otherwise highly successful surgical procedure. Research has determined that interaction of ultrahigh molecular weight polyethylene (PE) wear particles and macrophages results in the secretion of proinflammatory mediators, leading to a state of chronic inflammation, periprosthetic osteolysis, implant loosening, and failure.[1–3] Numerous patient factors such as gait, activity levels, and quality of bone influence the mechanical wear of the prosthesis. In addition, sterilization and manufacturing processes may have a profound influence on PE surface oxidation and, therefore, wear particle generation.[9] Finally, an individual's biologic response to the wear particle ultimately may determine implant failure. Regardless of the mechanism or rate of PE wear and particulate creation, all implants generate wear debris; the question is why some implant prostheses fail while others survive, despite the presence of PE particulate.

The link between genetic polymorphisms in inflammatory cytokine genes and disease progression[4,5] suggests that genetic background may play a role in an individual's response to wear debris. These genetic differences may result in elevated cytokine secretion during the inflammatory response to wear particulate and patients with osteolysis may secrete higher inflammatory cytokines than patients with well fixed implants. Other research studies have shown the cellular response to an implant material under identical conditions to be extremely heterogeneous,[8,10] suggesting that patient characteristics may be an important factor in determining the outcome of implant surgery.

We therefore ascertained the peripheral blood monocyte response to varying particle volume ratios through cytokine quantification in two patient populations: patients having revision surgery for failed THRs (failed implant group) and patients having primary THRs for osteoarthritis of the hip (primary implant group).

MATERIALS AND METHODS

Polyethylene particles obtained from Stryker Orthopaedics (Kalamazoo, MI) were generated using a wear simulator and retrieved from the serum surrounding the apparatus. Using scanning electron microscopy (SEM), the average particle size was

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characterized to be 1.86 ± 0.87 μm in length and 0.75 ± 0.25 μm in width (range, 0.1–2.2 μm), and shapes included rods, spheres, and fragments. Polyethylene particles were sterilized with 2.5 Mrad γ-irradiation and determined to be endotoxin-free using an E-TOXATE detection kit (Sigma, St Louis, MO). Particle-collagen solutions were prepared as previously described by Xing et al.[19] Briefly, particles were suspended in 0.01% collagen Type I solution (from calf skin, C-8919; Sigma) at a concentration determined using the particle weight to volume ratio for PE ($1\ \mu m^3 = 1 \times 10^9$ mg). A serial dilution of the particle solution resulted in a range of particle volumes (100:1, 50:1, 20:1, and 10:1 particles to cell). Round, 15-mm diameter glass coverslips (Fisher Scientific, Whitby, ON) then were coated with 3.6 μL of the particle suspension. Coverslips coated with the collagen solution alone were used as negative controls.

Two experimental groups were set up: patients having revision surgery for a failed THRs (n = 7); and patients having primary THRs for osteoarthritis of the hip (n = 7). Primary indication for hip replacement was osteoarthritis in all patients in the primary implant and failed implant groups. Revision surgery was done for aseptic loosening with ballooning osteolysis in the patients with failed implants. Patients with failed implants having rapid osteolysis and implant failure were specifically selected. Patients in both experimental groups consented to the procedure before being admitted to the hospital (University of Toronto; ethical protocol #2015). Patients were admitted to the hospital the day of surgery and blood was collected shortly after sedation in the operating room.

Human blood was collected in heparinized vacutainers (Becton Dickinson, Franklin Lakes, NJ) from the two patient groups. Lymphocytes were isolated using Ficoll-Paque (Pharmacia Biotech, San Francisco, CA) density gradient centrifugation and monocytes, then isolated with adherence. Cell viability at 24 hours was determined to be greater than 95% with the trypan blue exclusion assay. Cells were cultured in 24-well tissue culture plates with RPMI 1640 media (R7509, Sigma), supplemented with 10% heat-inactivated fetal bovine serum (Gibco BRL, Burlington, ON), 100 units/mL penicillin-streptomycin (Gibco BRL), 68 mmol/LL-glutamine (Gibco BRL), and incubated at 37° C in 5% CO_2 atmosphere. Two hours after cell seeding, the tissue culture well contents were aspirated, removing the nonadherent cells, and replaced with fresh media; this was defined as Time zero. Fourteen separate cultures were done with the seven patients in the failed implant group and seven patients in the primary implant group. Patient information is shown in Table 1.

At 18 hours, the supernatant was removed and adherent cells were washed three times with PBS buffer with calcium and magnesium. Cells were fixed using a 2:1 ratio of 100% methanol and 10% formalin for 2 hours and stained with hematoxylin and eosin. Coverslips were mounted on microscope glass slides and photographed under transmitted and polarized light (histologic examination was done by KP).

Deoxyribonucleic acid contents of each well were quantified to normalize cytokine secretion to the amount of DNA.[17,18] At Time zero, adherent cells were rinsed with PBS and incubated with lysis buffer (0.05% Triton X-100/10 mmol/L EDTA/PBS). Deoxyribonucleic acid content was determined with a fluorometric assay using Hoechst dye (Fisher H33258) as previously outlined by Labow et al.[7] Each DNA sample was assayed in duplicate.

Conditioned media were collected at 18 and 24 hours after incubation, centrifuged in a microcentrifuge at 2500 rpm for 5 minutes, and stored as 200 μL aliquots at -70º C until analysis by enzyme-linked immunosorbant assay (ELISA). Commercially available ELISA kits were used to quantify the cytokine levels: human interleukin-1beta (IL-1β) (KHC0012; Biosource International, Camarillo, CA), and human interleukin-6/tumor necrosis factor-alpha (IL-6/TNF-α) Flexia™ (Biosource International). The absorbance was read at 450 nm using a VersaMax microplate reader (Molecular Devices, Sunnyvale, CA) with SoftMax Pro software (supplied by Molecular Devices, version 3.1.1). All samples were analyzed in triplicate and the standards in duplicate. Cytokine values then were normalized with the DNA value obtained at Time zero.

Data were normally distributed and expressed as the mean ± standard deviation. Failed implant and primary implant cytokine results can be further divided into high and low secretors, based on the median cytokine value. Those designated high secretors have cytokine levels greater than the median and low secretors have levels less than the median. Analyze It© (Leeds, England), a program plug-in for Microsoft Excel, was used for the statistical analyses. A paired Student's t test was applied to analyze the differences in cytokine secretion in experimental groups. One-way analysis of variance (ANOVA) was used to compare the means between experimental groups with Scheffe's post hoc analysis to account for multiple comparisons. The relationships between cytokine levels were evaluated with the Pearson's Prod-

TABLE 1. Patient Data

Patient	Implant Type	Description	Primary Surgery Date*	Reason for Revision
1	St Michael's beaded cup	Cementless	1999	Instability, cup only
2	Unknown	Cementless	1991	Polyethylene wear
3	Unknown	Cementless	1992	Loose cup
4	St Michael's beaded cup	Cementless	1990	Loose cup
5	St Michael's beaded cup	Cementless	1989	Loose cup
6	St Michael's screw ring	Cementless	1988	Loose cup
7	Unknown	Cemented	1991	Loose stem and femur

*All revision surgeries were done in 2000.

uct Moment, a test to evaluate the linear relationship between variables. Statistical significance was determined at the 0.05 level ($p < 0.05$).

RESULTS

Monocytes exposed to PE were highly eosinophilic with granular cytoplasm when compared with the collagen control (Fig 1). Examination of the cells under polarized light revealed birefringent particles of PE in greater than 95% of the PE-challenged cells (Fig 1).

A particle dose response in the level of cytokine secretion was seen for all three proinflammatory cytokines analyzed: IL-6, IL-1β, and TNF-α. Column plots of the measured cytokine levels versus particulate volume were bell-shaped with the maximum cytokine response occurring at the 10:1 ratio (data not shown). As cytokine secretion seemed to peak between the 100:1 and the 10:1 particle volume to cell number ratio, the 10:1 ratio was selected to be the focus of this work.

No significant increase in cytokine secretion in response to PE exposure was seen in the pooled primary implant data ($n = 7$); however, individual patients did have significantly elevated cytokine levels with PE exposure (Table 2). When individual patient cytokine levels were evaluated at 18 hours, three of seven patients had increased IL-6 and TNF-α secretion in response to the 10:1 PE ratio, and one patient had elevated ($p < 0.05$) IL-1β (Table 2). The cytokine levels had a large range in values, showing the heterogeneity present in the patients in the primary implant group (Fig 2).

Significant elevation of cytokine secretion in response to PE was seen in the pooled data (IL-6, TNF-α, IL-1β $p<0.05$) of patients with failed implants. When cytokine levels of individual patients were evaluated at 18 hours, five of seven patients had increased IL-6 and TNF-α levels in response to a 10:1 PE ratio (Table 2). Cytokine levels of individual patients evaluated at 18 hours, also showed that four of five patients had increased IL-1β levels in response to 10:1 PE ratio (Table 2). Following the trend seen in the primary implant group, the absolute level of cytokine secretion varied greatly between the patients with failed implants (Fig 2).

Patients with failed implants had greater ($p < 0.05$) mean values of all three cytokines tested when compared with patients with primary implants in all experimental conditions tested (10:1 PE ratios; collagen control) at 18 and 24 hours.

High and low secretor types were found in the patients with failed implants and with primary implants in all three inflammatory cytokines evaluated (Fig 3). The high and low secretor designations remained constant for all experimental conditions tested: collagen control and 10:1 PE particle to cell ratios. Therefore, if a patient showed a high response to the PE group, they also showed a high response to the collagen control.

Interleukin-6 levels correlated ($p < 0.05$) with Il-1 levels in both patient groups (Fig 4). This correlation was seen for all the experimental conditions evaluated: collagen control, 100:1 PE, and 10:1 PE ratios.

DISCUSSION

Generation of wear debris is a normal result of implant usage; yet, not all implant recipients develop aseptic loosening. The difference may be attributable to surgical technique, patient activity levels, or unique genetic characteristics of the patients. This study was designed to compare monocyte cytokine secretion after submicron PE exposure in patients with osteoarthritis who had primary implants with patients with ballooning osteolysis. Cytokine levels were heterogeneous in the patients with primary implants and patients with failed implants, with cytokine levels differing by as much as 10-fold between the lowest and highest secretions. When cytokine results of patients with primary implants were examined independently, some patients had an elevated cytokine response to PE particle exposure; yet, when the cytokine level data were pooled, no differences were seen in IL-1β, IL-6, or TNF-α in response to PE exposure. The majority of the

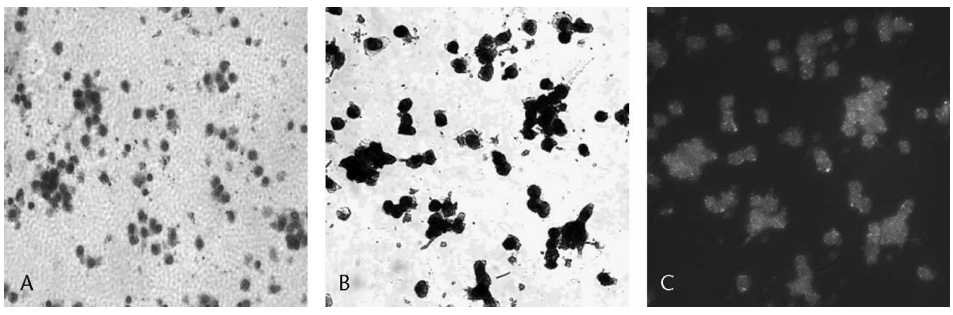

Fig 1A–C. Human monocytes were incubated for 18 hours. (A) Monocytes exposed to collagen alone have less eosinophils with granular cytoplasm than (B) monocytes in the PE-exposed group (100:1 ratio) (Stain, hematoxylin and eosin, magnification, ×400). When the same microscope field as in Figure 1B is viewed under polarized light, (C) the diffuse birefringence of PE particulate throughout the cellular cytoplasm is visible.

TABLE 2. Summary of Patients' Cytokine Responses

	Patients with Primary Implants							Patients with Failed Implants						
Cytokine	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
TNF-α	*	X	*	X	X	X	*	*	*	X	*	X	*	*
IL-6	X	*	*	X	X	X	*	X	X	*	*	*	*	*
IL-1β	*	X	X	X	X	nt	nt	X	*	*	*	*	nt	nt

*Significant elevation of cytokine ($p < 0.05$), Student's t test; X = no significant difference; nt = not tested

patients with failed implants (five of seven patients) showed an elevation in inflammatory cytokine secretion in response to PE when compared with the collagen control. Overall, patients with failed implants had three to four times greater absolute levels of IL-1β, IL-6, and TNF-α when compared with patients with primary implants. However, in each experimental group there were high and low secretors, suggesting that inflammatory cytokine response to a stimulus may be an inherent characteristic.

The results of our study must be interpreted with some caution because of the composition of the experimental groups. Patients in the failed implant group were not truly randomly selected by design (patients with rapid aseptic loosening were specifically chosen), and therefore were not representative of all patients with implants. Although care was taken to exclude patients with preexisting inflammatory conditions, such as rheumatoid arthritis, patients in the failed implant group all experienced aseptic loosening and therefore, their results may be skewed toward higher cytokine responses than would be seen in patients with well-fixed implants. Patients in the primary implant group were randomly selected, and accordingly were more representative of patients with primary implants. Therefore, it is possible that some of these patients may have osteolysis develop. In fact, two of seven patients in the primary implant group had elevated cytokine secretion in at least two cytokines (Table 2). It is not known at this time whether these patients will develop osteolysis. The addition of a third patient population, patients with well-fixed implants, would greatly enhance the value of our study. However, our purpose was to determine whether any differences exist between the extreme groups of patients outlined above. The results raise some valuable points regarding the concept of a genetic predisposition to elevated inflammatory response to wear particles, and the differences observed between these patient populations will serve as a basis for future studies.

An additional limitation of our study is the use of peripheral blood monocytes and not site-specific cells. The results from this study is relevant as peripheral blood cells are present in the implant site; however, an area that should be considered when investigating aseptic loosening is the pseudocapsule. The pseudocapsule is a synovial-like tissue and therefore rich in macrophage-like and fibroblast-like cells.[6,13] The cell mass in the pseudocapsule is

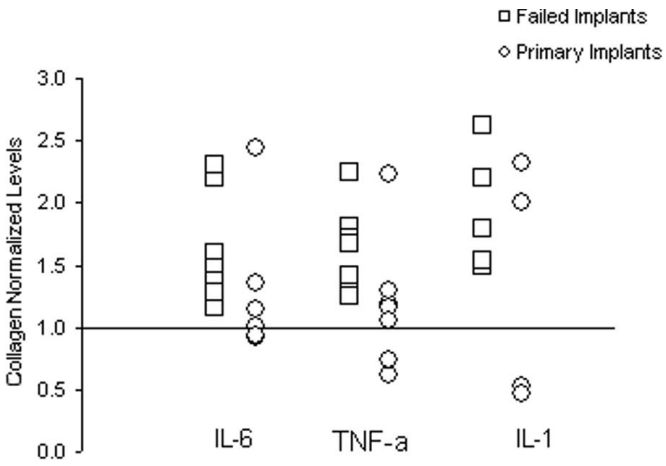

Fig 2. Collagen control levels have been set to equal one (collagen = 1); therefore the points on the chart represent the level of monocyte cytokine release elicited by PE exposure. The heterogeneity of cytokine response in and between the experimental groups can be seen.

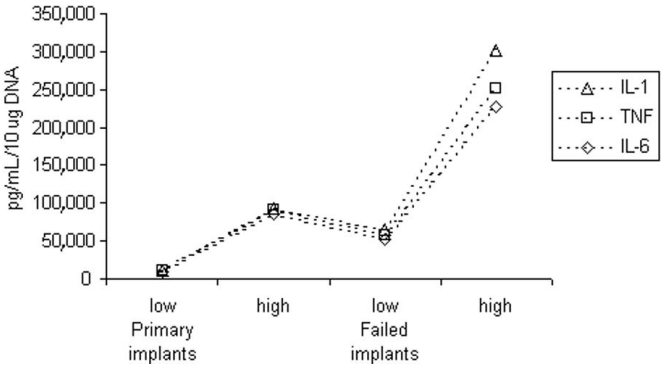

Fig 3. The patients with failed implants and primary implants were divided into high and low secretors, with high secretors having cytokine levels greater than the median and low secretors having values less than the median. This trend was observed in both patient populations and was present for all three cytokines evaluated.

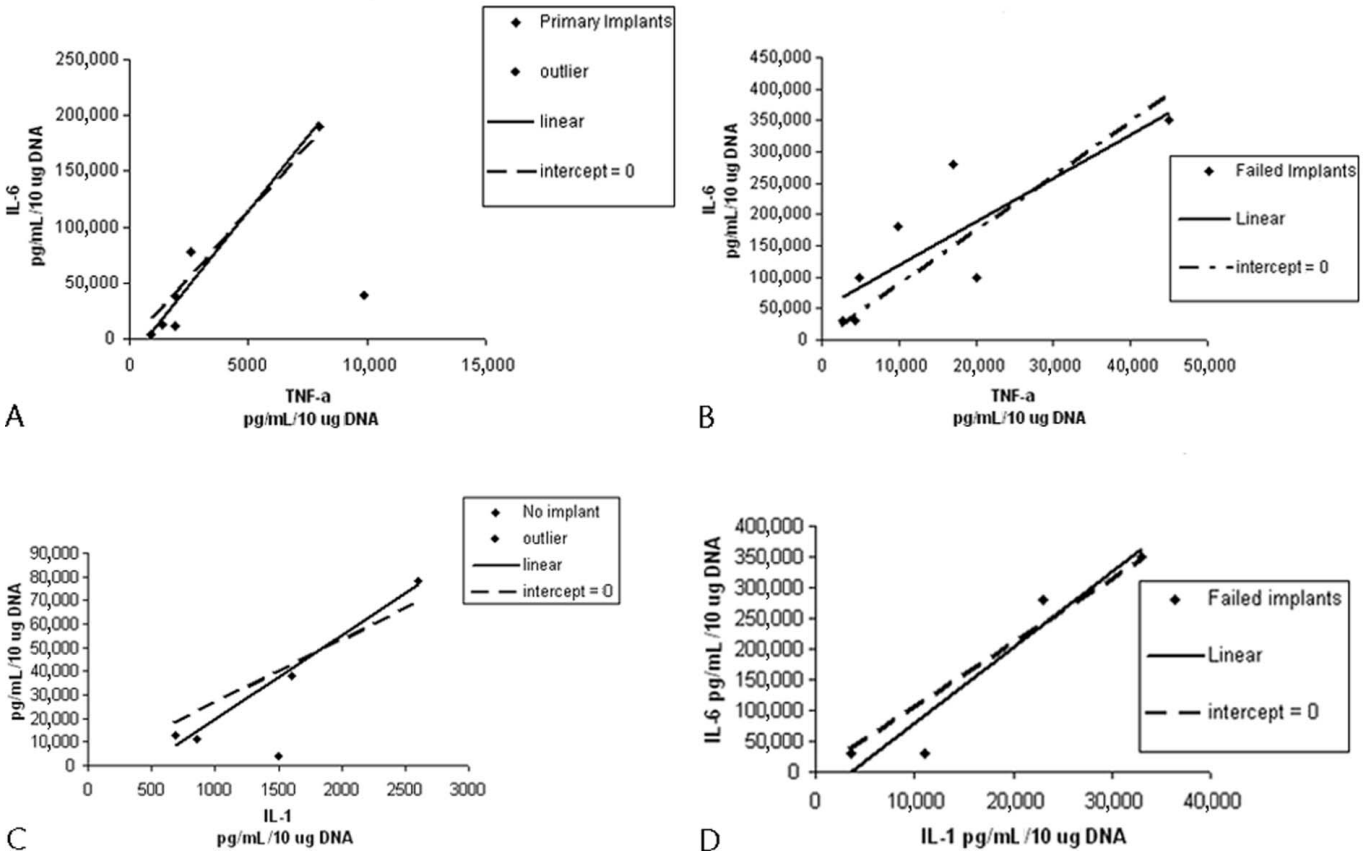

Fig 4A–D. Significant correlation ($p < 0.05$, Pearson Product Moment) was seen between all inflammatory indicators evaluated; XY scatter plots for IL-6β versus TNF-α in the patients in the (A) primary implant group and (B) failed implant group; and for IL-1 versus TNF-α in patients in the (C) primary implant group and the (D) failed implant group.

far greater than that in the implant interface lining; additionally, an histologic study of the pseudocapsule revealed it to be rich in proliferating cells.[14] Therefore, this is a highly active tissue that probably plays a significant role in aseptic loosening. Additional investigations using cells derived from the pseudocapsule and this model could be relevant to the clinical problem of aseptic loosening. Future work would include more complicated in vitro or in vivo systems that incorporate other cells observed at the bone-implant interface.

The majority of research done in the biomaterial field is done with human or animal cell lines or in vivo animal studies. These studies benefit from using a well-characterized cell line or model, with reproducible and predictable cellular responses. However, the most biologically relevant information will be derived from research with primary human cells and tissues. The major drawback of the use of human tissues is interdonor variability; studies using primary human cells have shown the in vitro cytokine response to a stimulus to be very heterogeneous with reproducible cytokine differences between individuals.[10,15] This suggests that inherent biologic differences may exist in patients, influencing their response to particulate wear debris and ultimately impacting on implant survival time. Genetic differences have been linked to disease progression; correlations have been found between polymorphisms in IL-1β, TNF-α, and IL-6 genes and conditions or diseases such as low bone mineral density, predisposition to osteoporosis, periodontal bone loss, and juvenile chronic arthritis.[4,5,16] In addition to the heterogeneity found in the general population, researchers have observed experimental differences in the biologic activities of cells and tissues derived from patients with THRs when compared with patients with primary implants.[8,12] The heterogeneity of cytokine results seen here may be attributable to inherent biologic characteristics; therefore, patients will have a unique response to PE wear debris, which may affect the longevity of orthopaedic implants. It is not known whether the inherent differences in monocyte cytokine secretion exist at the initial response to the wear

particle challenge (ie, immediate high absolute cytokine secretion) or whether this develops as a form of sensitization to a particle challenge postimplantation.

To define the differences in absolute cytokine secretion, the patients were divided into high and low secretors, with high secretors being greater than the median value and low secretors being less the median (Fig 3). High and low secretor types were found in each experimental group, suggesting it was not a developed response to PE exposure. This presentation of the data raises several interesting questions regarding predisposition to amplified inflammatory responses. It is possible that patients who are high secretors will have osteolysis develop, or that patients who are low secretors will have greater cytokine secretion as time increases postimplantation and the immune system becomes exposed to greater volumes of wear particulate. In the clinical situation, a diagnostic test for increased risk of osteolysis would greatly improve the efficacy of THR. Presently, aseptic loosening cannot be detected radiologically until approximately 50% of skeletal calcium already is lost[11]; therefore, anything allowing for prophylactic treatment of periimplant osteolysis would be of great benefit. Patients who had tested as a high responder before THR then could have more intense followups and pharmacologic treatment to prevent or decrease wear debris-induced osteolysis.

For our study, the levels of three inflammatory cytokines were evaluated for each patient. Although this was possible for a preliminary study such as ours, its expense and the amount of labor involved would be prohibitive for a large scale, longitudinal study. A study of this kind, which could compare cytokine levels before surgery to levels taken at various after surgery would be ideal for investigating the pathologic features of aseptic loosening. The ability to contrast cytokine levels, as correlated with radiographic failure, would help to elucidate the root mechanisms of inflammation induced by wear debris. The identification of one predictive factor of inflammation would greatly enhance any study of inflammatory-related disorders. Our results showed a positive correlation between all three cytokines investigated (Fig 4). That is, in the patients with failed implants and the patients with primary implants, as the levels of IL-6 increased so did the levels of TNF-α and IL-1β. This increase in cytokine response was true for all the experimental conditions: PE challenge ratios and the collagen control. With additional investigation of the relationships between inflammatory cytokines, it is possible that one cytokine could be used as a predictive factor preoperatively, to determine which patients are predisposed to rapid implant failure.

It is clear from the results of these experiments that patient characteristics influence the individual inflammatory response to a stimulus. To determine if genetic predisposition to inflammation plays a role in aseptic loosening, a study designed to evaluate cytokine levels before and after exposure to implant-derived wear debris should be done. Results of our study show that PE is capable of eliciting a significant increase in inflammatory cytokine secretion; when this is combined with an amplified inflammatory response (seen in the high secretors), the result may be advanced aseptic loosening with subsequent implant failure. A followup study on the patients with primary implants to see if the high and low secretor status is maintained with time, and to see if the high secretors have implant failure at an increased rate would be interesting.

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