Induction of macrophage C-C chemokine expression by titanium alloy and bone cement particles

Y. Nakashima, D.-H. Sun, M. C. D. Trindade, L. E. Chun, Y. Song, S. B. Goodman, D. J. Schurman, W. J. Maloney, R. L. Smith

From Stanford University School of Medicine, USA

Particulate wear debris is associated with periprosthetic inflammation and loosening in total joint arthroplasty. We tested the effects of titanium alloy (Ti-alloy) and PMMA particles on monocyte/macrophage expression of the C-C chemokines, monocyte chemoattractant protein-1 (MCP-1), monocyte inflammatory protein-1 alpha (MIP-1 α), and regulated upon activation normal T expressed and secreted protein (RANTES). Periprosthetic granulomatous tissue was analysed for expression of macrophage chemokines by immunohistochemistry. Chemokine expression in human monocytes/macrophages exposed to Ti-alloy and PMMA particles in vitro was determined by RT-PCR, ELISA and monocyte migration.

We observed MCP-1 and MIP-1 α expression in all tissue samples from failed arthroplasties. Ti-alloy and PMMA particles increased expression of MCP-1 and MIP-1 α in macrophages in vitro in a dose- and time-dependent manner whereas RANTES was not detected. mRNA signal levels for MCP-1 and MIP-1 α were also observed in cells after exposure to particles. Monocyte migration was stimulated by culture medium collected from macrophages exposed to Ti-alloy and PMMA particles. Antibodies to MCP-1 and MIP-1 α inhibited chemotactic activity of the culture medium samples.

Release of C-C chemokines by macrophages in response to wear particles may contribute to chronic inflammation at the bone-implant interface in total joint arthroplasty.

J Bone Joint Surg [Br] 1999;81-B:155-62.

Received 2 March 1998; Accepted after revision 28 July 1998

The longevity of total joint arthroplasty is significantly influenced by the onset of periprosthetic bone resorption or osteolysis. The latter is associated with the generation and accumulation of foreign-body wear debris at the prosthetic interface.¹⁻³ The wear debris incites the formation of granulomata localised to the sites of loosened implants.⁴⁻⁶ Histological analysis of the granulomatous tissue has shown the presence of abundant particulate debris surrounded by numerous macrophages, foreign-body giant cells and fibroblasts.^{7,8}

Phagocytosis of particulate debris by macrophages may serve as a stimulus for cellular activation with synthesis and secretion of bone-resorbing factors.⁹ Several studies have shown that macrophages release the proinflammatory mediators interleukin-1 (IL-1), interleukin-6 (IL-6), tumour necrosis factor- α (TNF- α) and prostaglandin E₂ in response to particulate debris.¹⁰⁻¹² Expression of these soluble factors adjacent to bone may contribute to the localised osteoclastic bone resorption.¹³

Recruitment and accumulation of monocytes to the foreign-body-induced inflammatory lesions occur in response to particles.¹⁴ This recruitment depends in part on chemotactic agonists generated by inflammatory cells. It is currently recognised that a superfamily of chemoattractant peptides, the chemokines, support the migration of monocytes to sites of inflammation.¹⁵⁻¹⁷

Chemokines are a family of proinflammatory cytokines that act primarily as chemoattractants for cells of the immune system. In man there are four subfamilies of chemokines which show homologous function and in some instances homologous protein structure.¹⁸ With the exception of lymphotactin and neurotactin, chemokines are subdivided based on the arrangement of the first two of four highly conserved cysteines.¹⁸ The C-C or β subfamily consists of monocyte chemoattractant proteins-1, 2, 3 and 4 (MCP-1, -2, -3, -4), macrophage inflammatory proteins-1 α ,

Y. Nakashima, MD, Postdoctoral Fellow
D.-H. Sun, MD, Postdoctoral Fellow
M. C. D. Trindade, BA, Graduate Student, Research Assistant
L. E. Chun, BS, Research Assistant
Y. Song, MD, Research Associate
S. B. Goodman, MD, Professor
D. J. Schurman, MD, Professor
R. L. Smith, PhD, Professor
Division of Orthopaedic Surgery, Stanford University Medical Centre,
Orthopaedic Research Laboratory, R144, 300 Pasteur Drive, Stanford,
California 94305-5341, USA.

W. J. Maloney, MD, Associate Professor
Department of Orthopaedic Surgery, Washington University School of
Medicine, St Louis, Missouri, USA.

Correspondence should be sent to Dr R. L. Smith.

©1999 British Editorial Society of Bone and Joint Surgery
0301-620X/99/18884 \$2.00

Fig. 1

Histological analysis of periprosthetic granulomatous tissue showing haematoxylin and eosin staining (A) and immunohistochemical staining with CD11b antibody for the detection of macrophages (B) and with antibody for the detection of MCP-1 (C) and MIP-1 α (D). Similar staining profiles were observed with membrane preparations obtained from six patients during revision total joint arthroplasty. The arrows emphasise positive staining.

1 β , 3 α and 3 β (MIP-1 α , 1 β , 3 α , 3 β) and regulated upon activation normal T expressed and secreted protein (RANTES). Other recognised members of this subfamily include eotaxin, I309, HCC-1 and TARC. The members of the C-C chemokine subfamily are potent chemoattractants for monocytes, lymphocytes, basophils and eosinophils, while C-X-C chemokines act primarily on neutrophils.¹⁵⁻¹⁷ Lymphotactin, the sole member of the C subfamily, lacks two of the cysteine residues that are characteristic of chemokines.¹⁹ It is a specific attractant for T-lymphocytes but not monocytes. Neurtactin (fractalkine), a C-X-C chemokine, exists as either an integral membrane protein or a shed 95K glycoprotein, exhibiting a N-terminal chemokine domain.^{20,21}

We hypothesised that macrophages respond to particulate wear debris by releasing the selected C-C chemokines, MCP-1, MIP-1 α and RANTES. The persistence of these chemokines may then contribute to a chronic inflammatory response at the bone-implant interface. We have analysed the expression of MCP-1 and MIP-1 α in retrieved tissues from failed total hip replacements. In addition, we determined in vitro the effects of Ti-alloy and PMMA particles on the release of MCP-1, MIP-1 α and RANTES from human monocytes/macrophages. The chemotactic response

of monocytes to particle-induced C-C chemokine release was quantified using assays of cell migration.

Materials and Methods

Reagents and antibodies. A monoclonal antibody to human MCP-1 was purchased from BMA Biomedicals AG (Rheinstrasse, Switzerland) and neutralising antibody for MCP-1 and anti-human MIP-1 α polyclonal antibody from R&D Systems (Minneapolis, Minnesota). Rabbit anti-mouse and anti-goat IgG were obtained from Dako (Carpinteria, California) and lipopolysaccharide (*Escherichia coli* serotype 0111:B4) and an esterase staining kit from Sigma Chemical Co (St Louis, Missouri).

Tissue collection. Tissue specimens were obtained from six patients undergoing revision surgery for aseptic loosening of total hip replacements. We excluded patients with infection as determined by preoperative aerobic and anerobic cultures of aspirates and histological evaluation of tissue specimens. There were no clinical and radiological signs of infection in any tissue sample analysed. The tissue samples included three membranes surrounding the femoral stem and three surrounding loosened acetabular cups. Each specimen was immersed in saline and immediately transported

to the laboratory for processing as described below.

Immunohistochemistry. Tissue specimens approximately 5 mm in diameter were placed in capsules containing optimum cutting temperature medium (OCT; Miles, Elkhart, Indiana). The capsules were frozen in liquid nitrogen and stored at -70°C until use. Serial sections $6\text{ }\mu\text{m}$ thick were cut with a cryostat (Cambridge Instruments, Buffalo, New York) and mounted on positively-charged microscope slides. The mounted sections were fixed in absolute acetone at 4°C for ten minutes. The sections were washed in phosphate-buffered saline (PBS) for five minutes and subsequently treated with 3% H_2O_2 for five minutes to reduce background staining. Excess H_2O_2 was removed by washing in PBS for ten minutes. The sections were then incubated at room temperature with primary antibodies, mouse anti-human MCP-1 monoclonal antibody and mouse anti-human MIP-1 α polyclonal antibody (R&D Systems), for one hour and then washed in PBS for ten minutes. They were then treated with rabbit anti-mouse secondary antibodies (Dako) conjugated with peroxidase for one hour and subsequently washed in PBS for ten minutes. Positively-stained cells were visualised using 3,3'-diaminobenzidine tetrahydrochloride (DAB) tablets (Sigma Chemical Co). The slides were counterstained with Mayer's haematoxylin, dehydrated and mounted.

Particle preparation and characteristics. Ti-alloy particles were obtained from periprosthetic tissues around failed Ti-alloy prostheses using papain hydrolysis as has been previously described.²² The elemental composition of the particles was characterised as Ti-alloy using an energy-dispersive X-ray analysis²² and they had a mean size of $0.7\text{ }\mu\text{m}$. Isolated metal debris was suspended in Dulbecco's PBS (DPBS) and autoclaved. From a 1% volume per volume (v/v) stock suspension, three serial dilutions were performed using serum-free RPMI 1640 medium (Gibco BRL, Grand Island, New York) to obtain final particle concentrations of 0.075, 0.015 and 0.003% (v/v). Commercially available PMMA particles (spherical, 1 to $10\text{ }\mu\text{m}$) were obtained from Polysciences Inc (Warrington, Pennsylvania) and suspended in serum-free RPMI 1640 medium using the volumetric determinations described above.

Cell culture. Human monocytes/macrophages were isolated from seven healthy individuals using Ficoll-Hypaque density-gradient centrifugation. Monocytes were resuspended in RPMI containing 5% human serum and $25\text{ }\mu\text{g/ml}$ of gentamicin and plated on 100 mm tissue-culture dishes (Costar, Cambridge, Massachusetts) in 8ml of medium at a cell density of 5×10^5 cells per cm^2 .

After adhesion, the plates were washed with DPBS to remove non-adherent cells. More than 90% of the adherent cells were characterised as monocytes/macrophages based on non-specific esterase-positive staining using a commercially available kit (Sigma Chemical Co).

The monocytes/macrophages were exposed to three concentrations (0.003, 0.015 and 0.075% v/v) of Ti-alloy and PMMA particles in serum-free RPMI 1640 medium for 48

hours. A time course was also performed using a particle concentration of 0.075% v/v at 1, 6, 12, 24, 48 and 72 hours. Medium alone was used as a negative control. Lipopolysaccharide was added to the cells at a concentration of 100 ng/ml as a positive control. Supernatants were harvested at indicated time periods and stored at -20°C .

Detection of chemokines in the medium. Levels of MCP-1, MIP-1 α , and RANTES protein in the culture supernatants were measured using enzyme-linked immunosorbent assay kits following the manufacturer's instructions (Quantikine; R&D Systems).

Reverse transcription polymerase chain reaction(RT-PCR). Total RNA was extracted from cells using acid guanidine isothiocyanate-phenol-chloroform extraction.²³ The amount of RNA was quantified spectrophotometrically. Equal amounts of RNA from each sample (400 ng) were then reverse-transcribed to single-stranded cDNA according to the method of Noonan and Roninson.²⁴ The target sequence in a sample (1 μl) of a 1:10 dilution of cDNA was amplified by PCR using the following sequence-specific sense and antisense oligonucleotide primers:

MCP-1 5' (ATAGCAGCCACCTTCATTCG) and 3' (TTCC CCAAGTCTCTGTATCT), MIP-1 α 5' (ACCATGGCTCT CTGCAACCA) and 3' (TTAAGAAGAGTCCCACAGTG), RANTES 5' (TCATTGCTACTGCCCTCTGC) and 3' (CGT CGTGGTCAGAATCTGGG).²⁵⁻²⁸ β -Actin sense and antisense primers were included under nested PCR conditions to control differences in amplification or losses of cDNA sample during loading. The β -actin primer sequences were 5' (GGGAATTCAAACTGGAACGGTGAAGG) and 3' (GGAAGCTTATCAAAGTCTCGGCCACA). The thermocycle programme included one cycle at 95°C for three minutes for initial heating followed by repeated cycles at 95°C for one minute and 56°C for 1 minute. The final extension was carried out at 72°C for five minutes. The cycle number which we used was 30 cycles for chemokines and 25 cycles for β -actin. Amplified products from PCR were separated on 5% polyacrylamide gels which were directly subjected to autoradiography using Kodak X-Omat autoradiography film in the presence of an image-intensifying screen (Dupont, Wilmington, Delaware) and exposed at -70°C for 12 to 18 hours.

Chemotaxis assay. Medium samples from monocyte/macrophage cultures were assessed for monocyte chemotactic activity. Human monocytes were isolated from peripheral blood as already described. Chemotaxis assays were carried out in cell-culture inserts of pore size $8\text{ }\mu\text{m}$ (Transwell; Costar). Culture supernatants were placed in 24-well plates and 1×10^6 human monocytes freshly prepared from buffy coats were added to the culture inserts. Anti-human MCP-1 monoclonal antibody ($5\text{ }\mu\text{g/ml}$) and anti-human MIP-1 α polyclonal antibody ($30\text{ }\mu\text{g/ml}$) were used for neutralisation. After two hours of incubation, the inserts were removed. The cells which migrated through the filters were harvested by trypsinisation and counted in a haematocytometer.

Fig. 2

Levels of MCP-1 released from macrophages in response to particulate debris. Figures 2A and 2B – Macrophages were exposed to Ti-alloy particles and PMMA particles at concentrations of 0.003%, 0.015% and 0.075% for 48 hours. Lipopolysaccharide (LPS) was added for comparison. Figure 2C – Time course for the macrophage response to Ti-alloy and PMMA particles at 0.075% v/v or as unexposed cells (CTL) (* $p < 0.05$ v CTL; the data are presented as means and standard deviations for seven cell preparations from normal blood donors and each donor cell preparation was tested in triplicate).

Statistical analysis. We compared statistically significant changes using one-way analysis of variance and Student's two-sample *t*-test (two-tailed). Significance levels were set at $p < 0.05$ using Bonferroni's approximation to correct for multiple comparisons.

Results

MCP-1 and MIP-1 α expression in the periprosthetic granuloma. Histological examination of periprosthetic granulomatous tissue from failed arthroplasties showed a fibrous stroma containing lymphocytes, numerous macrophages and multinucleated giant cells (Fig. 1A). Particulate polyethylene, PMMA, and metal particles were observed inside the cells in most specimens. Immunohistochemical

analysis confirmed the presence of CD11b-positive macrophages in the tissue (Fig. 1B). MCP-1 protein was detected in the granuloma and was most prominent in areas of greatest cellularity (Fig. 1C). MIP-1 α protein was also detected in macrophage-rich areas (Fig. 1D).

Monocytes/macrophages release MCP-1 in response to particulate debris. Monocytes/macrophages challenged with Ti-alloy particles showed a dose-dependent release of MCP-1 at 48 hours, while with unchallenged control monocytes/macrophages (CTL) release was only minimal (Fig. 2A). Ti-alloy particles at concentrations of 0.015% and 0.075% v/v showed a 5-fold and 15-fold increase ($p < 0.05$), respectively, in MCP-1 release when compared with control cultures. The level of MCP-1 released from monocytes/macrophages with particles at the 0.075% v/v

Fig. 3

The levels of MIP-1 α released from macrophages in response to particulate debris. Figures 3A and 3B – Time course for the macrophage response to Ti-alloy and PMMA particles at 0.075% v/v or as unexposed cells (CTL) (* $p < 0.05$ v CTL). Figure 3C – The data are presented as means and standard deviations for seven cell preparations from normal blood donors and each donor cell preparation was tested in triplicate.

concentration (2600 ± 180 pg/ml) was 78% of the MCP-1 release induced by lipopolysaccharide (3300 pg/ml). PMMA particles also stimulated a comparable dose-dependent release of MCP-1 from monocytes/macrophages (Fig. 2B).

The increased production of MCP-1 from particle-stimulated monocytes/macrophages was dependent on the time of exposure (Fig. 2C). Statistically significant differences were noted between particle-challenged and control cultures of monocytes/macrophages at 6, 12, 24, 48 and 72 hours for Ti-alloy particles and at 24, 48 and 72 hours for PMMA particles. In the absence of particles, the monocytes/macrophages showed slowly increasing levels of MCP-1 during the time periods examined.

Monocytes/macrophages release MIP-1 α in response to particulate debris. Monocytes/macrophages challenged with Ti-alloy and PMMA particles showed a dose-dependent release of MIP-1 α at 48 hours, while unchallenged control monocytes/macrophages showed low levels of MIP-1 α (Fig. 3A). Exposure of monocytes/macrophages to particles at concentrations of 0.015% and 0.075% v/v increased MIP-1 α release by 7- and 14-fold ($p < 0.05$), respectively, when compared with control cultures. The release of MIP-1 α at a concentration of 0.075% v/v (2085 ± 90 pg/ml) was 76% of the release by the cells in response to lipopolysaccharide (2688 ± 397 pg/ml). PMMA particles also stimulated a dose-dependent release of MIP-1 α from monocytes/macrophages in a similar manner (Fig. 3B).

Fig. 4

RT-PCR amplification of MCP-1 and MIP-1 α mRNA expressed in macrophages in response to particulate debris (CTL, unchallenged control cells; Ti-alloy, Ti-alloy-particle-challenged cells; PMMA, PMMA-particle-challenged cells; LPS, lipopolysaccharide-challenged cells).

Analysis of the time-dependent release of MIP-1 α from particle-stimulated monocytes/macrophages showed significant differences from unchallenged cultures at 6, 12, 24, 48 and 72 hours for Ti-alloy particles and at 24, 48 and 72 hours for PMMA particles (Fig. 3C). Control macrophages showed relatively constant low levels of MIP-1 α release during the test periods (Fig. 3C).

Monocyte/macrophage RANTES release in response to particulate debris. Monocytes/macrophages did not release RANTES at any time or at any particle dosage tested. By contrast, lipopolysaccharide-stimulated macrophages showed measurable levels of RANTES (170 ± 20 pg/ml).

RT-PCR detection of MCP-1 and MIP-1 α mRNA signals. As observed at the protein level, the MCP-1 mRNA signal was clearly detectable in monocytes/macrophages after exposure to Ti-alloy and PMMA particles (Fig. 4), whereas that in control cells was only just detectable. The MIP-1 α mRNA signal also remained detectable after exposure of the monocytes/macrophages to Ti-alloy and PMMA particles, but was barely detectable in control cells.

Particulate debris stimulated release of monocyte chemotactic activity by monocyte/macrophages. Quantification of monocyte chemotaxis showed that supernatants from particle-challenged monocytes/macrophages significantly increased the number of cells migrating across membrane filters when compared with medium from unchallenged cells (Table I). Neutralising antibodies against MCP-1 and MIP-1 α also inhibited the chemotactic activity of medium from cells exposed to Ti-alloy particles (0.075% v/v) by 26% and 11%, respectively. Antibodies against MCP-1 and MIP-1 α also inhibited the chemotactic activity of medium from cultures exposed to PMMA particles (0.075%) by 17% and 13%, respectively. When tested in combination, the two antibodies inhibited cell migration by 33% for Ti-alloy and 23% for PMMA particles.

Discussion

We have focused on the effects of particulate wear debris on the expression of selected C-C chemokines in retrieved periprosthetic membranes *in vivo* and in primary human macrophages *in vitro*. Primary human monocytes/macrophages were chosen as the target cells for the *in vitro* analysis since they are precursors of tissue macrophages. Freshly prepared monocytes from peripheral blood may more closely represent the *in vivo* cellular response to particulate debris than transformed macrophage-like cell lines.¹² Serum-free medium was used to avoid confounding effects of serum-derived cytokines and growth factors.

Analysis of the periprosthetic membrane from patients with loosened prostheses using immunohistochemical staining confirmed the presence of numerous macrophages and abundant particulate debris and agreed with studies showing an association between the presence of particulate debris and abundant macrophages.⁵⁻⁸ In our study, expression of MCP-1 and MIP-1 α was evident in the periprosthetic tissues as shown by antibody recognition and confirms that chemokine release persists in peri-

Table I. Macrophage chemotaxis in response to culture medium from macrophages exposed to Ti-alloy and PMMA particles

Sample	Number	Mean ($\pm$ SD) cell number ($\times 10^3$)	p value
Control cultures	7	63.6 $\pm$ 4.8	
Titanium alloy	7	103.3 $\pm$ 8.8	0.001*
Titanium alloy + MCP-1 Ab	7	76.0 $\pm$ 7.1	0.001†
Titanium alloy + MIP-1 α Ab	7	91.7 $\pm$ 6.4	0.04+
Titanium alloy + MCP-1 Ab and MIP-1 α Ab	7	69.7 $\pm$ 7.9	0.001†
PMMA	7	88.2 $\pm$ 7.8	0.001*
PMMA + MCP-1 Ab	7	73.5 $\pm$ 7.3	0.02‡
PMMA + MIP-1 α Ab	7	77.0 $\pm$ 7.7	0.04+
PMMA + MCP-1 Ab and MIP-1 α Ab	7	68.2 $\pm$ 6.2	0.001†

* control v titanium alloy and control v PMMA

† titanium alloy v titanium alloy + MCP-1 Ab

+ titanium alloy v titanium alloy + MIP-1 α Ab and PMMA v PMMA + MIP-1 α Ab

‡ titanium alloy v titanium alloy + MCP-1 Ab and MIP-1 α Ab

§ PMMA v PMMA + MCP-1 Ab

† PMMA v PMMA + MCP-1 Ab + MIP-1 α Ab

prosthetic granulomatous tissue. The localised release of chemokines may contribute to the formation of the synovial-like membrane described in aseptic loosening.²⁹ The synovial-like membrane shows increased levels of profibrotic and immunosuppressive transforming growth factor- β ²⁹ and upregulation of the plasminogen activation system,³⁰ which are consistent with a chronic inflammatory response.

Our chemotactic studies confirm that particle-induced chemokine release from monocytes/macrophages increases monocyte migration across a membrane. Neutralising antibodies against MCP-1 and MIP-1 α added to the medium of particle-stimulated monocytes/macrophages significantly inhibited cell migration. When tested in combination, they showed additive inhibitory effects on cell migration. These results agree with studies confirming that monocyte chemotaxis involves redundant mechanisms.^{31,32}

The stimulus for the release of MCP-1 and MIP-1 α from macrophages may involve cellular processes that are activated after either adherence and/or phagocytosis of the particles. In a study in which monocytes were the primary source of MCP-1 and MIP-1 α , antibodies to intercellular adhesion molecule-1 (ICAM-1) suppressed expression of the C-C chemokines but not the C-X-C chemokine, IL-8.³¹ Adding back soluble proinflammatory cytokines, IL-1 or TNF- α , to the ICAM-1-blocked cells increased proliferation but did not restore MCP-1 or MIP-1 α release.³² Phagocytosis of zymosan particles and *Mycobacterium tuberculosis* by human monocytic cells also results in an increased MCP-1 mRNA signal within four hours, and continued elevation of mRNA levels over a 24-hour period.³³ The induction of MCP-1 mRNA by *Mycobacterium* was not prevented by antibody to TNF- α suggesting that phagocytosis was required.

Both Ti-alloy and PMMA particles showed dose- and time-dependent release of MCP-1 and MIP-1 α from monocyte/macrophage cultures. Cultures challenged by Ti-alloy particles had a consistently higher level of chemokine release in response to the same volumetric concentration when compared with those challenged by PMMA particles. This effect may be due to variations between the number of particles for the two materials since the range of particle size of the metal alloy was smaller than that of the PMMA. Several studies have confirmed that expression of proinflammatory cytokines by macrophages is dose-dependent with respect to the number of particles^{34,35} and the type of metal alloy.³⁶

Release of chemokines is associated with a number of diseases in which formation of granulomatous tissue occurs. Parasitic induction of the formation of granulomatous tissue in the liver increases the release of both MCP-1 and MIP-1 α .³⁷ Expression of MIP-1 α and MIP-1 β increases granuloma formation in the lung induced by silica particles and oxidants and in idiopathic lung fibrosis.³⁸⁻⁴¹ Chemokines may also influence total joint arthroplasty. Ishiguro et al⁴² reported increased expression of MIP-1 α , MCP-1 and

interleukin-8 (IL-8) in periprosthetic granulomatous tissue when compared with fibrous scar tissue.⁴² Frokjar et al⁴³ showed induction of MCP-1 in synovial mononuclear cells and IL-8 in endothelial cells by the injection of polyethylene particles into rabbit knees. In our study, expression of RANTES was not observed in the monocyte/macrophage cultures with any particle concentration tested, but was stimulated by lipopolysaccharide. Chiba et al⁴⁴ also detected gene expression of MCP-1 in granulomatous tissues from loose prostheses but not that of RANTES.

The regulation of chemokine expression appears to be coupled to the presence of proinflammatory cytokines. IL-1 and TNF- α induce gene expression of MCP-1 in macrophages and fibroblasts and stimulate expression of MIP-1 α in macrophages, T-lymphocytes, fibroblasts, and endothelial cells.^{39,45} Conversely, MCP-1 stimulates IL-1 and IL-6 release from monocytes.³² MIP-1 α stimulates the production of IL-1, IL-6, and TNF- α from macrophages.³⁹ Ti-alloy and PMMA particles stimulate the release of MCP-1 and MIP-1 α in a manner similar to that of IL-1, IL-6, and TNF- α from human macrophages.^{34,35} Particulate debris may contribute to periprosthetic bone resorption through release from macrophages of MCP-1 and MIP-1 α and the subsequent recruitment of inflammatory cells.

This work was supported in part by an Educational Gift from Wright Medical Technology, Inc, Memphis, Tennessee and the Uehara Memorial Foundation, Tokyo, Japan.

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

References

1. Goldring SR, Clark CR, Wright TM. Editorial: The problem in total joint arthroplasty: aseptic loosening. *J Bone Joint Surg [Am]* 1993; 75-A:799-801.
2. Harris WH, Schiller AL, Scholler JM, Freiberg RA, Scott R. Extensive localized bone resorption in the femur following total hip replacement. *J Bone Joint Surg [Am]* 1976;58-A:612-8.
3. Jasty MJ, Floyd WE, Schiller AL, Goldring SR, Harris WH. Localized osteolysis in stable non-septic total hip replacement. *J Bone Joint Surg [Am]* 1986;68-A:912-9.
4. Maloney WJ, Jasty M, Harris WH, Galante JO, Callaghan JJ. Endosteal erosion in association with stable uncemented femoral components. *J Bone Joint Surg [Am]* 1990;72-A:1025-34.
5. Maloney WJ, Smith RL. Periprosthetic osteolysis in total hip arthroplasty: the role of particulate wear debris. *J Bone Joint Surg [Am]* 1995;77-A:1448-61.
6. Schmalzried TP, Jasty M, Harris WH. Periprosthetic bone loss in total hip arthroplasty: polyethylene wear debris and the concept of the effective joint space. *J Bone Joint Surg [Am]* 1992;74-A:849-63.
7. Kim KJ, Chiba J, Rubash HE. *In vivo* and *in vitro* analysis of membranes from hip prostheses inserted without cement. *J Bone Joint Surg [Am]* 1994;76-A:172-80.
8. Yokohama Y, Matsumoto T, Hirakawa M, et al. Production of matrix metalloproteinases at the bone-implant interface in loose total hip replacements. *Lab Invest* 1995;73:899-911.
9. Shanbhag AS, Jacobs JJ, Black J, Galante JO, Glant TT. Human monocyte response to particulate biomaterials generated in vivo and in vitro. *J Orthop Res* 1995;13:792-801.
10. Goodman SB, Knoblich G, O'Connor M, et al. Heterogeneity in cellular and cytokine profiles from multiple samples of tissue surrounding revised hip prostheses. *J Biomed Mater Res* 1996;31:421-8.
11. Glant TT, Jacobs JJ, Molnar G, et al. Bone resorption activity of particulate-stimulated macrophages. *J Bone Miner Res* 1993;8: 1071-9.

12. Glant TT, Jacobs JJ. Response of three murine macrophage populations to particulate debris: bone resorption in organ cultures. *J Orthop Res* 1994;12:720-31.
13. Sabokbar A, Pandey R, Quinn JM, Athanasou NA. Osteoclastic differentiation by mononuclear phagocytes containing biomaterial particles. *Arch Orthop Trauma Surg* 1998;117:136-40.
14. Horowitz SM, Purdon MA. Mechanisms of cellular recruitment in aseptic loosening of prosthetic joint implants. *Calcif Tissue Int* 1995; 57:301-5.
15. Taub DD, Oppenheim JJ. Review of the chemokine meeting the third international symposium of chemotactic cytokines. *Cytokine* 1993;5: 175-9.
16. Murphy PM. The molecular biology of leukocyte chemoattractant receptors. *Annu Rev Immunol* 1994;12:593-33.
17. Miller MD, Krangel MS. Biology and biochemistry of the chemokines: a family of chemotactic and inflammatory cytokines. *Crit Rev Immunol* 1992;12:17-46.
18. Rollins BJ. Chemokines. *Blood* 1997;90:909-28.
19. Kelner GS, Kennedy J, Bacon KB, et al. Lymphotactin: a cytokine that represents a new class of chemokine. *Science* 1994;266:1395-9.
20. Bazan JF, Bacon KB, Hardman G, et al. A new class of membrane-bound chemokine with a CX₃C motif. *Nature* 1997;385:640-4.
21. Pan Y, Lloyd C, Zhou H, et al. Neurotactin, a membrane-anchored chemokine upregulated in brain inflammation. *Nature* 1997;387:611-7.
22. Maloney WJ, Smith RL, Schmalzried TP, et al. Isolation and characterization of wear particles generated in patients who have had failure of a hip arthroplasty without cement. *J Bone Joint Surg [Am]* 1995;77-A:1301-10.
23. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987;162:156-9.
24. Noonan KE, Roninson IB. mRNA phenotyping by enzymatic amplification of randomly primed cDNA. *Nucleic Acids Res* 1988;16: 10366.
25. Zhou LJ, Tedder TF. A distinct pattern of cytokine gene expression by human CD83+ blood dendritic cells. *Blood* 1995;86:3295-301.
26. Saiki RK, Gelfand DH, Stoffel S, et al. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 1988;239:487-91.
27. Vu TH, Hoffman AR. Promoter-specific imprinting of human insulin-like growth factor II gene. *Nature* 1994;371:714-7.
28. Vu TH, Yballe C, Boonyanit S, Hoffman AR. Insulin-like growth factor II in uterine smooth-muscle tumors: maintenance of genomic imprinting in leiomyomata and loss of imprinting in leiomyosarcomata. *J Clin Endocrinology and Metabolism* 1995;80:1670-6.
29. Kontinen YT, Waris V, Xu J-W, et al. Transforming growth factor - β 1 and 2 in the synovial-like interface membrane between implant and bone in loosening of total hip arthroplasty. *J Rheumatol* 1997;24:694-701.
30. Nordsletten L, Bu L, Takagi M, et al. The plasminogen activation system is upregulated in loosening of total hip prostheses. *Acta Orthop Scan* 1996;67:143-8.
31. Lukacs NW, Kunkel SL, Burdick MD, Strieter RM. The production of chemotactic cytokines in an allogeneic response: the role of intercellular adhesion molecule-1 and lymphocyte function-associated antigen-3. *Am J Pathol* 1993;143:1179-88.
32. Jiang Y, Beller DI, Frendl G, Graves DT. Monocyte chemoattractant protein-1 regulates adhesion molecule expression and cytokine production in human monocytes. *J Immunol* 1992;148:2423-8.
33. Friedland JS, Shattock RJ, Griffin GE. Phagocytosis of Mycobacterium tuberculosis or particulate stimuli by human monocyte cells induces equivalent monocyte chemotactic protein-1 gene expression. *Cytokine* 1993;5:150-6.
34. Maloney WJ, James RE, Smith RL. Human macrophage response to retrieved titanium alloy particles in vitro. *Clin Orthop* 1996;322: 268-78.
35. Gonzalez O, Smith RL, Goodman SB. Effect of size, concentration, surface area and volume of polymethylacrylate particles on human macrophages in vitro. *J Biomed Mater Res* 1996;30:463-73.
36. Lee SH, Brennan FR, Jacobs JJ, et al. Human monocyte/macrophage response to cobalt-chromium corrosion products and titanium particles in patients with total joint replacements. *J Orthop Res* 1997; 15:40-9.
37. Lukacs NW, Kunkel SL, Strieter RM, Warmington K, Chensue SW. The role of macrophage inflammatory protein 1 alpha in Schistosoma mansoni egg-induced granulomatous inflammation. *J Exp Med* 1993;177:1551-9.
38. Lukacs NW, Chensue SW, Smith RE, et al. Production of monocyte chemoattractant protein-1 and macrophage inflammatory protein-1alpha by inflammatory granuloma fibroblasts. *Am J Pathol* 1994; 144:711-8.
39. Driscoll KE. Macrophage inflammatory proteins: biology and role in pulmonary inflammation. *Exp Lung Res* 1994;20:473-90.
40. Smith RE, Strieter RM, Zhang K, et al. A role for C-C chemokines in fibrotic lung disease. *J Leukoc Biol* 1995;57:782-7.
41. Iyonaga K, Takeya M, Saita N, et al. Monocyte chemoattractant protein-1 in idiopathic pulmonary fibrosis and other interstitial lung diseases. *Hum Pathol* 1994;25:455-63.
42. Ishiguro N, Kojima T, Kurokouchi K, Iwase T, Iwata H. mRNA expression of chemokines in interface tissue around of loosening total hip arthroplasty components. *Trans Orthop Res Soc* 1997;22:735.
43. Frokjaer J, Deleuran B, Lind M, et al. Polyethylene particles stimulate monocyte chemotactic and activating factor production in synovial mononuclear cells in vivo. *Acta Orthop Scand* 1995;66: 303-7.
44. Chiba J, Oyama M, Sugawara S, Inoue K, Rubash HE. The role of chemokine, adhesion molecules, and cytokine receptor in femoral osteolysis after cementless total hip arthroplasty. *Trans Orthop Res Soc* 1996;21:514.
45. Brieland JK, Flory CM, Jones ML, et al. Regulation of monocyte chemoattractant protein-1 gene expression and secretion in rat pulmonary alveolar macrophages by lipopolysaccharide, tumor necrosis factor-alpha, and interleukin-1 beta. *Am J Respir Cell Mol Biol* 1995; 12:104-9.