Cytokine receptor profile of arthroplasty macrophages, foreign body giant cells and mature osteoclasts

Susan D Neale[1] and Nicholas A Athanasou[2]

In the arthroplasty pseudomembrane surrounding a loose prosthesis there is a marked macrophage and foreign body giant cell (FBGC) response to implant-derived wear particles. These cells contribute to the osteolysis of loosening by releasing cytokines and growth factors which influence the formation and activity of osteoclasts. Using a panel of monoclonal antibodies directed against known cytokine/growth factor receptors, we have determined by immunohistochemistry whether arthroplasty macrophages, FBGCs and osteoclasts express receptors for cytokines and growth factors that are known to modulate osteolysis.

All these cell types reacted with antibodies directed against the following cytokine/growth factor receptors: gp130, IL-1R type 1, IL-2R, IL-4R, IL-6R, TNFR, M-CSFR, GM-CSFR and SCFR but not with antibodies directed against IL-3R and IL-8R. Arthroplasty macrophages, FBGCs and osteoclasts thus show a similar pattern of cytokine/growth factor receptor expression. This reflects the fact that arthroplasty macrophages are capable of osteoclast differentiation and that these cell types form part of the mononuclear phagocyte system. As regards the osteolysis of aseptic loosening, it also indicates that these cells are targets for numerous cytokines and growth factors which influence osteoclast formation and bone resorption.

[1]Nuffield Department of Orthopaedic Surgery, University of Oxford, [2]Department of Pathology, Nuffield Orthopaedic Centre, Windmill Road, Headington, Oxford OX3 7LD, U.K. Tel +44 1865 227–619. Fax –354.
E-mail: nick.athanasou@ndos.ox.ac.uk. Correspondence: Dr. N.A. Athanasou
Submitted 99-02-02. Accepted 99-07-01

The bone-implant interface often contains focal areas of osteolysis associated with large numbers of prosthesis-derived wear particles and a marked macrophage and foreign body macrophage polykaryon or giant cell (FBGC) response (Harris 1994). Cytokines such as interleukin-1((IL-1), tumor necrosis factor ((TNF) and interleukin-6 (IL-6) are potent stimulators of osteoclastic bone resorption (Bertolini et al. 1986, Gowen and Mundy 1986, Black et al. 1991) and have been identified in the arthroplasty pseudomembrane that surrounds failed implants (Chiba et al. 1994, Horikoshi et al. 1994). In vivo and in vitro studies have shown that these cytokines are released from wear particle-stimulated macrophages and fibroblastic cells (Glant et al. 1993, Haynes et al. 1993, Jiranek et al. 1993, Manlapaz et al. 1996).

The osteoclast is a highly specialized multinucleated cell which is responsible for bone resorption. It forms part of the mononuclear phagocyte system and is derived from the pluripotent haematopoietic stem cell; it shares a common marrow precursor with macrophages, and its mononuclear precursor is known to circulate in the monocyte fraction. Macrophage-colony stimulating factor (M-CSF) is an essential requirement for the proliferation and differentiation of osteoclast precursors (Tanaka et al. 1993, Fujikawa et al. 1996). Other cytokines and growth factors including interleukin-1 (IL-1), IL-3, IL-6, TNF, granulocyte/macrophage-colony stimulating factor (GM-CSF), and stem cell factor (SCF) have also been implicated in the recruitment, proliferation and differentiation of osteoclast progenitors (Pfeilschifter et al. 1989, Shinar et al. 1990, Takahashi et al. 1991, Demulder et al. 1992, Tamura et al. 1993).

It has been shown that wear particle-associated macrophages and human arthroplasty-derived macrophages are capable of differentiating into osteoclasts and that this process is influenced by growth factors (Quinn et al. 1992, Sabokbar et al. 1997). The effects of cytokines and growth factors

on cells are mediated following interaction with specific surface receptors. The distribution of these receptors and changes in their number and activity provide an important mechanism for regulating responses to cytokines/growth factors. In this study, we have examined a range of receptors for cytokines and growth factors found on cells of the mononuclear phagocyte system present in periprosthetic tissues.

Material and methods

Specimens examined

Periprosthetic tissues (pseudocapsule) were obtained from revision arthroplasties carried out on 2 patients. One was a 47-year-old woman who had a cementless JR1 Furlong polyethylene/ceramic/CoCr hip implant which showed radiographic signs of loosening. The implant had been in situ for 4 years prior to revision. The second specimen was obtained from a 48-year-old woman who had a cemented Oxford polyethylene/CoCr knee implant which showed radiographic signs of loosening. The implant had been in situ for 7 years prior to revision.

Giant cell tumor of bone (GCT) specimens were obtained from 2 patients: one, a 67-year-old woman with a tumour of the proximal femur, and the second, a 28-year-old woman with a tumour of the proximal tibia. The multinucleated giant cells present in these tissues have been shown to have all the characteristics of osteoclasts and are commonly employed to study human osteoclasts in vitro. They possess abundant calcitonin receptors and respond to calcitonin with a rise in cyclic adenosine monophosphate, are positive for tartrate-resistant acid phosphatase, have an antigenic phenotype identical with that of osteoclasts and are capable of lacunar bone resorption (Chambers et al. 1985, Horton et al. 1985, Athanasou et al. 1988).

Histological processing and immunohistochemical staining

The arthroplasty tissues were obtained fresh and snap-frozen in liquid nitrogen. With the use of a cryostat, frozen 6 µm sections were collected onto multiwell glass slides, fixed in cold (–20 °C) acetone for 20 minutes and air-dried at room temperature. Imprints of osteoclasts were prepared by pressing a freshly cut piece of giant cell tumour of bone tissue onto a multiwell glass slide. The imprints were air-dried, then fixed for 20 minutes in cold acetone.

Table 1. Cytokine receptor McAbs used for immunohistochemical staining

Monoclonal antibody [a]	Donor	Reactivity	Isotype
B-R9	Clement/Wijdenes	gp130	G1
B1	Brochier	gp130	G1
hIL-1R-M1	Armitage	IL-1R Type1	G1
7G7B6	Nelson	IL-2Rα	G2a
MEM-140, -145	Horejsi	IL-2Rα	M
H-31	Sugamura	IL-2Rα	G1
TUGh4	Sugamura	IL-2Rγ	G2b
9F5	Lopez	IL-3Rα	G1
S456C9	Agthoven	IL-4R	G1
hIL-4R-M57	Armitage	IL-4R	G1
B-N12, B-F19	Wijdenes	IL-6R	G1
M195	Brochier	IL-6R	G2a
R34.34	Agthoven	IL-7R	G
B-G20	Wijdenes	IL-8R	M
htr9	Lesslauer	TNFR/55kD	G1
utr1	Lesslauer	TNFR/75kD	G1
7-7A3 [b]		M-CSFR	G1
NU-SCF1	Nakamura	SCFR	G1
MTK1, MTK2	Morita	SCFR	G1
17F11	Agthoven	SCFR	M
SC04, SC06	Agthoven	GM-CSF-R	G1

[a] The monoclonal antibodies used were derived from the cytokine panel of monoclonal antibodies analysed in the 6th International Workshop on Human Leukocyte Differentiation Antigens.

[b] An antibody specific for the human CSF-1 receptor (M-CSFR) was purchased from Cambridge Bioscience, U.K. (Cat no. 13-2800).

An indirect immunoperoxidase method was used for monoclonal antibody staining. The monoclonal antibodies we used were diluted 1:10 in phosphate-buffered saline containing 2% bovine serum albumin (PBS/BSA). The antibodies and their source, listed in Table 1, were derived from the cytokine panel of monoclonal antibodies analysed in the 6th International Workshop on Human Leukocyte Differentiation Antigens (Kishimoto et al. 1998).

The identity of tissue macrophages and FBGCs was established using an antibody to CD14 (JML-H14), which reacts with macrophages, but not os-

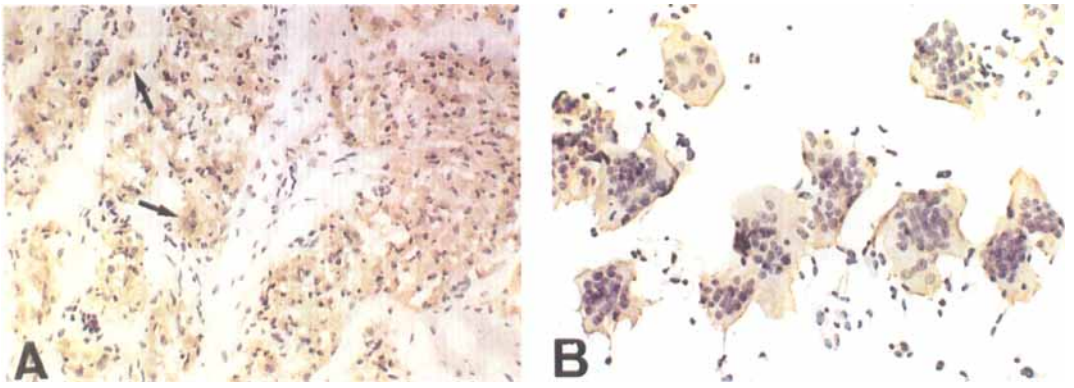

Figure 1. Immunohistochemical staining of (A) macrophages and FBGCs (arrows) in the arthroplasty pseudocapsule for the anti-macrophage CD68 antigen (×200) and (B) osteoclasts from GCT imprints for vitronectin receptor expression (×200). Counterstained with hematoxylin.

teoclasts (Athanasou and Quinn 1990). Osteoclasts were identified by the monoclonal antibody, 23C6, which is directed against the vitronectin receptor (Horton et al. 1985). Positive controls also included the anti-CD68 antibody, EBM/11, which reacts with all 3 cell types (Athanasou and Quinn 1990, Kadoya et al. 1994). Negative controls consisted of adding PBS/BSA alone.

Briefly, the acetone-fixed frozen sections and imprints were incubated with primary antibodies in a moist environment overnight at 4 °C. After incubation, the slides were gently washed in PBS for 10 minutes. This was followed by a 30-minute incubation in ethanol/3% hydrogen peroxide solution to block endogenous peroxidase activity. The slides were removed and rinsed in PBS for a further 10 minutes. The biotinylated secondary antibody (peroxidase-conjugated rabbit anti-mouse; Dako) was diluted 1:50 in PBS/BSA and the sections and imprints were incubated for 30 minutes at room temperature. The biotinylated tertiary antibody (peroxidase-conjugated swine anti-rabbit Ig; Dako) was diluted 1:100 in PBS/BSA and the sections and imprints incubated for 30 minutes at room temperature. A solution of 0.5 mg/mL of 3,3 diaminobenzidine (DAB) containing 0.05% hydrogen peroxide was applied to each section and imprint and the slides incubated for 3 minutes. The sections and imprints were washed thoroughly in running tap water, counterstained with haematoxylin, dehydrated through graded alcohols, brought to xylene, and finally mounted with DPX medium and examined by light microscopy.

Results

Histological features of specimens examined

The arthroplasty pseudocapsule was composed of cellular and collagenous connective tissue which contained a marked macrophage and FBGC response to numerous polymeric and metallic biomaterial wear particles. These cells stained strongly for the anti-macrophage markers, CD14 and CD68. Staining for CD14 was mainly evident on the membrane of tissue macrophages and FBGCs whereas staining for CD68 was largely cytoplasmic (Figure 1).

The GCT imprints contained scattered mononuclear cells and large numbers of osteoclasts, readily identifiable by their size and multinuclearity. All the osteoclasts and some mononuclear cells in the GCT imprints showed strong cytoplasmic staining (Figure 1) with the anti-CD68 antibody EBM/11. Osteoclasts also showed strong membrane staining with the monoclonal antibody 23C6 (directed against the α-chain of the vitronectin receptor) and were negative for CD14.

Antigenic phenotype of macrophages and FBGCs in revision arthroplasty tissue

A summary of the immunohistochemical staining reaction of wear particle-associated macrophages and FBGCs in periprosthetic tissues is shown in Table 2. Macrophages and FBGCs reacted strongly with antibodies directed against receptors for gp130, IL-1R type 1, IL-2Rα, IL-2Rγ, IL-6R, TNFR, M-CSFR, and SCFR. These cells showed

Table 2. Cytokine receptor profile of macrophages and foreign body giant cells in revision arthroplasty tissue, and mature osteoclasts

McAb name	Reactivity	Revision		GCT	
B-R9	gp130	+	+	+	+
B1	gp130	w	w	+	w
hIL-1R-M1	IL-1R Type1	+	+	w	w
7G7B6	IL-2Rα	w	w	+	+
MEM-140, -145	IL-2Rα	+	+	+	+
H-31	IL-2Rα	+	w	+	+
TUGh4	IL-2Rγ	+	w	+	+
9F5	IL-3Rα	–	–	–	–
S456C9	IL-4R	w	w	w	w
hIL-4R-M57	IL-4R	w	w	w	w
B-N12, B-F19	IL-6R	+	+	w	+
M195	IL-6R	+	w	+	+
R34.34	IL-7R	–	–	w	w
B-G20	IL-8R	–	–	–	–
htr9	TNFR/55kD	+	+	+	+
utr1	TNFR/75kD	+	+	+	+
7-7A3	M-CSFR	+	+	+	+
NU-SCF1	SCFR	+	+	w	+
MTK1, MTK2	SCFR	w	w	+	+
17F11	SCFR	+	+	+	+
SC04, SC06	GM-CSFR	w	w	w	w

+ strong positive staining, w weak staining, – no staining

mainly membrane staining for these cytokine receptors, but some cytoplasmic staining was also noted (Figure 2). Macrophages and FBGCs reacting with anti-IL-1R type 1 antibodies showed membrane staining only. Anti-TNFR antibodies reacted strongly with both the nucleus and cytoplasm of these cells (Figure 2). Weak staining was seen on macrophages and FBGCs with antibodies directed against receptors for IL-4 and GM-CSF. Blood vessels in the arthroplasty tissues also showed strong staining for SCFR, IL-7R and TNFR antibodies. Macrophages and FBGCs did not react with antibodies directed against IL-3R and IL-8R.

Antigenic phenotype of osteoclasts in giant cell tumours of bone

The pattern of staining of the cytokine receptor monoclonal antibodies on osteoclasts was almost identical to that of both inflammatory macrophages and FBGCs. Osteoclasts showed strong membrane staining with antibodies directed against IL-2Rα and IL-2Rγ, and strong membrane and cytoplasmic staining with antibodies directed against IL-6R, TNFR, M-CSFR and SCFR (Figure 3). Osteoclasts were not exclusively stained, as mononuclear cells present in the GCT imprints also reacted strongly with these antibodies. All of the above antibodies reacted mainly with the membrane of the mononuclear cells, except the TNFR antibodies which showed strong nuclear and membrane staining. Weak staining for gp130, IL-1R type 1, IL-4R, IL-7R and GM-CSFR was seen on osteoclasts. Osteoclasts did not react with antibodies directed against IL-3R and IL-8R.

Discussion

This is the first study to show that wear particle-associated macrophages and FBGCs in arthroplas-

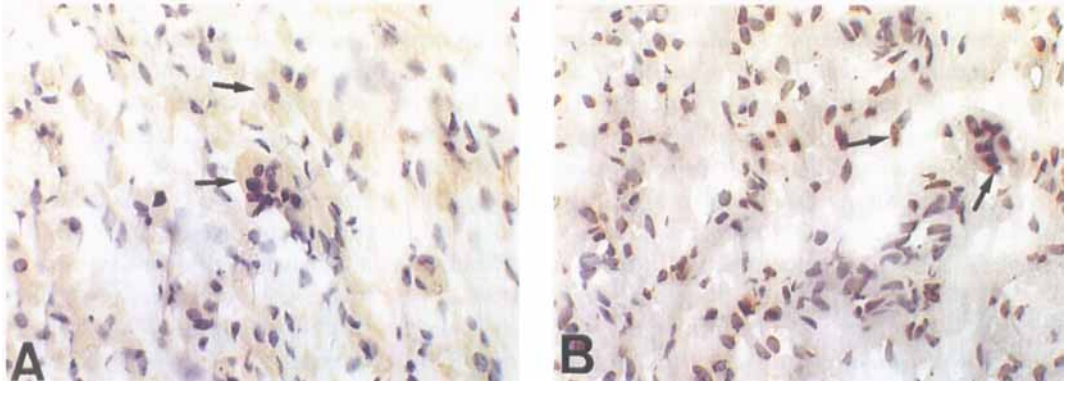

Figure 2. Cytokine receptor expression on macrophages and FBGCs in the arthroplasty pseudocapsule with monoclonal antibodies to (A) IL-6R (×400) and (B) TNFR (×400). Note the strong cytoplasmic staining in A (arrows) and the strong nuclear staining in B (arrows). Counterstained with hematoxylin.

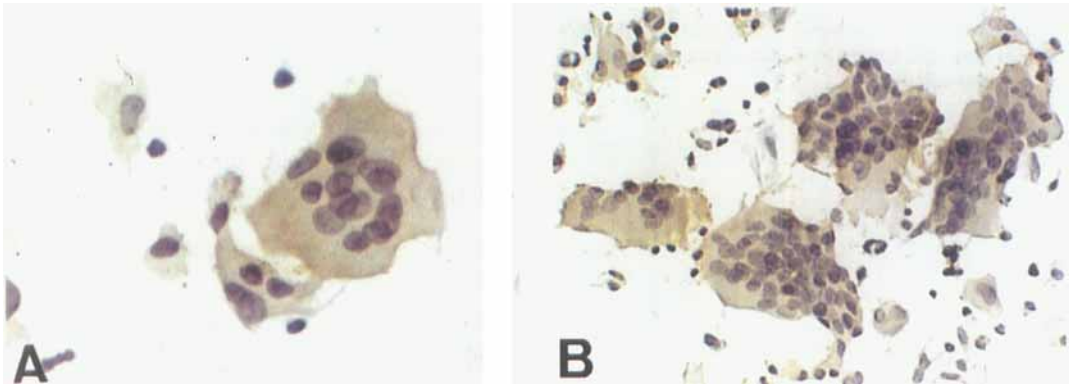

Figure 3. Cytokine/growth factor receptor expression on osteoclasts from GCT imprints with monoclonal antibodies to (A) IL-6R (×460) and (B) M-CSFR (×350). Counterstained with hematoxylin.

ty tissue exhibit a similar cytokine receptor profile to that of mature osteoclasts. The activity of such cells is likely to be influenced by these humoral factors, many of which are known to be present in periprosthetic tissues. It is widely accepted that FBGCs are formed by the fusion of mature macrophages, and it has recently been shown that human arthroplasty-derived macrophages are capable of osteoclast differentiation (Sabokbar et al. 1997). The strong correlation between cytokine receptor antigen expression on macrophages, FBGCs and osteoclasts thus reflects the common lineage of these cells and indicates that they are likely to represent targets for cytokines/growth factors known to modulate osteoclast formation and activity.

The presence of IL-1 and TNF receptors on arthroplasty macrophages and osteoclasts is consistent with the hypothesis that these cytokines play an important role in periprosthetic bone resorption and implant loosening. Both IL-1 and TNF are potent inducers of bone resorption in calvarial organ cultures (Bertolini et al. 1986, Gowen and Mundy 1986). In addition, IL-1 and TNF also stimulate osteoclast formation in long-term human marrow cultures (Pfeilshifter et al. 1989). These cytokines are believed to act via the osteoblast to promote osteoclast bone resorbing activity rather than to stimulate osteoclast function directly (Thomson et al. 1986, 1987). Both IL-1 and TNF are mitogenic for osteoclast precursors (Pfeilschifter et al. 1989) and IL-1 enhances osteoclast survival (Jimi et al. 1995).

Strong expression of IL-6 receptors was found on arthroplasty macrophages, FBGCs and mature osteoclasts. Previous studies have shown that IL-6 receptors are present on monocytes/macrophages (Munck-Petersen et al. 1990), and osteoclasts isolated from giant cell tumors of bone (Ohsaki et al. 1992). IL-6 induces bone resorption in foetal mouse calvaria which contains primitive osteoclast progenitors (Ishimi et al. 1990) and, in conjunction with other factors such as IL-1, can dramatically potentiate bone loss in vivo (Black et al. 1991). With regard to aseptic loosening, IL-1, TNF and IL-6 have all been identified in periprosthetic tissues surrounding loose implants (Chiba et al. 1994, Horikoshi et al. 1994). Several studies have shown that these cytokines are released from wear particle-stimulated macrophages and fibroblastic cells (Glant et al. 1993, Haynes et al. 1993, Manlapaz et al. 1996). Strong expression of gp130 was shown on arthroplasty macrophages, FBGCs and osteoclasts in this study. IL-6 is known to act via a cell surface receptor which consists of two components, a membrane-bound IL-6 receptor and gp130. When the IL-6 receptor is occupied, the ligand receptor complex binds to gp130 which then transduces the IL-6 signals (Taga et al. 1989).

M-CSF receptors have not previously been identified on macrophages in periprosthetic tissues. M-CSF is an essential co-factor for the proliferation and differentiation of osteoclast progenitors. In vitro studies using long-term mouse marrow culture systems (Tanaka et al. 1993) and more recently using human monocyte/macrophage and marrow culture systems (Fujikawa et al. 1996, Sarma and Flanagan 1996) have confirmed the importance of M-CSF in osteoclast formation.

Thus, our finding of M-CSF receptor expression on arthroplasty macrophages is in keeping with the concept of macrophage-osteoclast differentiation occurring in periprosthetic tissues. Consistent with our findings of M-CSF receptors on osteoclasts, it has been shown that M-CSF promotes osteoclast survival (Fuller et al. 1993, Jimi et al. 1995), and stimulates osteoclastic bone-resorbing activity (Yang et al. 1996, Sarma et al. 1997).

Weak staining for GM-CSF receptors on arthroplasty macrophages, FBGCs and osteoclasts was shown in this study. GM-CSF has recently been shown to be released from monocytes after wear particle stimulation (Horowitz and Purdon 1995). This response may be due to increased levels of IL-1, IL-6 and TNF, since such cytokines are known to stimulate GM-CSF production. The expression of IL-4 receptors on arthroplasty macrophages and osteoclasts is of interest as IL-4 inhibits osteoclast formation from both marrow and mononuclear phagocyte precursors (Shioi et al. 1991, Lacey et al. 1995).

In summary, macrophages and FBGCs in periprosthetic tissues were found to express receptors for cytokines which stimulate osteoclastic bone resorbing activity, including IL-1, TNF and IL-6, and receptors for cytokines/growth factors known to influence osteoclast formation, e.g., IL-1, IL-4, TNF, IL-6, M-CSF, GM-CSF and SCF. Receptors for these cytokines/growth factors were also found on osteoclasts, reflecting the fact that such cells are all part of the mononuclear phagocyte system and that both osteoclasts and FBGCs are of macrophage origin. Although many of these cytokines/growth factors are likely to produce their effects through common signal molecules, the widespread cellular distribution of these receptors may account for the diverse effects of these humoral factors on bone resorption associated with aseptic loosening.

The authors thank Barry Puddle for his advice on immunohistochemical staining. This study was supported by the Wellcome Trust.

Athanasou N A, Quinn J. Immunophenotypic differences between osteoclasts and macrophage polykaryons: immunohistological distinction and implications for osteoclast ontogeny and function. J Clin Path 1990; 43: 997-1003.

Athanasou N A, Quinn J, McGee J O. Immunocytochemical analysis of the human osteoclast: phenotypic relationship to other marrow-derived cells. Bone Miner 1988; 3: 317-33.

Bertolini D R, Nedwin G E, Bringman T S, Smith D D, Mundy G R. Stimulation of bone resorption and inhibition of bone formation in vitro by human tumour necrosis factor. Nature 1986; 319: 516-8.

Black K, Garrett I R, Mundy G R. Chinese hamster ovarian cells transfected with the murine interleukin-6 gene cause hypercalcaemia as well as cachexia, leukocytosis and thrombocytosis in tumor-bearing nude mice. Endocrinology 1991; 128: 2657-9.

Chambers T J, Fuller K, McSheehy P M J, Pringle J A S. The effects of calcium-regulating hormones on bone resorption by isolated human osteoclastoma cells. J Pathol 1985; 145: 297-305.

Chiba J, Rubash H E, Kim K J, Iwaki Y. The characterisation of cytokines in the interface tissue obtained from failed cementless total hip arthroplasty with and without femoral osteolysis. Clin Orthop 1994; 300: 304-12.

Demulder A, Suggs S V, Zsebo K M, Scarcez T, Roodman G D. Effects of stem cell factor on osteoclast-like cell formation in long-term human marrow cultures. J Bone Miner Res 1992; 7: 1337-44.

Fujikawa Y, Quinn J M W, Sabokbar A, McGee J O'D, Athanasou N A. The human osteoclast precursor circulates in the monocyte fraction. Endocrinology 1996; 137: 4058-60.

Fuller K, Owens J M, Jagger C J, Wilson A, Moss R, Chambers T J. Macrophage colony-stimulating factor stimulates survival and chemotactic behaviour in isolated osteoclasts. J Exp Med 1993; 178: 1733-44.

Glant T T, Jacobs J J, Molnar G, Shanbhag A S, Valyon M, Galante J O. Bone resorption activity of particulate-stimulated macrophages. J Bone Miner Res 1993; 8: 1071-9.

Gowen M, Mundy G R. Actions of recombinant interleukin-1, interleukin-2, and interferon gamma on bone resorption in vitro. J Immunol 1986; 136: 2478-82.

Harris W H. Osteolysis and particle disease in hip replacements (a review). Acta Orthop Scand 1994; 65: 113-23.

Haynes D R, Rogers S D, Hay S, Pearcy M J, Howie D W. The differences in toxicity and release of bone-resorbing mediators induced by titanium and cobalt-chromium-alloy wear particles. J Bone Joint Surg (Am) 1993; 75: 825-34.

Horikoshi M, Macaulay W, Booth R E, Crossett L S, Rubash H E. Comparison of interface membranes obtained from failed cemented and cementless hip and knee prostheses. Clin Orthop 1994; 309: 69-87.

Horowitz S M, Purdon M A. Mechanisms of cellular recruitment in aseptic loosening of prosthetic joint implants. Calcif Tissue Int 1995; 57: 301-5.

Horton M A, Lewis D, McNulty K, Pringle J A, Chambers T J. Monoclonal antibodies to osteoclastomas (giant cell bone tumors): definition of osteoclast-specific cellular antigens. Cancer Res 1985; 45: 5663-9.

Ishimi Y, Miyaura C, Jin C H, Akatsu T, Abe E, Nakamura Y, Yamaguchi A, Yoshiki S, Matsuda T, Hirano T, Kishimoto T, Suda T. IL-6 is produced by osteoblasts and induces bone resorption. J Immunol 1990; 145: 3297-303.

Jimi E, Shuto T, Koga T. Macrophage colony-stimulating factor and interleukin-1 alpha maintain the survival of osteoclast-like cells. Endocrinology 1995; 136: 808-11.

Jiranek W A, Machado M, Jasty M, Jevsevar D, Wolfe H J, Goldring S R, Goldberg M J, Harris W H. Production of cytokines around loosened cemented acetabular components: analysis with immunohistochemical techniques and in situ hybridization. J Bone Joint Surg (Am) 1993; 75: 863-79.

Kadoya Y, Al-Saffar N, Kobayashi A, Revell P A. The expression of osteoclast markers on foreign body giant cells. Bone Miner 1994; 27: 85-96.

Kishimoto T, Kikutani H, von dem Borne A E G Kr, Goyert S M, Mason D Y, Miyasaka M, Moretta L, Okumura K, Shaw S, Springer T A, Sugamura K, Zola H. Leukocyte typing VI. White cell differentiation antigens. Garland Publishing Inc., New York 1998.

Lacey D L, Erdmann J M, Teitelbaum S L, Tan M L, Ohara J, Shioi A. Interleukin 4, interferon-gamma, and prostaglandin E impact the osteoclastic cell-forming potential of murine bone marrow macrophages. Endocrinology 1995; 136: 2367-76.

Manlapaz M, Maloney W J, Smith R L. In vitro activation of human fibroblasts by retrieved titanium alloy debris. J Orthop Res 1996; 14: 465-72.

Munck-Petersen C, Davidsen O, Moestrup S K, Sonne O, Nykjaer A, Moller B K. Cellular targets and receptors for interleukin-6. II. Characterization of IL-6 binding and receptors in peripheral blood cells and macrophages. Eur J Clin Invest 1990; 20: 377-84.

Ohsaki Y, Takahashi S, Scarcez T, Demulder A, Nishihara T, Williams R, Roodman G D. Evidence for an autocrine/paracrine role for interleukin-6 in bone resorption by giant cells from giant cell tumors of bone. Endocrinology 1992; 131: 2229-34.

Pfeilschifter J, Chenu C, Bird A, Mundy G R, Roodman G D. Interleukin-1 and tumor necrosis factor stimulate the formation of human osteoclastlike cells in vitro. J Bone Miner Res 1989; 4: 113-8.

Quinn J, Joyner C, Triffitt J T, Athanasou N A. Polymethylmethacrylate-induced inflammatory macrophages resorb bone. J Bone Joint Surg (Br) 1992; 74: 652-8.

Sabokbar A, Fujikawa Y, Neale S, Murray D W, Athanasou N A. Human arthroplasty-derived macrophages differentiate into osteoclastic bone-resorbing cells. Ann Rheum Dis 1997; 56: 414-20.

Sarma U, Flanagan A M. Macrophage colony-stimulating factor induces substantial osteoclast generation and bone resorption in human bone marrow cultures. Blood 1996; 88: 2531-40.

Sarma U, Edwards M, Flanagan A M. Macrophage colony-stimulating factor stimulates bone resorption by isolated osteoclasts disaggregated from human foetal bones by increasing osteoclast survival. J Bone Miner Res (Suppl 1) 1997; 12: S126.

Shinar D M, Sato M, Rodan G A. The effect of hemopoietic growth factors on the generation of osteoclast-like cells in mouse bone marrow cultures. Endocrinology 1990; 126: 1728-35.

Shioi A, Teitelbaum S L, Ross F P, Welgus H G, Suzuki H, Ohara J, Lacey D L. Interleukin-4 inhibits murine osteoclast formation in vitro. J Cell Biochem 1991; 47: 272-7.

Taga T, Hibi M, Hirata Y, Yamasaki K, Yasukawa K, Matsuda T, Hirano T, Kishimoto T. Interleukin-6 triggers the association of its receptor with a possible signal transducer, gp130. Cell 1989; 58: 573-81.

Takahashi N, Udagawa N, Akatsu T, Tanaka S, Shiopnome M, Suda T. Role of colony-stimulating factors in osteoclast development. J Bone Miner Res 1991; 6: 977-85.

Tamura T, Udagawa N, Takahashi N, Miyaura C, Tanaka S, Yamada Y, Akatsu T, Koishihara Y, Ohsugi Y, Kumaki K, Taga T, Kishmoto T, Suda T. Soluble interleukin-6 receptor triggers osteoclast formation by interleukin-6. Proc Natl Acad Sci USA 1993; 90: 11924-8.

Tanaka S, Takahashi N, Udagawa N, Tamura T, Akatsu T, Stanley E R, Kurokawa T, Suda T. Macrophage colony-stimulating factor is indispensable for both proliferation and differentiation of osteoclast progenitors. J Clin Invest 1993; 91: 257-63.

Thomson B M, Saklatvala J, Chambers T J. Osteoblasts mediate interleukin-1 responsiveness of bone resorption by rat osteoclasts. J Exp Med 1986; 164: 104-12.

Thomson B M, Mundy G R, Chambers T J. Tumor necrosis factor α and ß induce osteoblastic cells to stimulate osteoclastic bone resorption. J Immunol 1987; 138: 775-9.

Yang S, Zhang Y, Rodriguiz R M, Ries W L, Key L L. Functions of the M-CSF receptor on osteoclasts. Bone 1996; 18: 355-60.