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Histological analysis and biological effects of granulation tissue around loosened hip prostheses in the development of osteolysis

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Abstract Although aseptic loosening of the prosthesis is a long-term complication after total joint replacement, the detailed mechanism of osteolysis remains unknown. We examined 82 samples from 40 patients with aseptic loosened hip prostheses histologically, and compared the distribution of particles, macrophages/histiocytes, and foreign body giant cells in the retrieved tissue from capsules and around prostheses. Furthermore, to investigate the mechanism of osteolysis, we cultured tissue from a patient with massive osteolysis and examined the effects of the conditioned medium on osteoblasts in vitro. Numerous multinucleated giant cells and histiocytes were present, and polyethylene particles ranging from medium to large were identified in the polarized light. However, the distribution was heterogeneous, and no particles were found microscopically in about 30%–40% of periprosthetic tissues, and in 60% of capsules. The amount of particles correlated with giant cells, but not with histiocytes. The conditioned medium of the granulation tissue culture stimulated osteoblasts to produce interleukin-6 in both protein and mRNA, and this was in part inhibited by anti-tumor necrosis factor-α or the interleukin-1β antibody, suggesting that interleukin-6 production is mediated by several cytokines. These findings suggest that interleukin-6, which is produced not only by macrophages but also by osteoblasts, is a contributing factor to aseptic loosening.

Key words Aseptic loosening · Osteolysis · Granulation tissue

Introduction

Although total joint replacement is beneficial for patients with joint disease, aseptic loosening of the prosthesis is a long-term complication. Many histological studies have shown the existence of polyethylene particles in the granulomatous tissue around the prosthesis, suggesting that this is the principal cause of osteolysis.[5,6,18]

It has been reported that particulate debris stimulates macrophages to release many chemical mediators such as cytokines, which induce bone resorption.[1,8] However, the detailed mechanisms of osteolysis remain unknown. Not only particulate debris but also mechanical stress contributes to loosening of the prosthesis.[17] These findings suggest heterogeneity in the histology of granulation tissue around the prosthesis, but an insufficient number of samples have been observed to date. Therefore, we investigated 82 samples from patients with aseptic loosened hip prostheses, and compared the histological findings of capsules and granulation tissue around the prostheses. To investigate their biological effects on osteolysis, we cultured tissue from a patient with massive osteolysis. Compared with those on macrophages or osteoclasts,[1,10,15,19,20,29] few studies have focused on the behavior of osteoblasts in osteolysis.[4,12] Therefore, in this study, we also examined the effects of conditioned medium from periprosthetic tissue on osteoblasts in vitro.

Materials and methods

Eighty-two samples were obtained from 40 patients at revision surgery for aseptic loosening of the hip. Informed consent was obtained after receiving approval from the local ethics committee. The samples were 23 capsules, 23 granulation tissues from around the stem (femoral), and 36 from around the socket (acetabular). The mean patient age was 67 years at revision. The mean interval between the initial surgery and revision surgery was 14 years. The first appearance of loosening could not be identified in 18 patients, because osteolysis was already present when they visited our hospital. Mechanical instability was determined by subsidence or

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Table 1. Patients and prostheses

Case number	Sex	Age	Duration of implant (y)	Duration of osteolysis (y)	Original disease	Type of fixation	Operation / Prosthesis	Mecahnical stability
(1)	f	77	5	Unknown	OA	Cementless	THA / unknown	Loose
(2)	m	75	15	Unknown	OA	Cemented	THA / Kyocera	Loose
(3)	f	78	15	Unknown	OA	Cemented	THA / Kyocera	Loose
(4)	f	76	24	Unknown	OA	Cemented	THA / Charnley	Loose
(5)	f	76	15	11	OA	Cemented	THA / Kyocera	Loose
(6)	f	60	19	Unknown	OA	Cemented	THA / Kyocera	Loose
(7)	f	76	15	Unknown	OA	Cemented	THA / Kyocera	Loose
(8)	m	71	4	Unknown	OA	Cemented	THA / Omniflex	Loose
(9)	f	67	17	Unknown	OA	Cemented	THA / Kyocera	Loose
(10)	f	59	3	Unknown	OA	Cemented	THA / Kyocera	Loose
(11)	f	67	14	Unknown	OA	Cementless	THA / Harris-Galante	Well fixed
(12)	f	71	20	Unknown	OA	Cemented	THA / Kyocera	Loose
(13)	m	71	13	Unknown	OA	Cemented	THA / Charnley	Loose
(14)	f	76	14	Unknown	RA	Cemented	THA / Kyocera	Loose
(15)	f	73	10	Unknown	OA	Cemented	THA / Kyocera	Loose
(16)	f	45	19	Unknown	OA	Cemented	THA / Kyocera	Loose
(17)	f	65	5	Unknown	Fracture	Cementless	BHA / Aesculap	Loose
(18)	m	61	16	Unknown	Fracture	Cemented	THA / Kyocera	Loose
(19)	m	56	13	6	AN	Cementless	BHA / Omnifit	Loose
(20)	f	77	26	22	OA	Cemented	THA / Charnley	Loose
(21)	m	75	9	8	Fracture	Cementless	BHA / Omnifit	Loose
(22)	f	60	13	2	OA	Cementless	BHA / Omnifit	Loose
(23)	m	74	17	6	OA	Cementless	THA / Lord	Loose
(24)	m	64	6	5	OA	Cemented	THA / Harris-Galante	Loose
(25)	f	60	21	19	OA	Cemented	THA / Charnley	Loose
(26)	m	70	16	6	OA	Cementless	THA / Lord	Loose
(27)	f	69	13	9	OA	Cemented	THA / Charnley	Loose
(28)	f	61	9	4	OA	Cemented	THA / Charnley	Loose
(29)	f	66	10	9	OA	Cementless	THA / Harris-Galante	Loose
(30)	f	67	18	3	OA	Cementless	THA / Lord	Loose
(31)	m	60	11	6	AN	Cementless	BHA / Omniflex	Loose
(32)	f	34	12	3	AN	Cementless	BHA / Omniflex	Loose
(33)	m	63	7	5	AN	Cementless	THA / Omniflex	Well fixed
(34)	f	70	17	Unknown	Fracture	Cementless	BHA / Harris-Galante	Well fixed
(35)	f	62	15	2	AN	Cementless	BHA / Bateman	Loose
(36)	f	70	14	10	OA	Cemented	THA / Charnley	Loose
(37)	f	72	17	15	OA	Cemented	THA / Charnley	Loose
(38)	f	73	23	21	OA	Cemented	THA / Charnley	Loose
(39)	f	65	17	15	OA	Cemented	THA / Charnley	Loose
(40)	f	67	10	3	OA	Cementless	BHA / Omnifit	Loose

f, female; m, male; y, years; OA, osteoarthritis; AN, avascular necrosis; THA, total hip arthroplasty; BHA, bipolar hip arthroplasty

migration of the prosthesis, and only three cases were well fixed. No highly cross-linked polyethylene acetabular liners were used in this study. Table 1 gives details of the patients and of the original implants.

Histological study

Tissue specimens were immediately fixed in 10% formalin, embedded in paraffin, cut into 5-μm-thick sections, and stained with hematoxylin and eosin. Immunohistochemical staining for CD68, interleukin-6 (IL-6), and tumor necrosis factor (TNF)-α, was performed using a Vectastain kit (Vector Laboratories, Burlingame, CA, USA). Monoclonal anti-CD68 antibody was obtained from ZYMED (ZYMED Laboratories, San Francisco, CA, USA). Monoclonal anti-human TNF-α and monoclonal anti-human IL-6 antibodies were purchased from DAKO (Kyoto, Japan). Nonimmune mouse immunoglobulin was used as a negative control for nonspecific binding reactivity. Polyethylene particles were examined under polarized light. Histological grading was evaluated using a quantitative five-grade (0–4) system for the presence of giant cells, histiocytes, and polyethylene particles as described by Goodman et al.:[6] 0 = absent; 1 = rare, <10% of fields; 2 = occasional, 10–30% of fields; 3 = moderate, 30%–50% of fields; and 4 = frequent, >50% of fields.

Table 2. Primer sequences for reverse transcriptase-polymerase chain reaction

Gene	Sequences	Size
GAPDH	5′-GCTCTCCAGAACATCATCCCTGCC-3′ 5′-CGTTGTCATACCAGGAAATGAGCTT-3′	346
IL-6	5′-ATGAACTCCTTCTCCACAAG-3′ 5′-GTGCCTGCAGCTTCGTCAGCA-3′	546
TFN-α	5′-TCAGATCATCTTCTCGAACC-3′ 5′-CAGATAGAGATGGGCTCATACC-3′	361
ODF	5′-ATAGAATATCAGAAGATGGCACTC-3′ 5′-TAAGGAGGGGTTGGAGACCTCG-3′	668
OPG	5′-TGCTGTTCCTACAAAGTTTACG-3′ 5′-CTTTGAGTGCTTTAGTGCCTG-3′	435
Collagen I	5′-AGCGCTGGTTTCGACTTCAGCTTCC-3′ 5′-CATCGGCAGGGTCGGAGCCCT-3′	340
MMP-13	5′-GACTTCACGATGGCATTGCTG-3′ 5′-GCATCAACCTGCTGAGGATGC-3′	491

GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IL-6, interleukin-6; TNF, tumor necrosis factor; ODF, osteoclast differentiation factor; OPG, osteoprotegerin; MMP, matrix metalloproteinase

Organ culture

Granulation tissue obtained from a patient with massive osteolysis was cut into small pieces and cultured in Dulbecco modified Eagle's medium (DMEM) without serum for 48 h according to the method described by Goldring et al.[5] The cultures were incubated at 37°C in an atmosphere of 5% CO_2 and 95% air. The conditioned medium was collected and used for the following study.

Osteoblast culture

Human osteoblasts (Cambrex Bio Science Walkersville, Walkersville, MD, USA) were cultured in DMEM containing 10% fetal calf serum. At confluence, the medium was replaced with serum-free medium and cultured for an additional 24 h. The conditioned medium of granulation tissue previously collected was then added to the medium at various dilutions and cultured for an additional 24 h.

Enzyme-linked immunosorbent assay

The concentration of IL-6 and TNF-α in conditioned medium was measured by enzyme-linked immunosorbent assay (ELISA, BioSource International, Camarilo, CA, USA). The minimal detectable doses of IL-6 and TNF-α were <2 pg/ml and 0.5 pg/ml, respectively.

Reverse transcriptase–polymerase chain reaction

Total RNA was extracted from retrieved tissues or cultured osteoblasts using Easy Prep RNA (Takara Biochemicals, Tokyo, Japan). Reverse transcriptase–polymerase chain reaction (RT-PCR) was performed as follows using a Onestep RT-PCR kit (QIAGEN, Tokyo, Japan). Briefly, total RNA (0.1 μg) was reverse-transcribed into cDNA and subjected to PCR. After initial denaturation at 95°C for 15 min, PCR for IL-6, TNF-α, osteoclast differentiation factor (ODF), osteoprotegerin (OPG), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was performed for 35 cycles at 94°C for 1 min, 62°C for 1 min, and 72°C for 1 min. PCR for collagen I was performed for 30 cycles at 95°C for 30 s, at 60°C for 30 s, and at 60°C for 30 s. PCR for matrix metalloproteinase (MMP)-13 was performed for 35 cycles at 90°C for 1 min, at 54°C for 2 min, and at 72°C for 1 min. All final elongation steps were performed at 72°C for 1 min. PCR products were electrophoresed on 2% agarose gel. The primer sequences are shown in Table 2.

Statistical analysis

The Mann-Whitney *U* test was used to compare the differences between samples. Spearman's rank correlation was used to analyze the relationship among different parameters.

Results

The histological findings of tissues showed numerous multinucleated giant cells and histiocytes, which were immunohistochemically detected by a CD68 antibody. Various sizes of polyethylene particles were identified in the polarized light (Fig. 1). In addition, these histiocytes were immunostained with an IL-6 or TNF-α antibody (Fig. 2). To clarify the heterogeneity in the distribution of histiocytes, giant cells, and polyethylene

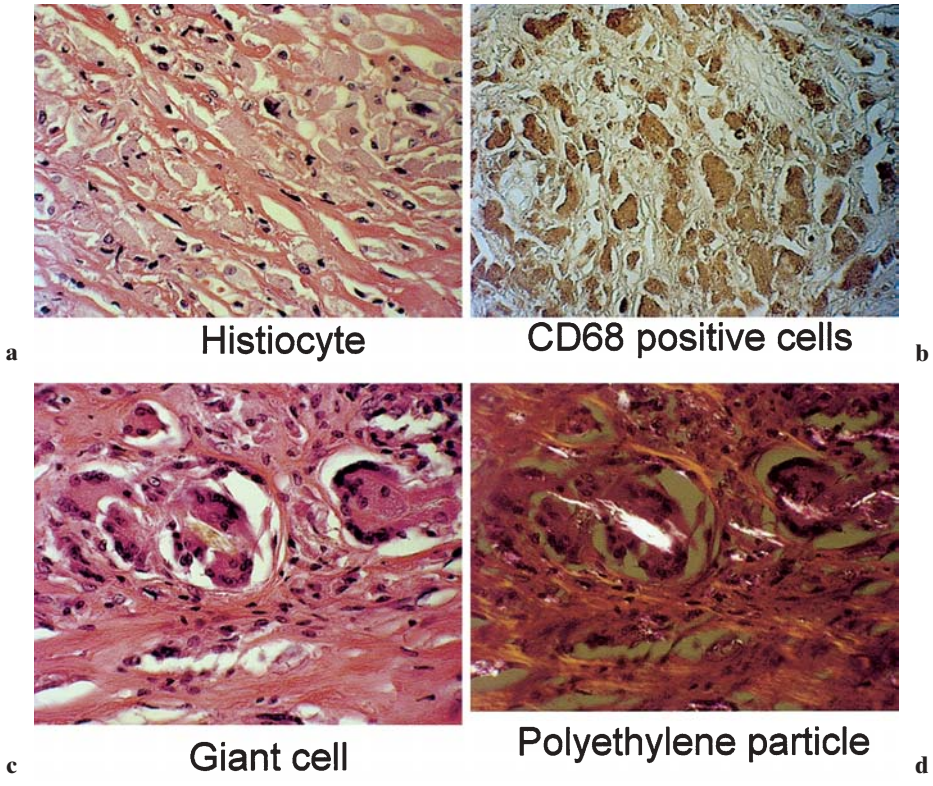

Fig. 1a–d. Photomicrograph of granulation tissue around a loosened prosthesis. **a** Histiocyte (hematoxylin and eosin, ×200); **b** immunostaining for macrophages using anti-CD68 antibody (×200); **c** multinuclear giant cells (hematoxylin and eosin, ×200); **d** polarized light photomicrograph (hematoxylin and eosin, ×200)

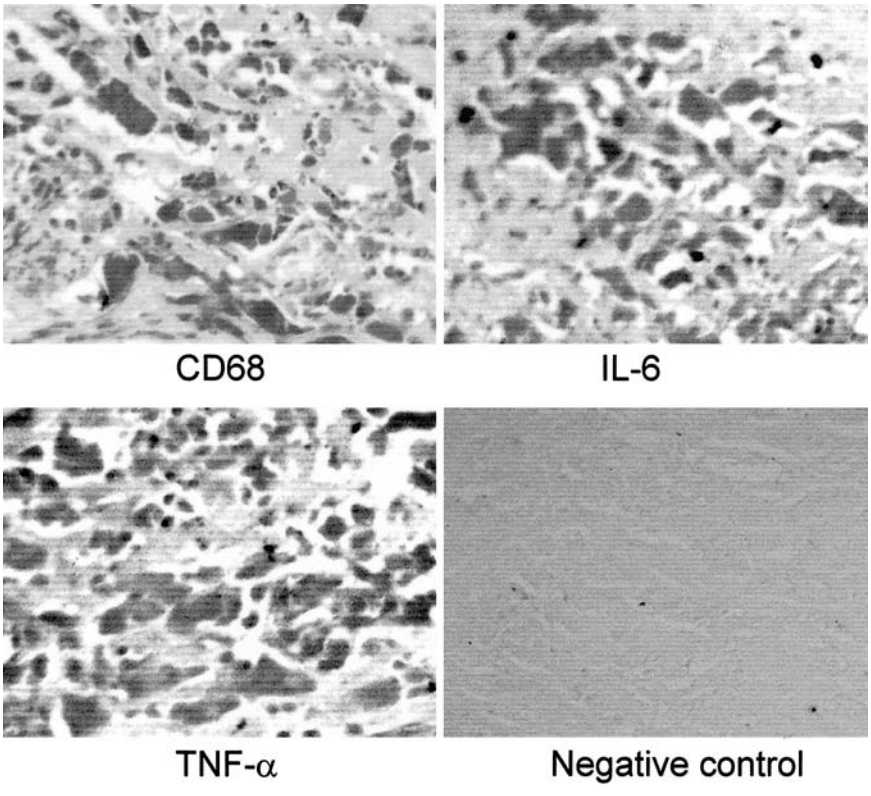

Fig. 2. Immunohistochemical staining for *CD68*, interleukin-6 (*IL-6*) and tumor necrosis factor (*TNF*)-α. The negative control was immunostained for nonimmune mouse immunoglobulin (×100)

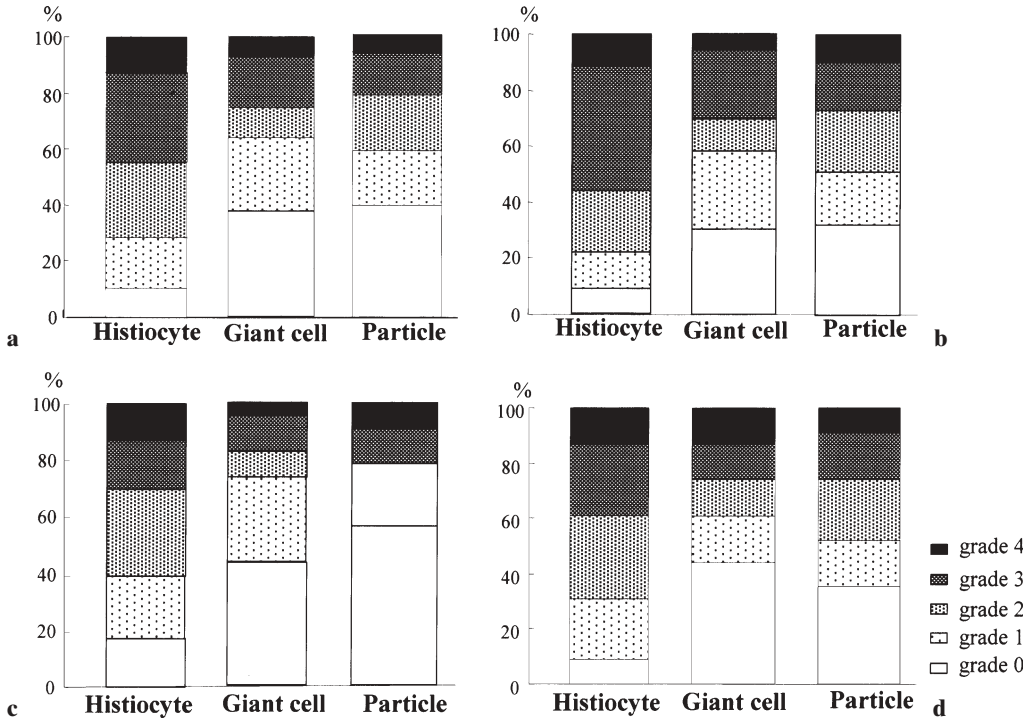

Fig. 3a–d. Distribution of histologic grading of histiocytes, giant cells, and polyethylene particles in all samples from aseptic loosening. **a** Total samples (sample number 82); **b** capsule (sample number 23); **c** granulation tissue around sockets (sample number 36); and **d** granulation tissue around stems (sample number 23). Histologic grading was as follows: 0 = absent; 1 = rare, <10%; 2 = occasional, 10%–30%; 3 = moderate, 30%–50%; and 4 = frequent, >50% of fields

particles, we examined their distribution semiquantitatively in all samples of capsules and periprosthetic granulation tissues. The histological findings were similar in tissue specimens from around the stem and the socket, as well as in a capsule. Histiocytes were more abundant than giant cells. No particles were identified in about 30%–40% of periprosthetic tissues, and in 60% of capsules (Fig. 3). We compared the relationship between giant cells and polyethylene particles and found that the amount of particles correlated with giant cells ($r = 0.797$, $P < 0.0001$), but not with histiocytes (Fig. 4). Among the clinical findings such as age and duration of implantation, the type of fixation was strongly related to the histological features. The tissues around the loosened noncemented socket contained significantly more particles and concomitantly more giant cells (Fig. 5). Although little difference was seen between Kyocera and Charnley type prostheses fixed with cement, the prosthesis without cement (Omniflex/Omnifit) resulted in more particles. The histological data from all samples are shown in Table 3.

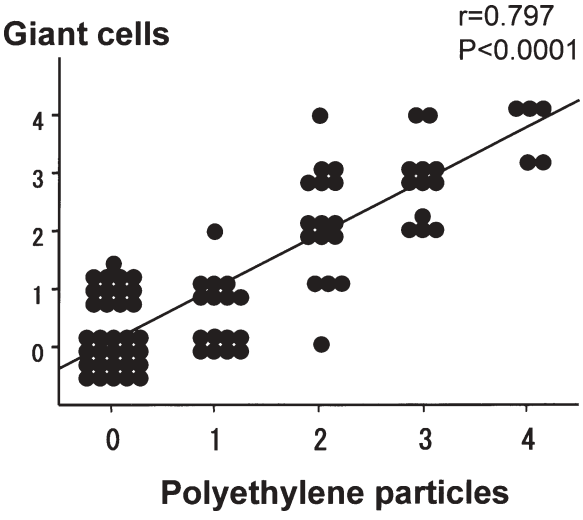

Fig. 4. Correlation between the histological grade of polyethylene particles and giant cells in the retrieved granulation tissue. Spearman's rank correlation was 0.797

To determine the mechanism of osteolysis, we examined the granulation tissues around the stem of a patient with massive osteolysis. The patient was a 56-year-old man (case 19) who had received an Omnifit femoral head replacement for avascular necrosis 13 years earlier. A radiolucent area around the stem was noticed 7 years after the primary operation. A prerevision radiograph showed massive osteolysis, and numerous giant cells engulfing polyethylene particles were demonstrated in the retrieved periprosthetic granulation tissue (Fig. 6). The granulation tissue expressed IL-6, TNF-α, OPG, ODF, and MMP-13 mRNA in RT-PCR (Fig. 7). To determine the biological effects of granulation tissue in osteolysis, 1 g of granulation tissue was organ cultured in 10 ml of DMEM for 2 days. The concentrations of IL-6 and TNF-α were 2700 pg/ml and 4 pg/ml, respectively. Ten or 20 μl of this medium was added to 1 ml of osteoblast culture medium, and was thus diluted 100 or

Table 3. Histological data of retrieved tissues from 40 loosened hip prostheses assessed semiquantitatively

Case number	Tissue location	Histological grading			Case number	Tissue location	Histological grading		
		Histiocyte	Giant cell	Particle			Histiocyte	Giant cell	Particle
(1)	Capsule	3	3	2	(17)	Acetabular	2	0	0
(2)	Acetabular	3	0	0		Femoral	2	0	0
	Femoral	3	1	0		Capsule	1	0	1
(3)	Acetabular	1	0	0	(18)	Acetabular	1	0	0
	Femoral	0	0	0		Femoral	1	0	0
	Capsule	1	1	0		Capsule	1	0	0
(4)	Acetabular	4	0	0	(19)	Femoral	1	4	4
	Capsule	4	1	0	(20)	Acetabular	4	0	0
(5)	Acetabular	0	3	3	(21)	Femoral	2	3	3
	Femoral	0	3	3		Capsule	3	0	0
	Capsule	0	3	2	(22)	Acetabular	0	4	4
(6)	Acetabular	3	0	1		Capsule	2	4	3
	Femoral	4	0	1	(23)	Acetabular	3	2	3
	Capsule	4	0	1		Femoral	3	2	2
(7)	Acetabular	3	2	2		Capsule	1	1	1
	Femoral	4	0	2	(24)	Femoral	3	0	0
	Capsule	2	2	2		Capsule	4	1	0
(8)	Acetabular	1	1	1	(25)	Acetabular	3	0	0
	Capsule	0	0	0	(26)	Acetabular	3	1	0
(9)	Acetabular	3	3	2	(27)	Acetabular	3	1	1
	Femoral	3	2	3	(28)	Acetabular	4	1	2
	Capsule	2	1	1	(29)	Acetabular	3	3	3
(10)	Acetabular	3	1	1		Femoral	1	3	3
	Femoral	2	0	1	(30)	Acetabular	2	2	3
	Capsule	2	0	0	(31)	Acetabular	2	3	4
(11)	Acetabular	3	1	1		Femoral	1	4	4
	Femoral	2	1	1	(32)	Acetabular	3	3	2
	Capsule	0	0	0	(33)	Acetabular	2	1	2
(12)	Acetabular	2	3	3		Femoral	2	1	2
	Capsule	0	0	0	(34)	Acetabular	1	3	4
(13)	Acetabular	2	1	0	(35)	Acetabular	2	3	2
	Femoral	1	0	0	(36)	Acetabular	2	1	0
	Capsule	2	1	0		Femoral	2	1	0
(14)	Acetabular	0	0	1	(37)	Acetabular	4	0	0
	Femoral	3	4	2		Femoral	3	0	0
	Capsule	3	0	0	(38)	Acetabular	3	3	2
(15)	Acetabular	3	1	0	(39)	Acetabular	3	2	2
	Capsule	3	1	0		Femoral	4	2	2
(16)	Acetabular	3	0	1		Capsule	2	3	3
	Femoral	2	0	1	(40)	Acetabular	1	4	3
	Capsule	2	0	0		Capsule	1	2	1

50 times. IL-6 was not detected in the medium just before osteoblast culture. After adding the organ culture medium and incubating the osteoblasts for 24 h, the IL-6 level in the medium significantly increased in a dose-dependent manner (Fig. 8). This IL-6 production was in part inhibited by 10 μg/ml of antibody for IL-1β or for TNF-α. Adding 10^{-4} M indomethacin to the culture also inhibited IL-6 production by half, while 10^{-6} M dexamethasone completely inhibited it (Fig. 9). Twenty-four h after the organ culture medium was added, total RNA was extracted from the osteoblasts, and RT-PCR was carried out. The expression of IL-6 mRNA increased, while that of collagen I mRNA was unchanged (Fig. 10).

Discussion

In this study, we analyzed periprosthetic tissues from patients with aseptic loosening and demonstrated the presence of macrophage/histiocytes, foreign body giant cells, and polyethylene particles in various grades. These findings were consistent with previous histological studies on osteolysis in joint replacement[5,6,18]

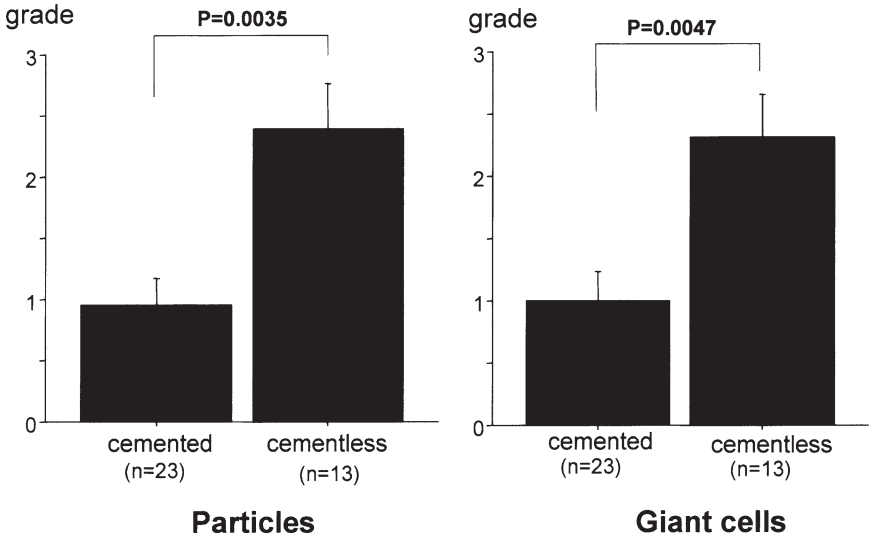

Fig. 5. Relationship of the histological grade of particles or giant cells in the granulation tissue around the socket between prostheses with and without cement. Bars represent the mean ± SEM

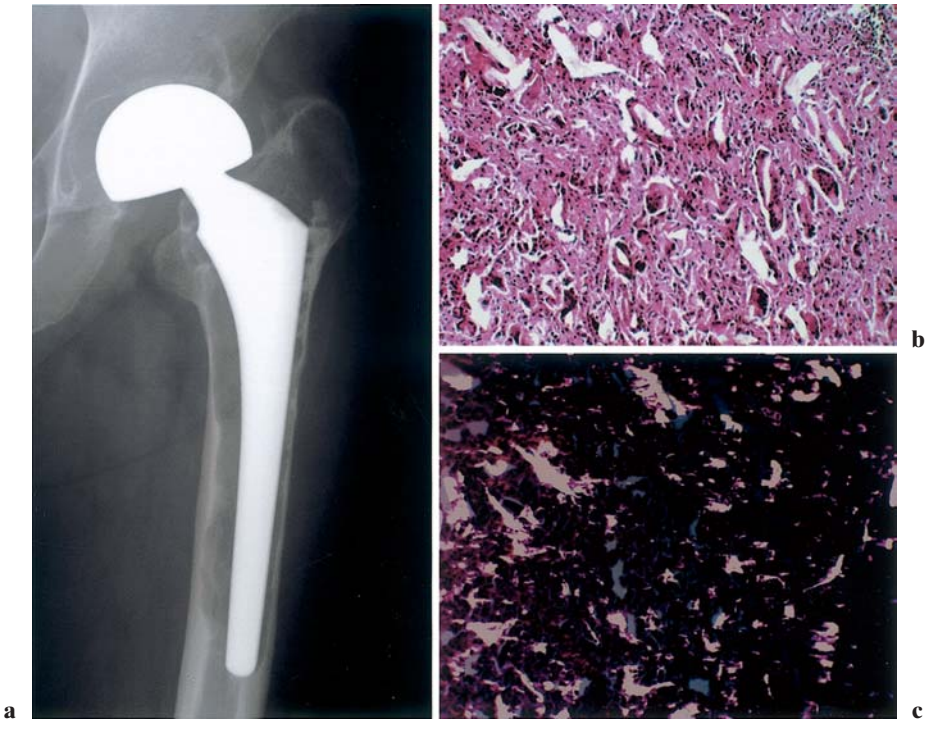

Fig. 6a–c. A case of massive osteolysis (case 19). A 56-year-old man had received an Omnifit bipolar femoral head replacement 13 years earlier. **a** Roentgenogram of the failed femoral component before the revision; **b** photomicrograph of granulation tissue around the loosened prosthesis (hematoxylin and eosin, ×100); **c** polarized light photomicrograph (hematoxylin and eosin, ×100)

and experimental animal models.[13,16] Unexpectedly, polyethylene particles were not always present in our samples, similar to the results shown by Kim et al.[14] that the percentage of specimens with polyethylene particles graded as moderate or severe was 52%–63%. This may be explained as follows: osteolysis was caused by other mechanisms such as mechanical stress,[17] or there were submicron polyethylene particles that were too small to be detected by light microscopy.[2] Since most cases involved in this study showed mechanical instability, both factors were related to osteolysis. It is known that particles smaller than 8–10 μm are phagocytosed by histiocytes, while bigger particles induce giant cell reaction and are associated with such cells.[23] In support of this, the number of giant cells significantly correlated with polyethylene particles. These results may indicate larger particles, but not submicron particles. However, it has been recently reported that contact between wear particles and macrophages without phagocytosis is also important for the signal transduction of cytokines.[20]

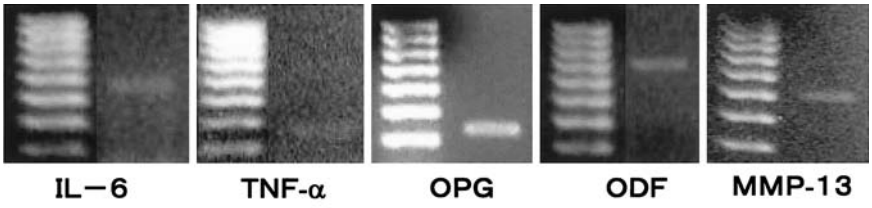

Fig. 7. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of interleukin (*IL*)-6, tumor necrosis factor (*TNF*)-α, osteoprotegerin (*OPG*), osteoclast differentiation factor (*ODF*), and matrix metalloproteinase (*MMP*)-13 in the granulation tissue of massive osteolysis. Molecular weight markers (bp) are shown on the left

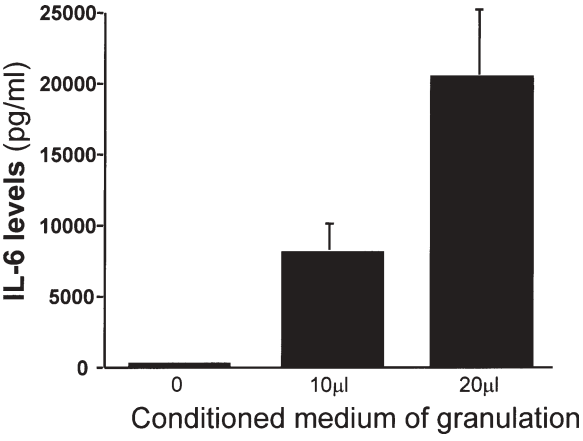

Fig. 8. Effect of conditioned medium from granulation organ culture on interleukin (IL)-6 production by osteoblasts. Ten or 20 μl of conditioned medium of the granulation tissue was added to 1 ml of osteoblast culture medium. IL-6 was not detected in the medium before the culture. After 24 h of culture, the concentration of IL-6 was measured. Values are the mean ± SEM

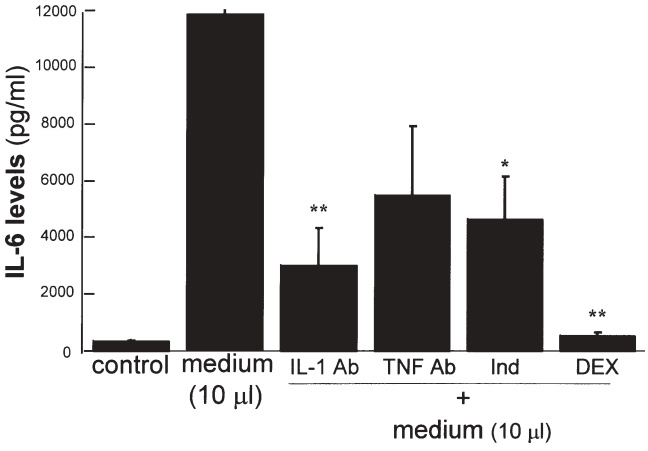

Fig. 9. Effects of the antibodies for interuleukin (IL)-1β or tumor necrosis factor (TNF)-α, indomethacin, and dexamethasone on the production of IL-6 from osteoblasts stimulated by conditioned medium from granulation tissue culture. Ten microliters of conditioned medium was added to 1 ml of osteoblast culture medium, alone or in combination with 10 μg/ml antibody for IL-1β (*IL-1 Ab*) or for TNF-α (*TNF Ab*), 10^{-4}M indomethacin (*Ind*), or 10^{-6}M dexamethasone (*DEX*). IL-6 was not detected in the medium before the culture. After 24 h of culture, the IL-6 levels were measured. Values are the mean ± SEM. *Asterisks* denote a significant difference compared with treatment by conditioned medium alone ($*P < 0.05$, $**P < 0.01$)

We compared the histological grading with the clinical findings and found more particles in tissue around a noncemented socket. Although the histological differences between cemented and noncemented implants are controversial,[7] Jones et al.[12] showed that immunohistochemical staining for cytokines is similar in both groups. Several cytokines, such as IL-6, TNF-α, and IL-1β, have been identified immunohistologically or by in situ hybridization.[7,11,12,25] These cytokines are considered to have been produced by particle-stimulated macrophages and to have played a role in bone resorption. In agreement with this, we also demonstrated the expression of IL-6 and TNF-α in protein and mRNA levels. MMPs are known to degrade the extracellular bone matrix, and several types have been shown in loosened hips.[9,21,24,26] We detected the expression of MMP-13 mRNA, which is known to degrade interstitial collagens, especially of the bone and cartilage matrix. Recently, it was reported that polymethylmethacrylate (PMMA) particles stimulate bone marrow cells to secrete ODF, which induces osteoclast genesis in vitro and is inhibited by OPG.[3] Consistent with this, we demonstrated mRNA expression for ODF and OPG in the sample. Thus, many cytokines related to bone resorption were expressed in the retrieved tissue. Neale et al.[22] have shown that IL-6 released by periprosthetic tissues plays important roles in the early stages of osteoclast formation from mononuclear precursors. On the other hand, osteoblasts are known to produce IL-6 in response to polyethylene particles,[28] PMMA,[30] or titanium particles.[27] However, several factors such as particles, cells, and cytokines in tissue seem to interplay in vivo. We therefore examined the effect of granulation tissue on the osteoblasts and found that IL-6 was produced not only by macrophages but also by osteoblasts.

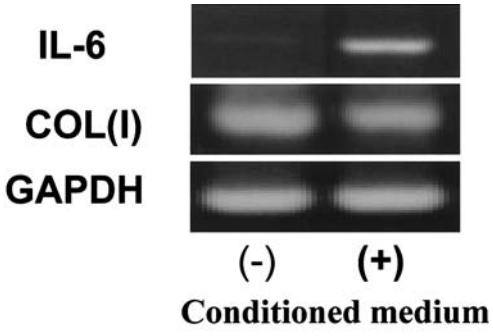

Fig. 10. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of interleukin (*IL*)-6 and collagen I (*COL I*) in osteoblasts. Total RNA was extracted 24 h after treatment with (+) or without (−) conditioned medium from granulation tissue culture. Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was an internal control

In summary, macrophages in granulation tissues were stimulated by particles or mechanical stress to produce several kinds of cytokines, MMPs, and other chemical mediators. These may affect the activity of both osteoblasts and osteoclasts, leading to osteolysis.

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References

1. Chiba J, Inoue K. Enhanced cytokine production from human macrophages stimulated by polyethylene particles retrieved from interface membranes after failed total hip arthroplasty. Mod Rheumatol 2000;10:24–30.
2. Chun L, Yoon J, Song Y, et al. The characterization of macrophages and osteoclasts in tissues harvested from revised total hip prostheses. J Biomed Mater Res 1999;48:899–903.
3. Clohisy JC, Frazier E, Hirayama T, et al. RANKL is an essential cytokine mediator of polymethylmethacrylate particle-induced osteoclastogenesis. J Orthop Res 2003;21:202–12.
4. Dean DD, Schwartz Z, Liu Y, et al. The effect of ultra-high molecular weight polyethylene wear debris on MG63 osteosarcoma cells in vitro. J Bone Joint Surg Am 1999;81:452–61.
5. Goldring SR, Schiller AL, Roelke M, et al. The synovial-like membrane at the bone–cement interface in loose total hip replacements and its proposed role in bone lysis. J Bone Joint Surg Am 1983;65:575–84.
6. Goodman SB, Chin RC, Chiou SS, et al. A clinical-pathologic biochemical study of the membrane surrounding loosened and non loosened total hip arthroplasties. Clin Orthop 1989;244:182–7.
7. Goodman SB, Huie P, Song Y, et al. Cellular profile and cytokine production at prosthetic interfaces. Study of tissues retrieved from revised hip and knee replacements. J Bone Joint Surg Br 1998;80:531–9.
8. Green TR, Fisher J, Stone M, et al. Polyethylene particles of a "critical size" are necessary for the induction of cytokines by macrophages in vitro. Biomaterials 1998;19:2297–302.
9. Imai S, Konttinen YT, Jumppanen M, et al. High levels of expression of collagenase-3 (MMP-13) in pathological conditions associated with a foreign-body reaction. J Bone Joint Surg Br 1998;80:701–10.
10. Itonaga I, Sabokbar A, Murray DW, et al. Effect of osteoprotegerin and osteoprotegerin ligand on osteoclast formation by arthroplasty membrane derived macrophages. Ann Rheum Dis 2000;59:26–31.
11. Jiranek WA, Machado M, Jasty M, et al. 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.
12. Jones LC, Frondoza C, Hungerford DS. Immunohistochemical evaluation of interface membranes from failed cemented and uncemented acetabular components. J Biomed Mater Res 1999; 48:889–98.
13. Jones LC, Frondoza C, Hungerford DS. Effect of PMMA particles and movement on an implant interface in a canine model. J Bone Joint Surg Br 2001;83:448–58.
14. Kim KJ, Chiba J, Rubash HE, et al. In vivo and in vitro analysis of membranes from hip prostheses inserted without cement. J Bone Joint Surg Am 1994;76:172–80.
15. Kim KJ, Itoh T, Tanahashi M, et al. Activation of osteoclast-mediated bone resorption by the supernatant from a rabbit synovial cell line in response to polyethylene particles. J Biomed Mater Res 1996;32:3–9.
16. Kim KJ, Kobayashi Y, Itoh T. Osteolysis model with continuous infusion of polyethylene particles. Clin Orthop 1998;352:46–52.
17. McEvoy A, Jeyam M, Ferrier G, et al. Synergistic effect of particles and cyclic pressure on cytokine production in human monocyte/macrophages: proposed role in periprosthetic osteolysis. Bone 2002;30:171–7.
18. Mirra JM, Amstutz HC, Matos M, et al. The pathology of the joint tissues and its clinical relevance in prosthesis failure. Clin Orthop 1976;117:221–40.
19. Nakashima Y, Sun DH, Maloney WJ, et al. Induction of matrix metalloproteinase expression in human macrophages by orthopaedic particulate debris in vitro. J Bone Joint Surg Br 1998;80: 694–700.
20. Nakashima Y, Sun DH, Trindade MCD, et al. Signaling pathways for tumor necrosis factor-α and interleukin-6 expression in human macrophages exposed to titanium-alloy particulate debris in vitro. J Bone Joint Surg Am 1999;81:603–15.
21. Nawrocki B, Polette M, Burlet H, et al. Expression of gelatinase A and its activator MT1-MMP in the inflammatory periprosthetic response to polyethylene. J Bone Miner Res 1999;14:288–94.
22. Neale SD, Sabokbar A, Howie DW, et al. Macrophage colony-stimulating factor and interleukin-6 release by periprosthetic cells stimulates osteoclast formation and bone resorption. J Orthop Res 1999;17:686–94.
23. Pizzoferrato A, Stea S, Sudanese A, et al. Morphometric and microanalytical analyses of alumina wear particles in hip prostheses. Biomaterials 1993;14:583–7.
24. Rudzki Z, Otfinowski J, Stachura J. The histological appearance of the periprosthetic capsule in failed total hip arthroplasty differs depending on the presence of polyethylene acetabulum destruction, iliac bone damage and presence of infection. Pol J Pathol 1996;47:19–25.
25. Stea S, Visentin M, Granchi D, et al. Wear debris and cytokine production in the interface membrane of loosened prostheses. J Biomater Sci Polym Ed 1999;10:247–57.
26. Takagi M, Santavirta S, Ida H, et al. Matrix metalloproteinases and tissue inhibitors of metalloproteinases in loose artificial hip joints. Clin Orthop 1998;352:35–45.
27. Takei H, Pioletti DP, Kwon SY, et al. Combined effect of titanium particles and TNF-α on the production of IL-6 by osteoblast-like cells. J Biomed Mater Res 2000;52:382–7.

28. Vermes C, Chandrasekaran R, Jacobs JJ, et al. The effects of particulate wear debris, cytokines, and growth factors on the functions of MG-63 osteoblasts. J Bone Joint Surg Am 2001;83:201–11.
29. Wang JY, Wicklund BH, Gustilo RB, et al. Prosthetic metals interfere with the functions of human osteoblast cells in vitro. Clin Orthop 1997;339:216–26.
30. Zambonin G, Colucci S, Cantatore F, et al. Response of human osteoblasts to polymethylmetacrylate in vitro. Calcif Tissue Int 1998;62:362–5.