Bone-resorbing cytokines in serum of patients with aseptic loosening of hip prostheses

Donatella Granchi, Elisabetta Verri, Gabriela Ciapetti, Susanna Stea, Lucia Savarino, Alessandra Sudanese, Michele Mieti, Roberto Rotini, Dante Dallari, Gianfranco Zinghi, Lucio Montanaro

From the Rizzoli Orthopaedic Institute, Bologna, Italy

Our aim was to determine if the serum levels of bone-resorbing cytokines (IL-1 β , TNF- α , IL-6, GM-CSF) are altered in patients with aseptic loosening of a total hip prosthesis, and if such levels are influenced by the type of implant. We determined cytokine levels in sera from 35 patients before revision for failed total hip arthroplasty and compared them with those in 25 healthy donors. We also assessed the soluble receptor of interleukin-2 (sIL-2r) in serum as an indication of a specific immune reaction against the implant.

Our findings showed that the sIL-2r and TNF- α serum level did not change. The IL-6 level was not significantly altered, but was higher in patients with TiAlV prostheses than in those with a CrCoMo implant and in patients with cemented prostheses. The IL-1 β level was found to be higher in those with a TiAlV cemented prosthesis than in the control group ($p = 0.0001$) and other groups of patients ($p = 0.003$ v uncemented TiAlV, $p = 0.01$ v cemented CrCoMo, $p = 0.001$ v uncemented CrCoMo). The GM-CSF level significantly increased in patients compared with healthy subjects ($p = 0.008$), and it was higher in those with cemented than with uncemented implants ($p = 0.01$). Only patients with cementless CrCoMo prostheses had levels of GM-CSF similar to those of

the control group. The highest GM-CSF concentrations were observed in patients treated with non-steroidal anti-inflammatory drugs (NSAIDs) in the last months before revision ($p = 0.04$). In addition, when massive osteolysis was observed, the level of GM-CSF tended to decrease to that of the control group.

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Cytokines play a fundamental role in bone remodelling¹ by inhibiting and stimulating both bone cells and the extra-cellular compartment of bone. Most of those involved in bone resorption are proinflammatory cytokines. The two isoforms of interleukin-1 (IL-1 α and IL-1 β) are potent stimulators of bone resorption and act synergistically with tumour necrosis factor- α (TNF- α) to induce the differentiation of osteoclasts and the production of prostaglandins. TNF- α induces the decrease in collagen synthesis and alkaline phosphatase activity in osteoblasts.² Interleukin-6 (IL-6) stimulates bone resorption either by recruiting mature osteoclasts or by activating them through an auto-crine mechanism.³ The colony-stimulating factors (CSFs), particularly GM-CSF, have a role in bone remodelling, and participate in bone resorption by acting on the bone cellular component along with other factors.⁴

Several in vitro studies have shown that cytokines are involved in the loosening of hip prostheses. Culture of the membranes surrounding failed prostheses has shown high activities of IL-1, TNF- α and IL-6.⁵ In situ hybridisation of the membranes has also shown high levels of IL-1 β mRNA in macrophages.⁶ In addition, in vitro studies have indicated that both cobalt-chromium (CoCrMo) and titanium-aluminium-vanadium (TiAlV) particles can favour the release of inflammatory cytokines involved in the osteolytic process.⁷

Our aim was to evaluate the serum levels of IL-1 β , TNF- α , IL-6 and GM-CSF in patients with aseptic loosening of a hip prosthesis. In addition, we have analysed the cytokine levels in patients according to either the features of the implant or the severity of osteolysis. Since the examined cytokines can also increase during a specific immune response, we assayed the serum level of the sol-

D. Granchi, MD, PhD, Researcher
E. Verri, BSc, Researcher
G. Ciapetti, BSc, Researcher
S. Stea, BSc, Researcher
L. Savarino, BSc, Researcher
L. Montanaro, MD, PhD, Head of Laboratory
Laboratory for Biocompatibility Research on Implant Materials, Rizzoli Orthopaedic Institute, Via di Barbiano 1/10, 40136 Bologna, Italy.

A. Sudanese, MD, Orthopaedic Surgeon
M. Mieti, MD, Orthopaedic Surgeon
VII Orthopaedic Department
R. Rotini, MD, Orthopaedic Surgeon
I Orthopaedic Department
D. Dallari, MD, Orthopaedic Surgeon
IV Orthopaedic Department
G. Zinghi, MD, PhD, Orthopaedic Surgeon, Head of Department
III Orthopaedic Department
Rizzoli Orthopaedic Institute, Via Pupilli 1, 40136 Bologna, Italy.

Correspondence should be sent to Dr D. Granchi.

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Table 1. Details of the 35 patients with aseptic loosening

Case	Age (yr)	Gender	Months to failure	Diagnosis	NSAIDs therapy*	Metal component	Cemented/ uncemented	Paprosky classification			Osteolysis	
								Acetabulum	Femur		Acetabulum	Femur
1	63	M	100	OA†	Yes	CrCoMo	Uncemented	1	2a		<2 mm	<2 mm
2	63	F	251	OA	Yes	CrCoMo	Uncemented	2b	2a		2 areas	<3 areas
3	64	M	144	OA	Yes	CrCoMo	Cemented	3b	3		2 areas	3 to 5 areas
4	66	F	56	OA	Yes	TiAlV	Uncemented	2b	3		1 area	3 to 5 areas
5	70	M	106	OA	Yes	TiAlV	Uncemented	2b	1		2 areas	<3 areas
6	68	F	92	Trauma	No	TiAlV	Cemented	3a	1		3 areas	<2 mm
7	67	F	52	OA	No	TiAlV	Uncemented	2a	1		1 area	<2 mm
8	40	F	241	Trauma	No	CrCoMo	Uncemented	2b	1		3 areas	<2 mm
9	44	M	168	OA	Yes	CrCoMo	Cemented	2b	2a		1 area	<2 mm
10	66	F	74	Trauma	Yes	CrCoMo	Uncemented	3b	2c		1 area	3 to 5 areas
11	68	F	180	OA	No	CrCoMo	Uncemented	2b	2b		3 areas	>5 areas
Endoprosthesis	12	F	112	Trauma	No	CrCoMo	Uncemented	-	2a		-	<3 areas
	13	F	37	Trauma	No	CrCoMo	Uncemented	2a	1		1 area	<2 mm
	14	M	21	Trauma	No	CrCoMo	Uncemented	3b	1		2 areas	<2 mm
	15	F	74	Trauma	Yes	TiAlV	Cemented	2b	2a		2 areas	3 to 5 areas
16	66	F	24	OA	Yes	TiAlV	Uncemented	1	1		<2 mm	<2 mm
17	63	F	150	OA	No	CrCoMo	Cemented	2c	2a		1 area	<2 mm
18	52	F	162	OA	Yes	CrCoMo	Cemented	2a	2a		2 areas	<3 areas
19	60	F	48	OA	Yes	TiAlV	Uncemented	2c	2a		2 areas	<3 areas
Endoprosthesis	20	F	22	Trauma	Yes	CrCoMo	Cemented	-	1		-	3 to 5 areas
	21	M	35	OA	No	CrCoMo	Uncemented	2c	2a		2 areas	3 to 5 areas
	22	F	70	Trauma	Yes	CrCoMo	Uncemented	2c	2a		2 areas	<3 areas
	23	F	25	OA	No	TiAlV	Cemented	1	1		<2 mm	<2 mm
24	51	F	21	OA	Yes	TiAlV	Uncemented	2a	2a		1 area	3 to 5 areas
25	74	F	92	OA	Yes	CrCoMo	Cemented	3b	2c		2 areas	>5 areas
26	78	M	108	OA	Yes	TiAlV	Uncemented	2c	3		3 areas	3 to 5 areas
27	76	F	156	Trauma	Yes	CrCoMo	Cemented	2a	1		<2 mm	<2 mm
28	61	F	92	Trauma	Yes	CrCoMo	Cemented	2a	1		<2 mm	<2 mm
29	57	F	96	OA	Yes	CrCoMo	Cemented	2a	2a		<2 mm	<3 areas
30	71	M	104	OA	No	CrCoMo	Uncemented	2c	1		2 areas	<2 mm
31	60	M	23	OA	No	TiAlV	Uncemented	1	1		<2 mm	<2 mm
32	60	F	96	OA	No	CrCoMo	Uncemented	2a	2a		1 area	<3 areas
33	73	M	96	OA	No	TiAlV	Cemented	2a	2a		1 area	<3 areas
34	64	M	21	Trauma	Yes	CrCoMo	Uncemented	2a	2a		1 area	<3 areas
35	76	F	74	OA	No	TiAlV	Cemented	2b	2b		2 areas	<3 areas

* prolonged therapy in the last months
† osteoarthritis

Table II. Source and sensitivity of the reagents used for the cytokine assay

Cytokine test kit	Source	Detection limit
h-Interleukin-1 β	Boehringer Mannheim Biochemica, Germany	1 pg/ml
h-Interleukin-2-receptor	Boehringer Mannheim Biochemica, Germany	5 pmol/l
Cytoscreen human IL-6	Biosource International, Camarillo, California	2 pg/ml
Cytoscreen human TNF- α	Biosource International, Camarillo, California	<1 pg/ml
Cytoscreen human GM-CSF	Biosource International, Camarillo, California	< 1pg/ml

Table III. Mean serum levels of cytokines (pg/ml $\pm$ SEM; range, median) and sIL-2r (pmol/l $\pm$ SEM; range, median) in patients with aseptic loosening and in the control group

	Failed prosthesis (n = 35)	Control group (n = 25)
IL-1 β	0.33 $\pm$ 0.1 (0 to 4.2; 0)	0.37 $\pm$ 0.1 (0 to 1.6; 0)
IL-6	28.9 $\pm$ 11 (0 to 386.7; 15.6)	35.3 $\pm$ 9 (3.0 to 177.1; 19.2)
TNF- α	0.06 $\pm$ 0.06 (0 to 2; 0)	0
GM-CSF	29.6 $\pm$ 7 (0 to 142.1; 8.4)*	4.4 $\pm$ 2 (0 to 57.2; 0)
sIL-2 receptor	86.6 $\pm$ 6 (49.8 to 168.8; 81.6)	83.9 $\pm$ 7 (37.9 to 158; 80.0)

* p < 0.01 compared with control group

uble receptor of interleukin-2 (sIL-2r) as an indicator of a hypersensitivity reaction against the implant. The IL-2 receptor mediates the activity of IL-2 and is exposed onto lymphocyte T and B membranes when they are stimulated by antigens. A small fraction of this protein, named sIL-2r, is released, and the serum concentration of this molecule is a marker of a specific immune response.

Patients and Methods

We collected serum samples from 35 patients with aseptic loosening of a hip prosthesis before revision. Details of the patients are given in Table I. We excluded patients with rheumatoid arthritis, allergy, autoimmune diseases and those having immunosuppressive therapy. Bone defects were evaluated according to the classification of Paprosky, Bradford and Younger.⁸ The extent of bone resorption was expressed as areas of osteolysis greater than 2 mm around both the acetabular and stem components.^{9,10} A control group consisted of 25 healthy subjects (9 male, 16 female) in the same age range as that of the patients (33 to 78 years).

Sample preparation. All testing procedures used in our laboratory are validated according to the quality assurance criteria in the EU accepted standard for the operation of testing laboratories (EN 45001 standard, 1989).¹¹ Samples were coded on arrival at the laboratory, so that the examiner did not know if the source of the sample was a patient or a healthy donor. We collected 7 ml of whole peripheral blood by venipuncture. This was centrifuged at 3000 rpm to separate off the serum which was then aliquoted and frozen at -70°C until use. We excluded haemolysed and lipaemic sera. Before testing, the serum aliquots were thawed and centrifuged at 3000 rpm for five minutes to remove any particulate residue.

Immunoenzymatic assay. For the quantitative determination of cytokines we used commercially available kits,

based on enzyme immunoassay methods in 96-well microplates (Table II). Each standard and sample were tested in duplicate. The absorbance of each sample was recorded using a microplate reader, after blank subtraction. A standard curve was constructed by plotting the absorbance of each standard v the corresponding standard concentration. The linear regression coefficient of the standard curve was calculated by the software: only R^2 values higher than 0.95 were accepted. The cytokine concentration of unknown samples was automatically calculated and expressed as pg/ml, or pmol/l for sIL-2r. The concentrations of cytokines lower than the detection limit were considered as 0 pg/ml. **Statistical analysis.** The results were expressed as the arithmetic mean $\pm$ SEM, the minimum to maximum range and the median of the cytokine concentration measured in each group. The cytokine levels of the groups were compared by the Mann-Whitney U test for unpaired data with a p value of less than 0.05 considered as statistically significant. We used the StatView 4.5 software for Macintosh (Abacus Concepts, Berkeley, California).

Results

The serum levels of TNF- α , IL-1 β , IL-6 and sIL-2r in patients with a failed hip arthroplasty did not differ from those found in the control group, whereas the GM-CSF concentration was significantly increased (p = 0.008) (Table III). The age of the patients, gender, diagnosis and time to failure did not correlate with the cytokine levels.

When we classified the failed arthroplasties on the basis of their metal component, either CrCoMo or TiAlV (Table IV), no significant differences were seen between the two groups. Patients with CrCoMo prostheses had a significant increase in their GM-CSF level (p = 0.03) compared with the control group. The TiAlV group also showed a significantly higher GM-CSF level (p = 0.01) than the control group; in these patients high levels of IL-1 β and IL-6 were found, but the

Table IV. Mean serum levels of cytokines (pg/ml $\pm$ SEM; range, median) and sIL-2r (pmol/l $\pm$ SEM; range, median) in patients according to the metal component of the implant and in the control group

	CrCoMo (n = 22)	TiAlV (n = 13)	Control group (n = 25)
IL-1 β	0.14 $\pm$ 0.09 (0 to 1.7; 0)	0.65 $\pm$ 0.4 (0 to 4.2; 0)	0.37 $\pm$ 0.1 (0 to 1.6; 0)
IL-6	19.5 $\pm$ 4 (0 to 69.5; 13.9)	45.3 $\pm$ 31 (0 to 386.6; 15.8)	35.3 $\pm$ 9 (3.0 to 177.1; 19.2)
TNF- α	0.1 $\pm$ 0.1 (0 to 2; 0)	0	0
GM-CSF	24.5 $\pm$ 8 (0 to 127.2; 2.7)*	37.6 $\pm$ 14 (0 to 142.1; 13.2)*	4.4 $\pm$ 2 (0 to 57.2; 0)
sIL-2 receptor	79.8 $\pm$ 6 (49.8 to 151.3; 80.8)	97.3 $\pm$ 11 (56.7 to 168.8; 87.9)	83.9 $\pm$ 7 (37.9 to 158; 80.0)

* p < 0.05 compared with control group

Table V. Mean levels of cytokines (pg/ml $\pm$ SEM; range, median) and sIL-2r (pmol/l $\pm$ SEM; range, median) in patients according to the presence of cement and in the control group

	Cemented (n = 14)	Uncemented (n = 21)	Control group (n = 25)
IL-1 β	0.86 $\pm$ 0.4 (0 to 4.2; 0)	0.05 $\pm$ 0.05 (0 to 1.1; 0)	0.37 $\pm$ 0.1 (0 to 1.6; 0)
IL-6	48.8 $\pm$ 29 (0 to 386.7; 15.9)	16 $\pm$ 3 (0 to 50.2; 13.1)	35.3 $\pm$ 9 (3.0 to 177.1; 19.2)
TNF- α	0	0.1 $\pm$ 0.1 (0 to 2; 0)	0
GM-CSF	50.4 $\pm$ 14 (0 to 142.1; 33.5)*†	17.7 $\pm$ 7 (0 to 131.5; 0)	4.4 $\pm$ 2 (0 to 57.2; 0)
sIL-2 receptor	87.5 $\pm$ 10 (49.8 to 168.8; 81.6)	85.9 $\pm$ 7 (52.5 to 165.2; 82.3)	83.9 $\pm$ 7 (37.9 to 158; 80.0)

* p < 0.05 compared with control group

† p < 0.05 compared with uncemented group

Table VI. Mean serum levels of cytokines (pg/ml $\pm$ SEM; range, median) and sIL-2r (pmol/l $\pm$ SEM; range, median) in patients grouped according to both the metal component and the presence of cement and in the control group

	CrCoMo		TiAlV		Control group (n = 25)
	Cemented (n = 9)	Uncemented (n = 13)	Cemented (n = 5)	Uncemented (n = 8)	
IL-1 β	0.2 $\pm$ 0.2 (0 to 1.7; 0)	0.09 $\pm$ 0.09 (0 to 1.1; 0)	2.6 $\pm$ 1.3 (0 to 4.2; 3.6)*†	0	0.37 $\pm$ 0.1 (0 to 1.6; 0)
IL-6	23.2 $\pm$ 9 (0 to 69.5; 15.6)	16.8 $\pm$ 4 (0 to 50.2; 13.1)	106.4 $\pm$ 93 (0 to 386.7; 19.4)	14.9 $\pm$ 6 (0 to 43.8; 12.8)	35.3 $\pm$ 9 (3.0 to 177.1; 19.2)
TNF- α	0	0.2 $\pm$ 0.2 (0 to 2; 0)	0	0	0
GM-CSF	52.3 $\pm$ 15 (0 to 128.2; 46.9)*	6.0 $\pm$ 4 (0 to 33.1; 0)	30.1 $\pm$ 19 (0 to 142.1; 8.3)*	33.7 $\pm$ 16 (0 to 131.5; 13.2)*	4.4 $\pm$ 2 (0 to 57.2; 0)
sIL-2 receptor	80.8 $\pm$ 11 (49.8 to 151.3; 68.4)	78.9 $\pm$ 7 (52.5 to 129.4; 82.3)	102.7 $\pm$ 23 (66 to 168.8; 87.9)	94.7 $\pm$ 13 (56.7 to 165.2; 90.0)	83.9 $\pm$ 7 (37.9 to 158; 80.0)

* p < 0.05 compared with the control group

† p < 0.05 compared with the patient group

Table VII. Mean serum levels of cytokines (pg/ml $\pm$ SEM; range, median) and sIL-2r (pmol/l $\pm$ SEM; range, median) in patients according to the amount of osteolysis around the acetabular cavity

	0; <2 mm (n = 7)	1 area (n = 10)	2 areas (n = 12)	3 areas (n = 4)
IL-1 β	0.98 $\pm$ 0.7 (0 to 4.2; 0)	0	0.32 $\pm$ 0.32 (0 to 3.6; 0)	0
IL-6	19.1 $\pm$ 9 (0 to 54.9; 7.9)	23.1 $\pm$ 10 (0 to 69.5; 14.7)	11.9 $\pm$ 4 (0 to 33.7; 5.5)	19.0 $\pm$ 7 (0.2 to 33.8; 21.1)
TNF- α	0	0	0.2 $\pm$ 0.2 (0 to 2; 0)	0
GM-CSF	40.6 $\pm$ 17 (0 to 128.2; 31.8)	30.3 $\pm$ 13 (0 to 87.9; 11.2)	40.8 $\pm$ 12 (0 to 142.1; 19.4)	4.2 $\pm$ 3 (0 to 18.9; 0.7)
sIL-2 receptor	100.2 $\pm$ 15 (58.7 to 151.3; 94.3)	100.4 $\pm$ 15 (51 to 165.2; 92.2)	68.7 $\pm$ 5 (49.8 to 99.2; 67.2)	98.3 $\pm$ 26 (57 to 168.8; 83.6)

differences were not significant. Patients with cemented implants had an increased GM-CSF level when compared with both the control group (p = 0.002) and the group with uncemented prostheses (p = 0.01) (Table V); IL-6 and IL-1 β levels were also moderately increased in these patients.

When the patients were grouped according to both the metal component and the presence of cement (Table VI), those with TiAlV cemented prostheses had higher levels of IL-1 β than both the control (p = 0.0001) and the other

groups (p = 0.003 v uncemented TiAlV, p = 0.01 v cemented CrCoMo, p = 0.001 v uncemented CrCoMo). High levels of GM-CSF were found in all groups except for that with CrCoMo uncemented implants which had levels similar to those of the control group.

When the patients were classified according to the areas of osteolysis around the cup and stem (Tables VII and VIII) the levels of IL-6 and TNF- α showed no difference whereas those of IL-1 β and GM-CSF tended to decrease as bone

Table VIII. Mean serum levels of cytokines (pg/ml $\pm$ SEM; range, median) and sIL-2r (pmol/l $\pm$ SEM; range, median) in patients classified according to the amount of osteolysis around the stem

	0; <2 mm (n = 14)	<3 areas (n = 11)	3 to 5 areas (n = 8)	>5 areas (n = 2)
IL-1 β	0.32 $\pm$ 0.32 (0 to 4.2; 0)	0.24 $\pm$ 0.24 (0 to 1.7; 0)	0.51 $\pm$ 0.51 (0 to 3.6; 0)	0
IL-6	15.8 $\pm$ 6 (0 to 69.5; 8.9)	20.9 $\pm$ 7 (0 to 54.9; 19.6)	14.2 $\pm$ 4 (0 to 33.7; 12.7)	19.4 $\pm$ 14 (4.8 to 33.8; 19.3)
TNF- α	0	0.28 $\pm$ 0.28 (0 to 2; 0)	0	0
GM-CSF	28.8 $\pm$ 11 (0 to 128.2; 3.6)	28.6 $\pm$ 15 (0 to 131.5; 16.2)	49.1 $\pm$ 19 (0 to 142.1; 33.1)	6.7 $\pm$ 6 (0 to 19.4; 0.7)
sIL-2 receptor	83.2 $\pm$ 10 (51 to 168.8; 86.6)	79.2 $\pm$ 13 (49.8 $\pm$ 151.3; 71.6)	104.1 $\pm$ 12 (63.1 to 165.2; 102.2)	65.2 $\pm$ 3 (62 to 68.4; 65.2)

Table IX. Mean serum levels of cytokines (pg/ml $\pm$ SEM; range, median) and sIL-2r (pmol/l $\pm$ SEM; range, median) in patients according to whether they were receiving NSAIDs

	NSAID (n = 25)	No NSAID (n = 10)
IL-1 β	0.33 $\pm$ 0.2 (0 to 3.6; 0)	0.32 $\pm$ 0.32 (0 to 4.2; 0)
IL-6	34.8 $\pm$ 18 (0 to 386.6; 15.6)	18.6 $\pm$ 5 (0 to 50.2; 14.1)
TNF- α	0.1 $\pm$ 0.1 (0 to 2; 0)	0
GM-CSF	40.4 $\pm$ 10 (0 to 142.1; 19.2)*	13 $\pm$ 7 (0 to 87.9; 0)
sIL-2 receptor	87.8 $\pm$ 7 (49.8 to 165.1; 80.8)	84 $\pm$ 10 (52.2 to 168.8; 84.9)

* $p < 0.05$ compared with no NSAID

resorption increased. Patients with the highest number of areas of osteolysis therefore had GM-CSF levels similar to those observed in healthy subjects. Six patients with areas of osteolysis <2 mm around the cup and stem had a high GM-CSF concentration (42.0 ± 20 pg/ml; median 24.2 pg/ml) compared with those with at least one osteolytic area around the implant (28.6 ± 8 pg/ml; median 9.4 pg/ml).

Grouping of the patients according to whether or not they were receiving non-steroidal anti-inflammatory drugs (NSAIDs) showed that the highest cytokine concentrations were in those who had had prolonged treatment with NSAIDs, with a statistically significant difference for GM-CSF ($p = 0.04$) (Table IX).

Discussion

In recent years the main causes of failure of hip prostheses have mostly been solved. The incidence of postoperative infections has been reduced and the design of implants and surgical techniques have been improved. Nevertheless, there is still a considerable incidence of inexplicable loosening of prostheses. The 'effect' of loosening is likely to be determined by different 'causes'. Among the biological causes, a crucial role has been attributed to chemical mediators, namely the cytokines, which regulate bone remodelling.¹²

Since loosening is associated with the radiological observation of osteolytic areas, it has been suggested that there is some mechanism which favours the release of bone-resorbing cytokines such as IL-1 α and IL-1 β , TNF- α , IL-6, and CSFs. Possible mechanisms are: 1) an inflammatory action of wear particles which induces monocytes and macrophages surrounding the implant to release these cytokines; or 2) a sensitising activity of the metals released from the prosthesis producing a type-IV hypersensitivity reaction which then induces the release of cytokines.¹³

The first hypothesis is supported by many studies on

tissue samples^{6,14} and in vitro cultured cells,^{5,7} which have shown the presence of cytokines involved in bone resorption at the bone-prosthesis interface and therefore suggest a role for some mediators such as IL-1 α and IL-1 β , TNF- α and IL-6 in the pathogenesis of aseptic loosening. There have been some studies on the possibility of a specific immune reaction against the implant,¹⁵⁻¹⁸ but with no clear evidence of such a reaction in the failure of hip arthroplasties.

Our findings have shown that levels of TNF- α and IL-6 did not significantly differ in patients with a loose prosthesis. Those with a TiAlV cemented prosthesis showed a significant increase in levels of IL-1 β and also had the highest levels of IL-6. These data agree with the results of other authors, who showed that TiAlV^{7,18,19} and polymethylmethacrylate particles²⁰ in vitro induced the release of inflammatory cytokines in monocyte/macrophages.

The highest cytokine concentrations were observed in the group of patients treated with NSAIDs. About 70% of our patients were so treated in the last months before revision demonstrating that the clinical symptoms which necessitate drug treatment are probably related to an increase in the serum level of inflammatory cytokines. The large variations found in the serum concentration of the inflammatory cytokines may be explained by the different ability of NSAIDs to inhibit the production of these molecules,²¹ so that in some patients drug treatment was more effective. It was not possible to verify this hypothesis since our patients had been treated with different types of drug at varying concentrations and for different periods of time. It has been suggested that the assay of the inflammatory cytokines may be used to evaluate the efficacy of therapeutic protocols for NSAIDs in patients with hip arthroplasty. In vitro experiments have shown that selected drugs can reduce the bone resorption induced by IL-1 β and TNF- α released from the pseudomembranes surrounding the implant; such drugs could be used for the prevention of

the resorption occurring in failure of a prosthesis.²²

In patients with a loosened hip prosthesis significantly high levels of GM-CSF were found which seemed to be related to the presence or absence of cement rather than to the metal component. All groups had significantly higher levels of GM-CSF except for patients with an uncemented CrCoMo prosthesis. GM-CSF favours osteoclast differentiation in bone resorption. Other authors have described the potential role for this molecule in cellular recruitment in aseptic loosening of implants, and have shown in vitro that exposure of macrophages to polymethylmethacrylate particles leads to the release of TNF, which in turn stimulates the osteoblasts to release GM-CSF, IL-6 and PGE₂.²³ Although the examined cytokines are involved in bone resorption, we did not find any correlation between the extent of osteolysis and the cytokine concentration. Surprisingly, the highest GM-CSF levels were found when bone resorption was absent. When a massive osteolysis was observed, the levels of GM-CSF tended to decrease to the values of healthy individuals.

Our findings suggest that the increase in the GM-CSF level could be an early event in the pathogenesis of loosening, because it seems to rise before osteolysis becomes evident. The concentration of sIL-2r was not changed in patients with aseptic loosening which indicates that a specific immune response is not likely to be involved in the

increase of the cytokines tested. The absence of such a response is supported also by previous experiences which have shown that patients with aseptic loosening, mainly with CrCoMo prostheses, have a reduced number and function of lymphocytes.²⁴ Moreover, in vitro experiments have shown that non-cytotoxic concentrations of chromium are able to inhibit significantly the release of sIL-2r in mononucleated cells stimulated with phytohaemagglutinin.²⁵

Conclusions. Aseptic loosening of an implant is probably due to the inflammatory activity of wear particles which induce the release of bone-resorbing cytokines from the monocytes and macrophages surrounding the implant. The serum levels of inflammatory cytokines involved in bone resorption are increased in patients with TiAlV prostheses or cemented implants. The significant steady increase in GM-CSF levels indicates the role for this cytokine in the pathogenesis of loosening and suggests that it could be an early marker of implant failure. The assay of cytokines could be used as a method of monitoring after implantation.

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