

Pro-inflammatory and anti-inflammatory circulating cytokines and periprosthetic osteolysis

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We investigated the circulating levels of the main cytokines involved in bone resorption (IL-1 β , IL-6, TNF- α), prostaglandins (PGE₂) and metalloproteases (MMP-1), as possible early markers of osteolysis, in the serum of eight patients with periprosthetic osteolysis and ten patients without osteolysis. All had received a cementless hip prosthesis (ABG-1). We also assessed the serum levels of IL-11 and TGF- β anti-inflammatory cytokines exerting protective effect on bone resorption. The mean serum levels of IL-1 β , IL-6, TNF- α , TGF- β , MMP-1, and PGE₂ in patients with periprosthetic osteolysis did not differ significantly from those of patients without osteolysis or from those of normal controls. IL-11 serum levels were not detectable at all in any of the patients, while they were detected within normal reference values in the control subjects (significant inverse correlation).

We believe that circulating cytokines cannot be regarded as markers of osteolysis, a condition characterised by a local inflammation without systemic signs of inflammation. On the contrary, the undetectable levels of IL-11 in implanted patients could provide evidence for a lack of balance between pro- and anti-inflammatory cytokines in these patients.

J Bone Joint Surg [Br] 2003;85-B:1202-6.

Received 6 September 2001; Accepted after revision 12 June 2003

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doi:10.1302/0301-620X.85B8.12799 \$2.00

Periprosthetic osteolysis is one of the most important complications of total hip arthroplasty.¹ Although many studies have contributed to the understanding of the biological processes underlying osteolysis, it remains only partially understood.¹⁻³

Evidence has been provided that cytokines, enzymes and other mediators which are released by activated cells in response to an inflammatory stimulus play a fundamental role in bone remodelling by stimulating bone resorption and enzymatic digestion of the collagenous matrix of bone.² The crucial role played by these chemical mediators and their importance in the resorption of periprosthetic bone have been highlighted by different authors. High levels of interleukin (IL)-1 β , tumour necrosis factor (TNF)- α and IL-6 have been shown in many *in vivo* studies in cultured membranes surrounding failed prostheses.³⁻⁵ Macrophages and fibroblasts at the prosthesis-bone interface when activated by phagocytosed wear debris release significant amounts of these bone-resorbing cytokines.⁶⁻¹⁰ The supernatants from cultures of synovial cell wear debris stimulate osteoclastic bone resorption.^{6,11,12} Recently increased levels of these cytokines have been demonstrated in the synovial fluid of patients with a well-functioning loose prosthesis.¹³ IL-1 α and 1 β are highly inflammatory cytokines produced by macrophages which are responsible for bone resorption by stimulating the production of prostaglandin E₂ (PGE₂) and collagenase or matrix metalloproteases (MMPs) by synoviocytes and chondrocytes^{14,15} and by inducing the differentiation of osteoclasts.^{16,17} IL-6 is one of the essential factors in the acute-phase systemic reaction to inflammatory stimuli or tissue injury.¹⁸ This cytokine stimulates bone resorption by recruiting mature osteoclasts and by activating them through an autocrine mechanism.^{19,20} TNF- α is regarded as the major cytokine in the osteolytic process^{12,13} in that it stimulates bone resorption by enhancing the recruitment and the activation of osteoclasts.^{21,22} Collagenases are produced by macrophages and fibroblasts which have been activated by IL-1 and TNF- α . They cause osteolysis by degrading the connective-tissue elements in the connective tissues.^{23,24}

Many authors have searched for serum markers of osteolysis but the relevance of serum cytokines is controversial.^{25,26} We have investigated the circulating levels of the main cytokines involved in bone resorption (IL-1 β , IL-6,

Table I. Mean (\pm SD) values of serum cytokine levels (pg/ml) in the patients with and without osteolysis and in the control group

Group	Mean age (yrs)	IL-1 β	IL-6	IL-11	TNF- α	TGF- β	PGE ₂	MMP-1
1 (with osteolysis) (n = 8)	61.8	2.15 \pm 1.37	2.86 \pm 1.95	0.0	4.32 \pm 5.2	23175 \pm 8773.58	1330 \pm 1097.44	3.69 \pm 1.75
2 (without osteolysis) (n = 16)	66.3	2.26 \pm 0.89	4.58 \pm 4.02	1.22 \pm 2.57	3.84 \pm 1.13	21120 \pm 13657	2021 \pm 1046	4.10 \pm 1.44
3 (normal controls) (n = 17)	62.7	2.02 \pm 1.25	3.88 \pm 2.72	18.69 \pm 10.55	3.42 \pm 2.12	23615 \pm 10681	2893 \pm 782	3.31 \pm 1.7
p value								
Group 1 v group 3		0.79	0.36	0.001*	0.95	0.88	0.005*	0.52
Group 2 v group 3		0.94	0.36	0.001*	0.63	0.45	0.02*	0.15
Group 1 v group 2		0.96	0.24	0.27	0.62	0.50	0.14	0.50
Implanted patients (18) v normal controls (17)		0.83	0.94	0.001*	0.79	0.69	0.002*	0.18

*inverse correlation

TNF- α) and the circulating levels of MMP-1 and PGE₂ as possible early markers of osteolysis in the serum of patients with a cementless hip prosthesis (ABG-I; Stryker Howmedica Osteonics, Mahwah, New Jersey) with and without osteolysis. We also determined the serum levels of IL-11 and of transforming growth factor- β (TGF- β) both of which are considered to be anti-inflammatory cytokines which exert protective effects on bone resorption. IL-11 is a member of the IL-6 superfamily which is produced by cells from the mesenchymal lineage as bone-marrow stromal cells and osteoblasts, but not by T lymphocytes and monocytes.²⁷ This cytokine has a potent anti-inflammatory activity in animal models, both *in vitro* and *in vivo*. It suppresses the synthesis of the pro-inflammatory cytokines TNF- α and IL- β and the production of nitric oxide in activated macrophages.²⁸ It also stimulates the synthesis of the tissue-inhibitor of MMP-1 (TIMP) by fibroblasts of the rheumatoid synovial membrane and down-regulates the production of MMP-1 and 3 (collagenase and stromelysin) by the same cells.²⁹ IL-11 balances IL-1 β extracellular or matrix breakdown and bone resorption and may have a protective role against the destruction of joint connective tissues by enzymatic digestion of collagenase.³⁰ TGF- β stimulates bone formation *in vivo* by facilitating the formation of extracellular bone matrix by osteoblasts and inhibiting the function of osteoclasts.³¹ Given the anti-inflammatory and bone-protective properties of both of these cytokines, we measured their serum levels to investigate their possible protective role in patients with osteolysis.

Patients and Methods

We studied 18 patients with severe osteoarthritis who had a cementless ABG (Anatomic Benoist-Girard) type-I hip prosthesis (Stryker Howmedica Osteonics) implanted through a posterolateral approach by the same surgeon (CG). The ABG prosthesis is made of TiA16V4 titanium alloy. The acetabular component is a hemispherical, hydroxyapatite (HA) fully-coated cup made of titanium

alloy, which is designed to be fixed by two screws. The socket is made of ultra-high molecular weight polyethylene (UHMWPE). The diameter of the cobalt-chrome (Co-Cr) head is 28 mm. The anatomic femoral stem is made of titanium alloy which is HA-coated in its proximal third.

All the participating patients had been chosen at random from those who had been clinically examined over a period of two months. After implantation, our patients had a clinical and radiological examination at three months, one year and then every two years. They were subdivided into two groups. Group 1 consisted of eight patients (four men and four women) with a mean age of 61.8 years (50 to 72) in whom the mean time from implantation was 78 months (48 to 120). They had radiological evidence of osteolysis and a polyethylene wear rate of 0.21 mm/year. Femoral osteolysis was always seen in Gruen zones 1A and 7A without any diaphyseal involvement. This was explained by the HA coating of only the proximal third of the stem. HA coatings have been shown to achieve a strong bond with the living bone, without any fibrous interface.³² Acetabular osteolysis was localised within Charnley zones 1, 2 or 3, but particularly at zone 2. In group 2 there were ten patients (eight men and two women) with a mean age of 66.3 years (47 to 82). The mean time from implantation was 58.8 months (12 to 120). They had minimal signs of osteolysis and a rate of polyethylene wear of less than 0.12 mm/year (0.05 mm/year). None of the patients had autoimmune allergic infectious disease. Group 3 was a control group consisting of 17 normal, healthy subjects, seven women and ten men, with a mean age of 57.2 years (27 to 60), who were blood donors from the Blood Transfusion Department.

Evaluation of osteolysis. The extent of bone resorption was measured on the anteroposterior and the lateral radiographs. While the degree of osteolysis around the stem can be precisely defined, that around the acetabular component is more difficult to measure as small osteolytic areas may be masked by the cup. A more precise estimation of bone resorption around the acetabular component can be made using oblique projections.³³ Computerised scanning images

are disturbed by the metallic cup. Thus it remains difficult to quantify with accuracy the degree of acetabular osteolysis.

Measurement of polyethylene wear. The wear rate of polyethylene was established by measuring the distance between the centres of the two circles representing the acetabulum and the femoral head on the anteroposterior radiographs. This was obtained using a magnifying graduated rule to correct for the radiographic enlargement. The mean wear rate for each patient was obtained by dividing the total wear by the number of years after implantation.

Preparation of serum samples. We collected samples of whole peripheral blood by venipuncture. They were centrifuged at 3000 rpm to separate the serum which was frozen at -20°C until use.

Determination of cytokines. The serum levels of IL-1 β , IL-6, IL-11, TNF- α and TGF- β were measured by a sandwich quantitative enzyme immunoassay technique (R&D Systems, Abingdon, UK). The standard curves are respectively: 0 to 250 pg/ml for IL-1 β ; 0 to 10 pg/ml for IL-6; 0 to 2000 pg/ml for IL-11; 0 to 32 pg/ml for TNF- α and 0 to 2000 pg/ml for TGF- β . The normal values, as determined by the manufacturer, were less than 3.9 pg/ml for IL-1 β , 10.1 pg/ml for IL-6, less than 31.2 pg/ml for IL-11 and lower than 3.62 pg/ml for TNF- α and 63.9 ng/ml for TGF- β . The serum levels of MMP-1 were evaluated by a sandwich enzyme immunoassay technique (Oncogene, Cambridge, Massachusetts). The standard curve was 0 to 3.6 ng/ml and the mean serum level measured by the manufacturer in 36 healthy volunteers was 13 ng/ml. Serum levels of PGE₂ were measured by a competitive binding enzyme immunoassay (R&D Systems). The standard curve is 0 to 5000 pg/ml and no normal reference value is given by the manufacturer.

Statistical analysis. The data were analysed using the Mann-Whitney non-parametric U-test, the Wilcoxon test and calculation of the confidence intervals for the ROC area. A p value <0.05 was considered to be significant. All values are given as the mean \pm SD.

Results

The mean serum levels of IL-1 β , IL-6, TNF- α , TGF- β , PGE₂ and MMP-1 in the patients with periprosthetic osteolysis (group 1) did not differ from those in the patients without osteolysis (group 2) and from the normal control subjects (group 3) (Table I). No correlation was observed between age, gender, length of time after implantation, degree of osteolysis and the serum levels of cytokines. Seven of the 18 patients who had an implant had an increase in serum collagenase. But, when combined, both groups of implanted patients showed no significant difference ($p = 0.18$) *versus* normal controls. It was surprising to find that the anti-inflammatory cytokine IL-11 was not detectable at all in the patients with periprosthetic osteolysis and only at very low levels in those without osteolysis. There was a sig-

nificant difference between the patients and the control groups (Table I).

Discussion

The regulation of the production of bone-resorbing cytokine by macrophages and fibroblasts activated by wear debris, located in the membranous tissue at the interface between the implant and bone, is not completely understood. Undoubtedly, individual factors of the host modulate the inflammatory response to wear particles. These host-specific responses are variable depending on age, gender and the level of immune tolerance of immunocompetent cells.

It may be possible to prevent or delay the onset of the inflammatory reaction to wear debris by diagnosing the activation of cells involved in the development of the granulomatous tissue before clinical signs have developed. For this reason many researchers have looked for a serum marker of osteolysis which precedes the onset of its clinical and/or radiological signs. Some have found significantly higher levels of colony-stimulating factors (GM-CSF) in patients with cemented implants but not of IL-6 and TNF- α . They did not find any correlation between the extent of osteolysis and the concentration of cytokine, while the GM-CSF levels seemed to be related only to the presence or absence of cement.²⁵ Others have detected increased levels of IL-6 which correlated with the severity of osteolysis, but did not find significantly increased circulating levels of IL-1 β , IL-8 or TNF- α .²⁶

We did not demonstrate a significant relationship between the circulating levels of the pro-inflammatory cytokines IL-1 β , IL-6, TNF- α and osteolysis. The serum levels of MMPs were increased but not significantly. IL-1 β , IL-6, TNF- α and PGE₂ are all produced in large amounts at the site of activity of disease, and it seems likely that their activity is restricted to the microenvironment around the implant and does not reach the systemic vascular bed. Indeed, most of the patients did not have either clinical or laboratory signs of systemic inflammation.

We observed a statistically significant inverse relationship between PGE₂ serum levels in our patients with implants compared with the control group ($p < 0.005$). PGE₂ is known to be a potent stimulator of bone resorption and has been found to be elevated in peri-implant tissues. It is a product of the metabolism of arachidonic acid, is secreted at inflammatory sites and is metabolised and excreted from the blood in urine. This may be why it is sequestered in the periprosthetic tissues and its circulating serum levels in patients with an artificial implant are lower than those in control subjects. We showed undetectable levels of IL-11 in the sera in the two study groups, which agrees with the findings of other authors,^{34,35} but found a statistically significant inverse relationship between the levels of IL-11 in the serum of implanted patients and those in the normal healthy control group. The levels were

almost undetectable in the patients but were normal in the control subjects. Only limited data are available on the circulating levels of IL-11 in various inflammatory rheumatic diseases. Some authors have found detectable levels of this cytokine in the serum of patients with rheumatoid arthritis,²⁹ but others have failed to detect it in the serum of patients with either rheumatoid arthritis or osteoarthritis.^{34,35} However, none of these studies has determined the level of IL-11 in normal healthy subjects and there are no data on the serum levels of this cytokine in patients affected with periprosthetic osteolysis. This could be because of injury to the bone marrow during implantation or the existence of circulating antagonists downregulating its synthesis.

Pro-inflammatory and anti-inflammatory cytokines act together in an ordered temporal and spatial sequence and are often referred to as a network, a term which emphasises not just the interactive but also the dynamic nature of their expression and function. The integration of the activities of the individual cytokines within the network provides a system by which biological processes can be regulated through the balance of inhibitory and stimulatory effects.³⁶ Cytokines form an integral part of the normal immune response in which numerous control mechanisms are in place to prevent them from becoming destructive. Thus it is not surprising, given the dynamic nature of the cytokine network, that different cytokine patterns can prevail at any time in the course of a disease and different results can be obtained in different study groups.

Periprosthetic osteolysis is a pathological condition characterised by the *in situ* expression of bone-resorbing and inflammatory cytokines secreted by activated cells of mesenchymal tissues around the implant. Systemic signs of inflammation are very rarely found in these patients, thus our finding that serum cytokine levels are not increased in patients with osteolysis could be consistent with this situation. Considering the small number of patients in our study groups, we could not draw definite conclusions about the usefulness of cytokines as serum markers of osteolysis. In fact, even though there is not a statistically significant difference between osteolytic and non-osteolytic patients, the analysis of the ROC area and the assessment of the p values by the Wilcoxon test do not allow the role of these cytokines to be ruled out.

We thank Dr Irene Schettino-Nobile and Dr Claudio Angeletti for their helpful collaboration in statistical analysis.

One or more of the authors have received or will receive benefits for personal or professional use from a commercial party related directly or indirectly to the subject of this article.

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