Interleukin 1 α and β production by cells isolated from membranes around aseptically loose total joint replacements

Carole I Westacott, Graham Taylor, Roger Atkins, Chris Elson

Abstract

Aseptic loosening of joint prostheses is accompanied by local osteolysis. To determine whether local production of interleukin 1 might contribute to such lysis, the number of interleukin 1 secreting cells in the pseudo-synovial membrane surrounding prostheses was measured. Interleukin 1 α and β secreting cells were identified by ELISPOT, a sensitive cytokine secreting assay. The proportion of interleukin 1 β secreting cells in pseudo-synovial membrane was comparable with the proportion occurring in the synovium of patients with rheumatoid arthritis and higher than that in normal subjects and patients with osteoarthritis. The proportion of interleukin 1 α producing cells was higher in pseudo-synovial membrane than in diseased synovium. Overall, higher numbers of interleukin 1 β than interleukin 1 α secreting cells were detected. A correlation was observed between the number of cells in pseudosynovial membrane producing interleukin 1 β and those producing interleukin 1 α . When divided into area of origin, tissue samples from the femoral area contained a higher proportion of interleukin 1 β producing cells than tissue in the acetabular or capsular regions, though due to variance within each group this difference did not reach significance.

Aseptic loosening, characterised by prosthetic migration and progressive radiographic osteolysis at the bone-cement interface,¹ is the major cause of failure of total hip joint replacements. In the early stages, the process is often asymptomatic, though as lysis progresses the prosthesis becomes mechanically unstable and the patient experiences pain. In later stages the localised lysis around the prosthesis may be sufficiently severe to compromise bone stock and make revision hip replacement technically difficult.

The cause of aseptic loosening remains controversial. It was thought to be a mechanical phenomenon² and changes in prosthetic design have been shown to alter the rate of loosening.³ There is a membrane, however, which is invariably present within the zone of lysis which is histologically and histochemically similar to synovium⁴ and is rich in macrophages. This membrane has been called pseudosynovial membrane.⁴ When cultured, cells from pseudosynovial membrane resemble synoviocytes and have been shown to produce collagenase and prostaglandin E₂.⁴ Pseudosynovial membrane cell supernatants also contain bone resorbing

activity as assessed by the release of calcium-45 ions (⁴⁵Ca²⁺) from mouse calvarial explants.⁵

Prostaglandin E₂ is known to be a potent bone resorbing agent and it has been suggested that it plays an active part in local osteolysis.⁴ Other bone resorbing agents have also been identified and these include products from stimulated macrophages such as interleukin 1⁶ and tumour necrosis factor α .⁷ In addition, these molecules have been shown to induce the proliferation of synoviocytes^{8,9} and enhance synoviocyte production of prostaglandin E₂ and proteases,^{10,11} thereby potentially exacerbating bone destruction. Although interleukin 1 like molecules have been detected in supernatants from cultured pseudosynovial membrane cells and tissue fragments using the lymphocyte activating factor assay,^{12,13} this assay does not differentiate between interleukin 1 α and interleukin 1 β and also detects interleukin 2 and interleukin 6.^{14,15} Thus it remains to be established that interleukin 1 is actively secreted by pseudosynovial membrane and whether it contributes to the observed osteolysis.

The primary aim of this work was therefore to show that cells isolated from pseudosynovial membrane actively secrete interleukin 1 and to determine the proportion secreting interleukin 1 α and β . The secondary objective was to determine whether the amount of interleukin 1 produced by pseudosynovial membrane was the same as that produced by tissues associated with local osteolysis. We compared the proportion of interleukin 1 α and β secreting cells in pseudosynovial membrane with the proportion found in synovium from patients with rheumatoid arthritis (RA) and synovium from non-arthritic subjects and patients with osteoarthritis (OA).

Patients and methods

TISSUE STUDIED

Tissue samples were obtained at the time of surgery from 10 patients with OA (six women, four men, mean age 69 years, range 57-86) undergoing revision operations for clinical failure after total hip replacement. All patients had radiographic and clinical evidence of aseptic prosthetic loosening. Membranous material was obtained from the bone-cement interface at sites along the femur adjacent to areas with radiographic evidence of bone destruction. In some patients samples of pseudocapsule or pseudosynovial membrane from the acetabular bone-cement interface were also obtained. To ascertain that loosening was not due to infection, samples of tissue were subjected to histological and bacteriological examination. Serum samples

Department of Pathology, University of Bristol Medical School, Bristol, United Kingdom
C I Westacott
C Elson

Department of Orthopaedic Surgery, University of Bristol Medical School, Bristol, United Kingdom
G Taylor
R Atkins

Correspondence to:
Dr C J Elson,
Department of Pathology,
University of Bristol
Medical School,
University of Bristol,
Bristol BS8 1TD,
United Kingdom.

Accepted for publication
13 September 1991

obtained before the operation were assayed for C reactive protein and the plasma viscosity was measured.

To determine whether cytokine production occurred only adjacent to prostheses in the presence of active osteolysis, tissue was obtained from a patient with OA (a 47 year old woman) undergoing revision of a malpositional acetabular component of a total hip prosthesis for recurrent dislocation. In this patient there was no clinical nor radiographic evidence of loosening. During the operation the prosthesis was found to be soundly fixed. Material was also obtained from within the medullary canal in two patients (one woman, one man, aged 29 and 40 years respectively) at the time of removal of the intra-medullary nails which had been inserted at least 18 months previously for the treatment of femoral shaft fracture. In these patients the fracture was soundly united and there was no evidence of osteolysis.

For comparison, samples of diseased synovium were obtained from 26 patients undergoing total joint arthroplasty. Of these, 17 patients (11 women, six men, mean age 70 years, range 26–86) presented with OA and nine (all women, mean age 60 years, range 43–77) with RA. Samples of synovium for use as controls were also obtained from three non-arthritic subjects undergoing diagnostic arthroscopy of the knee (one woman, two men, mean age 51 years, range 37–64).

PREPARATION OF CELL SUSPENSIONS

All tissue samples were washed in sterile phosphate buffered saline (PBS, 0.15 M, pH 7.4), cut into small pieces (3 mm²) and the fragments were incubated in PBS containing 5 mg/ml collagenase 1A (Sigma) for two hours at 37°C with shaking. Dissociated cells were harvested and washed twice in PBS before resuspension in RPMI 1640 tissue culture medium (Gibco; supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, 25 mM HEPES, 5% heat inactivated human serum). Cell suspensions were filtered through sterile metal gauze (pore size 40 µm) to remove cell debris. Cells were counted and the viability checked using trypan blue dye exclusion.

CYTOKINE SECRETING CELL ASSAY

The assay used was a modification of that developed by Hutchings *et al*¹⁶ and used antibodies to both capture and visualise secreted cytokines, thereby enabling cytokine secreting cells to be identified. Briefly, 96 well plates

were coated (overnight, 4°C) with mouse monoclonal antihuman interleukin 1α or interleukin 1β (10 µg/ml; RU 280–2–13 and RU 609 respectively). The plates were washed and blocked with 1% w/v PBS/BSA (bovine serum albumin in phosphate buffered saline) and the test cells (1×10³–1×10⁴/well) in RPMI 1640 complete medium were incubated overnight (14 hours, 37°C, 5% carbon dioxide) alone or in the presence of 200 ng/ml lipopolysaccharide from *Escherichia coli* 055 B5 (Sigma). Cells were removed by washing three times with cold PBS containing 0.5% (w/v) Tween 20 (PBS/T).

Polyclonal sheep antihuman interleukin 1α or β diluted 1/500 and 1/1500 respectively in PBS/BSA containing 0.5% Tween 20 were added and plates incubated for one and a half hours at 37°C. After washing three times with PBS/T, alkaline phosphatase conjugated anti-sheep IgG (Sigma) was added and the plates were incubated for a further one and a half hours at 37°C. The enzyme substrate, 5-bromo-4-chloro-3-indoyl phosphate (Sigma) containing 0.6% agarose was added after further washing to remove excess conjugate. The agarose was allowed to solidify and the plates were incubated at 37°C overnight (14 hours) to allow the development of the discrete spots of insoluble blue colour which were counted under an inverted microscope. Results were the mean number of spots in triplicate wells expressed as a percentage of the number of cells (1000) added to each well.

STATISTICAL ANALYSIS

The significance in the difference in the proportion of cytokine secreting cells in tissue samples from different groups of subjects was determined using the Mann Whitney U test. Correlations between the proportion of cells producing interleukin 1β and those producing interleukin 1α were examined by Spearman's rank correlation coefficient.

Results

Histological investigation showed the presence of foreign body type giant cells in association with polyethylene and cement particles in five of the six pseudosynovial membrane samples examined; evidence of inflammatory activity was absent. Samples of pseudosynovial membrane showed no bacterial growth after anaerobic and aerobic culture for 48 hours. C reactive protein measurements in serum samples obtained before the operation were within the normal range (<10 mg/l) as were the plasma viscosities (1.5–1.72 mP) before the operation.

Cells secreting interleukin 1β and α were determined in various tissue samples using the ELISPOT assay and these results are given in the table and figs 1 and 2. Cells producing interleukin 1β were detected in 73% (38/52) of the synovial and pseudosynovial membrane tissue samples examined, including 1/3 (33%) samples from non-arthritic subjects (table). Figure 1 shows that the proportion of cells producing interleukin 1β was significantly (p<0.05) higher in samples of synovium from

Numbers of interleukin 1α and β producing cells isolated from synovium and pseudosynovial membrane

Source of tissue	Number (%) of samples containing interleukin 1β producing cells*	Number (%) of samples containing interleukin 1α producing cells*
Non-arthritic subjects (n=3)	1/3 (33)	0/1
Patients with OA (n=17)	13/17 (76)	3/10 (30)
Patients with RA (n=9)	7/10 (70)	2/9 (22)
Revision patients (n=10)	17/22 (77)	11/17 (65)

RA=rheumatoid arthritis; OA=osteoarthritis.
*Counted in triplicate at 1000 cells/test wall.

Figure 1 Proportion of cells producing interleukin 1 β (IL-1 β) in samples of synovium and pseudosynovial membrane. Mean (SD) values were 0.006 (0.01)% in non-arthritic synovium, 0.18 (0.17)% in osteoarthritic synovium, 1.67 (2.33)% in rheumatoid arthritis synovium, and 1.4 (2.0)% in pseudosynovial membrane.

Figure 2 Proportion of cells producing interleukin 1 α (IL-1 α) in samples of synovium and pseudosynovial membrane. Mean (SD) values were 0.03 (0.07)% in osteoarthritic synovium, 0.11 (0.24)% in rheumatoid synovium, 0.46 (0.95)% in pseudosynovial membrane.

patients with RA (1.67 (2.33)) and pseudosynovial membrane (1.4 (2.0)) than in samples of tissue from patients with OA (0.18 (0.17)) and non-arthritic subjects (0.006 (0.01)). The proportion of interleukin 1 β producing cells in pseudosynovial membrane and diseased synovium was significantly ($p < 0.05$) higher than that in tissue from non-arthritic subjects. Figure 3 shows that samples of pseudosynovial membrane from different patients and from different regions in the same patient were found to contain various proportions of interleukin 1 β producing cells. When divided into area of origin, tissues from sites around the femur were found to contain higher proportions of interleukin 1 β producing cells (2.09 (2.26)) than capsular (1.4 (2.4)) or acetabular material (0.9 (1.8)), though due to the variance within each group this difference did not reach significance.

Figure 3 Proportion of cells producing interleukin 1 β (IL-1 β) in samples of pseudosynovial membrane divided into area of tissue origin. Mean (SD) values were 0.9 (1.8)% in tissue from around the acetabulum, 1.4 (2.4)% in tissue from the capsular area, and 2.09 (2.26)% in tissue from the femoral region.

Interleukin 1 α producing cells were detected in 16 of 37 (43%) tissue samples, 11 (69%) of which were samples of pseudosynovial membrane (table). The proportion of interleukin 1 α producing cells was significantly ($p < 0.01$) higher in pseudosynovial membrane (mean (SD) 0.46 (0.95)) than in other tissues examined (RA 0.11 (0.24); OA 0.03 (0.07)). A weak but significant correlation was observed between the numbers of cells in pseudosynovial membrane producing interleukin 1 β and those producing interleukin 1 α ($r = 0.54$, $n = 17$, $p = 0.025$).

To determine whether the introduction of foreign material *per se* stimulated cytokine production, the presence of interleukin 1 secreting cells was determined in samples of tissue from around a well fixed prosthesis and in tissue from within intermedullary nails used to repair fractures. In none of these subjects was there evidence of bone resorption. Cells actively secreting interleukin 1 were found in both types of membrane used as controls. Capsule-like material from around the non-loosened prosthesis contained 0.5% interleukin 1 β producing cells. In one sample from within one intermedullary nail the proportion of interleukin 1 β and interleukin 1 α producing cells was 0.1 and 0% respectively, whereas in the other it was 0.2 and 0.3% respectively.

Discussion

These results extend previous work on the part played by cytokines in aseptic loosening by showing unequivocally the presence of interleukin 1 α and interleukin 1 β secreting cells in pseudosynovial membrane. A comparative investigation of the proportion of interleukin 1 β secreting cells in pseudosynovial membrane and in diseased and normal synovium showed that the proportion of cells secreting interleukin 1 β

was similar to that found in samples of synovium from patients with rheumatoid arthritis and higher than that in samples of normal synovium or synovium from patients with osteoarthritis. Moreover, the proportion of interleukin 1 α producing cells was significantly higher in pseudosynovial membrane than in synovium from patients with either OA or RA. As bone erosion is a feature of RA, but not OA, and is associated with the local production of cytokines in RA,^{6,17} these results are consistent with interleukin 1 production by pseudosynovial membrane contributing to local osteolysis.

These results led us to expect that the proportion of cytokine secreting cells in membranes adjacent to radiographic evidence of bone destruction would be higher than in tissues from other areas. The results showed that the highest proportion of interleukin 1 secreting cells was observed in pseudosynovial membrane from around the femur (i.e. the membrane closest to the site of most aggressive bone destruction). A relatively high proportion of interleukin 1 producing cells, however, was also found in pseudosynovial membrane from the acetabulum and pseudocapsule samples. In these patients both acetabular and femoral components of the prosthesis were loosened, as judged by radiographic evidence and intra-operative mechanical testing. Thus a high proportion of interleukin 1 secreting cells was again associated with bone destruction.

Two major questions are posed by these data. The first is what stimulates cytokine secretion around the prostheses. One explanation is that it is due to microorganisms. Against this possibility, serum C reactive protein levels and plasma viscosities before the operation were normal and bacteriological and histological investigations of tissue samples showed no evidence of infection. A second possibility is that the underlying disease itself may be responsible for cytokine production. Synovium samples from patients with osteoarthritis undergoing arthroplasty, however, contained a smaller proportion of interleukin 1 producing cells than pseudosynovial membrane from patients with osteoarthritis undergoing revision operation for failed total hip replacement. In addition, only small amounts of interleukin 1 β have been detected in synovial fluids from patients with osteoarthritis.¹⁸ These findings, together with the fact that synovium from non-arthritic subjects contains relatively few interleukin 1 producing cells, a comparison not made in previous studies, also shows it to be unlikely that the techniques used to isolate membrane cells were responsible for stimulating cytokine production. Finally, the introduction of foreign material, particularly in a particulate physical state, may trigger cytokine secretion by phagocytic cells. In this study interleukin 1 secreting cells were found in pseudosynovial membrane together with polyethylene wear debris and cement particles, suggesting that these may be the stimulus for cytokine production. This suggestion is supported by evidence from animal experiments which showed that polyethylene debris¹⁹ and polymethylmethacrylate particles,²⁰ but not the bulk material, induce

bone resorption. Such debris presumably forms as a result of mechanical action. The trigger for cytokine production is therefore likely to consist of the interaction of a complex set of mechanical and biochemical events.

The second question is whether cytokine production alone is sufficient to cause osteolysis. As already discussed, an association exists between increasing levels of interleukin 1 production and bone destruction. In this respect it may be significant that tissue from within intermedullary nails and from around a non-loosened prosthesis were found to contain a relatively low proportion of interleukin 1 secreting cells. Whether tissue damage ensues, however, may depend on the interaction between interleukin 1 and other cytokines as well as between interleukin 1 and other potentially destructive molecules such as proteases and prostaglandins. Supernatants from pseudosynovial membrane fragments have been shown to contain bone resorbing activity.⁵ It will be interesting to determine the contribution of interleukin 1 β and α , tumour necrosis factor α , prostaglandin E₂, and proteases to this activity.

We are grateful to Drs M A Cousin and D Lando (Rousell Uclaf, France) for generously supplying the monoclonal antibodies to interleukin 1 α and β and recombinant interleukin 1 β , Dr M Carrier (Wellcome Research Laboratories, UK) and Dr S D Poole (National Institute for Biological Standards and Controls, UK) for the gift of polyclonal antibodies to interleukin 1 α and interleukin 1 β , and Dr A Stern, Dr P Lomedico (Hoffman LaRoche, USA) for the gift of interleukin 1 α . This work was supported by a grant from the Arthritis and Rheumatism Council.

- 1 Paterson M, Fulford P, Denham R. Loosening of the femoral component after total hip replacement. The thin black line and the sinking hip. *J Bone Joint Surg [Br]* 1986; **68**: 392-7.
- 2 Linder L, Lindberg L, Carlsson A. Aseptic loosening of hip prostheses. A histological and enzyme histochemical study. *Clin Orthop* 1975; **175**: 93-104.
- 3 Bannister G. Mechanical failure in the femoral component in total hip replacement. *Orthop Clin North Am* 1988; **19**: 567-73.
- 4 Goldring S R, Schiller A L, Roelke M, Rourke C M, O'Neil D A, Harris W H. The synovial like membrane of 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.
- 5 Goldring S R, Jasty M, Roelke M S, Rourke C M, Bringhurst F R, Harris W H. Formation of a synovial-like membrane at the bone-cement interface. Its role in bone resorption and implant loosening after total hip replacement. *Arthritis Rheum* 1986; **29**: 836-41.
- 6 Gowen M, Wood D D, Ihrie E J, McGuire M K B, Russell R R G. An interleukin-1-like factor stimulates bone resorption in vitro. *Nature* 1983; **306**: 378-80.
- 7 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.
- 8 Emery P, Williamson D J, MacKay I R. Role of cytokines in rheumatological inflammation. *Concepts Immunopathol* 1987; **4**: 171-9.
- 9 Browning J. Interferons and rheumatoid arthritis: insight into interferon biology. *Immunol Today* 1987; **8**: 372-4.
- 10 di Giovine F S, Symons J A, Manson J, Duff G W. Soluble mediators of immunity: interleukins. In: Goodacre J, Dick C, eds. *Immunopathogenic mechanisms in arthritis*. Lancaster MTP Press, 1988: 101-21.
- 11 Dayer J M, Beutler B, Cerami A. Cachectin/tumour necrosis factor- α stimulates collagenase and prostaglandin production by human synovial cells and dermal fibroblasts. *J Exp Med* 1985; **161**: 2163-5.
- 12 Kim K J, Wilson S C, Rubash H E. Comparative study of interface tissues in cementless and cemented prostheses. *Transactions of the 36th Annual Meeting Orthopaedic Research Society*: Chicago (IL). Adept Printing, 1990: 236.
- 13 Appel A M, Sowder W G, Siverhus S W, Hopson C N, Herman J H. Prosthesis-associated pseudomembrane-induced bone resorption. *Br J Rheumatol* 1990; **29**: 32-6.
- 14 Helle M, Boeije L, Arden L. Functional discrimination between interleukin-1 and interleukin-6. *Eur J Immunol* 1988; **18**: 1535-40.

- 15 Le J, Frederickson G, Pollack M, Vilcek J. Activation of thymocytes and T cells by interleukin-6. *Ann N Y Acad Sci* 1989; 557: 444–53.
- 16 Hutchings P, Cambridge G, Tite G P, Meager T, Cooke A. The detection and enumeration of cytokine-secreting cells in mice and man and the clinical application of these assays. *J Immunol Methods* 1989; 120: 1–8.
- 17 Alwan W H, Dieppe P A, Elson C J, Bradfield J. Bone resorbing activity in synovial fluids in destructive osteoarthritis and rheumatoid arthritis. *Ann Rheum Dis* 1988; 47: 198–205.
- 18 Westacott C I, Whicher J T, Barnes I C, Thompson D, Swan A J, Dieppe P A. Synovial fluid concentration of five different cytokines in rheumatic diseases. *Ann Rheum Dis* 1990; 49: 676–81.
- 19 Howie D W, Vernon-Roberts B, Oakesgitt R, Manthey B. A rat model of resorption of bone at the cement-bone interface in the presence of polyethylene wear particles. *J Bone Joint Surg* 1988; 70: 257–63.
- 20 Goodman S, Fornasier V L, Kei J. The effect of bulk versus particulate polymethylmethacrylate on bone. *Clin Orthop* 1988; 232: 255–62.