Aberrant cytokine production from tenosynovium in dialysis associated amyloidosis

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Abstract
Culture supernatants of tenosynovial tissues from patients with carpal tunnel syndrome undergoing chronic haemodialysis contained interleukin (IL) 1-like and IL6-like activity. These culture supernatants also induced active proliferation of rheumatoid synovial cells. Immunohistochemical analysis of tenosynovial tissues showed the accumulation of mononuclear cells bearing CD14 and HLA-DR antigens adjacent to the deposition of amyloid protein (β_2 microglobulin). These cells also reacted with antibodies to IL1 and IL6 respectively. These data suggest that multiple cytokines, including IL1 and IL6, produced from tenosynovial tissues in patients with dialysis associated amyloidosis might induce the proliferation of synovial cells that, together with deposition of amyloid protein, might cause carpal tunnel syndrome.

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that cannot be removed by conventional haemodialysis.
In addition to amyloid deposition, however, we found the proliferation of synovial cells in biopsy samples of tenosynovial tissues from the carpal tunnel area of patients undergoing long term haemodialysis and therefore reconsidered whether carpal tunnel syndrome is solely attributable to the local deposition of β_2 microglobulin. We report here the augmented production of cytokines with activities of interleukin (IL) 1 and IL6 from biopsy samples of tenosynovial tissues from the carpal tunnel area and speculate that cytokines produced in situ might be responsible for the proliferation in tenosynovial tissues which subsequently causes carpal tunnel syndrome. We also suggest that local accumulation of β_2 microglobulin might stimulate synovial cells to induce constitutive production of cytokines from tenosynovial tissues.

Patients and methods

PATIENTS
Fifteen patients with carpal tunnel syndrome undergoing long term haemodialysis (mean duration 10.4 years) were selected for the study (table 1). Informed consent was obtained from all patients. Surgical decompression of the median nerve was performed and biopsy samples of tenosynovial tissues were taken at the same time from the carpal tunnel area without complications. These samples were used for assays of cytokine activity and immunohistochemical staining. Tenosynovial tissues obtained from eight patients with trauma were used as controls.

CULTURES

Biopsy samples were processed as reported previously.⁷ Briefly, biopsy specimens were dissected into fragments 3 mm in diameter, washed extensively with RPMI 1640 (Gibco, Grand Island, NY, USA) and a fragment was plated into each well in 24 well culture plates (Sumitomo, Japan) as an organ culture with RPMI 1640 supplemented with 10% heat inactivated fetal calf serum (Gibco), 100 U/ml penicillin, 100 µg/ml streptomycin, and 10 mM HEPES. Cultures for IL1 and IL6 were performed as described in the following.

MEASUREMENT OF IL1 ACTIVITY

Interleukin 1 activity was measured by a growth inhibition assay using the melanoma cell line

Carpal tunnel syndrome is often found in patients undergoing long term haemodialysis.¹ There is a positive correlation between the incidence of carpal tunnel syndrome and the duration of haemodialysis.² Patients who undergo haemodialysis for more than 15 years are always affected by carpal tunnel syndrome.³ Deposition of amyloid protein in the carpal tunnel area is believed to cause compression of the median nerve resulting in carpal tunnel syndrome,⁴ a clinical condition referred to as dialysis associated amyloidosis. The major component of amyloid protein in this situation was found by Gejyo *et al*⁵ to be β_2 microglobulin, a low molecular weight amyloidogenic protein (molecular weight 11.8 kilodaltons)⁶

Table 1 Cytokine production from tenosynovial tissues of patients with haemodialysis associated amyloidosis and carpal tunnel syndrome

Patient No	Age (years)	Sex	Cause of renal failure*	Cytokine production (U/ml)	
				IL1†	IL6‡
1	42	F	CGN	97	165
2	69	M	CGN	137	115
3	64	M	CGN	37	180
4	52	F	CGN	62	110
5	63	M	DM	12	100
6	37	M	CGN	12	115
7	65	M	CGN	21	150
8	48	M	CGN	0	20
9	55	M	CGN	10	0
10	58	F	CGN	0	57
11	66	M	CGN	0	58
12	49	F	CGN	128	81
13	47	M	CGN	135	28
14	54	F	CGN	30	123
15	59	M	CGN	42	55

*(CGN) chronic glomerulonephritis; (DM) diabetes mellitus.
†Measured using A375 melanoma cell line.
‡Measured using SKW-C1-4 cell line.

A375.⁸⁻⁹ Briefly, A375 cells were plated into 96 well flat bottom culture plates (1×10^4 /well) in Eagle's minimal essential medium supplemented with 10% fetal calf serum with various dilutions of test samples or recombinant IL1 β . After four days' incubation at 37°C, 0.5% neutral red was added to each well. Neutral red incorporated in viable cells was extracted with ethanol after two hours of culture. The absorbance of each well was measured at 540 nm by a multiscan spectrophotometer. One unit of growth inhibitory factor per millilitre represented the reciprocal of the dilution of samples causing 50% cytostasis or cytolysis after four days of culture, which is equivalent to the activity exerted by 50 pg/ml of recombinant IL1 β . In preliminary experiments, recombinant IL1 α activity was blocked by antibodies to human IL1 α , and recombinant IL1 β activity was neutralised by antibodies to human IL1 β . The specificity of the assay was further confirmed by using recombinant human tumour necrosis factor α , IL2, and IL6. With this assay, 2–1000 pg/ml of IL1 was detectable. In addition, a thymocyte proliferation assay was used to confirm IL1 activity.¹⁰ One IL1 unit in this assay is defined as the half maximum response of thymocytes to recombinant human IL1 β . An enzyme linked immunosorbent assay (ELISA) for IL1 β was also performed in some of the samples using a commercial kit (Ohtsuka Assay Co. Ltd, Japan).¹¹

In part of the experiments using the A375 cell line, samples with known IL1-like activity were added to A375 cells with either polyclonal antibodies to human IL1 α (1:400 dilution), antibodies to IL1 β (1:400), or an equivalent dose of normal rabbit IgG to determine whether their IL1-like activity in the samples was neutralised by these antibodies. These antibodies have been described in detail elsewhere.¹¹

In addition, part of the samples with increased IL1 activity were preincubated for two hours at 37°C in the presence or absence of 5 μ g of polymyxin B (Pfizer, New York, NY, USA) to remove any lipopolysaccharide in the samples.

MEASUREMENT OF IL6 ACTIVITY

Interleukin 6 activity was determined with an Epstein-Barr virus transformed B cell line, SKW-C1-4, as described previously.¹² Briefly, 1×10^4 cells/200 μ l/well were cultured with test samples or recombinant IL6 for four days, and the concentration of IgM in the culture supernatants was determined by an ELISA. Interleukin 6 activity was also measured using the IL6 dependent murine hybridoma MH60.BSF2 cell clone.¹³ The growth of MH60.BSF2 cells was dependent on IL6; none of the other lymphokines examined (human IL1 α , IL1 β , IL3, IL4, interferon γ , interferon α , or granulocyte colony stimulating factor supported the growth of this clone. MH60.BSF2 cells (1×10^4 cells/200 μ l/well) were cultured with various concentrations of test samples or recombinant IL6 for 48 hours, and DNA synthesis was measured by a spectrophotometric assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.¹⁴ In some of the experiments,

MH60.BSF2 cells were cultured with the test sample together with the IgG fraction of antibodies to IL6 or preimmune rabbit serum to determine whether IL6-like activity was neutralised.

ASSAY FOR SYNOVIAL CELL GROWTH PROMOTING ACTIVITY

A source of synovial cells was derived from rheumatoid synovium as sufficient amounts of synovial cells were not obtained from normal synovium. The synovial tissue obtained by arthroscopic synovectomy from the knee joints of a patient with rheumatoid arthritis was minced, washed extensively with phosphate buffered saline (PBS), and treated with PBS plus 0.25% trypsin for 40 minutes at 37°C.¹⁵ The cells were washed three times with PBS and finally suspended in HAM F-12 medium (Gibco) supplemented with 10% fetal calf serum, 5×10^{-5} M 2-mercaptoethanol, 100 U/ml penicillin, and 100 μ g/ml streptomycin. The cells were incubated in culture flasks until confluence, then treated with 0.05% trypsin for five minutes at room temperature. Morphologically, most of these cells were fibroblast-like cells. After they were adjusted to 2×10^4 cells/ml with 10% fetal calf serum-RPMI 1640, 100 μ l of the cell suspension was plated in 96 well culture plates with various concentrations of test samples or recombinant IL1 β for 72 hours, and neutral red uptake was measured by the method described earlier.

IMMUNOHISTOCHEMICAL ANALYSIS

Immunohistochemical staining was performed as described previously.⁷ In brief, specimens embedded in OCT medium (Miles, Naperville, IL, USA) were frozen in liquid nitrogen and stored at -70°C . Frozen tissues were fixed with acetone and stained by monoclonal or polyclonal antibodies as described below. Antibodies to CD2, CD4, CD8, CD20, CD21 (reactive with B cells), I $_2$ (reactive with HLA-DR antigens), CD13 (reactive with myelocytes, granulocytes, and monocytes), and CD14 (reactive with monocytes and macrophages) were all from Coulter Immunology (Hialeah, FL, USA). The tissue sections were incubated with a specific antibody or a control antibody of the same isotype. After rinsing, the sections were reacted with a serum sample containing biotinylated goat antimouse (IgG plus IgM) antibodies (Tago, Burlingame, CA, USA), followed by avidin-biotin complex (Vector Laboratories, Burlingame, CA, USA) and then the substrate, 3',3'-diaminobenzidine. When rabbit antibodies to human IL1 β (OCT204) or IL6 was used, the sections were reacted with the serum sample containing biotinylated goat antirabbit (IgG plus IgM) antibodies, followed by avidin-biotin complex.

STATISTICAL ANALYSIS

Statistical analysis was performed by Wilcoxon's rank sum test.

Results

INTERLEUKIN 1-LIKE ACTIVITY IN THE CULTURE SUPERNATANTS FROM BIOPSY SAMPLES OF TENOSYNOVIAL TISSUES FROM PATIENTS WITH DIALYSIS ASSOCIATED AMYLOIDOSIS

Culture supernatants from tenosynovial tissues harvested on day 1 of culture strongly inhibited the growth of the A375 human melanoma cell line in a dose dependent manner (fig 1). The mean (SE) value was 44.8 (11.9) U/ml, suggesting that the culture supernatants contained IL1-like activity (table 1). This activity was significantly increased compared with that in culture supernatants obtained from patients with trauma (2.0 (0.3) U/ml; $p<0.01$). The IL1-like factor was also produced by tenosynovial tissues cultured for 24 hours in medium with polymyxin B, indicating that contamination by lipopolysaccharide is not responsible for the production of IL1-like factor. The IL1-like activity was greater on day 1 of culture than on day 4 or 7. Furthermore, the IL1-like activity in the supernatants was significantly blocked by antibodies to IL1 β , but not by normal rabbit IgG, indicating that most of the IL1-like activity could be ascribed to the IL1 β molecule (fig 2). As the amounts of tenosynovial tissues obtained were small, not enough sample was available for further testing. The IL1-like activity was further confirmed, however, by thymocyte

proliferation assay and an ELISA in the samples tested (table 2).

INTERLEUKIN 6-LIKE ACTIVITY IN CULTURE SUPERNATANTS FROM TENOSYNOVIAL TISSUES

Culture supernatants from tenosynovial tissues in patients with dialysis associated amyloidosis harvested on day 1 of culture induced IgM production in SKW6-C1-4 cells. The mean (SE) value was 86.1 (13.9) U/ml, which was equivalent to the activity exerted by 17.2 pg/ml of recombinant IL6 (table 1). In contrast, no significant IL6 activity was detected in culture supernatants from patients with trauma (3.1 (0.4) U/ml; $p<0.01$). This activity was confirmed to be ascribed to IL6 by the IL6 dependent murine hybridoma clone MH60.BSF2. Table 3 shows that a culture supernatant from a representative patient induced the proliferation of MH60.BSF2 cells in a dose dependent manner. Furthermore, this activity was neutralised by polyclonal antibodies to IL6 but not by non-immune rabbit IgG, indicating that the active molecule was actually IL6.

Table 2 Detection of interleukin 1 (IL1) in culture supernatants from tenosynovial tissues by various assays

	IL1 activity		
	GIF (U/ml)*	LAF (U/ml)†	ELISA (pg/ml)‡
Sample 1	42	6.1	82
Sample 2	12	2.0	15

*GIF assay using A375 cell line.
†LAF assay using murine thymocytes.
‡Enzyme linked immunosorbent assay for IL1.

Table 3 Interleukin (IL) 6 activity in a culture supernatant from tenosynovial tissue and its inhibition by polyclonal antibodies to IL6

Sample	Antibody to IL6*	Absorbance†
Culture supernatant (1:200)	—	0.140
Culture supernatant (1:800)	+	0.028
Culture supernatant (1:800)	+	0.075
No culture supernatant	—	0.022
No culture supernatant	—	0.015

*Final concentration of antibody 12.5 g/ml.
†Absorbance of a culture supernatant of the SKW-Cl-4 cell line cultured with each sample.

Figure 1 Interleukin 1-like activity in culture supernatants of tenosynovial tissues from long term haemodialysis patients with carpal tunnel syndrome. A375 cells were cultured with various dilutions of either (A) recombinant IL1 β or (B) four representative culture supernatants for four days as described under methods.

Figure 2 Neutralisation of IL1 activity in culture supernatants from tenosynovial tissues by antibodies to IL1. A375 cells were cultured with a culture supernatant from a tenosynovial tissue sample (final concentration 1.5% vol/vol) together with antibodies to IL1 or preimmune rabbit IgG for four days. Bars indicate standard errors of the experiment. A representative result is shown in repeated experiments.

Figure 3 Rheumatoid synovial cells (1×10^4 /well) were cultured with various dilutions of samples: (○) a culture supernatant of a tenosynovial tissue (patient No 1), (●) recombinant IL1 β (1 ng/ml), (▲) IL6 (20 U/ml), or (■) culture medium alone for four days as described under methods. The results of a representative experiment are shown.

SYNOVIAL CELL GROWTH PROMOTING ACTIVITY IN CULTURE SUPERNATANTS FROM TENOSYNOVIAL TISSUES

Synovial cells obtained from rheumatoid synovial tissue as described under methods were cultured for approximately two weeks. These cultured synovial cells were plated (2×10^4 cells/well) and incubated with various concentrations of culture supernatants from tenosynovial tissues or recombinant IL1 β for 72 hours at 37°C. Figure 3 shows that these supernatants promoted the growth of synovial cells in a dose dependent manner; this capacity was more potent than that obtained with 1 ng/ml of recombinant IL1 β . In addition, this activity was also blocked by antibodies to IL1 β .

IMMUNOHISTOCHEMICAL STAINING OF TENOSYNOVIAL TISSUES

Haematoxylin-eosin staining of the tenosynovial tissues obtained from haemodialysis patients with carpal tunnel syndrome showed prominent

Figure 4 Haematoxylin-eosin stain of the tenosynovial tissue obtained from a patient with carpal tunnel syndrome undergoing haemodialysis shows proliferation of synovial cells.

Figure 5 Immunohistochemical analysis of synovial cells in tenosynovial tissues. Most of the cells showed cytoplasmic staining with monoclonal antibodies to CD14.

Figure 6 Immunohistochemical analysis of IL1 producing cells. Synovial cells showed cytoplasmic staining with polyclonal antibodies to IL1.

proliferation of synovial cells and scattered deposition of amorphous materials (fig 4). These amorphous materials were positive for Congo red with a typical green birefringence observed under a polarising microscope.

None of the cells infiltrating the tenosynovial tissues reacted with antibodies to T cells (CD2) or B cells (CD20). Most proliferating cells were positive for CD14 (fig 5) and approximately one third of CD14 positive cells expressed CD13 on their surfaces. They also had HLA-DR antigens on their surfaces. These data suggest that the proliferating synovial cells could have originated as macrophages. Moreover, these cells reacted strongly with polyclonal antibodies to human IL1 β (fig 6) and antibodies to human IL6. Preincubation of specific antibodies with the corresponding recombinant cytokines completely abolished these stainings (data not shown). These data indicate that there might be IL1 and IL6 producing synovial cells in tenosynovial tissues.

Discussion

Carpal tunnel syndrome is now recognised to be one of the major complications of long term haemodialysis, and a significant correlation has been found between carpal tunnel syndrome and the duration of haemodialysis.² The deposition of amorphous materials that have a high affinity to Congo red and are sensitive to potassium permanganate treatment is invariably found in the tenosynovial tissues of these patients. This amyloid protein appears to be intact β_2 microglobulin,^{5, 16} a low molecular weight protein associated with major histocompatibility complex class I antigens. The deposition of β_2 microglobulin is itself believed to induce compression of the median nerve, resulting in carpal tunnel syndrome. Marked proliferation of synovial cells is found in the tenosynovial tissues of these patients, however, and can also be responsible for compression of the median nerve.

In this study, we showed a potent IL1-like activity in culture supernatants of biopsy

samples of tenosynovial tissues from patients with carpal tunnel syndrome undergoing long term haemodialysis. This organ culture system overcomes the difficulties of obtaining enough cells from the limited amount of tissue for functional studies. The cytokine activity detected here can be ascribed to IL1 β , as most of it was neutralised by the antibody against human IL1 β . In addition, the IL1-like activity was confirmed by thymocyte proliferation assay and an ELISA in the sample tested. Interleukin 1 produced in the culture supernatants is speculated to be constitutively secreted from synovial cells and not to be induced during the culture, as immunohistochemical analysis using antibodies to IL1 showed cytoplasmic staining corresponding to synovial cells. In addition, polymyxin B did not alter the IL1 activity in the culture supernatants. It has been shown that IL1 β is secreted, whereas IL1 α remains associated with cells.¹⁷ It is therefore necessary to measure the amount of intracellular IL1 to clarify this point further. Interleukin 6 activity was also found in these culture supernatants using the SKW-CI-4 and MH60.BSF2 cell lines. This activity was also blocked by a specific antibody against IL6. Antibodies to IL6 reacted with synovial cells in immunohistochemical staining suggesting that these are the IL6 producing cells in tenosynovial tissues. Moreover, these culture supernatants induced active proliferation of rheumatoid synovial cells in vitro. The experiment performed simultaneously showed that IL1, but not IL6, is capable of inducing proliferation of synovial cells in vitro. These data suggest that cytokines, including IL1, produced in situ might contribute to the proliferation of synovial cells, resulting in entrapment of the median nerve in the carpal tunnel.

The so called 'interleukin hypothesis' has been proposed to explain acute and chronic complications observed in patients undergoing long term haemodialysis.¹⁸ Among the interleukins, IL1 is thought to play a central part in the pathogenetic changes seen in haemodialysis patients.¹⁹ Symptoms such as fever, lassitude, wasting, sleepiness, and possibly hypotension can be ascribed to the acute production of IL1 in vivo. The significance of the chronic production of IL1 in long term haemodialysis patients remains to be clarified. Local production of IL1 can affect many complications seen in haemodialysis, however, including destructive arthropathy and carpal tunnel syndrome. We have already reported augmented IL1 production in the synovium of patients with rheumatoid arthritis.⁷ Interleukin 1 induces the production of prostaglandins and collagenase from synovial cells.²⁰ Interleukin 1 also promotes the growth of fibroblasts,²¹ and activates osteoclasts.²² Interleukin 1 upregulates the production of IL2²³ and triggers IL6 production.²⁴ Thus cytokines constitutively produced in tenosynovial tissue could evoke destructive arthropathy on the one hand and synovial proliferation which causes carpal tunnel syndrome in long term haemodialysis patients on the other. In this respect, we previously showed that cloned rheumatoid synovial cells spontaneously liberate

both IL1¹⁵ and IL6²⁵ in culture. Cells that belong to the macrophage lineage are known to produce IL1 and IL6 in response to the correct stimuli—for example, silica and lipopolysaccharide.²⁶ The stimulus that produces IL1 and IL6 from proliferating synovial cells in this instance is not known at present. Multiple mechanisms including contamination of endotoxin and complement activation may contribute to the constitutive production of IL1 in long term haemodialysis patients. It is interesting to speculate, however, that amyloid protein deposited locally in tenosynovial tissue might activate synovial cells. Further studies will be necessary to clarify this point.

This is the first study to examine the relationship between in vitro cytokine production and the pathogenesis of carpal tunnel syndrome in haemodialysis associated amyloidosis.

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- Warren D J, Otieno L S. Carpal tunnel syndrome in patients on intermittent hemodialysis. *Postgrad Med J* 1975; 51: 450-2.
- Schwarz A, Keller F, Seyfert S, Poll W, Molzahn M, Distler A. Carpal tunnel syndrome: a major complication in long-term hemodialysis patients. *Clin Nephrol* 1984; 22: 133-7.
- Bardin T, Kuntz D, Zingraff J, Voisin M, Zelman A, Lansman J. Synovial amyloidosis in patients undergoing long-term hemodialysis. *Arthritis Rheum* 1985; 28: 1052-8.
- Bardin T, Zingraff J, Noel L, et al. Hemodialysis-associated amyloidosis and beta-2 microglobulin. Clinical and immunohistochemical study. *Am J Med* 1987; 83: 419-24.
- Gejyo F, Yamada T, Odani S, et al. A new form of amyloid protein associated with chronic hemodialysis was identified as microglobulin. *Biochem Biophys Res Commun* 1985; 129: 701-6.
- Gorevic P D, Casey T T, Stone W J, DiRaimondo C R, Prelli F C, Frangione B. Beta-2 microglobulin is an amyloidogenic protein in man. *J Clin Invest* 1985; 76: 2425-9.
- Miyasaka N, Sato K, Goto M, et al. Augmented interleukin-1 production and HLA-DR expression in the synovium of rheumatoid arthritis patients. Possible involvement in joint destruction. *Arthritis Rheum* 1988; 31: 480-6.
- Nakai S, Mizuno K, Kaneta M, Hirai Y. A simple, sensitive bioassay for the detection of interleukin-1 using human melanoma A375 cell line. *Biochem Biophys Res Commun* 1988; 154: 1189-96.
- Lachman L B, Dinarello C A, Leans N D, Fidler I J. Natural and recombinant IL-1 is cytotoxic for human melanoma cells. *J Immunol* 1986; 136: 3098-102.
- Lachman L B, Hacker M P, Blyden G T, Handschumacher R E. Preparations of lymphocyte-activating factor from continuous murine macrophage cell lines. *Cell Immunol* 1977; 34: 416-9.
- Tanaka K, Ishikawa E, Ohmoto Y, Hirai Y. In vitro production of human interleukin 1 and interleukin 1 by peripheral blood mononuclear cells examined by sensitive sandwich enzyme immunoassay. *Eur J Immunol* 1987; 17: 1527-30.
- Hirano T, Taga T, Nakano N, et al. Purification to homogeneity and characterization of human B cell differentiation factor (BCDF or BSFp-2). *Proc Natl Acad Sci USA* 1985; 82: 5490-4.
- Matsuda T, Hirano T, Kishimoto T. Establishment of interleukin-6/B cell stimulatory factor 2 (BSF-2)-dependent cell line and preparation of anti-IL-6/BSF-2 monoclonal antibodies. *Eur J Immunol* 1988; 18: 951-4.
- Mosman T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assay. *J Immunol Methods* 1983; 65: 55-63.
- Goto M, Sasano M, Yamanaka H, et al. Spontaneous production of an interleukin-1-like factor by cloned rheumatoid synovial cells in long-term culture. *J Clin Invest* 1987; 80: 786-96.
- Gejyo F, Odani S, Yamada T, et al. Beta 2-microglobulin: a

- new form of amyloid protein associated with chronic hemodialysis. *Kidney Int* 1986; **30**: 385-90.
- 17 Conlon P J, Grabstein K H, Alpert A, Prickett K S, Hopp T P, Gillis S. Localization of human mononuclear cell interleukin 1. *J Immunol* 1987; **139**: 98-102.
 - 18 Dinarello C A, Koch K M, Shaldon S. The interleukin hypothesis. Interleukin-1 and its relevance in patients treated with hemodialysis. *Kidney Int* 1988; **33** (suppl 24): S21-6.
 - 19 Bingel M, Lonnemann G, Shaldon S, Koch K M, Dinarello C A. Human interleukin-1 production during hemodialysis. *Nephron* 1986; **43**: 161-3.
 - 20 Dayer J M, de Rochemonteix B, Burrus B, Demczuk S, Dinarello C A. Human recombinant interleukin 1 stimulates collagenase and prostaglandin E production by human synovial cells. *J Clin Invest* 1986; **77**: 645-8.
 - 21 Schmidt J A, Mizel S B, Cohen D, Green I. Interleukin 1: a potential regulator of fibroblast proliferation. *J Immunol* 1982; **128**: 2177-82.
 - 22 Dewhirst F E, Stashenko P P, Mole J E, Tsurumachi T. Purification and partial sequence of human osteoclast-activating factor: identity with interleukin 1 β . *J Immunol* 1985; **135**: 2562-8.
 - 23 Dinarello C A, Ikejima T, Warner S J C, *et al.* Interleukin 1 induces interleukin 1. I. Induction of circulating interleukin 1 in rabbits in vivo and human mononuclear cells in vitro. *J Immunol* 1987; **139**: 1902-10.
 - 24 Content J, De Wit L, Poupart P, Opdenakker G, Van Damme J, Billiau A. Induction of a 26-kDa-protein mRNA in human cells treated with an interleukin-1-related, leukocyte-derived factor. *Eur J Biochem* 1985; **152**: 253-7.
 - 25 Miyasaka N, Sato K, Hashimoto J, *et al.* Constitutive production of interleukin 6/B cell stimulatory factor-2 from inflammatory synovium. *Clin Immunol Immunopathol* 1989; **52**: 238-47.
 - 26 Kishimoto T, Hirano T. Molecular regulation of B lymphocyte response. *Ann Rev Immunol* 1988; **6**: 485-512.