Gene expressions of antiinflammatory mediators in THR retrieved interfacial membranes

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We investigated gene expression of antiinflammatory mediators in the interfacial membranes retrieved at hip revision arthroplasty using reverse transcription-polymerase chain reaction (RT-PCR). Levels of RT-PCR products were compared with those of synovial tissue from patients with osteoarthrosis or rheumatoid arthritis. Antiinflammatory mediators such as type II interleukin (IL)-1 receptor, IL-4, IL-10, IL-1 receptor antagonist, and transforming growth factor-ß1 (TGF-ß1) were expressed in the interfacial membrane. In interfacial tissue, the level of IL-10 was lower, but that of the IL-1 receptor antagonist higher than in diseased synovial tissue.

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Current evidence indicates that wear THR implant particles initiate an inflammatory process that results in bone loss and contributes significantly to implant failure. The primary cell involved in the biologic reaction to THR wear debris appears to be the macrophage. Macrophages phagocytose small debris particles and may fuse to form foreign body-type multinucleated giant cells that phagocytose larger particles (Wright and Goodman 1996). Numerous in vitro studies suggest that activated macrophages release proinflammatory mediators such as interleukin-1 (IL-1), but the regulatory mechanisms are complex and at present poorly understood (Horowitz et al. 1993, Jiranek et al. 1993). Among factors identified in inflammatory tissue, IL-1 appears to be especially important because it enhances secretion of metalloproteinases, prostaglandins, and cytokines. However, the effects of proinflammatory mediators may be modified by antiinflammatory cytokines such as IL-4, IL-10, and TGF-ß or other antiinflammatory factors. Proinflammatory inhibitory mechanisms are important in inflammatory arthritis leading to joint destruction (Isomäki et al. 1996, Tucci et al. 1997). Likewise, an imbalance between the factors stimulating osteolysis and osteogenesis seems to be involved in aseptic loosening (Konttinen et al. 1997b).

We investigated the expression of 6 antiinflammatory factors, namely IL-4, IL-10, TGF-ß1, secretory IL-1 receptor antagonist (sIL-1ra), intracellular IL-1ra (icIL-1ra), and type II IL-1 receptor (IL-1R), together with proinflammatory factors such as IL-1ß, IL-6, and type I IL-1R, in the interfacial membrane, and compared the levels of these mediators with those of diseased joint synovium.

Material and methods

Retrieval of tissues

We obtained the interfacial membranes from 6 patients at the time of revision surgery for aseptic loosening of implants (Table 1). Patients' mean age was 64 (28–82) years. The interval between primary and revision surgery was mean 10 (6–16) years.

Synovial tissue from 6 RA and 6 OA patients was obtained in a separate series of primary total knee arthroplasty (5 patients, 2 men), total hip arthroplasty (5 patients, 2 men) or total ankle arthroplasty (2 women), for comparison.

The interfacial tissues were taken without inclusion of bone tissue, and synovial tissues without inclusion of fibrous capsule. They were washed with physiological saline and immediately preserved in liquid nitrogen. Some portions of the interfacial tissues were fixed in neutral buffered formalin for immunohistochemical staining.

Total RNA extraction

Total RNA was isolated by the acid guanidinium-isothiocyanate-phenol-chloroform method, with a slight modification. Briefly, the sliced interface or synovium specimen, preserved in liquid nitrogen, was homogenized in 10 volumes of 4 M guanidinium isothiocyanate/50 mM Tris-HCl (pH 7.5)/10 mM

Table 1. Clinical characteristics of the patients undergoing revision surgery

Patient	Age	Sex	Diagnosis	Primary surgery	Cement	Metal	Site of retrieved tissue	Time to revision years
1	82	F	OA	THA	+	CoCrMo	stem	15
2	71	F	OA	THA	+	CoCrMo	cup	12
3	70	F	OA	THA	+	CoCrMo	cup	16
4	28	M	OA	BE	-	CoCrMo/TiAlV	stem	8
5	66	M	OA	THA	-	CoCrMo/TiAlV	stem	10
6	68	F	RA	THA	-	CoCrMo	stem	6

OA osteoarthrosis, RA rheumatoid arthritis, THA total hip arthroplasty, BE bipolar endoprosthesis, CoCrMo cobalt-chromium-molybdenum alloy, TiAlV titanium-aluminum-vanadium alloy.

EDTA/2% sodium dodecyl sarcosinate/150 mM 2-mercaptoethanol using a homogenizer (HG-30, Hitachi Koki, Chiyoda-ku, Tokyo, Japan). An equal volume of 0.1 M sodium acetate buffer (pH 5.2)-saturated phenol/chloroform was then added and the mixture was vigorously shaken at 60 °C. The aqueous phase was recovered, then an equal volume of 0.1 M sodium acetate buffer-saturated phenol/chloroform was added, and the mixture was shaken at room temperature until the aqueous phase became clear. This phase was recovered, chloroform was added and the mixture was shaken again. 2 volumes of absolute ethanol were then added and the mixture was allowed to stand for 18 hours at –20 °C. After centrifugation (12000 rpm, 20 minutes at 4 °C), the pellet was dissolved in 1 mL of 0.1 M Tris-HCl(pH 7.5)/50 mM NaCl/10 mM EDTA/0.2% SDS containing proteinase K (100 μg/mL) and incubated for 2 hours at 37 °C. The solution was treated with saturated phenol/chloroform and the aqueous phase was transferred to a fresh tube containing 2 volumes of absolute ethanol. After centrifugation (12000 rpm, 20 min. at 4 °C), the pellet was dissolved in 3 mM sodium acetate buffer (pH 5.2).

To remove genomic DNA contamination, each sample was treated with DNase I (RNase-free, Nippongene, Toyama, Japan) in the presence of RNase inhibitor (Wako Pure Chemical, Osaka, Japan) for 1 hour at 37 °C, followed by proteinase K digestion, phenol extraction, and ethanol precipitation. The pellet was dissolved in 3 mM sodium acetate buffer (pH 5.2) again, and quantified spectrophotometrically at 260 nm.

RT-PCR (reverse transcription-polymerase chain reaction)

RT-PCR was performed using a RNA PCR kit (Takara Shuzo, Otsu, Shiga, Japan). Briefly, reverse transcription of RNA into cDNA was performed by incubating 2 μg of RNA with AMV reverse transcriptase, random primers, dNTP, and RNase inhibitor at 30 °C for 10 min, followed by 42 °C for 30 min. 1 μL of each cDNA sample from the above extractions was amplified by PCR in a total volume of 100 μL using 2.5 units of Ex Taq polymerase with the accompanying buffer (Takara Shuzo, Otsu, Shiga, Japan) and 0.2 μM each of specific oligonucleotide primers. The oligonucleotide primers of the following substances were prepared according to the published sequence of the human gene: glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Tang et al. 1995), IL-1ß, IL-6 (Butch et al. 1993), type I IL-1R, type II IL-1R (Sadouk et al. 1995), IL-4 (Heuer et al. 1996), IL-10 (Esnault et al. 1996), TGF-ß1 (Mulheron et al. 1992), sIL-1ra and icIL-1ra (Krzesicki et al. 1993). Amplifications were performed at from 25 to 45 cycles and each cycle consisted of denaturation at 94 °C for 30 min, annealing at 55–65 °C for 1 min, and extension at 72 °C for 1 min. The amplified products were separated by electrophoresis using 1% agarose gels. To determine the size of the fragments, 100 bp DNA ladder (Gibco BRL, Rockville, MD, USA) was used as the marker. The gels were stained using ethidium bromide (0.5 μg/mL) and the cDNA bands were photographed under UV illumination. Electrophoresis of PCR products revealed a dominant size for each set of cytokine primers. Each experiment included negative controls. The PCR procedure was performed with sample RNA that had not been reverse-transcribed, and with distilled water instead of cDNA. The strength of the expected bands was semi-quantified using computer analysis. Briefly, the photographs were scanned and analyzed with Adobe Photoshop software (Adobe Systems Inc., Mountain View, CA, USA). Results were normalized for GAPDH mRNA and expressed in arbitrary units.

Immunostaining using antimacrophage antibody (MAC387)

Some portions of the interface tissues were fixed in neutral buffered formalin, embedded in paraffin, and cut into 4-μm thick sections. Immunostaining was

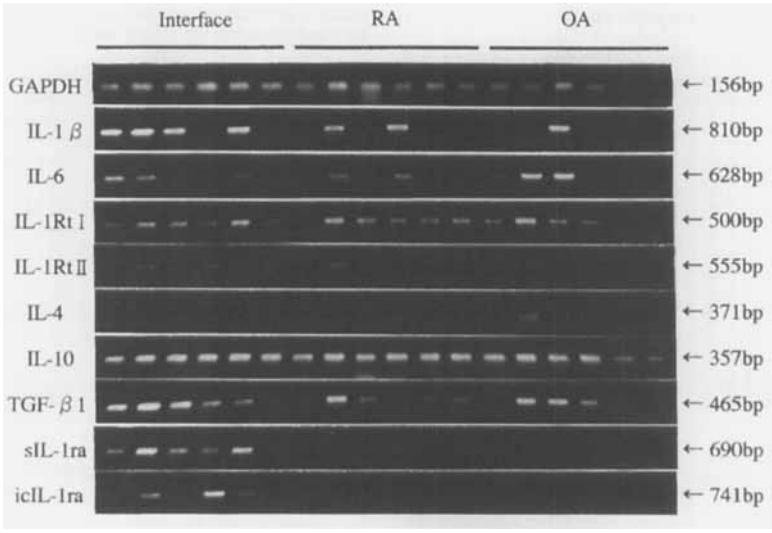

Figure 1. Expression of pro- and antiinflammatory factors. RA rheumatoid arthritis, OA osteoarthrosis, IL-1Rt I type I IL-1 receptor; IL-1Rt II type II IL-1 receptor; bp: base pair.

performed using a Vectastain ABC kit (Vector Laboratories, Burlingame, CA, USA). Briefly, after trypsinization following deparaffinization, endogenous peroxidase was blocked by placing the sections in hydrogen peroxidase solution for 30 min. They were then incubated in the following reagents with appropriate phosphate-buffered saline (PBS: 0.1 M, pH 7.4) washes: normal goat serum for 30 min, primary antibody (MAC 387, DAKO A/S, Glostrup, Denmark) for 1 hour, biotinylated antimouse antibody for 30 min, and avidin-biotin complex for 30 min. The reaction was visualized with chromogen substrate solution (diaminobenzidine, hydrogen peroxidase, PBS), and sections were then counterstained with methyl green, dehydrated, and mounted. Normal immunoglobulin instead of the primary antibody was used as the negative control.

One section for each patient was chosen and examined at a magnification of 40× under a microscope with a square grid of 0.2 × 0.2 mm. The number of MAC 387-positive cells was counted in 5 grid squares of each section. The mean number of positive cells in the 5 grid squares was divided by the area (0.2 × 0.2 mm^2), and this value (number of macrophages/mm^2) was defined as the macrophage count of each patient.

Statistics

Fisher's exact probability test, one-way analysis of variance, and Spearman's rank correlation coefficient were used for statistical analysis. Statistical significance was inferred when the P value was less than 0.01.

Results

In each PCR procedure, the amount of PCR product increased for up to 45 cycles, and increased almost linearly as a function of the amount of total RNA used (data not shown). No PCR product was observed in the procedures with sample RNA that had not been reverse-transcribed with distilled water, instead of cDNA, showing that the amplified product was mRNA specific and that there was no cDNA contamination. Figure 1 demonstrates that specific DNA fragments of various mediators and enzymes in the interfacial membrane were amplified. The numbers of positive expression of each mediator in 6 interfacial membranes, and 6 RA and 6 OA synovial tissues, are summarized in Table 2. The proinflammatory mediators, IL-1ß, IL-6, and type I IL-1R, were nearly always expressed in the interfacial tissues. Among antiinflammatory mediators, type II IL-1R, IL-10, TGF-ß1 and sIL-1ra were always expressed in the interfacial tissues, whereas IL-4 could be detected in 2 subjects and icIL-1ra in 3 of 6 subjects.

Normalized intensity of RT-PCR products of each mediator was compared between three tissue groups, using one-way analysis of variance (Table 3). A difference was evident in intensity of IL-10 ($p = 0.003$) and of sIL-1ra ($p = 0.0006$). In interfacial tissue, intensity of IL-10 was the lowest, whereas that of sIL-1ra was the highest. With Fisher's least significant difference method, intensity of IL-10 in interfacial tissue was lower than in OA synovium ($p = 0.0007$), and tended to be lower than in RA synovium ($p = 0.09$). Similarly, sIL-1ra was higher than in OA ($p =$

Table 2. Numbers of positive expression

Tissue	Interfacial membrane n 6	RA synovium n 6	OA synovium n 6
IL-1ß	5	3	4
IL-6	5	3	4
IL-1Rt I	6	6	6
IL-1Rt II	6	5	4
IL-4	2	1	2
IL-10	6	6	6
TGF-ß1	6	6	6
sIL-1ra	6	3	3
icIL-1ra	3	0	0

RA rheumatoid arthritis, OA osteoarthrosis, IL-1Rt I type I IL-1R, IL-1Rt II type II IL-1R

Table 3. Normalized intensity of RT-PCR products of mediators and macrophage count, Mean (SD)

	Interfacial membrane	RA synovium	OA synovium
IL-1ß	2.81 (2.14)	1.09 (1.61)	0.96 (1.33)
IL-6	0.93 (1.05)	0.50 (0.77)	1.57 (1.84)
IL1Rt I	1.71 (0.64)	1.89 (0.68)	2.11 (1.49)
IL1Rt II	0.75 (0.21)	0.49 (0.39)	0.49 (0.54)
IL-4	0.30 (0.63)	0.72 (1.75)	1.92 (3.58)
IL-10	3.83 (0.36)	4.79 (1.27)	6.06 (0.89)
TGF-ß1	2.67 (1.47)	1.39 (0.70)	2.17 (1.54)
sIL-1ra	2.03 (1.08)	0.25 (0.32)	0.30 (0.41)
icIL-1ra	0.89 (1.07)	0	0
M count [a]	230 (126)	-	-

Abbreviations, see Table 2, [a] macrophages/mm^2

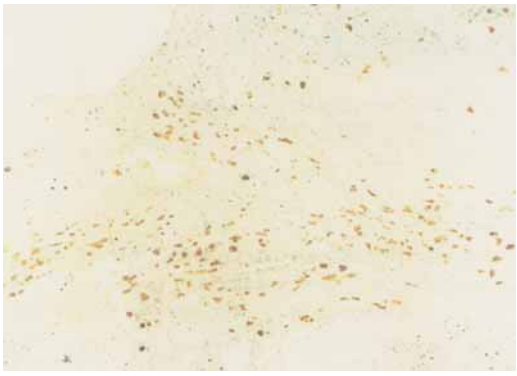

Figure 2. Immunohistochemical staining with MAC 387 (anti-macrophage antibody) of the interfacial membrane obtained from patient No. 4. Although the specimen had the highest macrophage count (410/mm^2), the levels of both proinflammatory and antiinflammatory cytokines were not high except for icIL-1ra (× 40).

0.0006) and RA synovium (p = 0.0005). There was no significant difference with regard to other mediators, although intensity of icIL-1ra tended to be high in interfacial tissue (p = 0.04).

Immunohistochemical studies of interfacial membranes using monoclonal mouse antibody showed the localization of macrophages (Figure 2). Sheets of macrophages were seen in fibrous tissue. However, the appearance of these sheets tended to be partial features of a whole section. Macrophage counts in the interfacial membrane varied from 95/mm^2 to 410/mm^2 (average 230, SD 126/mm^2). We therefore examined the relationship between macrophage count and normalized intensity of RT-PCR product of each factor using Spearman's rank correlation coefficient, with the assumption that production of these mediators may be affected by the amount of macrophages in the interfacial membrane. However, no significant relation was evident (Table 4). Similarly, correlation coefficients between normalized intensities of RT-PCR products of each mediator were evaluated with the assumption that some mediators may regulate expression of other mediators. Again, no significant relation was found between these mediators with the statistical power of $p < 0.01$, although IL-1ß tended to have a positive relation with TGF-ß1 (p = 0.03), type I IL-

Table 4. Correlation coefficients between intensity of RT-PCR products of each mediator and macrophage count in interfacial membrane

	IL-1ß	IL-6	IL-1Rt I	IL-1Rt II	IL-4	IL-10	TGF-ß1	sIL-1ra	icIL-1ra	M count
IL-1ß	-	0.71	0.43	–0.09	0.14	–0.03	1.00 [a]	0.60	–0.15	–0.35
IL-6	-	-	0.49	–0.26	0.37	–0.03	0.71	0.43	–0.27	–0.46
IL-1Rt I	-	-	-	0.26	0.85	0.49	0.43	0.89 [b]	–0.03	–0.03
IL-1Rt II	-	-	-	-	0.54	–0.43	–0.09	0.54	–0.88	0.93 [c]
IL-4	-	-	-	-	-	0.10	0.14	0.78	0.40	0.38
IL-10	-	-	-	-	-	-	–0.03	0.14	–0.70	–0.55
TGF-ß1	-	-	-	-	-	-	-	0.60	–0.15	–0.35
sIL-1ra	-	-	-	-	-	-	-	-	0.27	0.23
icIL-1ra	-	-	-	-	-	-	-	-	-	0.96 [d]

IL-1Rt I type I IL-1R, IL-1Rt II type II IL-1R, M macrophage
[a] p = 0.025
[b] p = 0.048
[c] p = 0.038
[d] p = 0.033

1R with sIL-1ra ($p = 0.05$), type II IL-1R with macrophage count ($p = 0.04$), and icIL-1ra with macrophage count ($p = 0.03$). Correlation coefficients between RT-PCR products from synovium were also calculated, but no significant relation was evident in OA or in RA synovium (data not shown).

Discussion

We found that antiinflammatory factors such as IL-4, IL-10, TGF-ß1, sIL-1ra, icIL-1ra, and type II IL-1R are expressed at the interfacial membrane of a failed THR. The level of IL-10 in the interfacial membranes was lower, but that of sIL-1ra was higher than in OA and RA synovia. The presence of antiinflammatory factors such as TGF-ß1 (Konttinen et al. 1997a), IL-4 (Weyand et al. 1998) and basic FGF implicated in bone formation (Waris et al. 1996) in the interfacial membrane has already been shown by others. Our findings established that a variety of antiinflammatory mediators are synthesized at the bone-implant interface.

IL-10 has potent inhibitory effects on monocyte and T cell functions. IL-10 inhibits both the production of proinflammatory cytokines such as IL-1, IL-6, IL-8, TNFα, and GM-CSF by human monocytes in vitro (De Waal Malefyt et al. 1991a, b). Based on animal models, IL-10 has potent antiinflammatory effects in vivo. Neutralization of endogenous IL-10 by anti-IL-10 antibodies results in more severe collagen-induced arthritis in mice (Kasama et al. 1995). Administration of IL-10 suppresses induction of experimental allergic encephalomyelitis in Lewis rats (Rott et al. 1994). It has been suggested that IL-10 is endogenously produced in rheumatoid synovial joints but at suboptimal levels (Isomäki et al. 1996). In addition, administration of high doses of IL-10 is safe in humans (Chernoff et al. 1995). At present, we do not know whether the endogenously produced IL-10 in the interfacial membrane is suboptimal and leads to bone resorption around the implant. However, the lower level of IL-10 in the interfacial tissue shown in the present study raises the hypothesis that IL-10 might be useful in the prevention of aseptic loosening.

The effect of IL-1 is mediated by specific receptors (IL-1R). Two types of IL-1R, type I and type II, have been identified and cloned (Sims et al. 1989, McMahan et al. 1991). The type I receptor appears to be responsible for transducing signals, whereas type II may serve as a precursor for a shed soluble receptor (Sims et al. 1993), functioning as a receptor antagonist. IL-1ra is one of the antiinflammatory factors which is a specific inhibitor of IL-1. It acts by blocking the binding of IL-1 to its receptors, without exhibiting IL-1 bioactivity (Dripps et al. 1991). Two structural variants of IL-1ra have been described: sIL-1ra, which is a major product of monocytes and macrophages and icIL-1ra, which remains intracellular (Haskill et al. 1991, Bigler et al. 1992).

The most remarkable characteristic of the interfacial tissue was the higher level of sIL-1ra expression. This was 6- to 8-fold higher than in the diseased joint synovium. The level of icIL-1ra also tended to be higher. The endogenous production of IL-1ra has been examined in synovial fluid and tissue from patients with RA (Firestein et al. 1994, Chomarat et al. 1995). These studies suggested that IL-1ra is not sufficient to suppress the disease process. It is to be noted that target cells are exquisitely sensitive to binding of only a few IL-1 molecules per cell, and relatively large numbers of IL-1 receptor are present on most cells. Thus endogenous IL-1ra must be produced in vast amounts to inhibit IL-1 effects in the cell microenvironment in tissues. The possible biological importance of IL-1ra in vivo is influenced by the requirement for amounts of this protein 10- to 100-fold greater than normal to inhibit biological responses to IL-1 in vitro (Firestein et al. 1994). A higher level of IL-1ra in the interfacial membrane could therefore still be inadequate to suppress bone resorption.

In our study, we failed to demonstrate a significant positive or inverse relationship between proinflammatory and antiinflammatory factors, or between these factors and the macrophage count, probably due to the limited sample size. However, bone resorption induced by proinflammatory mediators is functionally neutralized by antiinflammatory factors, and bone resorption is determined by the overall balance of these factors. Given that antiinflammatory factors are induced in the interfacial membrane, a better understanding of the factors that most effectively block bone resorption, and that cells that produce such factors will enhance the ability to mitigate the deleterious effects of particulate wear debris, leading to an increase in the longevity of reconstructed joints.

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Bigler C F, Norris D A, Weston W L, Arend W P. Interleukin-1 receptor antagonist production by human keratinocytes. J Invest Dermatol 1992; 98 (1): 38-44.

Butch A W, Chung G-H, Hoffmann J W, Nahm M H. Cytokine expression by germinal center cells. J Immunol 1993; 150 (1): 39-47.

Chernoff A E, Granowitz E V, Shapiro L, Vannier E, Lonnemann G, Angel J B, Kennedy J S, Rabson A R, Wolff S M, Dinarello C A. A randomized, controlled trial of IL-10 in humans. J Immunol 1995; 154 (10): 5492-9.

Chomarat P, Vannier E, Dechanet J, Rissoan M C, Banchereau J, Dinarello C A, Miossec P. Balance of IL-1 receptor antagonist/IL-1ß in rheumatoid synovium and its regulation by IL-4 and IL-10. J Immunol 1995; 154 (3): 1432-9.

De Wall Malefyt R, Haanen J, Spits H, Roncarolo M-G, te Velde A, Figdor C, Johnson K, Kastelein R, Yssel H, de Vries JE. Interleukin 10 (IL-10) strongly reduces antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via downregulation of class II major histocompatibility complex expression. J Exp Med 1991a; 174 (4): 915-24.

De Wall Malefyt R, Abrams J, Bennett B, Figdor C G, de Vries J E. Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: An autoregulatory role of IL-10 produced by monocytes. J Exp Med 1991b; 174 (5): 1209-20.

Dripps D J, Brandhuber B J, Thompson R C, Eisenberg S P. Interleukin-1 (IL-1) receptor antagonist binds to the 80-kDa IL-1 receptor but does not initiate IL-1 signal transduction. J Biol Chem 1991; 266 (16): 10331-6.

Esnault S, Benbernou N, Lavaud F, Shin H C, Potron G, Guennounou M. Differential spontaneous expression of mRNA for IL-4, IL-10, IL-13, IL-2 and interferon-gamma (IFN-γ) in peripheral blood mononuclear cells (PBMC) from atopic patients. Clin Exp Immunol 1996; 103 (1): 111-8.

Firestein G S,Boyle D L,Yu C, Paine M M, Whisenand T D, Zvaifler N J, Arend W P. Synovial interleukin-1 receptor antagonist and interleukin-1 balance in rheumatoid arthritis. Arthritis Rheum 1994; 37 (5): 644-52.

Haskill S, Martin G, van Le L, Morris J, Pease A, Bigler C F, Jaffe G J, Hammerberg C, Sporn S A, Fong S, Arend W P. cDNA cloning of an intracellular form of the human interleukin 1 receptor antagonist associated with epithelium. Proc Natl Acad Sci USA 1991; 88 (9): 3681-5.

Heuer M, Aust G, Ode-Hakim S, Scherbaum W A. Different cytokine mRNA profiles in Graves' disease, Hashimoto's thyroiditis, and nonautoimmue-thyroid disorders determined by quantitative reverse transcriptase polymerase chain reaction (RT-PCR). Thyroid 1996; 6 (2): 97-106.

Horowitz S M, Doty S B, Lane J M, Burstain A H. Studies of the mechanism by which the mechanical failure of polymethylmethacrylate leads to bone resorption. J Bone Joint Surg (Am) 1993; 75 (6): 802-13.

Isomäki P, Luukkainen R, Saario R,.Toivanen P, Punnonen J. Interleukin-10 functions as an antiinflammatory cytokine in rheumatoid synovium. Arthritis Rheum 1996; 39 (3): 386-95.

Jiranek W A, Machado M, Jasty M, Jevsevar D, Wolfe H J, Goldring S R, Goldberg M J, Harris W H. Production of cytokines around loosened cemented acetabular components. Analysis with immunohistochemical techniques and in situ hybridization. J Bone Joint Surg (Am) 1993; 75 (6): 863-79.

Kasama T, Strieter R M, Lukacs N W, Lincoln P M, Burdick M D, Kumkel S L. Interleukin-10 expression and chemokine regulation during the evolution of murine type II collagen-induced arthritis. J Clin Invest 1995; 95 (6): 2868-76.

Konttinen Y T, Waris V, Xu J-W, Jiranek W A, Sorsa T, Virtanen I, Santavirta S. Transforming growth factor-ß1 and 2 in the synovial-like membrane between implant and bone in loosening of total hip arthroplasty. J Rheumatol 1997a; 24 (4): 694-701.

Konttinen Y T, Xu J W, Pätiälä H, Imai S, Waris V, Li T F, Goodman S B, Nordsletten L, Santavirta S. Cytokines in aseptic loosening of total hip replacement. Current Orthop 1997b; 11: 40-7.

Krzesicki R F, Hatfield C A, Bienkowski M J, McGuire J C, Winterrowd G E, Chapman D L, Berger A E, McEwan R N, Carter D B, Chosay J G, Tracey D E, Chin J E. Regulation of expression of IL-1 receptor antagonist protein in human synovial and dermal fibroblasts. J Immunol 1993; 150 (9): 4008-18.

McMahan C J, Slack J L, Mosley B, Cosman D, Lupton S D, Brunton L L, Grubin C E, Wignall J M, Jenkins N A, Brannan C I, Copeland N G, Huebner K, Croce C M, Cannizzarro L A, Benjamin D, Dower S K, Spriggs M K, Sims J E. A novel IL-1 receptor, cloned from B cells by mammalian expression, is expressed in many cell types. EMBO J 1991; 10 (10): 2821-32.

Mulheron G W, Bossert N L, Lapp J A, Walmer D K, Schomberg D W. Human granulosa-luteal and cumulus cells express transforming growth factors-beta type 1 and type 2 mRNA. J Clin Endocrinol Metab 1992; 74 (2): 458-60.

Rott O, Fleischer B, Cash E. Interleukin-10 prevents experimental allergic encephalomyelitis in rats. Eur J Immunol 1994; 24 (6): 1434-40.

Sadouk M, Pelletier J-P, Tardif G, Kiansa K, Cloutier J-M, Martell-Pelletier J. Human synovial fibroblasts coexpress IL-1 receptor type I and type II mRNA. The increased level of the IL-1 receptor in osteoarthritic cells is related to an increased level of the type I receptor. Lab Invest 1995; 73(3): 347-55.

Sims J E, Acres R B, Grubin C E, McMahan C J, Wingnall J M, March C J, Dower S K. Cloning the interleukin 1 receptor from human T cells. Proc Natl Acad Sci USA 1989; 86 (22): 8946-50.

Sims J E, Gayle M A, Slack J L, Alderson M R, Bird T A, Giri J G, Colotta F, Re F, Mantovani A, Shanebeck K, Grabstein K H, Dower S K. Interleukin 1 signaling occurs exclusively with the type 1 receptor. Proc Natl Acad Sci USA 1993; 90 (13): 6155-9.

Tang P-Z, Gannon M J, Andrew A, Miller D. Evidence for oestrogenic regulation of heat shock protein expression in human endometrium and steroid-responsive cell lines. Eur J Endocrinol 1995; 133 (5): 598-605.

Tucci M A, Baker R, Mohamed A, Tsao A K, Hughes J. Synovial tissues collected from rheumatoid patients undergoing total joint arthroplasty express markers for acute inflammation. Biomed Sci Instrum 1997; 34: 169-74.

Waris V, Xu J W, Nordsletten L, Sorsa T, Santavirta S, Konttinen Y T. Basic fibroblast growth factor (bFGF) in the synovial-like membrane around loose total hip prostheses. Scand J Rheumatol 1996; 25(4): 257-62.

Weyand C M, Geisler A, Brack A, Bolander M E, Goronzy J J. Oligoclonal T-cell proliferation and interferon-gamma production in periprosthetic inflammation. Lab Invest 1998; 78 (6): 677-85.

Wright T M, Goodman S B (eds). Implant wear: The future of total joint replacement. American Academy of Orthopaedic Surgeons, Illinois 1996.